NESTMATE RECOGNITION: THE ROLE OF CUTICULAR HYDROCARBONS IN THE ANT *CAMPONOTUS VAGUS* SCOP.

A. Bonavita-Cougourdan,[1] J. L. Clément,[2] and C. Lange[3]
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ABSTRACT

Both behavioral and chemical analyses demonstrated that in the ant *Camponotus vagus* the colony recognition signal is strongly correlated with the composition of cuticular hydrocarbons. Variation of relative proportions of dimethylalkanes characterize the chemical signatures in different colonies.

Key Words: *Camponotus vagus*, cuticular hydrocarbons, nestmate recognition.

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INTRODUCTION

In ants as in most social insects, members of a colony are able to discriminate nestmates and alien individuals of the same species belonging to other colonies. This discrimination is one of the principal elements in the social organization. It is connected in many species with a very strong aggressive behavior towards alien individuals.

The paramount problem of nestmate recognition is to determine what perceptual mechanisms and recognition cues are involved. Various experiments suggest that in ants the cues are chemical (cf. Hölldobler and Michener 1980). Some hypotheses have been proposed about the nature and the origin of compounds responsible for the nest odor: volatile compounds incorporated in cuticular wax (Jaffé and Marcuse 1983; Wilson 1971), mandibular gland secretions (Jaffé 1980; Jaffé and Sanchez 1984) and cuticular hydrocarbons (Howse 1975). In termites, the importance of cuticular hydrocarbons has been demonstrated in species recognition (Howard et al. 1982; Clément and Lange 1984; Clément et al. 1985). It has been suggested that similar cuticular hydrocarbons allowed integration of termitophiles and myrmecophiles into the termite and ant colonies (Howard et al. 1980; Vander Meer et al. 1982). However, no experimental analysis using both behavioral bioassays directly testing chemicals and determination of these chemicals has been presented to substantiate these hypotheses. The aim of this paper is to determine the role of cuticular hydrocarbons in nestmate recognition of the ant *Camponotus vagus*, using both behavorial and chemical analyses. This ant is an european monogynous species living in stumps or fallen trees whose cuticular hydrocarbon proportions vary between nests (Bonavita-Cougourdan and Clément 1986).

[1] C.N.R.S./Ethologie, 31, Chemin Joseph Aiguier, 13402 Marseille Cedex 9, France and Université de Provence, Marseille France.

[2] Laboratoire d'Evolution (C.N.R.S., UA 681), Université Pierre et Marie Curie, 105, bd Raspail, 75006 Paris, France.

[3] Laboratoire de Chimie Organique Structurale (C.N.R.S., UA 455), Université Pierre et Marie Curie, 4, place Jussieu, 75005 Paris, France.

MATERIALS AND METHODS

Chemistry

We extracted 60 samples of cuticular hydrocarbons by immersing, for each, four heads or four thoraces or two heads and two thoraces of foraging workers, previously killed by freezing, in 3 ml of pentane for 3 minutes. The samples were dried under pure nitrogen. The solid part was analyzed by gas-liquid chromatography (G.C.) and by gas chromatography/mass spectrometry (G.C./M.S.). We used a DELSI 300 gas chromatograph equipped with a flame ionization detector and a CPSIL 5 capillary (WCOT) column CHROMPACK 25 m, 0.22 mm diameter. G.C. analyses for cuticular hydrocarbons utilized temperature programming from 140°C to 320°C at 1°C/min. Retention times were compared to those of alkane standards and coinjected. An UNICA 10 integrator gave the relative proportion of each peak. The quantity of variation between nests and between head and thorax was evaluated by the following formula where A and B are relative proportions of the same peak, and A > B:

$$F = \frac{[A - B]}{[B]} \times 100$$

Gas chromatography/mass spectrometry analyses were performed on NERMAG R1010C, using a SIDAR 8 computer. Ionisation was performed under electronic impact (70 ev) and chemical ionisation with CH4 (0,05 t). The extracts were separated by thin layer chromatography (T.L.C.) on 10% silver nitrate silica gel plates developed in benzene (8), hexane (92) and scraped into vials followed by the addition of 50 μl of benzene. We compared on G.C. (temperature programming from 40°C to 320°C at 1°C/mn): 1) head and thorax extract without desiccation (cuticular hydrocarbons and volatile compounds from exocrine glands), 2) head and thorax desiccated extract (non-volatile compound only), 3) mandibular gland undesiccated extract.

Ethology

We conducted ethological analysis on 21 colonies collected in southeastern France and we chose 6 nests (three couples) who exhibited a strong aggressive behavior when a dead or living individual from one colony was introduced into the foraging area of the other. We reported here partial analyses of behavior for two couples and a complete analysis for one pair of colonies only. The number of aggressive acts exhibited by resident ants towards alien workers varies between colonies; so the numeric data of the 3 experiments could not be summed.

Ethological tests consisted of various random introduction of either foraging workers or chemically treated surrogates or lures into the foraging area of one nest (colony R). Workers (alien A or resident R) were either alive or killed by freezing. For some experiments we used workers deprived of their heads or thoraces or abdomens. Lures were made of dead resident workers immersed with three successive changes of acetone (6 ml) for 5 min and five changes of pentane (6 ml) for 10 min, then air-dried for 10 min followed by immersion for 3 min in the experimental sample (extract of head or thorax or head and thorax of alien or resident ant). The lure was then air-dried for 10 min before testing. Eleven categories of tests were performed with ants or lures:

1. Alien alive worker: A
2. Alien dead worker: DA
3. Resident alive worker: R
4. Resident dead worker: DR
5. Resident washed worker: W
6. Lure covered with alien worker head extract: WAH
7. Lure covered with alien thorax extract: WAT
8. Lure covered with alien head and thorax extract: WAHT
9. Lure covered with resident worker head extract: WRH
10. Lure covered with resident thorax extract: WRT
11. Lure covered with resident head and thorax extract: WRHT

Different kinds of behavioral responses of resident ants were noted and divided into two types: mandibular opening, nibbling, snatching up, snatching with traction (agonistic behavioral units, type I), abdominal curving with venom spraying (agonistic behavioral unit, type II). The most aggressive responses are those of type II. Behavioral responses were observed for 15 minutes, each 30 seconds for type I and continuously for type II. Ten tests were performed for each category. A statistical analysis using the Mann-Whitney U-test compared the responses of resident workers. We consider, according to the quantity of resident workers in the area of foraging, that workers responsible of aggressive behavior are different from one test to another.

RESULTS

Chemistry

Combined G.C., T.L.C. and G.C./M.S. analyses indicated that samples were composed of saturated hydrocarbons only. Comparison of G.C. traces of the three extracts (undesiccated head and thorax extract, desiccated head and thorax extract and mandibular extract) showed that the volatile compounds synthetised by the exocrine glands were completely eliminated by the nitrogen desiccation.

These analyses indicated that 32 linear and branched saturated hydrocarbons (25 to 35 carbons) constituted the major components of the blend (Table 1). The relative proportions of each component vary from one nest to the other (Fig. 1) and from head to thorax of workers from the same nest. These differences are stable. Relative proportions of the heaviest hydrocarbons are higher in the head than in the thorax. Cuticular hydrocarbons proportions are significantly different between societies in each couple where aggression is high, especially in dimethylalkanes.

The most variable compounds between colonies used in the complete ethological analysis (F more than 100) are 1°) dimethylalkanes with 32 carbons (peak 15:11, 15 diMe C30; 13, 17 diMe C30), 33 carbons (peak 20:5, 11 diMe C31, peak 22:5, 17 diMe C31; 5, 19, diMe C31), 34 carbons (peak 27:11, 20 diMe C32) and 35 carbons (peak 31:5, 15 diMe C33; 5, 17 diMe C33, peak 32:5, 19 diMe C33; 5, 21 diMe C33. 2°) monomethylalkanes with 27 carbons (peak 2:2 Me C26), 32 carbons (peak 19:7 Me C31, 9 Me C31), and 3°) two n alkanes (peak 1:nC25, peak 2:nC27).

Table 1. Cuticular hydrocarbons of *Camponotus vagus* head and thorax for colonies N1 or R (resident) and N2 or A (alien) in couple C1: identification and percent composition.

Peak	Component	MW	Diagnostic IE-MS ions	(i)	Diagnostic IC-CH$_4$-MS ions	Percent composition: Head Society A (N1)	Head Society R (N2)	Thorax Society A (N1)	Thorax Society R (N2)	*: F up to 100: Between societies in Head	Between societies in Thorax	Between head and thorax in society A (N1)	Between head and thorax in society R (N2)
1	nC$_{25}$	352		351	, , , , ,	11,43	0, 3	7,01	1,33	*	*		
2	nC27†	380		379		2,56	0,85	2,26	1,60	*			
	2Me-C26	380	43,364/365	379	365								
3	4MeC28	408	70/71,364/365	407	365,393	1,27	0,66	0,64	1,35				
4	nC29	408		407		3,38	1,70	3,15	3,65				
5	11MeC29†	422	168/169,280/281	421	169,365,407	3,23	2,00	2,26	2,46				
	13MeC29†	422	196/197,252/253	421	197,365,407								
	15MeC29	422	224/225	421	225,407								
6	7MeC29	422	112/113,336/337	421	113,337,407	0,72	1,18	0,49	1,49		*		
7	11,15diMeC29†	436	168,224,239,295	435	169,225,239,295,421	0,40	0	1,94	0	*	*	*	
	13,17diMeC29	436	196,267	435	197,267,421								
8	5,15diMeC29†	436	84,224,239,379	435	225,239,365,421								
	5,17diMeC29†	436	84,196,267,379	435	197,267,365,421	0,40	0,28	0,40	0,83				
	5,19diMeC29	436	84,168,295,379	435	169,295,365,421								
9	3MeC29†	422	56/57,392/393	421	393,407	0	0,23	0,25	0,77	*	*	*	*
	6MeC29	422	84/85,350/351	421	351,407								
10	nC30	422		421		1,75	1,17	2,27	1,67				
11	11MeC30†	436	168/169,294/295	435	169,295,421	0,38	0	4,81	0,33	*	*	*	*
	13MeC30	436	196/197,266/267	435	197,267,421								
12	xMeC30	436		435		1,21	1,04	1,22	1,14				
13	yMeC30	436		435		0,85	0,20	7,90	0,88	*	*	*	*
14	4MeC30	436	70/71,392/393	435	393,421	21,13	8,75	21,32	16,48	*			
15	11,15diMeC30†	450	168,238,239,309	449	169,239,309,435	1,01	2,19	0,57	1,92	*	*		
	13,17deMeC30	450	196,210,267,281	449	197,211,267,281,435								
16	nC31	436		435		3,71	2,60	4,18	4,53				
17	3,25diMeC31	464	56,112,379,435	463	113,379,435,449	0	1,01	0,67	1,29	*		*	
	4,26diMeC31	464	70,98,393,421	463	393,421,449								
18	11MeC31†	450	168/169,308/309	449	169,309,435	12,61	18,77	8,33	16,69		*		
	13MeC31†	450	196/197,280/281	449	197,281,435								
	15MeC31	450	224/225,252/253	449	225,253,435								
19	7MeC31†	450	112/113,364/365	449	113,365,435	0,86	2,30	1,06	2,46	*	*		
	9MeC31	450	140/141,336/337	449	141,337,435								
20	5,11diMeC31	464	84,183,308,407	463	183,309,407,449	0,92	4,49	2,34	3,96	*			
21	5,13diMeC31†	464	84,211,280,407	463	211,281,407,449	1,86	1,80	7,96	1,79		*	*	
	5,15diMeC31	464	84,239,252,407	463	239,253,407,449								
22	5,17diMeC31†	464	84,224,267,407	463	225,267,407,449	0	2,84	0	1,16	*	*		*
	5,19diMeC31	464	84,196,295,407	463	197,295,407,449								

Table 1. Continued.

23	5,21diMeC31 †	464	84,168,323,407	463	169,323,407,449	0,78	1,48	0,32	1,23		*	*
	5,23diMeC31	464	84,140,351,407	463	141,351,407,449							
24	5,25diMeC31 †	464	84,112,379,407	463	113,379,407,449	0	2,69	0,52	2,18	*	*	*
	5,27diMeC31	464	84,407	463	407,449							
25	11MeC32 †	464	168/169,322/323	463	169,323,449	1,97	2,58	1,29	2,01			
	13MeC32	464	196/197,294/295	463	197,295,449							
26	7MeC32 †	464	112/113,378/379	463	113,379,449	2,41	1,65	2,12	1,85			
	5,20diMeC32 †	478	84,196,309,421	477	197,309,421,463							
	5,23diMeC32	478	84,154,351,421	477	155,351,421,463							
27	11,20diMeC32	478	168,196,309,337	477	169,197,309,337,463	1,31	3,51	1,03	2,64	*	*	
28	11MeC33 †	478	168/169,336/337	477	169,337,463	8,33	14,65	5,34	10,62			
	13MeC33 †	478	196/197,308/309	477	197,309,463							
	15MeC33	478	224/225,280/281	477	225,281,463							
29	2,19diMeC33 †	492	224,295,477	491	225,295,477	13,97	15,15	7,49	9,75			
	3,21diMeC33 †	492	56,323,196,463	491	197,323,463,477							
	4,23diMeC33	492	70,168,351,449	491	169,351,449,477							
30	5,11diMeC33 †	492	84,183,336,435	491	183,336,435,477	1,45	1,55	0,86	0,86			
	5,13diMeC33	492	84,211,308,435	491	211,309,435,477							
31	5,15diMeC33 †	492	84,239,280,435	491	239,281,435,477	0	1,07	0	0,61	*	*	
	5,17diMeC33	492	84,252,267,435	491	253,267,435,477							
32	5,19diMeC33 †	492	84,224,295,435	491	225,295,435,477	0	0,68	0	0,47	*	*	
	5,21diMeC33	492	84,196,323,435	491	197,323,435,477							

† (i) Ion (M-H)

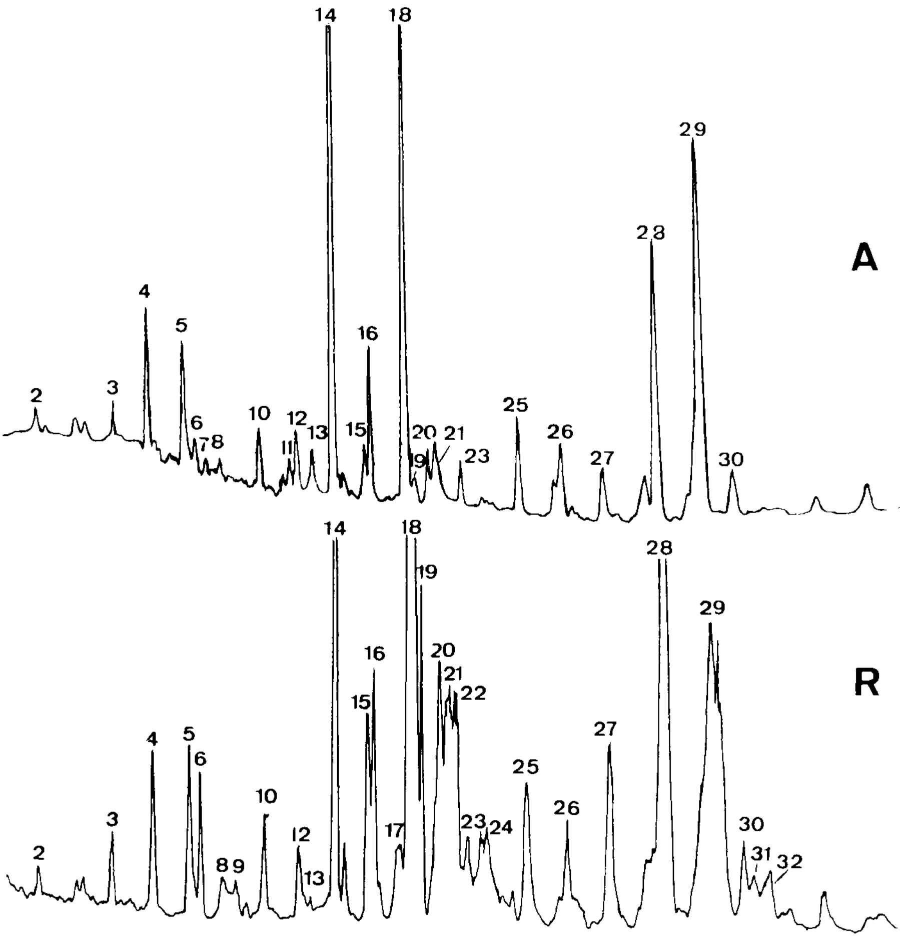

Fig. 1. Gas chromatograph traces of the total cuticular hydrocarbons of *Camponotus vagus* workers heads of colony N1 or R (resident) and N2 or A (alien) in couple C1.

Those dimethylalkanes which were shown to vary between aggressive colonies in each of the three couples are: 5, 11 diMe C31 (peak 20); 5, 17 diMe C31, 5, 19 diMe C31 (peak 22); 5, 15 diMe C33, 5, 17 diMe C33 (peak 31). Peaks 9, 11, 17 are branched alkanes and their relative proportions vary between head and thorax in the same nest and probably have no significance in nest recognition.

Ethology

The same level of aggressive responses appeared either with alien worker *in toto* or deprived of its head or thorax or abdomen. This indicates that the chemical signature is not carried by a single part of the body.

Resident workers (Fig. 2 and Tables 2 and 3) dead or alive, reintroduced in their own foraging area elicited no or very few aggressive responses and absolutely

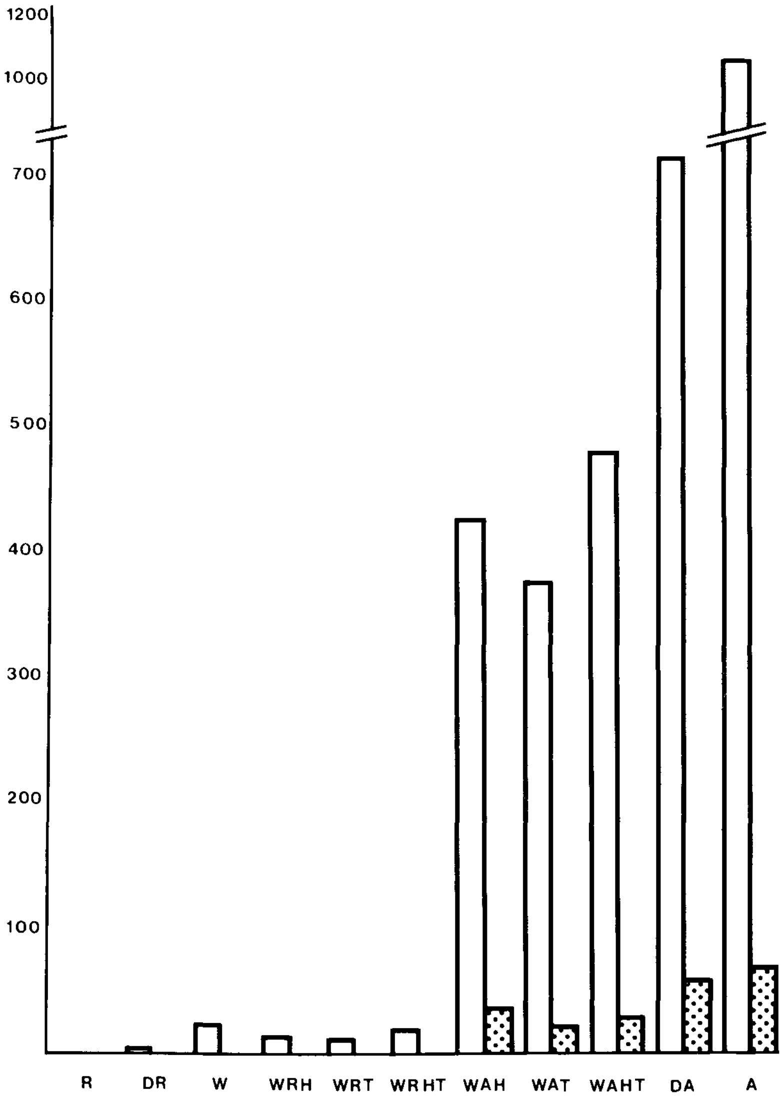

Fig. 2. Responses of resident workers against introduced worker or lure: total numbers of agonistic behavioral units for each category for 10 tests. White: type I units; stippled: type II units. R: resident alive worker; DR: resident dead worker; W: resident washed worker; WRH: lure covered with resident worker head extract; WRT: lure covered with resident thorax extract; WRHT: lure covered with resident head and thorax extract; WAH: lure covered with alien worker head extract; WAT: lure covered with alien thorax extract; WAHT: lure covered with alien head and thorax extract; DA: alien dead worker; A: alien living worker.

Table 2. Aggressive behavioral responses (couple C1) of resident workers towards other resident workers living (R) or dead (DR), dead (DA) or living (A) alien workers, and lures (W: resident washed worker; WRH, lure covered with resident worker head extract; WRT, lure covered with resident thorax extract; WRHT, lure covered with alien worker head extract; WAH, lure covered with alien worker head extract; WAT, lure covered with alien thorax extract; WAHT, lure covered with alien head and thorax extract.

Workers or lures categories	Aggressive behavioral units: TYPE I X†	TYPE I T‡ (me)§	TYPE I Significance level	TYPE II X	TYPE II T (me)	TYPE II Significance level
R	0	0		0	0	
DR	4	4 (0)	**	0	0	
W	9	24 (3)	** ns¶	0	0	
WRH	9	16 (1,5)	ns ***	0	0	
WRT	8	13 (1)		0	0	
WRHT	10	20 (2)		0	0	
WAH	10	426 (41,5)	ns	10	38 (4)	
WAT	10	376 (37)	ns ** * **	10	23 (2,5)	ns *** ns
WAHT	10	481 (45,5)	**	10	30 (2)	**
DA	10	712 (74,5)	**	10	60 (6)	ns
A	10	1069 (109)		10	70 (7)	

† X = number of tests in which a response was noted.
‡ T = total number of acts for 10 tests.
§ me = median
¶ ns = nonsignificant.
* $p < 0.05$ (two-tailed Mann-Whitney U-test).
** $p < 0.02$ (two-tailed Mann-Whitney U-test).
*** $p < 0.002$ (two-tailed Mann-Whitney U-test).

no abdominal curving nor venom spraying from their sisters. Lures without cuticular wax or covered with head or thorax or head and thorax extracts of their sisters, gave the same low level of agonistic behavior. Nevertheless, workers were more aggressive towards lures than towards dead workers.

On the other hand, introduction of alien workers, dead or alive or lures with cuticular hydrocarbons from alien workers' head or thorax, or head and thorax, elicited a very strong behavioral response from resident workers: very high number

Table 3. Total number of agonistic behavioral acts for 10 tests of resident workers against a dead resident (DR), a resident washed worker (W), a lure covered with resident head and thorax extract (WRHT), a lure covered with alien head and thorax extract (WAHT) and a dead alien worker (DA). The median is in parentheses. N is the colony and C the couple.

Lures categories	DR		W		WRHT		WAHT		DA	
Aggressive behavioral units	I	II	I	II	I	II	I	II	I	II
Societies in confrontation										
C1 (N1/N2)	4	0	24	0	20	0	481	30	712	60
	(0)		(3)		(2)		(45)	(2)	(74)	(6)
C2 (N3/N4)	0	0	30	0	22	0	828	14	1090	30
			(2)		(2)		(93)	(1)	(95)	(2)
C3 (N5/N6)	0	0	10	0	10	0	316	18	862	98
			(1)		(1)		(32)	(2)	(86)	(9)

of type I units and presence of type II units. Some quantitative differences were nevertheless observed between these categories. Concerning type II units, there was no difference between the three types of lures. Concerning type I units, the lures with alien thoracic extract released less aggressive responses than lures with alien head and thorax extracts. On the other hand, total aggressive behavior or resident workers was lower towards lures than towards dead alien workers.

CONCLUSIONS

These results demonstrated that we can transfer the colony chemical label or signature on a worker: a resident worker deprived of its own odor and covered with alien worker cuticular wax release a strong aggressive behavior from her sisters. In *C. vagus* the chemical signature is composed of cuticular hydrocarbons and the relative proportions of some of them, especially dimethylalkanes are responsible of the nestmate recognition.

These hydrocarbons may be produced either by hypodermic cuticular cells or by the postpharyngeal gland (which contains hydrocarbons: Vander Meer and Wojcik 1982; Attygalle et al. 1985). In preliminary experiments, we laid crushed alien postpharyngeal glands or postpharyngeal gland extracts (in pentane) on resident workers thorax. Reintroduced into the foraging area of their colony, these workers release a high level of aggressive behavior. In contrast, if the products came from homocolonial workers, no aggressive behavior was aroused. Thus, postpharyngeal hydrocarbons may intervene in the colony odor. They are the same as those extracted from head or thorax, with relative proportions resembling more the head than the thorax and may spread on the worker's body during individual or interindividual grooming or contacts. It is also possible that secretions produced by the queen and transferred to the workers constitute a part of the colony odor (Carlin and Hölldobler 1983).

Taken together, our results demonstrated that hydrocarbons present on cuticle are responsible for nestmate recognition and constitute the label or each colony.

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