Pergamon

PII: S0305-1978(97)00025-2

Biochemical Systematics and Ecology, Vol. 25, No. 5, pp. 379–389, 1997

0305–1978/97 $17.00+0.00

Chemical Mimicry between Parasitic Ants of the Genus *Formicoxenus* and their Host *Myrmica* (Hymenoptera, Formicidae)

ALAIN LENOIR,*§ CHRISTIAN MALOSSE† and RYOHEI YAMAOKA‡
*Laboratoire d'Éthologie Expérimentale et Comparée, URA CNRS 2214, Université Paris Nord, 93430 Villetaneuse, France;
†Laboratoire de Phytopharmacie, INRA, Route de St Cyr, 78026 Versailles Cedex, France;
‡Department of Applied Biology, Kyoto Institute of Technology, Matsugasaki, Sakyoku, Kyoto 606, Japan

Key Word Index—*Myrmica*; *Formicoxenus*; Formicidae; Hymenoptera; cuticular hydrocarbons; social parasites; xenobiosis; chemical mimicry.

Abstract—Ants of the genus *Formicoxenus* are called *xenobiotic ants* because they live in close association with their *Myrmica* host; they cannot live independently of the host but they keep their brood apart. They forage in the host's galleries and obtain food from it through trophallaxies. They also very frequently lick the *Myrmica* workers and for that reason are called "shampoo ants". The chemical basis of the integration of the parasite into the host society is studied. In the two host–parasite pairs *F. provancheri*/*M. incompleta* and *F. quebecensis*/*M. alaskensis*, chemical mimicry in the cuticular hydrocarbons was found. With adoption experiments it is shown that this mimicry was not innate but is acquired during the first days of adult life. © 1997 Elsevier Science Ltd

Introduction

Howse (1975) proposed that social insects recognize nestmates through the mediation of non-volatile cuticular substances. It is now well known that cuticular lipids are species and colony specific (Howard, 1993). Among these substances hydrocarbons are dominant and are generally considered as the main nestmate recognition cues, constituting the chemical signature of the colony. For example, in *Cataglyphis cursor* a correlation appears between the similarity of the colonial hydrocarbon profile and the level of aggressive behaviour (Nowbahari *et al.*, 1990). This could explain the tolerance and amicable behaviour between nestmates and aggressive behaviour between non-nestmates. Several authors have confirmed the role of cuticular substances with lure experiments (review by Howard (1993) and Lorenzi *et al.* (1996)). Each ant possesses its own cuticular hydrocarbon profile which is influenced by its social and physical environment. Crozier and Dix (1979) hypothesized that this profile is shared between all the members of the colony, forming a "gestalt odour" characteristic of the colony. It was discovered recently that the postpharyngeal gland contains the same hydrocarbons as do the cuticles (Bagnères and Morgan, 1991; Do Nascimento *et al.*, 1993). By spreading the content of a postpharyngeal gland on an ant, it was possible to demonstrate that it is used in colonial recognition (Soroker *et al.*, 1994; Hefetz *et al.*, 1996). In

---

§Corresponding author. Present address: Laboratoire d'Éthologie et Pharmacologie du Comportement, Faculté des Sciences et Techniques, Parc de Grandmont, 37200 Tours, France (Tel: 33 2 47 36 69 95; Fax: 33 2 47 36 72 85; E-mail: lenoir@univ-tours.fr).

(*Received* 24 *May* 1996; *accepted* 27 *January* 1997)

Cataglyphis niger hydrocarbons are synthesized in the cuticular epithelium and concentrated in the postpharyngeal gland (possibly transported through the haemolymph) and the gestalt odour is achieved through an exchange of hydrocarbons between nestmates, mostly through trophallaxis and grooming (Soroker *et al.*, 1995a,b).

The cohabitation of two ant species in the same nest is accompanied, in most of the known cases, in a similarity in the cuticular hydrocarbon profiles between the allospecific workers. This chemical congruency was reported in artificial mixed groups where each species acquires the hydrocarbons of the other species (Errard and Jallon, 1987; Bagnères *et al.*, 1991; Vienne, 1993). Heterospecific hydrocarbons are found in the postpharyngeal gland (Hefetz *et al.*, 1992). Hydrocarbons are synthesized by each species and transferred to the other species where they are accumulated in the postpharyngeal gland (Vienne *et al.*, 1995). This confirms the role of the postpharyngeal gland in the formation of the colonial odour. This similarity of hydrocarbons was also found in natural heterospecific colonies, as well as in slave-making ants and their hosts (review by Howard (1993), Liepert and Dettner (1993), and Lorenzi *et al.* (1996)). Parabiotic associations are known in the ant-gardens from the tropical forests of America, where two or three ant species live in the same nest and share the same trails without aggression. They seem to keep their own cuticular specific profile (Orivel *et al.*, 1997). This cohabitation implies a familiarization period to learn to tolerate the other species. Such chemical mimicry can also be more diverse, for example the ant (*Pseudomyrmex*), the host-plant (*Acacia*) and a social wasp (*Parachartepus*) (Espelie and Hermann, 1988).

The objective of the present study was to analyse another example of parasitism, the case of xenobiosis, which is an association where the parasite lives independently inside the nest of the host. This way of life is intermediate between cleptobiosis where ants steal food (and brood) from the host, and a true social parasitism where ants form mixed colonies with their hosts. We studied two associations found in North America: *Formicoxenus provancheri*/*Myrmica incompleta* and *Formicoxenus quebecensis*/*Myrmica alaskensis* (see Francoeur *et al.* (1985) for taxonomic position and biology). It was observed that *F. provancheri* is very attracted by the host which is licked for long periods, mainly on the head (Lenoir *et al.*, 1992). This typical behaviour led Wheeler (1910) to name this ant the "shampoo ant", belonging to a group of insects that "lick the surface of the ants and seem to feed very largely, if not exclusively, on the cutaneous secretions, and the thin coating of saliva with which the ants cover one another" (p. 393).

Material and Methods

Myrmica incompleta is widespread in North America. Colonies were collected in Quebec, Canada, from 1987 to 1992. *Formicoxenus provancheri* is frequent south of the Laurentides mountains, *M. alaskensis* and its parasite *F. quebecensis* are rare and were found near Tadoussac. Voucher specimens are in the A. Francoeur collection at the University of Quebec at Chicoutimi.

The ants were reared in Petri dishes according to the technique developed by Francoeur *et al.* (1985) at 20°C and subjected to a photoperiod L:D = 12:12 h. Workers were taken from the nests for chemical analysis.

Chemical analysis. Cuticular substances were extracted from whole individuals, previously killed by freezing, by immersing them 10 min in 1 ml of pentane. *Myrmica* workers were analysed alone whereas *Formicoxenus* were analysed in groups of five, due to their small size. Global data were obtained with a pooled extract of 50 workers. The samples were dried under nitrogen and readjusted to 50 µl. Then, 2 µl of that solution were injected into a Delsi gas chromatograph equipped with a CPSil 5 capillary WCOT Chrompack column (25 m×0.25 mm) and a flame ionization detector (FID). The temperature increase was programmed from 100 to 300°C at 3°C min^{-1} (carrier gas was helium at 10 psi). The data were collected on an Enica 21 integrator. Gas chromatography–mass spectrometry (GC–MS) was carried out with a Nermag R-10-10C quadrupole mass spectrometer (electron impact at 70 eV) coupled with a Girdel 32 gas chromatograph equipped with a Ross injector. The system was fitted with a 25 m×0.32 mm ID fused silica capillary column WCOT CPSil8CB (Chrompack) heated from 200 to 300°C at 3°C min^{-1} (carrier gas: helium at 0.3 bar pressure). Direct location of the alkenes double bond was measured with methyl-vinyl-ether (MVE) as reactant gas at 92.5 eV (Malosse *et al.*, 1994). *Myrmica rubra* hydrocarbons have been previously identified (Bagnères and Morgan, 1991; Vienne *et al.*, 1990; Vienne, 1993) and as the chromatograms were identical, we assumed that the substances were the same.

The Nei index of similarity was calculated to compare the hydrocarbon profiles, computed with the relative percentages of the components (Ferguson, 1980; Dettner and Liepert, 1994). A dendrogram was constructed with euclidian distances by single linkage on the same data.

TABLE 1. HYDROCARBON COMPOSITION OF CUTICULAR EXTRACTS OF Mi (*Myrmica incompleta*), Fp (*Formicoxenus provancheri*), Ma (*Myrmica alaskensis*) AND Fq (*Formicoxenus quebecensis*)

Peak No.	Component	ECL	Mi	Fp	Ma	Fq	Mol. wt	Diagnostic EI ions	Diagnostic CH_4/CI ions
Alkanes									
2	n-C_{19}	19	+	+			268		267
5	n-C_{21}	21	+	+	+		296		295
7	n-C_{22}	22	+	+	+		310		309
10	n-C_{23}	23	+	+	+	+	324		323
16	n-C_{24}	24	+	+	+	+	338		337
21	n-C_{25}	25	+	+	+	+	352		351
28	n-C_{26}	26	+	+	+	+	366		365
35	n-C_{27}	27	+	+	+	+	380		379
42	n-C_{28}	28	+	+	+	+	394		393
49	n-C_{29}	29	+	+	+	+	408		407
54	n-C_{30}	30	+	+	+	+	422		421
60	n-C_{31}	31	+	+	+	+	436		435
Monomethyl alkanes									
11	9-Me C_{23}	23.35	+	+	+	+	339	140/141, 224/225	337, 140, 224
11	11-Me C_{23}	23.35	+	+	+	+	338	168/169, 196/197	337, 168, 196
12	7-Me C_{23}	23.41	+	+	+	+	338	112/113, 252/253	33, 253
13	5-Me C_{23}	23.50	+	+	+	+	338	84, 280/281	337, 281
15	3-Me C_{23}	23.80	+	+			338	56, 308/309	337/309
17	9-Me C_{24}	24.35	+				352	140/141, 238/239	351, 141, 239
17	10-Me C_{24}	24.35	+	+			352	154/155, 224/225	351, 154, 224
17	11-Me C_{24}	24.35	+	+			352	168/169, 210/211	351, 168, 211
17	12-Me C_{24}	24.35	+	+			352	182/183, 196/197	351, 182, 196
22	11-Me C_{25}	25.35	+	+	+	+	366	168/169, 224/225	365, 168, 224
22	13-Me C_{25}	25.35	+	+	+	+	366	196/197	365, 196
22	9-Me C_{25}	25.35	+	+	+	+	366	140/141, 252/253	365, 140, 253
23	7-Me C_{25}	25.42	+	+	+	+	366	112/113, 294/295	365, 295
24	5-Me C_{25}	25.51	+	+	+	+	366	84, 308/309	365, 309
26	3-Me C_{25}	25.74	+	+	+	+	366	56, 336/337	365, 337
30	10-Me C_{26}	26.34	+	+	+	+	380	154/155, 252/253	379, 154, 253
30	11-Me C_{26}	26.34	+	+	+	+	380	168/169, 238/239	379, 168, 238
30	12-Me C_{26}	26.34	+	+	+	+	380	182/183, 224/225	379, 182, 224
30	13-Me C_{26}	26.34	+	+	+	+	380	196/197, 210/211	379, 196, 210
30	9-Me C_{26}	26.34			+	+	380	140/141, 266/267	379, 140, 267
32	4-Me C_{26}	26.63	+	+			380	70, 336/337	379, 337
36	9-Me C_{27}	27.33	+	+	+	+	394	140/141, 280/281	393, 140, 281
36	11-Me C_{27}	27.33	+	+	+	+	394	168/169, 252/253	393, 168, 252
36	13-Me C_{27}	27.33	+	+	+	+	394	196/197, 224/225	393, 196, 224
37	7-Me C_{27}	27.41	+	+	+	+	394	112/113, 308/309	393, 309
38	5-Me C_{27}	27.52	+	+	+	+	394	84, 336/337	393, 337
40	3-Me C_{27}	27.75	+	+	+	+	394	56, 364/365	393, 365
44	10-Me C_{28}	28.33	+	+	+	+	408	154/155, 280/281	407, 154, 281
44	11-Me C_{28}	28.33	+	+	+	+	408	168/169, 266/267	407, 168, 267

TABLE 1—*CONTINUED*

Peak No.	Component	ECL	Mi	Fp	Ma	Fq	Mol. wt	Diagnostic EI ions	Diagnostic CH_4/CI ions
44	12-Me C_{28}	28.33	+	+	+	+	408	182/183, 252/253	407, 183, 252
44	13-Me C_{28}	28.33	+	+	+	+	408	196/197, 238/239	407, 196, 238
44	14-Me C_{28}	28.33	+	+	+	+	408	210/211, 224/225	407, 211, 225
46	4-Me C_{28}	28.62	+	+			408	70, 364/365	407, 365
50	9-Me C_{29}	29.32	+	+	+	+	422	140/141, 308/309	421, 140, 309
50	11-Me C_{29}	29.32	+	+	+	+	422	168/169, 280/281	421, 168, 281
50	13-Me C_{29}	29.32	+	+	+	+	422	196/197, 252/253	421, 196, 252
50	15-Me C_{29}	29.32	+	+	+	+	422	224/225	421, 224
50b	5-Me C_{29}	29.51			+	+	422	84, 364/365	421, 365
52	3-Me C_{29}	29.73	+	+	+	+	422	56, 392/393	421, 393
56	10-Me C_{30}	30.33	+				436	154/155, 308/309	435, 154, 309
56	11-Me C_{30}	30.33	+	+			436	168/169, 294, 295	435, 168, 295
56	12-Me C_{30}	30.33	+	+			436	182/183, 280/281	435, 182, 280
56	13-Me C_{30}	30.33	+	+			436	196/197, 266/267	435, 197, 267
56	14-Me C_{30}	30.33	+	+			436	210/211, 252/253	435, 211, 253
56	15-Me C_{30}	30.33	+	+			436	224/225, 238/239	435, 224, 239
58	4-Me C_{30}	30.61	+	+			436	70, 392/393	435, 393
61	9-Me C_{31}	31.33	+				450	140/141, 336/337	449, 140, 337
61	11-Me C_{31}	31.33	+	+	+	+	450	168/169, 308/309	449, 168, 308
61	13-Me C_{31}	31.33	+	+	+	+	450	196/197, 280/281	449, 196, 280
61	15-Me C_{31}	31.33	+	+			450	224/225, 252/253	449, 225, 253
64	3-Me C_{31}	31.76	+				450	56, 421/422	449, 421
65	11-Me C_{32}	32.32	+	+			464	168/169, 322/323	463, 168, 323
65	12-Me C_{32}	32.32	+				464	182/183, 308/309	463, 182, 309
65	13-Me C_{32}	32.32	+	+			464	196/197, 294/295	463, 196, 295
65	14-Me C_{32}	32.32	+				464	210/211, 280/281	463, 210, 281
65	15-Me C_{32}	32.32	+	+			464	224/225, 266/267	463, 224, 267
67	11-Me C_{33}	33.32	+	+			478	168/169, 336/337	477, 168, 337
67	13-Me C_{33}	33.32	+	+			478	196/197, 308/309	477, 196, 308
67	15-Me C_{33}	33.32	+	+			478	224/225, 280/281	477, 224, 281
67	17-Me C_{33}	33.32	+				478	252/253	477, 253
69	11-Me C_{35}	35.32?	+				506	168/169, 364/365	505, 168, 365
69	13-Me C_{35}	35.32?	+				506	196/197, 336/337	505, 196, 337
69	15-Me C_{35}	35.32?	+				506	224/225, 308/309	477, 224, 309
69	17-Me C_{35}	35.32?	+				506	252/253, 280/281	477, 253, 281
72	11-Me C_{37}	37.32?	+				534	168/169, 364/365	533, 168, 365
72	13-Me C_{37}	37.32?	+				534	196/197, 336/337	533, 196, 337
72	15-Me C_{37}	37.32?	+				534	224/225, 308/309	533, 224, 309
72	17-Me C_{37}	37.32?	+				534	252/253, 280/281	533, 281
Dimethyl alkanes									
25	9, 15-diMe C_{25}	25.65	+	+	+	+	380	140, 168, 239, 267	379, 141, 168, 239, 267
27	5, 15-diMe C_{25}	25.88			+	+	380	84, 168, 239, 323	379, 168, 239, 323
27	5, 17-diMe C_{25}	25.88	+	+	+	+	380	84, 140, 267, 323	379, 140, 267, 323
29	4, 13-diMe C_{25}	26.06			+	+	380	70, 196, 225, 351	379, 196, 225, 351
29	4, 15-diMe C_{25}	26.06			+	+	380	70, 168, 253, 351	379, 168, 253, 351
32	10, 14-diMe C_{26}	26.63	+				395	154, 196, 224, 268	393, 154, 197, 225, 268
39	9, 14-diMe C_{27}	27.61	+	+			408	140, 211, 224, 295	407, 140, 211, 225, 295
39	11, 15-diMe C_{27}	27.65	+	+			408	168, 196, 239, 267	407, 168, 196, 239, 267
40	7, 11-diMe C_{27}	27.70			+	+	408	112, 183, 252, 323	407, 183, 253, 323
41	5, 17-diMe C_{27}	27.84	+	+	+	+	408	84, 168, 267, 351	407, 168, 267, 351
43	3, 14-diMe C_{27}	28.06			+	+	408	56, 210, 224, 379	407, 211, 224, 379
43	3, 15-diMe C_{27}	28.06			+	+	408	56, 196, 239, 379	407, 196, 239, 379
46	10, 14-diMe C_{28}	28.62	+	+			422	154, 224, 295	421, 154, 225, 295
51	11, 15-diMe C_{29}	29.60	+	+			436	168, 224, 239, 295	435, 168, 224, 239, 295
52	7, 17-diMe C_{29}	29.73	+	+	+	+	436	112, 196, 267, 351	435, 196, 267, 351
53	5, 17-diMe C_{29}	29.82			+	+	436	84, 196, 267, 379	435, 196, 267, 379
55	3, 15-diMe C_{29}	30.04			+	+	436	56, 224, 239, 407	435, 224, 239, 407
55	3, 16-diMe C_{29}	30.04			+	+	436	56, 210, 252, 407	435, 211, 252, 407

TABLE 1—*CONTINUED*

Peak No.	Component	ECL	Mi	Fp	Ma	Fq	Mol. wt	Diagnostic EI ions	Diagnostic CH_4/CI ions
58	10, 16-diMe C_{30}	30.61	+	+			450	154, 225, 253, 323	449, 154, 225, 235, 323
62	11, 19-diMe C_{31}	31.47			+	+	464	168, 196, 295, 323	463, 168, 196, 295, 323
63	11, 15-diMe C_{31}	31.59	+	+			464	168, 239, 252, 323	463, 168, 239, 252, 323
63	13, 17-diMe C_{31}	31.59	+	+			464	196, 224, 267, 295	463, 196, 225, 267, 295
66	10, 18-diMe C_{32}	32.59	+				478	154, 224, 280, 351	477, 154, 225, 281, 351
68	11, 15-diMe C_{33}	33.57	+	+			492	168, 239, 280, 351	491, 168, 239, 280, 351
68	13, 17-diMe C_{33}	33.57	+	+			492	196, 252, 267, 23	491, 196, 253, 267, 323
70	11, 15-diMe C_{35}	35.57	+				520	168, 239, 308, 379	519, 168, 239, 309, 379
70	13, 17-diMe C_{35}	35.57	+				520	196, 252, 295, 351	519, 196, 253, 295, 351
73	11, 15-diMe C_{37}	37.57	+				548	168, 239, 336, 407	547, 168, 239, 337, 407
73	13, 15-diMe C_{37}	37.57	+				548	196, 253, 309, 479	547, 196, 253, 309, 379
Monoenes									Diagnostic MVE/CI ions
1	x-$C_{19:1}$	18.75?	+	+			280		
3	x-$C_{21:1}$	20.75	+	+			294		
6	x-$C_{22:1}$	21.75?	+	+			308		
8	x-$C_{23:1}$	22.75	+	+			322		
9	x-$C_{23:1}$	22.79	+	+			322		
14	x-$C_{24:1}$	23.80	+	+			336		
19	9-$C_{25:1}$	24.75	+	+	+	+	350		409(M + mve) +, 170, 268
20	x-$C_{25:1}$	24.81	+	+			350		
33	9-$C_{27:1}$	26.72	+	+	+	+	378		437(M + mve) +, 170, 296
34	x-$C_{27:1}$	26.80	+	+	+	+	378		
47	9-$C_{29:1}$	28.77	+	+	+	+	406		465(M + mve) +, 170, 324
48	x-$C_{29:1}$	28.85	+	+			406		
59	x-$C_{31:1}$	30.85?	+	+	+	+	434		
Dienes									
31	x, y-$C_{27:2}$	26.54	+		+	+	376		376, 377
45	x, y-$C_{29:2}$	28.51			+	+	404		404, 405
4	Unknown	19.95			+	+			
18	Unknown	24.70	+						
57	Unknown	30.45	+		+	+			
71	Unknown	35.80			+	+			

Results

Hydrocarbon composition (Tables 1 and 2, Fig. 1)

The hydrocarbons found in the four species are very similar to those found in other ants. A great number of hydrocarbons were found, including all the linear alkanes from C_{23} to C_{31}, C_{23} and C_{25} were major peaks of more than 10 and 5%, respectively. Many monomethyl alkanes were found from C_{23} to C_{37}, the main ones being Me-C_{25}, C_{27}, C_{29} and C_{31}. Dimethyl alkanes were found from C_{25} to C_{37}. The monoene 9-$C_{25:1}$ was a major peak with generally more than 25% while 9-$C_{29:1}$ was abundant in *Myrmica alaskensis* and *Formicoxenus quebecensis*. *Myrmica incompleta* hydrocarbons have been studied by Howard *et al.* (1990). Our results, based on a high sensitivity mass spectrometer, are more complete than Howard *et al.*'s, but we find the same three major peaks: n-C_{23} (peak 10), 9-$C_{25:1}$ (peak 19) and n-C_{25} (peak 21).

Comparison of hydrocarbon profiles (Figs 1 and 2)

Figure 1 presents the hydrocarbon relative composition for the 15 major peaks. It appears that the two host/parasite pairs have very similar profiles. Some differences appear for example in peaks 22 and 26 (monomethyl C_{25}), and peak 27 (dimethyl C_{27}) which are more abundant in *Myrmica incompleta/Formicoxenus provancheri*; the quantities are inverted for peaks 35 (n-C_{27}), 36 (monomethyl C_{27}), 41 and 43 (dimethyl C_{27}), and 47 (C_{29} alkane) which are more abundant in *Myrmica alaskensis/Formicoxenus quebecensis*.

The Nei index of similarity was 0.96 between the host and parasite in the two pairs. They appear very connected in the tree diagram (Mi and Fp; Ma and Fq; Fig. 2) but are clearly differentiated, due to the differences indicated before.

Hydrocarbon profiles of adopted Formicoxenus provancheri (Fig. 2)

In order to assess the origin of the cuticular hydrocarbons we reared newly emerged *F. provancheri* with non-natural hosts. It is known that artificial mixed groups can be made with young ants of various species (Errard, 1984) so we introduced five young *F. provancheri* into groups of 30 young *Myrmica alaskensis* or *M. rubra*. This latter species originates from Europe and so cannot be a potential host for *F. provancheri*. Three replicates were made for each association. It was not possible to repeat the experiment with *F. quebecensis* which is rare. The adoptions of mature *Formicoxenus* by a non-natural species seems to be impossible (Lenoir, personal observations).

Formicoxenus provancheri reared with *Myrmica alaskensis* or *Myrmica rubra* were analysed at one month old. It appears that they acquired the hydrocarbon profile of their adoptive host. The Nei index between *M. alaskensis* and adopted *F. provancheri* was 0.96, that is identical to a normal host/parasite pair. By contrast, the Nei index for control ants of *M. alaskensis* and *F. provancheri* living with its natural host *M. incompleta* was only 0.86.

Myrmica rubra was very different chemically. Analysis of the hydrocarbon composition was made according to Vienne (1993). The Nei index between the two North American *Myrmica* species and *M. rubra* was about 0.50 on each case. This indicates that they belong to very different groups of species. The Nei index between *M. rubra* and the adopted *F. provancheri* was only 0.91 indicating a similarity which is not so high as with North American *Myrmica*, but still highly congruent. These similarities appear in Fig. 2:

TABLE 2. RELATIVE PROPORTIONS (%) OF HYDROCARBONS OF Mi, Fp, Ma, Fq (SEE TABLE 1)

Peak No.	Name	ECL	Mi (%)	Fp (%)	Ma (%)	Fq (%)
1	x-C19:1	18.75	t	t		
2	n-C19	19.00	t	t		
3	x-C21:1	20.75	t	t	0.31	
4	unknown	19.95	0	t	0.61	
5	n-C21	21.00	0.27	0.15	0.47	
6	x-C22:1	21.75	t	t	0.57	
7	n-C22	22.00	0.09	0.27	0.32	
8	x-C23:1	22.75	0.31	1.22		
9	x-C23:1	22.79	0.32	0.33		
10	n-C23	23.00	18.47	9.92	9.40	15.15
11	11+13 Me C23	23.35	0.38	0.42	0.10	0.34
12	7-Me C23	23.41	0.07	0.09	0.03	
13	5-Me C23	23.50	0.35	0.39		
14	x-C24:1	23.73	0.36	0.22	0.24	0.33
15	3-Me C23	23.80	0.30	0.43		
16	n-C24	24.00	0.99	0.56	0.52	0.68

TABLE 2—*CONTINUED*

Peak No.	Name	ECL	Mi (%)	Fp (%)	Ma (%)	Fq (%)
17	9+10+11+12Me C24	24.35	0.25	0.29		0.67
18	unknown	24.70	0.57	0.80	0.27	0.29
19	9-C25:1	24.75	27.38	26.30	18.77	26.37
20	3-C25:1	24.81	0.16	0.76	0.20	
21	n-C25	25.00	10.56	6.17	8.27	8.13
22	9+11+13-Me C25	25.35	4.06	4.58	0.73	1.28
23	7-Me C25	25.42	0.26	0.35	0.09	0.00
24	5-Me C25	25.51	1.09	1.23	0.48	0.23
25	9,15-diMe C25	25.65	0.66	0.58	0.29	
26	3-Me C25	25.74	4.95	4.06	0.59	1.69
27	5,15+5,17-diMe C25	25.88	2.54	3.47	0.69	
28	n-C26	26.00	0.24	0.25	0.59	0.00
29	4,13+4,15 diMeC26	26.06	0.16	0.19	0.48	0.39
30	9+10+11+12+13 Me C26	26.34	0.34	0.35	0.73	0.78
31	x,y-C27:2	26.54	0.13		1.73	1.29
32	10,14-diMe C26+4-Me C26	26.63	0.07		0.10	0.51
33	9-C27:1	26.72	0.20	0.12	4.89	3.87
34	3-C27:1	26.80	0.34	0.20	0.31	
35	n-C27	27.00	0.63	1.25	5.95	4.41
36	9+11+13 Me C27	27.33	1.41	1.84	5.44	3.84
37	7-Me C27	27.41	0.13	0.14		0.23
38	5-Me C27	27.52	0.49	0.63	1.99	0.73
39	9,14+11,15 diMe C27	27.61	0.44	0.48	0.76	0.45
40	7,11-diMe C27+3-Me C27	27.70	0.54	0.49	2.52	2.32
41	5,17-diMe C27	27.84	0.60	0.78	4.46	1.80
42	n-C28	28.00	0.21	0.13	0.60	0.25
43	3,14+3,15 diMe C27	28.06	0.01	0.33	2.68	3.49
44	10+11+12+13+14 Me C28	28.33	0.40	0.59	2.96	2.16
45	x,y-C29:2	28.51	0.61			
46	10,14-diMe C28+4-Me C28	28.62	0.67	0.77	0.61	0.00
47	9-C29:1	28.77	0.67	1.60	4.78	6.20
48	3-C29:1	28.85	0.16	0.30	0.24	0.00
49	n-C29	29.00	0.46	1.84	1.33	2.15
50	9+11+13+15 Me C29	29.32	2.92	4.62	2.39	2.42
50b	5-Me C29	29.51				
51	11,15-diMe C29	29.60	2.64	3.28	1.06	0.94
52	3-Me C29+7,17-diMe C29	29.73	0.58	0.90	1.07	1.15
53	5,17-diMe C29	29.82	0.13	0.23	1.28	0.74
54	n-C30	30.00	0.14	0.31	0.30	0.47
55	3,15+3,16 diMe C29	30.04	0.04	0.08	1.75	2.46
56	10+11+12+13+14+15 Me C30	30.33	0.33	0.58	0.45	
57	unknown	30.45	0.44	0.48	0.91	
58	10,16-diMe C30+4-Me C30	30.61	0.28	0.49	0.35	
59	x-C31:1	30.85	0.14	0.60	0.30	
60	n-C31	31.00	0.09	0.25	0.27	
61	9+11+13+15 Me C31	31.33	2.75	4.16	0.69	0.96
62	11,19-diMe C31	31.47			0.82	
63	11,15+13,17 diMe C31	31.59	2.13	3.24	0.29	0.83
64	3-Me C31	31.76	0.14		0.16	
65	11+12+13+14+15 Me C32	32.32	0.19	0.30	0.09	
66	10,18-diMe C32	32.59	0.14	0.28		
67	11+13+15+17 Me C33	33.32	0.82	1.20	0.15	
68	11,15+13,17 diMe C33	33.57	0.98	1.73	0.20	
69	11+13+15+17 Me C35	35.32	0.18	0.25	0.10	
70	11,15+13,17 diMe C35	35.57	1.03	1.49	0.31	
71	unknown	35.80			0.31	
72	11+13+15+17 Me C37	37.32	0.09		0.15	
73	11,13+13,15 diMe C37	37.57	0.54	0.65	0.46	
			100%	100%	100%	100%

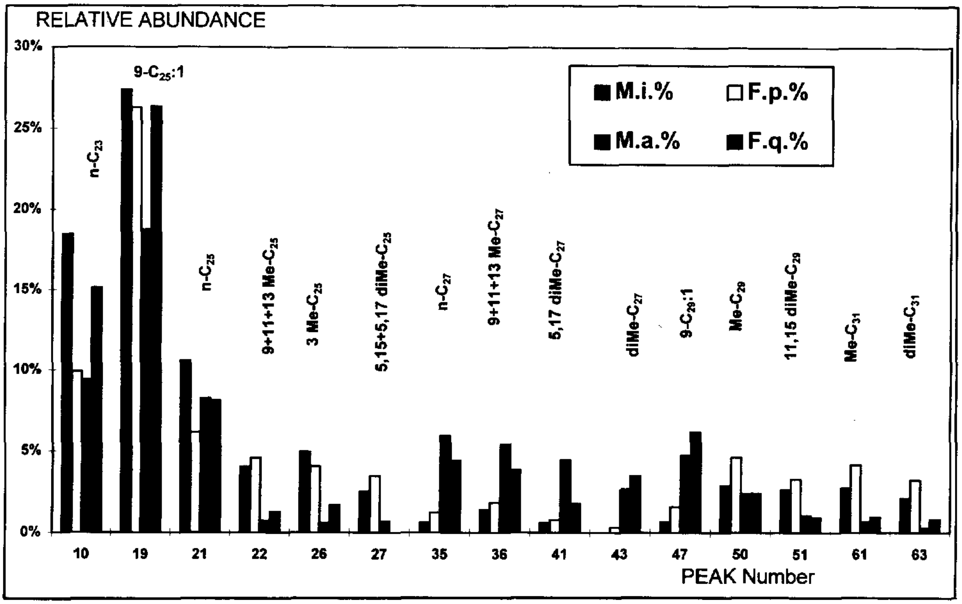

FIG. 1. RELATIVE ABUNDANCE OF MAJOR PEAKS FOR THE TWO PAIRS OF SPECIES Mi—*M. incompleta*/Fp—*F. provancheri* AND Ma—*M. alaskensis*/Fq—*F. quebecensis*.

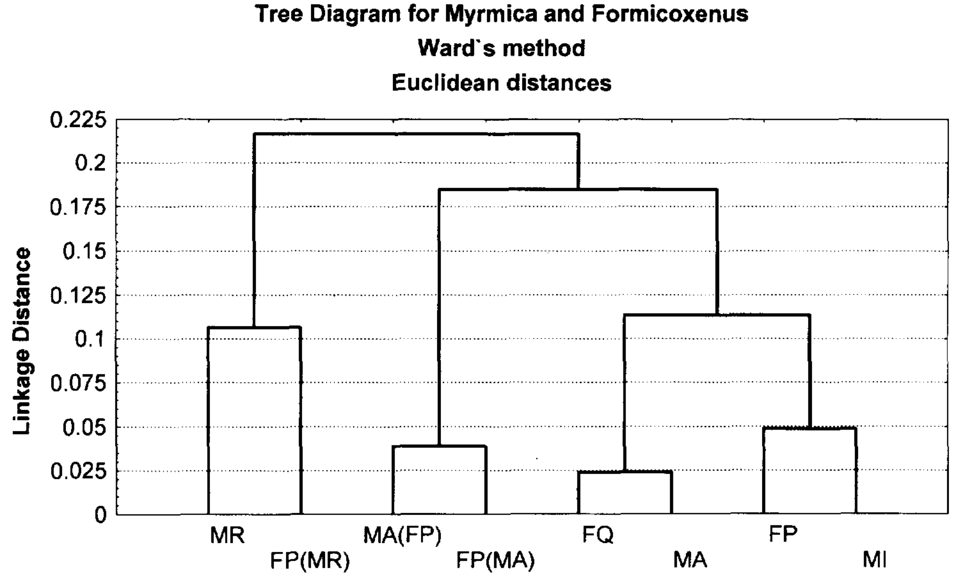

FIG. 2. TREE DIAGRAM FOR SIMILARITY OF CHEMISTRY BETWEEN *MYRMICA* AND *FORMICOXENUS* PAIRS.

the artificial pair *M. alaskensis*—Ma(Fp)/*F. provancheri*—Fp(Ma) is near the natural group *M. alaskensis* (Ma)/*F. quebecensis* (Fq), with a slightly longer distance. The other artificial pair *M. rubra* (Mr)/*F. provancheri*—Fp(Mr) is very distant from any other sample, indicating that the parasite acquired the hydrocarbons of the host.

Discussion

Many insect species live as inquilines within colonies of social insects. The chemical resemblance between inquilines and hosts may be referred to as mimicry or camouflage. Howard *et al.* (1990) considered that an organism uses chemical mimicry when it biosynthesizes the substances, whereas chemical camouflage is involved when the mimic acquires the substances from the model. Dettner and Liepert (1994) in their review of chemical deception do not refer to these definitions because they do not consider the operator and its reaction to the presence of the deceptive signals. Camouflage is therefore defined only as the simulation of the background, and therefore most of the cases of chemical resemblance known so far belong to the category of chemical mimicry *sensu lato*. We accept this assessment in agreement with Bagnères *et al.* (1991). Taking this perspective then a frequent way adopted to integrate into an ant colony is to mimic the brood cuticular recognition patterns of the host species. Adult ants may also serve as the model for the inquilines (review by Stowe (1988)). In a few cases chemical data on the mimicry are available, indicating both possibilities for the parasite (see reviews by Howard (1993), Dettner and Liepert (1994), and Lorenzi *et al.* (1996)). The first possibility is to biosynthesize the cuticular hydrocarbons of the host, for example in the syrphid fly larvae *Microdon* or three species of termitophile beetles. Inquilines can also produce substances which release brood tending behaviours and are treated as brood (for example lycaenid caterpillars or the sphinx caterpillar *Acherontia*).

The second possibility is to directly acquire the host-specific odour. This can be obtained through passive mechanisms (contact with the host and the walls of the nest) or actively (licking the host and obtaining trophallaxis) for example in the beetle *Martinezia duterteri* (=*Myrmecaphodius excavaticollis*) living with *Solenopsis* or *Iridomyrmex* (Vander Meer and Wojcik, 1982) and in *Orasema*, a parasitoid wasp of the fire ant (Vander Meer *et al.*, 1989). It is well known that many myrmecophilous insects perform intensive grooming of the worker ants, probably to acquire the host odour (Akre and Rettenmeyer, 1966; Akre and Torgerson, 1968). The cricket *Myrmecophila manni* is covered with enlarged hairs which adsorb the cuticular lipids coating the walls of the ant nest (Henderson and Akre, 1986). Wheeler wrote in 1910 that the galleries of the ant's nests are covered by the cuticular deposits of ants!; and the cricket "derives some benefit from the oily secretions covering the ant's body, and they can adapt immediately to a new nest or a new species". Yamaoka (1990) also found three species of myrmecophilous spiders that mimic the ant *Camponotus kiushiuensis*. Takada and Hashimoto (1985) observed the parasitoid females of *Paralipsis eikoae* riding *Lasius niger* workers and rubbing their legs on the ant. They were tolerated in their colony but attacked in other *L. niger* colonies. The cuckoo ant queens *Leptothorax kutteri* have cuticular hydrocarbons and fatty acids similar to those of the *L. acervorum* host workers (Franks *et al.*, 1990). The authors hypothesize that this is the result of intensive grooming. The adult *Harpagoxenus sublaevis* slave-makers also adopt the *Leptothorax* host odour (Kaib *et al.*, 1993), but the situation is probably more complex as Alloway and Keough (1990) observed that *H. americanus* (the American species) chemically mark their slaves to prevent them returning to their parent colony.

The chemical similarity between the parasite and the host explains that the parasite can live peacefully in the host nest. In the two studied associations (*Formicoxenus provancheri*/*Myrmica incompleta* and *F. quebecensis*/*M. alaskensis*) we can also conclude that chemical mimicry is responsible for mutual tolerance. Analyses of new-born *Formicoxenus* showed that they have very small quantities of hydrocarbons, as also noted in *Cataglyphis* by Soroker *et al.* (1995a,b). The mimicry is acquired during the first days after the emergence of parasite workers, and later maintained permanently through host grooming. We observed that *Formicoxenus* spend long periods in the host nest where they spend an average of 45% of their time licking workers; which 40% of the time is directed toward the host's head (Lenoir *et al.*, 1989). By licking the host they must obtain the host odour, necessary for the host's tolerance. We can hypothesize that the host odour is not present on the cuticle in great quantities, and as a consequence to collect enough of these substances by grooming requires a long time. This necessity may explain the special polyethism observed in this species where 75% of workers are foragers, which is exceptional in free living ants (Errard *et al.*, 1997).

Acknowledgements—We thank A. Francoeur for ant collection, X. Cerda and A. Dahbi for their help in performing Gas Chromatography, A. Hefetz for a critical reading of the manuscript and Andrea Dejean for revising the English translation.

References

Akre, R. D. and Rettenmeyer, C. W. (1966) Behavior of Staphylinidae associated with army ants (Formicidae: Ecitonini). *J. Kansas Entomol. Soc.* **39**, 745–782.

Akre, R. D. and Torgerson, R. L. (1968) The behavior of *Diploeciton nevermanni*, a staphylinid beetle associated with army ants. *Psyche* **75**, 211–215.

Alloway, T. M. and Keough, H. (1990) Slave marking by the slave-making ant *Harpagoxenus americanus* (Emery) (Hymenoptera: Formicidae). *Psyche* **97**, 55–64.

Bagnères, A. G. and Morgan, E. D. (1991) The postpharyngeal gland and the cuticule of Formicidae contain the same characteristic hydrocarbons. *Experientia* **47**, 106–111.

Bagnères, A. G., Errard, C., Mulheim, C., Joulie, C. and Lange, C. (1991) Induced mimicry of colony odors in ants. *J. Chem. Ecol.* **17**, 1641–1664.

Crozier, R. H. and Dix, M. H. (1979) Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. *Behav. Ecol. Sociobiol.* **4**, 217–224.

Dettner, K. and Liepert, C. (1994) Chemical mimicry and camouflage. *Ann. Rev. Entomol.* **39**, 129–154.

Do Nascimento, N. N. R., Billen, J. and Morgan, E. D. (1993) The exocrine secretions of the jumping ant *Harpegnathos saltator*. *Comp. Biochem. Physiol. B. Comp. Biochem.* **104**, 505–508.

Errard, C. (1984) Evolution en fonction de l'âge des relations sociales dans les colonies mixtes hétérospécifiques chez les fourmis des genres *Camponotus* et *Pseudomyrmex*. *Ins. Soc.* **31**, 185–198.

Errard, C. and Jallon, J. M. (1987) An investigation of the development of the chemical factors in ants intra-society recognition. In *Chemistry and Biology of Social Insects*, eds E. Eder and H. Rembolds, p. 478. Verlag J. Peperny, Munchen.

Errard, C., Fresneau, D., Francoeur, A., Heinze, J. and Lenoir, A. (1997) Social organization in the guest ant *Formicoxenus provancheri*. *Ethology* **103**, 149–159.

Espelie, K. E. and Hermann, H. R. (1988) Congruent cuticular hydrocarbons: biochemical convergence of a social wasp, an ant and a host plant. *Biochem. Syst. Ecol.* **16**, 505–508.

Ferguson, A. (1980) *Biochemical Systematics and Evolution*. Blackie, Glasgow.

Francoeur, A., Loiselle, R. and Buschinger, A. (1985) Biosystématique de la tribu Leptothoracini (Formicidae, Hymenoptera). 1—Le genre *Formicoxenus* dans la région holarctique. *Naturaliste Canadien* **112**, 343–403.

Franks, N., Blum, M. S. and Smith, R. K. (1990) Behavior and chemical disguise of cuckoo ant *Leptothorax kutteri* in relation to its host *Leptothorax acervorum*. *J. Chem. Ecol.* **16**, 1431–1444.

Hefetz, A., Errard, C. and Cocojaru, M. (1992) Heterospecific substances in the postpharyngeal gland secretion of ants reared in mixed groups. *Naturwissenschaften* **79**, 417–420.

Hefetz, A., Errard, C., Chambris, A. and Le Négrate, A. (1996) Postpharyngeal gland secretion as a modifier of aggressive behavior in the myrmicine ant *Manica rubida*. *J. Insect Behav.* **9**, 709–727.

Henderson, G. and Akre, R. D. (1986) Morphology of *Myrmecophila manni*, a myrmecophilous cricket (Orthoptera: Gryllidae). *J. Entomol. Soc. Brit. Columbia* **83**, 57–62.

Howard, R. W. (1993) *Insects Lipids: Chemistry, Biochemistry and Biology*, eds D. W. Stanley-Samuelson and G. J. Blomquist. Univ. Nebraska Press, Lincoln.

Howard, R. W., Akre, R. D. and Garnett, W. B. (1990) Chemical mimicry of an obligate predator of carpenter ants (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **83**, 607–616.

Howse, P. E. (1975) Chemical defenses of ants, termites and other insects: some outstanding questions. In *Pheromones and Defensive Secretions in Social Insects*, eds C. Noirot, P. E. Howse and G. Le Masne, p. 23. IUSSI, Dijon, France.

Kaib, M. J., Heinze, J. and Ortius, D. (1993) Cuticular hydrocarbon profiles in the slave-making ant *Harpagoxenus sublaevis* and its hosts. *Naturwissenschaften* **80**, 281–285.

Lenoir, A., Francoeur, A., Errard, C. and Jaisson, P. (1989) Résultats préliminaires sur le comportement de *Formicoxenus provancheri* en relation avec son hôte *Myrmica incompleta* (Hymenoptera Formicidae). *Actes Coll. Insectes Soc.* **5**, 225–232.

Lenoir, A., Errard, C., Francoeur, A. and Loiselle, R. (1992) Relations entre la fourmi parasite *Formicoxenus provancheri* et son hôte *Myrmica incompleta*. Données biologiques et éthologiques. *Ins. Soc.* **39**, 81–97.

Liepert, C. and Dettner, K. (1993) Recognition of aphid parasitoids by honeydew-collecting ants: the role of cuticular lipids in a chemical mimicry system. *J. Chem. Ecol.* **19**, 2143–2153.

Lorenzi, M. C., Bagnères, A.-G. and Clément, J. L. (1996) The role of cuticular hydrocarbons in social insects: is it the same in paper wasps? In *Natural History and Evolution of Paper Wasps*, eds S. Turillazzi and M. J. West-Eberhard, pp. 178–189. Oxford University Press, Oxford.

Malosse, C., Einhorm, J. and Lenoir, A. (1994) An application of ion–molecule reaction with vinyl methyl ether: direct location of double bond in C25 to C35 monoolefins of ant cuticular extracts. *13th Int. Mass Spectrometry Conf.*, Budapest, 29 August–2 September.

Nowbahari, E., Lenoir, A., Clément, J. L., Lange, C., Bagnères, 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**, 63–73.

Orivel, J., Errard, C. and Dejean, A. (1997) Ants gardens: interspecific recognition in parabiotic ant species. *Behav. Ecol. Sociobiol.* **40**, 87–93.

Soroker, V., Vienne, C. and Hefetz, A. (1994) The postpharyngeal gland as a "Gestalt" organ for nestmate recognition in the ant *Cataglyphis niger*. *Naturwissenschaften* **81**, 510–513.

Soroker, V., Hefetz, A., Cocojaru, M., Billen, J., Francke, S. and Francke, W. (1995a) Structural and chemical ontogeny of the postpharyngeal gland in the desert ant *Cataglyphis niger*. *Physiol. Entomol.* **20**, 323–329.

Soroker, V., Vienne, C. and Hefetz, A. (1995b) Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger*. *J. Chem. Ecol.* **21**, 365–378.

Stowe, M. K. (1988) Chemical mimicry. In *Chemical Mediation of Coevolution*, ed. K. C. Spencer, p. 513. Academic Press, San Diego.

Takada, H. and Hashimoto, Y. (1985) Association of the aphid parasitoids *Aclitus sappaphis* and *Paralipsis eikoe* (Hymenoptera Aphidiidae) with the aphid attending ants *Pheidole fervida* and *Lasius niger* (Hymenoptera Formicidae). *Kontyu* **53**, 155–160.

Vander Meer, R. K. and Wojcik, D. P. (1982) Chemical mimicry in the myrmecophilous beetle *Myrmecaphodius excavaticollis*. *Science* **218**, 806–808.

Vander Meer, R. K., Jouvenaz, D. P. and Wojcik, D. P. (1989) Chemical mimicry in a parasitoid (Hymenoptera: Eucharitidae) of fire ants (Hymenoptera: Formicidae). *J. Chem. Ecol.* **15**, 2247–2261.

Vienne, C. (1993) Organisation sociale et reconnaissance interindividuelle dans les colonies mixtes artificielles de fourmis. Unpublished Thesis, University Paris Nord.

Vienne, C., Bagnères, A. G., Lange, C. and Errard, C. (1990) Etude chimique de la reconnaissance interindividuelle chez *Myrmica rubra* et *Manica rubida* (Formicidae, Myrmicinae) élevées en colonies mixtes artificielles. *Actes Coll. Insectes Soc.* **6**, 261–265.

Vienne, C., Soroker, V. and Hefetz, A. (1995) Congruency of hydrocarbon patterns in heterospecific groups of ants: transfer and/or biosynthesis? *Ins. Soc.* **42**, 267–277.

Wheeler, W. M. (1910) *Ants*. Columbia University Press, New York.

Yamaoka, R. (1990) Chemical approach to understanding interactions among organisms. *Physiol. Ecol., Japan* **27**, 31–52.