

Hydrocarbon-released nestmate aggression in the Argentine ant, *Linepithema humile*, following encounters with insect prey

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Abstract

Argentine ants, *Linepithema humile*, were attacked by their nestmates following contact with a particular prey item, the brown-banded cockroach, *Supella longipalpa*. Contact with prey, as brief as 2 min, provoked nestmate aggression. Argentine ants contaminated with hydrocarbons extracted from *S. longipalpa* also released nestmate aggression behavior similar to that released by the whole prey item, confirming the involvement of hydrocarbons. In contrast to *S. longipalpa*, little or no nestmate aggression was induced by other ant prey from diverse taxa. A comparison of prey hydrocarbon profiles revealed that all hydrocarbons of *S. longipalpa* were very long chain components with 33 or more carbons, while other prey had either less, or none, of the very long chain hydrocarbons of 33 carbons or greater. We identified the hydrocarbons of *S. longipalpa* and some new groups of long chain hydrocarbons of *L. humile*. The majority of *S. longipalpa* hydrocarbons were 35 and 37 carbons in length with one to three methyl branches, and closely resembled two previously unidentified groups of compounds from *L. humile* of similar chain length. The hydrocarbons of *S. longipalpa* and *L. humile* were compared and their role in the Argentine ant nestmate recognition is discussed. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Nestmate recognition and discrimination against non-nestmates are critical components of social insect colony maintenance and defense. Members of different colonies of the same species are frequently aggressive towards each other (Hölldobler and Wilson, 1990), and it is generally

believed that nestmate recognition provides a basis for kin selection (Vander Meer and Morel, 1998). Signals used in nestmate recognition by social insects, including ants, are chemical in nature and genetically and/or environmentally based (Howard and Blomquist, 1982; Breed, 1983; Gamboa et al., 1986; Stuart, 1987; Nielsen et al., 1999). Recent direct evidence indicates that hydrocarbons mediate nestmate discrimination in several ant species (Lahav et al., 1999; Thomas et al., 1999; Boulay et al., 2000; Liang and Silverman, 2000).

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The Argentine ant, *Linepithema humile*, is a unicolonial, polygynous species distributed widely in regions with Mediterranean and mild temperate climates. It is an important agricultural and urban pest, and has negatively impacted on native communities by displacing many indigenous ants and other arthropods (Markin, 1970; Ward, 1987; Holway, 1999). Intra-specific aggression in Argentine ants is rare in non-native habitats, and this lack of intercolonial aggression is thought to contribute to the success of *L. humile* in its introduced range (Holway et al., 1998). Within its native range, intercolonial aggression in *L. humile* is common, genetic variation exceeds that in introduced populations, and the species does not appear to be ecologically dominant (Suarez et al., 1999; Tsutsui et al., 2000). We induced *L. humile* nestmate aggression by rearing fragments of an Argentine ant colony, collected from within its introduced range, on different insect diets (Liang and Silverman, 2000). These ants acquired the cuticular hydrocarbons from their insect prey. Workers from colonies reared in the laboratory, particularly those provided with brown-banded cockroaches, *Supella longipalpa*, as prey, displayed high levels of mutual aggression with workers from a field Argentine ant colony. Therefore, we were interested in determining whether *S. longipalpa*, in particular, transferred hydrocarbons to the Argentine ant, which may also be important in nestmate recognition.

In this report, we examined the degree of *L. humile* nestmate aggression following contact with *S. longipalpa* and various other prey. We also compared the hydrocarbon profiles of *S. longipalpa* and the other prey, with the profile of *L. humile*, to understand how relationships in chain length might contribute to aggression. Finally, we provided identifications of *L. humile* and *S. longipalpa* cuticular hydrocarbons, and based on hydrocarbon similarities between these species, we have suggested a possible explanation for the change in ant nestmate recognition following prey contact.

2. Materials and methods

2.1. Insects

Argentine ants used in behavioral experiments

were obtained from an indoor foraging trail of an outdoor field colony in Pleasanton, CA (Silverman and Nsimba, 2000). The ants from this foraging trail were provided with 0.25 M sucrose and live German cockroaches, *Blattella germanica*. Ants used for hydrocarbon identification were collected from a field colony that was not exposed to any supplied insect diets. Prey insects were procured as follows. Cockroaches, *Blattella germanica* and *Supella longipalpa* were reared in the laboratory and fed rat chow (Ralston Purina, St Louis, MO). Houseflies, *Musca domestica*, were reared in the laboratory and fed a diet of egg powder, yeast, milk powder and sucrose. Field-collected *Incisitermes minor* and *Zootermopsis nevadensis* were provided by V. Lewis (U.C. Berkeley). *Oncopeltus fasciatus*, *Acheta domesticus*, *Reticulitermes flavipes* and *Tenebrio molitor* were obtained from Carolina Biological Supply Co. (Burlington, NC). *Camponotus ferrugineus* was obtained from Connecticut Valley Biological (Southampton, MA). Workers were used for the termites, and larvae were used for *T. molitor*. For all other prey, adults were used in these studies.

2.2. Bioassay of prey-induced aggression behavior

Ants foraging on a 60 cm × 60 cm horizontal surface were presented with a single prey item within the center of a 15-cm diameter observation zone. The number of worker–worker aggressive encounters, defined as leg seizing and/or gaster flexing, occurring within this zone were counted over a 20-min period. Five replicates were performed for each prey species, with at least a 1-h interval between each replicate.

To evaluate the transfer rate of cuticular cues, 30 ants collected from foraging trails of a *B. germanica*-fed colony were confined in a jar with a freshly freeze-killed male *S. longipalpa* for 2, 5 and 30 min, after which the cockroach was removed. Also, a *S. longipalpa* was placed within this same ant colony, and when contacted by a worker the ant was immediately separated from the colony. In both situations, individual *S. longipalpa*-exposed ants were subsequently transferred to the nestmate foraging trail in the *B. germanica*-fed ant colony, and the number of *S. longipalpa*-contaminated workers attacked by nestmates within 20 encounters was recorded (13–15 replicates).

2.3. Bioassay of hydrocarbons

Twenty males, 20 females and 40 large nymphs of *S. longipalpa* were extracted twice with 10-ml hexane each. One-half of the extract (10 ml) was concentrated under a nitrogen stream to approximately 2 ml, then loaded onto a silica gel column (60–200 mesh, J.T. Baker, Phillipsburg, NJ) and the hydrocarbons were eluted with 10 ml hexane. The equivalent of one insect of crude extract or hydrocarbon elution (in 250 μ l hexane) was transferred into a 4-ml glass vial and dried under a nitrogen stream. Hexane was allowed to evaporate in a vial as a solvent control. We also considered that nestmate aggression elicited by *L. humile* following contact with *S. longipalpa* might be due to ants acquiring an excess of their own hydrocarbons, which were shared by *S. longipalpa*. To examine this possibility, a hexane extract from approximately 1000 *L. humile* workers was prepared as described above. Both insect extracts were assayed as follows. Argentine ant workers were collected from the foraging area. An individual ant was anesthetized by brief exposure to CO₂ and placed within the glass vial. The vial was tapped for approximately 5 s to maximize contact between the ant and the contaminated surface. The ant recovered before or during this agitation. It was then transferred back to the foraging area and observed for up to 20 encounters with nestmates. A positive attack response was recorded if the legs of the introduced ant were seized by one or more nestmates within 20 encounters. The contaminated ant and its attackers were subsequently removed from the foraging surface prior to the next replicate. Fifteen replicates were conducted for each extract and the solvent control.

2.4. Extraction, isolation and chemical analysis of cuticular hydrocarbons

Insects were extracted in hexane for 10 min followed by a brief secondary rinse. The extract was loaded onto a silica gel (60–200 mesh, J.T. Baker, Phillipsburg, NJ) mini-column and hydrocarbons were eluted with 5-ml hexane. The hydrocarbons were analyzed using 30 m \times 0.32 mm DB-5 capillary columns with an HP5890II or HP5880 GC, equipped with a flame-ionization detector, and interfaced with a HP GC ChemStation data acquisition system (Rev. A.05.02). Split-

less injection was made with temperature programmed from 80°C for 2 min, then increased to 270°C at 20°C/min and then increased to 310°C at 3°C/min. Both injector and detector were maintained at 320°C.

The extracted hydrocarbons of *L. humile* and *S. longipalpa* were further identified with a GC-Mass Spectrometer in EI mode (Finnigan 4023 interfaced with INCOS data system). The hydrocarbons were separated on a DB-5MS column (30 m \times 0.32 mm in diameter) with a temperature from 100 to 300°C at 5°C/min, then held at 300°C for 20 min. Hydrocarbons were identified based on fragment patterns.

3. Results

3.1. Nestmate aggression

Argentine ants immediately attacked a freshly-killed *S. longipalpa* placed in the foraging arena. Many workers contacting the cockroach were subsequently attacked by their nestmates. The aggression consisted of one or more workers seizing the legs of a nestmate. Attacking ants frequently were observed flexing their abdomen, appearing to deposit glandular materials. The ant being attacked was never observed to exhibit any counter-attack behavior. These intra-colony attacks were observed within 2 min of prey placement onto the foraging trail (Fig. 1 insert). Similarly, 30% of the ants elicited aggression from nestmates within 2 min when placed in direct contact with *S. longipalpa* (Fig. 2).

Although all prey species were attacked and consumed by *L. humile*, contact with *S. longipalpa* provoked significantly more intra-colony nestmate aggression than contact with other insect prey (Kruskal–Wallis test, $H = 26.53$, d.f. = 9, $P < 0.002$) (Fig. 1). Argentine ants contacting a hexane extract of *S. longipalpa* released nestmate aggression behavior similar to that after contact with a freshly-killed cockroach. A crude hexane extract elicited attacks in 14/15 (93.3%) replicates and silica gel-purified hydrocarbons released nestmate aggression in 13/15 (86.7%) replicates, while no nestmate attacks (0/15) were observed in the solvent control (chi-square = 33.89, d.f. = 2, $P < 0.0001$). Also, *L. humile* workers contaminated with an excess of their own hydrocarbons were not attacked by nestmates

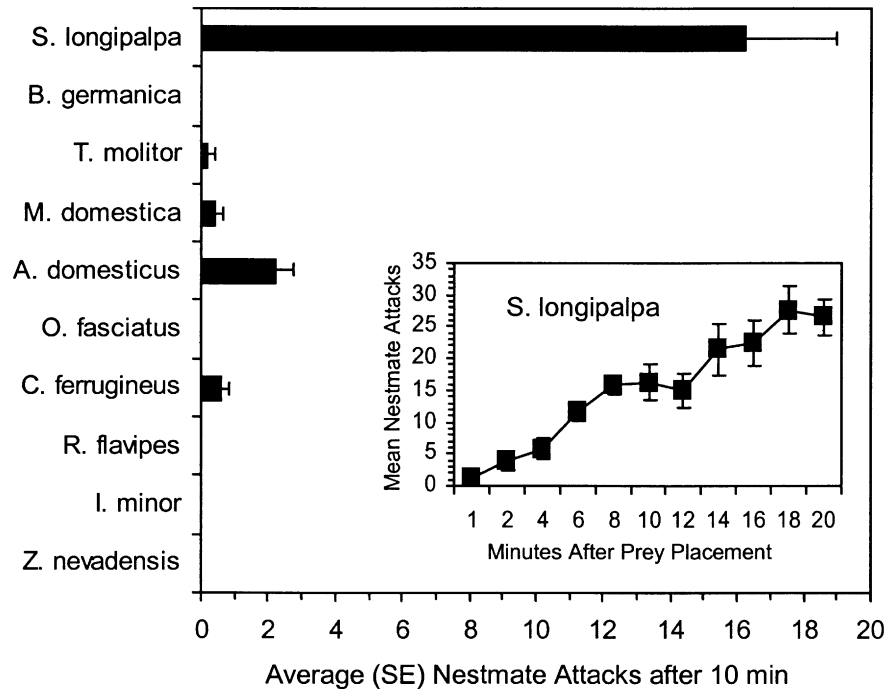


Fig. 1. Number (mean \pm S.E.) of Argentine ant, *Linepithema humile*, nestmate attacks observed within a 15-cm diameter observation zone 10 min after placement of a freshly-killed prey. The Argentine ants used for bioassay were from an indoor trail of an outdoor colony provided with sucrose water and live German cockroaches, *Blattella germanica*. Insert: number (mean \pm S.E.) of Argentine ant nestmate attacks over time observed within a 15-cm diameter observation zone after placement of a freshly-killed brown-banded cockroach, *Supella longipalpa*.

(0/20 aggressive responses). These results demonstrate that *L. humile* nestmate aggression was induced by contact with *S. longipalpa* cuticular hydrocarbons, which were acquired by the ants.

3.2. Comparison of prey hydrocarbon profiles

The hydrocarbon profiles of each insect prey were compared with that of *L. humile*. A broad range of different chain length hydrocarbons was present in the Argentine ant (Fig. 3). Unlike the hydrocarbon profile of field-collected Argentine ants (Fig. 4), the hydrocarbon profile of *L. humile* used in the bioassay (Fig. 3) also revealed hydrocarbons corresponding to those of *B. germanica*. This result was expected since these workers were obtained from an indoor trail of an outdoor colony that was provided with *B. germanica* as food (Liang and Silverman, 2000). Hydrocarbons of most prey species occurred within a narrow carbon chain length range (Fig. 3), however, *A. domesticus* and *O. fasciatus* contained a broad

range of hydrocarbons, including some with more than 35 carbons in length. *Acheta domesticus* released the second highest level of *L. humile* nestmate aggression, though substantially less than *S. longipalpa* (Fig. 1). While the hydrocarbons of all other insects corresponded primarily

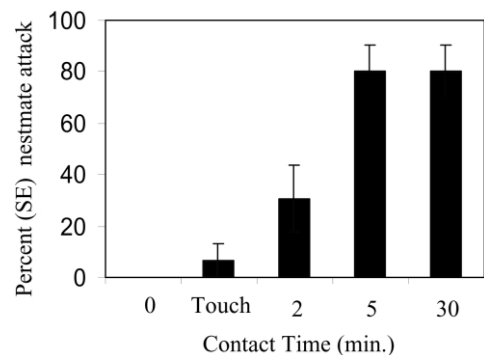


Fig. 2. Percent intra-colony nestmate aggression (\pm S.E.) elicited by individual Argentine ants which were in contact with *Supella longipalpa* for various durations. Touch = contact less than 5 s. The Argentine ants were from a colony provided with *Blattella germanica*.

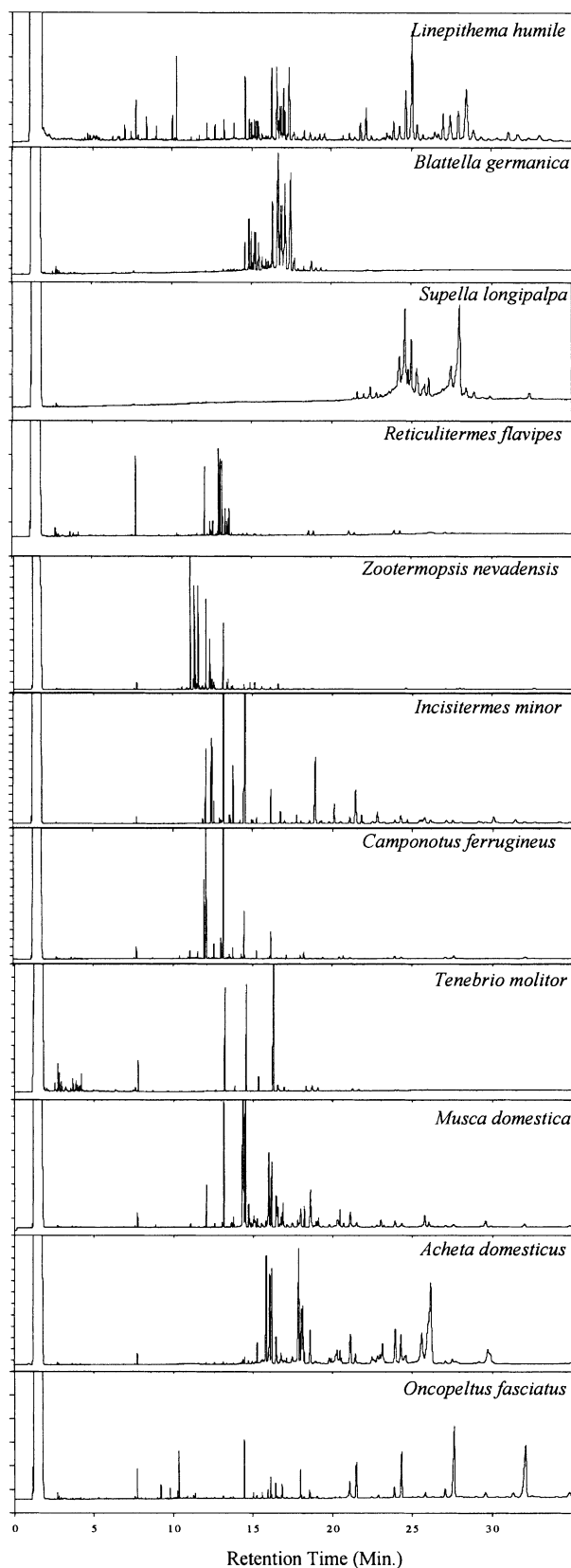


Fig. 3.

to the short or medium chain length portion of the *L. humile* hydrocarbon profile (< 20 min), *S. longipalpa* hydrocarbons overlapped the long chain hydrocarbon spectrum of *L. humile* (Fig. 3).

3.3. Hydrocarbon analysis

To further study the chemical basis of the aggression behavior, we analyzed the hydrocarbon composition of both the Argentine ant and the brown-banded cockroach. Field-collected *L. humile* contain many hydrocarbons between 14 and 37 carbons in length (Fig. 4; Table 1). The majority of the shorter chain hydrocarbons (< 30 carbons) are unbranched, whereas longer chain hydrocarbons are methyl-branched. Not reported previously in this species are compounds with chain lengths of 35 and 37 carbons, which include at least 10 compounds, all saturated and branched with one to three methyl groups. They comprise a significant proportion (approximately 40%) of the total hydrocarbons. Trimethyl hydrocarbons are more abundant than the mono- and di-methyl hydrocarbons combined. Trimethylpentatriacontane, the most abundant compound in *L. humile*, accounts for approximately 13% of the total hydrocarbons (Table 1).

Supella longipalpa hydrocarbons have chain lengths of 33, 35 and 37 carbons with one to three methyl branches (Fig. 5; Table 2). Compounds with chain lengths of 35 and 37 carbons represent the majority of the *S. longipalpa* hydrocarbons. Dimethyl hydrocarbons were more abundant than monomethyl and trimethyl components combined. 15,19-Dimethylpentatriacontane and 15,19- and 17,21-dimethylheptatriacontane were the most abundant compounds, comprising approximately 19 and 26% of the total hydrocarbons, respectively.

4. Discussion

4.1. Hydrocarbon transfer and nestmate aggression

Ants and other social insects use colony-specific odors to distinguish nestmates. We have demon-

Fig. 3. Hydrocarbon profiles of the Argentine ant, *Linepithema humile*, and of prey used in the aggression bioassay (Fig. 1). The Argentine ants were from an outdoor colony that was provisioned with German cockroaches.

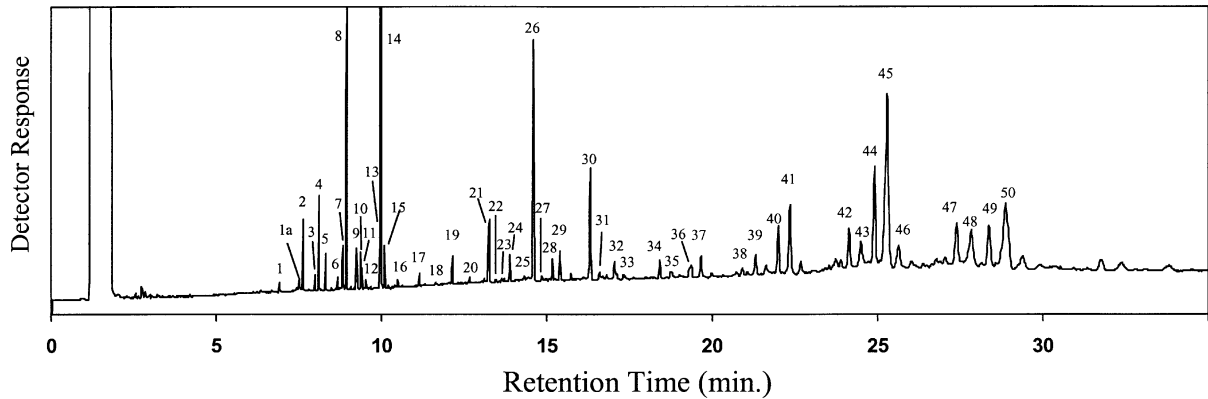


Fig. 4. Gas chromatogram of cuticular hydrocarbons of Argentine ant, *Linepithema humile*, workers. The ants were collected from a field colony that was not exposed to any supplied insect diets. Peaks and relative composition are listed in Table 1.

strated that *L. humile* nestmate recognition cues are altered following contact with *S. longipalpa*, thereby provoking nestmate aggression. The data, showing aggression when the *S. longipalpa* hydrocarbon was applied to ants, and no aggression with controls, provide strong evidence that hydrocarbons trigger the aggressive behavior. Allies et al. (1986) reported another example of intra-colonial aggression induction in *Leptothorax acervorum*, where the cuckoo ant, *L. kutteri*, provoked *L. acervorum* intra-nest attacks by depositing a secretion on the host, thereby diminishing its defensive response. Argentine ants contaminated with *S. longipalpa* hydrocarbons were naïve to the administered cuticular compounds, which probably explains why reciprocal aggression from the worker attacked by nestmates was never observed. However, earlier, Liang and Silverman (2000) and Silverman and Liang (2001) determined that *L. humile*, provided with *S. longipalpa* as a diet for an extended period (>1 month), incorporated *S. longipalpa* cuticular hydrocarbons, and displayed aggression towards workers that lacked these hydrocarbons.

Ants acquire odor labels from their environment that also serve in nestmate recognition. Nesting material strongly influences *Leptothorax nylanderi* colony odor (Heinze et al., 1996), while colony odor is affected by plant material retrieved by *Acromyrmex octospinosus* foragers (Jutsum et al., 1979). Diet, which includes insect prey, also contributes to colony-specific recognition odor and aggression towards non-nestmate conspecifics in: *Solenopsis invicta* (Obin and Vander Meer, 1988); *Rhytidoponera confusa* (Crosland, 1989);

and *L. humile* (Liang and Silverman, 2000). Hydrocarbons were found to be the chemical cues transferred from diet to *L. humile* ants resulting in aggression between colonies fed on different diets (Liang and Silverman, 2000). How the hydrocarbons were transferred from the diet to Argentine ants was not understood. We demonstrated that the hydrocarbons could be transferred from *S. longipalpa* to *L. humile* workers rather quickly. Following a 2-min contact with the prey, many Argentine ants obtained sufficient levels of the novel hydrocarbons to release aggression from their nestmates, suggesting that direct transfer through cuticular contact is an important route.

In contrast to previous observations of attacks by field-collected Argentine ants on Argentine ants reared on *B. germanica* (Liang and Silverman, 2000), the lack of aggression by resident workers towards nestmates contacting *B. germanica* may reflect either the low level of hydrocarbons transferred during the brief contact period or the origin of this resident colony which fed on *B. germanica*, in addition to sucrose and items in their outdoor environment. These ants acquired *B. germanica* hydrocarbons (Fig. 3) and, consequently, may not have recognized newly acquired *B. germanica* hydrocarbons as foreign.

4.2. Nestmate aggression and prey hydrocarbon profiles

Of the insect prey evaluated from diverse taxa, contact with *S. longipalpa* provoked the greatest number of nestmate attacks. *Supella longipalpa*

was also unique among these prey in that all its hydrocarbons have chain lengths of C33 or longer, which corresponds to the very long-chain hydro-

carbon portion of *L. humile*. In contrast, the other prey displayed less or no hydrocarbons within a similar range. It is interesting that the

Table 1
Hydrocarbons composition of *Linepithema humile* as identified by GC-MS

Peak no.	Compound	Average	S.E.	Identified by:
1	<i>n</i> -Tetradecane	0.5	0.4	Cavill and Houghton (1973)
1a	Pentadecene	< 0.5	–	Cavill and Houghton (1973)
2	<i>n</i> -Pentadecane	1.4	0.5	Cavill and Houghton (1973) Brophy et al. (1983)
3	5-Methylpentadecane	< 0.5	–	Cavill and Houghton (1973) Brophy et al. (1983)
4	3-Methylpentadecane	1.4	0.3	Cavill and Houghton (1973) Brophy et al. (1983)
5	<i>n</i> -Hexadecane	0.6	0.2	Cavill and Houghton (1973) Brophy et al. (1983)
6	2-Methylhexadecane	< 0.5	–	
7	Heptadecene	1.4	0.2	Cavill and Houghton (1973) Brophy et al. (1983)
8	<i>n</i> -Heptadecane	8.3	0.9	Cavill and Houghton (1973) Brophy et al. (1983)
9	5-Methylheptadecane	1.0	0.2	Cavill and Houghton (1973) Brophy et al. (1983)
10	3-Methylheptadecane	0.6	0.2	Cavill and Houghton (1973) Brophy et al. (1983)
11	Octadecene	< 0.5	–	Cavill and Houghton (1973) Brophy et al. (1983)
12	<i>n</i> -Octadecane	< 0.5	–	Cavill and Houghton (1973) Brophy et al. (1983)
13	Nonadecadiene	1.0	0.2	Cavill and Houghton (1973) Brophy et al. (1983)
14	Nonadecene	11.6	1.6	Cavill and Houghton (1973) Brophy et al. (1983)
15	<i>n</i> -Nonadecane	0.8	0.1	Cavill and Houghton (1973) Brophy et al. (1983)
16	3-Methylnonadecane	< 0.5	–	Cavill and Houghton (1973) Brophy et al. (1983)
17	<i>n</i> -Heneicosane	< 0.5	–	Cavill and Houghton (1973) Brophy et al. (1983)
18	<i>n</i> -Docosane	< 0.5	–	Cavill and Houghton (1973) Brophy et al. (1983)
19	<i>n</i> -Tricosane	0.8	0.0	Cavill and Houghton (1973) Brophy et al. (1983)
20	<i>n</i> -Tetracosane	< 0.5	–	Cavill and Houghton (1973) Brophy et al. (1983)
21	<i>n</i> -Pentacosane	1.8	0.3	Cavill and Houghton (1973) Brophy et al. (1983)
22	11- and 13-Methylpentacosane	< 0.5	–	Brophy et al. (1983)
23	3-Methylpentacosane	< 0.5	–	Brophy et al. (1983)
24	<i>n</i> -Hexacosane	0.6	0.0	Cavill and Houghton (1973) Brophy et al. (1983)
25	2-Methylhexacosane	< 0.5	–	
26	<i>n</i> -Heptacosane	8.3	0.9	Cavill and Houghton (1973) Brophy et al. (1983)
27	11- and 13-Methylheptacosane	< 0.5	–	Brophy et al. (1983)
28	3-Methylheptacosane	0.6	0.0	Brophy et al. (1983)
29	<i>n</i> -Octacosane	0.9	0.0	Brophy et al. (1983)
30	<i>n</i> -Nonacosane	3.4	0.1	Brophy et al. (1983)

Table 1 (Continued)

Peak no.	Compound	Average	S.E.	Identified by:
31	11- and 13- and 15-Methyl-nonacosane	< 0.5	–	Brophy et al. (1983)
32	3-Methylnonacosane	0.8	0.3	Brophy et al. (1983)
33	<i>n</i> -Triacosane	< 0.5	–	Brophy et al. (1983)
34	<i>n</i> -Hentriacosane	0.7	0.0	Brophy et al. (1983)
35	11- and 13- and 15-Methyl-hentriacosane	< 0.5	–	Brophy et al. (1983)
36	Dimethylhentriacosane	1.0	0.2	
37	Trimethylhentriacosane	1.0	0.1	
38	<i>n</i> -Tritriacosane	< 0.5	–	Brophy et al. (1983)
39	13- and 15-Methyltritriacosane	0.9	0.0	
40	5,15- and 5,17-Dimethyltritriacosane	2.1	0.0	
41	5,15,19-Trimethyltritriacosane	3.4	0.1	
42	13- and 15- and 17-Methylpentatriacontane	2.1	0.5	
43	15,19-Dimethylpentatriacontane	1.9	0.7	
44	5,15- and 5,17-Dimethylpentatriacontane	5.5	0.4	
45	5,13,17- and 5,15,19-Trimethylpentatriacontane	11.9	0.7	
46	3,13,17- and 3,15,17-Trimethylpentatriacontane	1.8	0.1	
47	13- and 15- and 17- and 19-Methylheptatriacontane	2.7	0.3	
48	15,19-Dimethylheptatriacontane	3.3	0.7	
49	5,15- and 5,17-Dimethylheptatriacontane	2.8	0.2	
50	5,15,19- and 5,13,17-Trimethylheptatriacontane	6.7	0.4	

The Argentine ants were collected from a field colony that was not exposed to any supplied insect diets. Average percentage was based on three separate runs of the same extract. See Fig. 4 for GC profile.

insect which gives the second highest response (although much lower than *S. longipalpa*) is *A. domesticus*, which has a small group of long-chain hydrocarbons similar to that of *S. longipalpa* hydrocarbons in length. The prey hydrocarbon pro-

files generated in this study were similar to those reported earlier for: *R. flavipes* (Howard et al., 1978); *B. germanica* (Augustynowicz et al., 1987; Jurenka et al., 1989); *A. domesticus* (Blomquist et al., 1976); *M. domestica* (Nelson et al., 1981); *Z.*

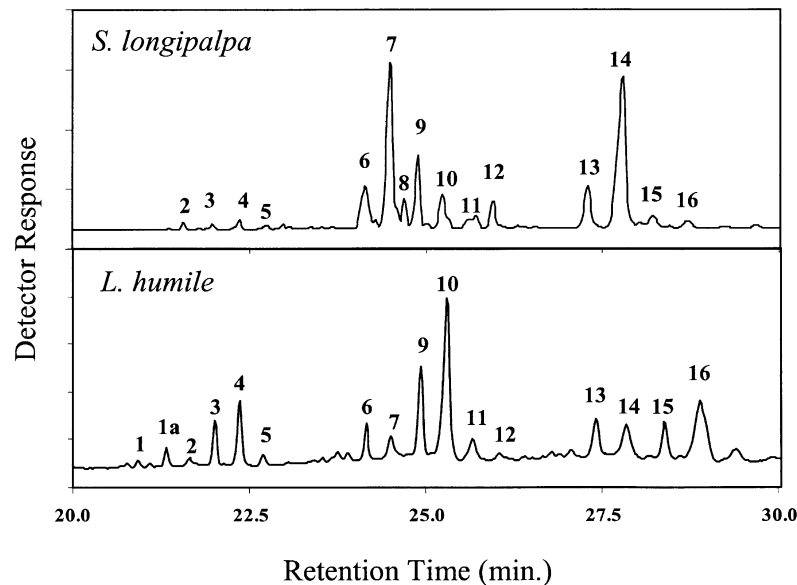


Fig. 5. Gas chromatogram of (a) cuticular hydrocarbons of brown-banded cockroach, *Supella longipalpa* and (b) long chain hydrocarbons of Argentine ant, *Linepithema humile*. Peaks and relative composition are listed in Table 2. See Figs. 3 and 4 for complete profiles.

Table 2
Hydrocarbon composition of *Supella longipalpa* in comparison with long chain length hydrocarbons of *Linepithema humile*

Peak no.	<i>Supella longipalpa</i>			<i>Linepithema humile</i>		
	Compound	Average	S.D.	Compound	Average	S.D.
1	None			<i>n</i> -Triacontane	< 1.0%	–
1a	Unknown	< 1.0%	–	13- and 15-Methyltriacontane	< 1.0%	–
2	5-Methyltriacontane	< 1.0%	–	Unknown		
3	5,9-Dimethyltriacontane	1.0	0.6	5,15- and 5,17-Dimethyltriacontane	2.1	0.0
4	3,7- and 3,9-Dimethyltriacontane	1.6	0.7	5,15,19-Trimethyltriacontane	3.4	0.1
5	3,7,23-Trimethyltriacontane etc.	1.3	1.2	Unknown	< 1.0%	–
6	7- and 9- and 11- and 13- and 15- and 17-Methyl-pentatriacontane	10.4	2.6	13- and 15- and 17-Methyl-pentatriacontane	2.1	0.5
7	15,19-Dimethylpentatriacontane	19.0	5.0	15,19-Dimethylpentatriacontane	1.9	0.7
8	7,11- and 7,13- and 7,15-Dimethyl-pentatriacontane	3.3	0.3	None	–	–
9	5,9- and 5,11-Dimethylpentatriacontane	9.1	0.9	5,15- and 5,17-Dimethylpentatriacontane	5.5	0.4
10	5,9,17- and 5,11,15-Trimethyl-pentatriacontane	6.2	0.8	5,13,17- and 5,15,19-Trimethylpentatriacontane	11.6	0.7
11	3,7,21-Trimethyl-pentatriacontane etc.	4.0	1.1	3,13,17- and 3,15,17-Trimethylpentatriacontane	1.8	0.1
12	<i>x,y,z</i> -Trimethylpentatriacontane	4.1	1.0	Unknown	< 1.0%	–
13	13- and 15- and 17- and 19-Methyl-heptatriacontane	8.5	2.7	13- and 15- and 17- and 19-Methylheptatriacontane	2.7	0.3
14	15,19- and 17,21-Dimethyl-heptatriacontane	25.7	3.8	15,19-Dimethylheptatriacontane	3.3	0.7
15	5,13- and 5,17- and 5,19-Dimethyl-heptatriacontane	2.9	0.8	5,15- and 5,17-Dimethylheptatriacontane	2.8	0.2
16	Unknown	2.4	2.2	5,15,19- and 5,13,17-Trimethylhepta- triacontane	6.7	0.4

Average percentage was based on three separate runs of the same extract. See Fig. 5 for GC profile.

nevadensis (Haverty et al., 1988); and *T. molitor* larvae (Bursell and Clements, 1967).

4.3. Comparative hydrocarbon analysis

The Argentine ant produces a large number of hydrocarbons ranging from 14 to 37 carbons in chain length. Our results (Fig. 4; Table 1) largely agree with earlier reports by Cavill and Houghton (1973) and Brophy et al. (1983), who reported hydrocarbons up to 33 carbons in length, except that no dotriacontane and tetratriacontane were found. However, by using capillary columns and a higher oven temperature for the GC analysis, better separation was obtained for long chain hydrocarbons and consequently, we identified 14 additional compounds of 31 carbons or more in chain length. The very long chain hydrocarbons with C35 and C37 in chain length also represent a large portion of the total hydrocarbons (approx. 40%). It is interesting to note that while all *L.*

humile hydrocarbons shorter than 33 carbons in length are either *n*-alkanes, alkenes or monomethylalkanes, hydrocarbons greater than 33 carbons in length are mostly alkanes with two or three methyl groups, with trimethylalkanes being the most abundant. This complexity may indicate important biological functions for these very long chain hydrocarbons.

The hydrocarbons of *S. longipalpa*, which released nestmate aggression, occur within a narrow size range with most being C35 or C37 in chain length. This narrow hydrocarbon range is consistent with the profiles of most other cockroach species examined (Augustynowicz et al., 1987; Carlson and Brenner, 1988; Jurenka et al., 1989; Everaerts et al., 1997) with the exception of *Macropanesthia rhinoceros* (Brown et al., 2000). *Supella longipalpa* hydrocarbons closely resembled the long chain hydrocarbons of *L. humile* yet revealed certain qualitative and quantitative differences (Fig. 5; Table 2). Most *S. longipalpa*

hydrocarbons were also present in *L. humile*, such as: some monomethylalkanes; 15,19-dimethylpentatriacontane; and 15,19-, and 5,17-dimethylheptatriacontane. Among them, 15,19-dimethylpentatriacontane and 15,19-dimethylheptatriacontane were abundant in *S. longipalpa* but much less abundant in *L. humile* (19.0 and 25.7% compared to 1.9 and 3.3%, respectively). The dominant hydrocarbons in *L. humile* were trimethylalkanes, while those in *S. longipalpa* were dimethylalkanes. Some hydrocarbons present in *S. longipalpa* were not found in *L. humile*, such as 7,*x*-dimethylpentatriacontane. Therefore, adding *S. longipalpa* hydrocarbons to *L. humile* ants would potentially alter the relative ratio of hydrocarbons already present as well as introduce novel compounds to *L. humile*.

4.4. Role of hydrocarbons in nestmate recognition

Hydrocarbons have been implicated as an important cue for nestmate recognition in ants (Hölldobler and Wilson, 1990) with direct evidence recently provided for: *Cataglyphis niger* (Lahav et al., 1999); *Iridomyrmex purpureus* (Thomas et al., 1999); and *Linepithema humile* (Liang and Silverman, 2000). Yet, little is known of how hydrocarbons are used as nestmate recognition cues. Our combined behavioral and chemical data suggest that the long chain hydrocarbons in *L. humile* may be more important for nestmate recognition than its shorter chain hydrocarbons. The long chain hydrocarbons of *L. humile* represent a large portion of the total amount of hydrocarbons and are more complex with one to three methyl branches, suggesting possible biological roles. Furthermore, some short chain hydrocarbons (C14–C24) found in a field population of *L. humile* were much reduced in quantity, or absent, in ant colonies reared in the laboratory (Liang and Silverman, 2000). While this does not indicate that those short chain hydrocarbons are not inherent to *L. humile*, being found in ants at different geographical locations and time (Cavill and Houghton, 1973; Brophy et al., 1983; Table 1), it suggests that the long chain hydrocarbons would provide more persistent chemical cues for nestmate recognition. Finally, *L. humile* workers contaminated with long chain prey hydrocarbons (C35 and C37) were recognized as alien and attacked while prey hydrocarbons of shorter chain

length did not interfere with nestmate recognition in this species.

Critical qualitative or quantitative changes to *L. humile* recognition cues must have occurred when the ants were contaminated with *S. longipalpa* hydrocarbons. These changes were apparently not due to excess ant hydrocarbons, since adding a complete blend of *L. humile* hydrocarbons did not induce any aggression. Yet, a few related compounds, particularly 15,19-dimethyl C35 and C37, present in much larger quantities in *S. longipalpa* than *L. humile*, may be critical to the recognition process by changing ant hydrocarbon ratios. The proportion of certain hydrocarbons was thought to be important in subcaste recognition in *Camponotus vagus* (Bonavita-Cougourdan et al., 1993) and in nestmate recognition in *C. fellah* (Boulay et al., 2000). However, quantitative differences in the major hydrocarbons between field and lab colonies of the red imported fire ant, *Solenopsis invicta*, failed to explain intercolony aggression (Obin, 1986). Alternatively, the aggression behavior may have been released by *S. longipalpa* hydrocarbons novel to *L. humile* such as 7,*x*-dimethylpentatriacontane. Our results did not distinguish between these two hypotheses, and further investigation is needed. *Supella longipalpa* hydrocarbons may release Argentine ant aggression because they are similar enough to *L. humile* hydrocarbons to be recognized (i.e. they contain receptors on their antennae to recognize them), but also qualitatively or quantitatively different enough to be perceived as originating from a competing colony.

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