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SOLID-PHASE MICROEXTRACTION AND CUTICULAR HYDROCARBON DIFFERENCES RELATED TO REPRODUCTIVE ACTIVITY IN QUEENLESS ANT *Dinoponera quadriceps*

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Abstract—We extracted the cuticular hydrocarbons from live *Dinoponera quadriceps* ants (10 colonies collected from Brazil) with the solventless solid-phase microextraction (SPME) technique. Gas chromatography of the SPME samples (N = 233 measurements) compared with pentane extracts (N = 10) resulted in similar profiles. Eighty-one compounds belonging to the main long-chain hydrocarbon families were identified by GC-MS. There is no morphologically specialized queen in *D. quadriceps* and only one aggressively dominant worker (alpha) mates and reproduces in each colony. The alpha ant (N = 26 individuals) always yielded higher amounts and percentages of 9-hentriacontene (9-C_{31} : 1) than her sterile nestmates (N = 47). Since SPME is not destructive, it allowed for the repeated extraction of the same individuals, demonstrating that the alpha ant (virgin or mated) always had higher levels of 9-hentriacontene. This difference appears related to ovarian activity and may function as a signal of the alpha's dominance status.

Key Words—Ant, Ponerinae, reproduction, dominance, cuticular hydrocarbons, solid-phase microextraction.

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INTRODUCTION

All insect societies are distinguished by the sterility of most group members. In species where reproductive and sterile individuals are morphologically specialized (termites, most ants, some bees and wasps), queens produce pheromones regulating the egg-laying of workers and their rearing of new female sexuals (Fletcher and Ross, 1985). However, queen pheromones have only been identified in honeybees, where they are produced in the mandibular glands (Winston, 1987; Plettner et al., 1996). In contrast, in social insects lacking physical castes, reproduction is regulated by aggressive interactions among a proportion of nestmates (Heinze et al., 1994). This aggression is highly directed and necessitates the olfactory recognition of dominance status (West-Eberhard, 1977; Downing and Jeanne, 1985).

About 100 species belonging to 10 genera of the ant subfamily Ponerinae have lost the queen caste (Peeters, 1993). All workers are morphologically similar, but according to species either one or several mate and lay fertilized eggs in each colony. In the monogynous *Dinoponera quadriceps*, only the worker having top-rank in the hierarchy ("alpha") can mate (Monnin and Peeters, 1998). Almost all subordinates have inactive ovaries. The alpha ant exhibits an exceptional dominance behavior. She bends her gaster forward, bites the tip of one antenna of a subordinate worker and rubs it against the intersegmental membranes between her abdominal tergites V and VII. This behavior is likely to involve the transfer of olfactory information. Our aim was to identify the cuticular hydrocarbons and determine whether they differ between the fertile alpha and sterile subordinate workers.

Solid-phase microextraction (SPME) (Berlardi and Pawliszyn, 1989; Arthur and Pawliszyn, 1990, Arthur et al., 1992) seemed an ideal technique to study cuticular hydrocarbons of *D. quadriceps*. Since a limited number of colonies were available for study (with only one alpha in each), a nondestructive technique was needed. SPME was recently used to document the daily production of a volatile pheromone in the curculionid beetle *Metamasius hemipterus* (Malosse et al., 1995) and to extract directly a pheromone from an exocrine gland in Lepidoptera, thereby avoiding solvent interaction (Frérot et al., 1997, Mozuraitis et al., 1996).

METHODS AND MATERIALS

Colonies of *Dinoponera quadriceps* (82 $\pm$ 29 workers; range 39–141; N = 17) were collected in Bahia state (along the road between Sambaiba and Tobias Barreto), Brazil, in October 1994 and January 1996. They were kept in artificial laboratory nests where live insects were provided daily as food. Ten

colonies were used for chemical analyses; all the workers had been individually marked with numbers glued onto their thorax, and aggressive interactions between nestmates were recorded in order to determine their rank in the hierarchy. Several workers eclosed from their cocoons in the laboratory, and thus their exact age was known. All workers were later dissected to check their ovarian activity and presence of sperm in the spermatheca (Monnin and Peeters, 1998). Since the alpha ant is able to oviposit before she has mated, we differentiate between virgin alphas and mated alphas (the latter are termed gamergates) (Peeters, 1993).

Cuticular hydrocarbons of individual workers were sampled with a Supelco 7-μm polydimethylsiloxane fiber, designed to extract compounds of high molecular weight. Live ants were immobilized with a nylon wire and entomological pins. The sting was seized with forceps to gently bend the abdomen underneath the thorax, thus exposing the sclerotized membrane between tergites VI and VII; this is where dominant workers normally rub the antennae of subordinates. The fiber was carefully rubbed against this membrane for 2 min, then desorbed in the injection port of a gas chromatograph for 5 min, for either GC or GC-MS analysis. *Dinoponera* workers are the largest known ants (roughly 3 cm in length), which facilitated our sampling procedure. There were no injuries because intersegmental membranes are very thick in ponerine species; in fact, the flexible SPME fiber broke readily. Seventy-three workers of different ages, ranks, and colonies were measured with SPME-GC. Since SPME is not destructive, we were able to sample most workers several times (Table 1).

We attempted to estimate the absolute quantities of hydrocarbons extracted. Addition of an internal standard to the SPME samples was impossible: rubbing the fiber on the ant after adding the standard would have changed the amount

TABLE 1. NUMBER OF SPME PERFORMED ON WORKERS FROM 10 COLONIES[a]

Functional group	Individuals (*N*)	Total SPME samples	SPME samples per individual	
			Mean ± SE	Min–Max
Mated alphas	7	60	8.6 ± 2.1	1–17
Virgin alphas	19	85	4.5 ± 0.8	1–14
Young sterile workers (less than 1 month-old)	11	25	2.3 ± 0.4	1–5
Older sterile workers (more than 1 month-old)	36	63	1.8 ± 0.2	1–6

[a] Alphas (mated or virgin) had this rank for more than two weeks.

of standard finally injected, and immersing the fiber in the standard after sampling the ant would have changed the amount of extract injected. Thus, we injected 3 μg of n-C_{31} in the GC-MS as an external standard ($N = 2$), and the area of the n-C_{31} peak allowed us to estimate the quantity corresponding to the area of each peak in the chromatograms. SPME of the head, petiole, and abdomen of the same alpha workers was done to compare the spatial distribution of hydrocarbons over the body. This was replicated with 12 alphas (mated or virgin) from six colonies. The Dufour's gland of five alphas and nine subordinate workers was dissected. Each gland was extracted in 200 μl of pentane for at least 24 hr, and 5 μl of the solution was injected for GC analysis.

Our sampling protocol is a novel application of SPME and thus needed validation. Classical pentane extractions of three alphas and seven subordinates were done for comparison. Each ant was cooled at 5°C for a few minutes and then totally immersed in 2 ml of pentane for 2 min. Five microliters of the extract were injected for GC or GC-MS analysis. We also compared the efficiency of SPME and pentane in extracting a reference mixture previously applied on a dead ant. The mixture was composed of 9-C_{23}:1, n-C_{23}, n-C_{26}, n-C_{28}, n-C_{30}, n-C_{32}, n-C_{34}, and n-C_{36} at 63, 160, 26, 21, 57, 77, 27 and 19 ng/μl, respectively. These standard compounds are present in low amounts on the ants' cuticle, thus avoiding interference with the compounds interest. The dead ant was washed with pentane, after which 10 μl of the reference mixture were applied on her abdomen. After a few seconds needed for solvent evaporation, the abdomen was sampled with the SPME fiber for 1 min and analyzed. This procedure was then repeated ($N = 11$), except that after some SPME measurements, the ant was washed with 500 μl of pentane for 2 min. This extract was evaporated to 50 μl, and 2 μl was injected for chemical analysis ($N = 4$).

GC analyses were conducted with a HP 5890 Series II chromatograph equipped with a split–splitless injector and a FID detector heated at 260°C. The nonpolar fused-silica capillary column (HP-5, Hewlett Packard, 30 m × 0.32 mm ID, 0.25-μm film phase) was programmed from 260°C (isothermal for 15 min) to 300°C at 5°C/min, then isothermal for 22 min, with helium as carrier gas at 15 psi. The integrations were realized with HP GC-ChemStation Software. Combined gas chromatography–mass spectrometry (GC-MS) was done with either SPME samples or pentane samples, using a Varian 3300 gas chromatograph equipped with a SPI injector heated at 280°C, and linked to a Nermag R10-10C quadrupole mass analyzer piloted by HP GC1034C ChemStation Software. Compounds were eluted on a 25-m × 0.32-mm-ID, 0.4-μm film-phase, nonpolar fused-silica capillary column (DB5-MS, J & W Scientific) programmed from 200 to 310°C at 5°C/min. Spectral data were obtained with electronic impact (EI, 40–550 amu). Chemical ionization (CI) was performed with methyl-vinyl-ether (MVE) (Matheson Gas Products) scanning from 130 to 550 amu.

To estimate the similarity of the hydrocarbon profiles obtained from different SPME-GC measurements of the same individuals, we computed the coefficients of variation (standard deviation divided by mean) for each of the major peaks. We selected six mated alphas that had been measured five times each (over a few weeks), and compared coefficients of variation within and between individuals.

The hydrocarbon profiles obtained by SPME-GC were compared among four functional groups: mated alphas, virgin alphas, young sterile workers, and older sterile workers (which includes foragers). Fourteen major peaks occurring regularly were selected for statistical analysis. The percentages of each peak were compared between groups by an analysis of variance (ANOVA). Since most workers had been sampled several times, we used the mean percentage of each peak. Differences between groups were further investigated with a discriminant analysis. Since relative proportions are dependent variables, they cannot be used for discriminant analysis. Thus, to avoid the limitations inherent to the analysis of compositional data, peak areas were standardized according to the formula of Reyment (1989):

$$Z_{i,j} = \ln [Y_{i,j}/g(Y_j)]$$

where $Y_{i,j}$ is the area of peak i for ant j, $g(Y_j)$ the geometric mean of the areas of all peaks for ant j, and $Z_{i,j}$ the standardized area of peak i for ant j. For workers measured several times, we used the mean standardized areas of each peak. Profiles where one of the peaks had a null area were removed, and thus the discriminant analysis was performed on 57 workers instead of 73 used for the ANOVA. Factor structure coefficients, which are the correlations between each peak and the factors of the discriminant analysis, were plotted to interpret which peak contributes most to the separation between the four groups of workers.

RESULTS

A GC-MS analysis of a pentane extract of the cuticle from an alpha worker is shown in Figure 1, where compounds are numbered in order of elution on the nonpolar column. Eighty-one compounds belonging to the main long-chain hydrocarbon families and ranging from 21 to 37 carbon atoms were identified (Table 2): saturated, mono- and diunsaturated, and methyl-branched alkanes. All methyl-branched alkanes were determined by comparison of equivalent chain lengths (ECL) and diagnostic ion fragments (Table 2) with that of alkanes in other ant species (Lenoir et al., 1997).

Hydrocarbon profiles obtained by SPME-GC are similar, except that there are fewer peaks (Figure 2). Alphas and subordinate workers differ remarkably

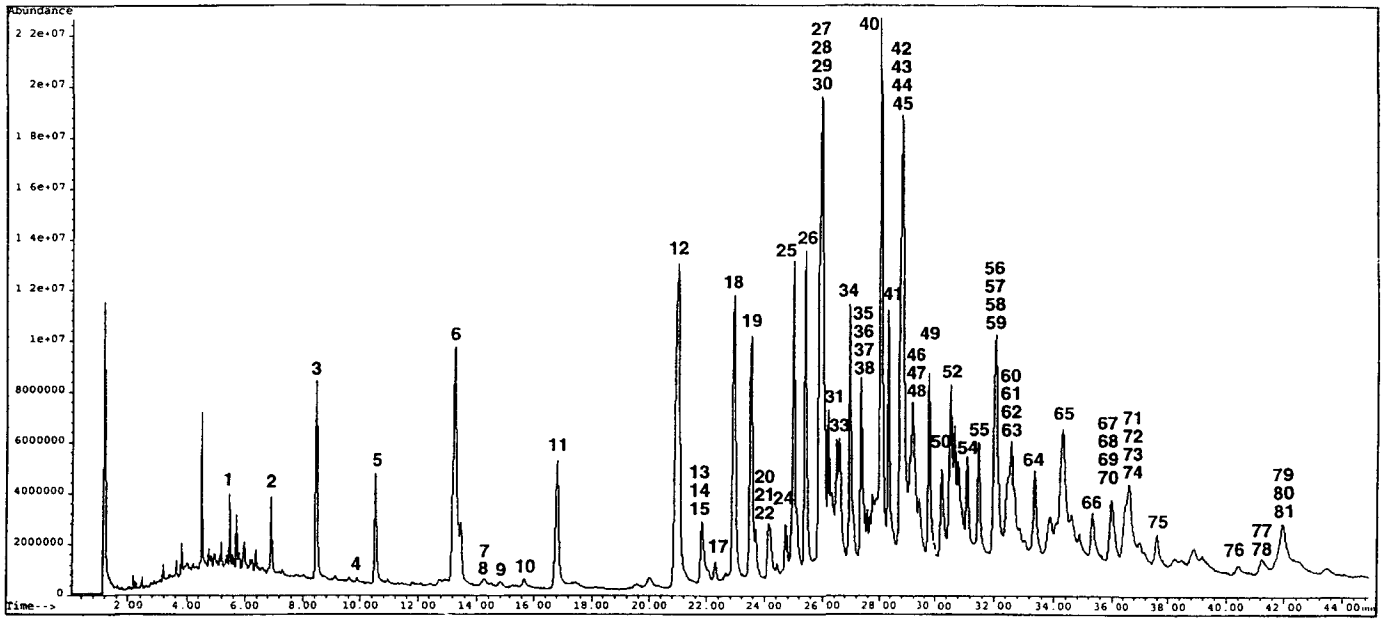

FIG. 1. GC-MS chromatogram of a virgin alpha extracted in pentane. Compounds are identified in Table 2.

TABLE 2. COMPOUNDS IDENTIFIED IN CUTICULAR PENTANE EXTRACT OF VIRGIN ALPHA WORKER, TOGETHER WITH PHYSICOCHEMICAL DATA[a]

No.	Component	ECL	Mol wt	Diagnostic EI ions
1	*n*-C_{21}	21.00	296	296
2	*n*-C_{22}	22.00	310	310
3	*n*-C_{23}	23.00	324	324
4	3-MeC_{23}	23.69	338	56, 308/309
5	*n*-C_{24}	24.00	338	338
6	*n*-C_{25}	25.00	352	352
7	11-MeC_{25}	25.28	366	168/169, 224/225
8	13-MeC_{25}	25.28	366	196/197
9	5-MeC_{25}	25.44	366	84, 308/309
10	3-MeC_{25}	25.69	366	56, 336/337
11	*n*-C_{26}	26.00	366	366
12	*n*-C_{27}	27.00	380	380
13	9-MeC_{27}	27.32	394	140/141, 280/281
14	11-MeC_{27}	27.32	394	168/169, 252/253
15	13-MeC_{27}	27.32	394	196/197, 224/225
16	7-MeC_{27}	27.41	394	112, 308/309
17	5-MeC_{27}	27.51	394	84, 336/337
18	3-MeC_{27}	27.77	394	56, 364/365
19	*n*-C_{28}	28.00	394	394
20	9-MeC_{28}	28.33	408	140/141, 294/295
21	11-MeC_{28}	28.33	408	168/169, 266/267
22	13-MeC_{28}	28.33	408	196/197, 238/239
23	5-MeC_{28}	28.48	408	84, 350/351
24	3-MeC_{28}	28.78	408	56, 378/379
25	9-C_{29}:1	28.79	406	138/170*, 292/324*
26	*n*-C_{29}	29.00	408	408
27	9-MeC_{29}	29.35	422	140/141, 308/309
28	11-MeC_{29}	29.35	422	168/169, 280/281
29	13-MeC_{29}	29.35	422	196/197, 252/253
30	7-MeC_{29}	29.41	422	112, 336/337
31	5-MeC_{29}	29.51	422	84, 364/365
32	11,15-diMeC_{29}	29.58	436	168, 295, 239, 224
33	13,17-diMeC_{29}	29.58	436	196, 267
34	3-MeC_{29}	29.73	422	56, 392/393
35	*n*-C_{30}	30.00	422	422
36	11-MeC_{30}	30.31	436	168/169, 294/295
37	12-MeC_{30}	30.31	436	182/183, 280/281
38	13-MeC_{30}	30.31	436	196/197, 266/267
39	14-MeC_{30}	30.31	436	210/211, 252/253
40	9-C_{31}:1	30.80	434	138/170*, 320/352*
41	*n*-C_{31}	31.00	436	436
42	9-MeC_{31}	31.32	450	140/141, 336/337
43	11-MeC_{31}	31.32	450	168/169, 308/309
44	13-MeC_{31}	31.32	450	196/197, 280/281

TABLE 2. CONTINUED

No.	Component	ECL	Mol wt	Diagnostic EI ions
45	15-MeC_{31}	31.32	450	224/225, 252/253
46	9,13-diMeC_{31}	31.57	464	140, 351, 211, 280
47	11,15-diMeC_{31}	31.57	464	168, 323, 239, 252
48	13,17-diMeC_{31}	31.57	464	196, 295, 267, 224
49	*n*-C_{32}	32.00	450	450
50	12-MeC_{32}	32.27	464	182/183, 308/309
51	14-MeC_{32}	32.27	464	210/211, 280/281
52	2Δ-C_{33}:2	32.44	460	460
53	2Δ-C_{33}:2	32.52	460	460
54	9-C_{33}:1	32.77	462	138/170*, 348/380*
55	*n*-C_{33}	33.00	464	464
56	11-MeC_{33}	33.32	478	168/169, 336/337
57	13-MeC_{33}	33.32	478	196/197, 308/309
58	15-MeC_{33}	33.32	478	224/225, 280/281
59	17-MeC_{33}	33.32	478	252/253
60	9,11-diMeC_{33}	32.58	492	140, 379, 211, 308
61	11,15-diMeC_{33}	32.58	492	168, 351, 239, 280
62	13,17-diMeC_{33}	32.58	492	196, 323, 267, 252
63	15,19-diMeC_{33}	32.58	492	224, 295
64	*n*-C_{34}	34.00	478	478
65	2Δ-C_{35}:2	34.48	488	488
66	*n*-C_{35}	35.00	492	492
67	11-MeC_{35}	35.29	506	168/169,364/365
68	13-MeC_{35}	35.29	506	196/197, 336/337
69	15-MeC_{35}	35.29	506	224/225, 308/309
70	17-MeC_{35}	35.29	506	252/253, 280/281
71	9,13-diMeC_{35}	35.57	520	140, 407, 211, 336
72	11,15-diMeC_{35}	35.57	520	168, 379, 239, 308
73	13,17-diMeC_{35}	35.57	520	196, 351, 267, 280
74	15,19-diMeC_{35}	35.57	520	224, 323, 295, 252
75	*n*-C_{36}	36.00	506	506
76	*n*-C_{37}	37.00	520	520
77	13-MeC_{37}	37.31	534	196/197, 364/365
78	15-MeC_{37}	37.31	534	224/225, 336/337
79	11,15-diMeC_{37}	37.58	548	168, 407, 239, 336
80	13,17-diMeC_{37}	37.58	548	196, 379, 267, 308
81	15,19-diMeC_{37}	37.58	548	224, 351, 295, 280

[a] Diagnostic ions marked with an asterisk were used to locate the double bond position.

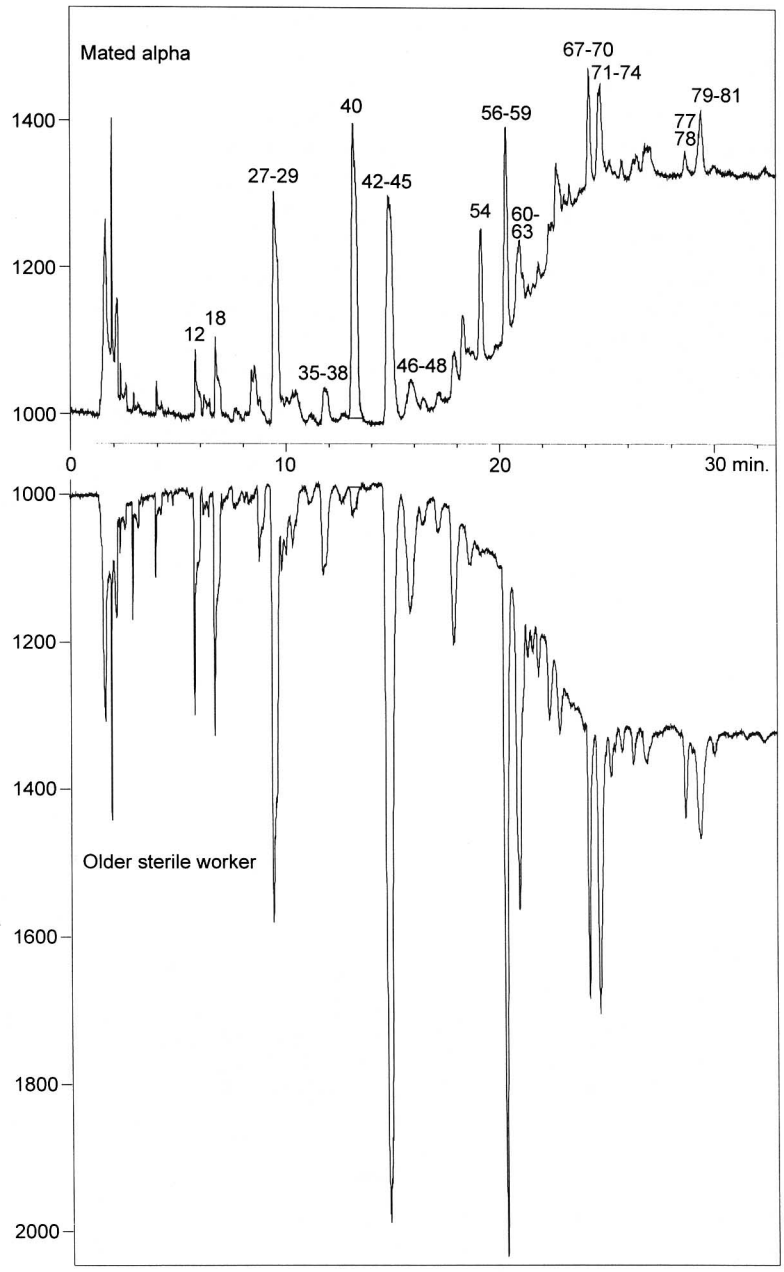

FIG. 2. SPME-GC chromatograms of a mated alpha (above) and an older sterile worker (below). Note the difference for peak 40 (9-hentriacontene), characteristic of alpha workers. Quantitative details are given in Table 3.

by the amount of peak 40. EI mass spectrum of this peak is characteristic of a monounsaturated alkane with 31 carbon atoms [ions at *m/z* 55, 69, 83, 97, 111, 434 (M^+)]. The double bond position was determined directly from the ion-molecule reaction with MVE (Ferrer-Correia et al., 1976; Malosse et al., 1994). In the GC-(MVE)-CI/MS mass spectrum, in addition to molecular ion species [*m/z* 432 $(M-2H)^+$ and 493 $(M+HMVE)^+$], diagnostic ions at *m/z* 138/170 and 320/352 locate unambiguously the double bond at a C_9 position (9-hentriacontene, or 9-C_{31}). These two pairs of ions, both separated by 32 amu, are related to the position of the double bond in the initial alkene. The C_{29} and C_{33} alkenes present in the extracts were identified in the same way (diagnostic ions marked with an asterisk in Table 2).

SPME is nondestructive and thus allowed for multiple extractions of the same individuals (Table 1). To examine the repeatability of SPME measurements, we compared coefficients of variation within and between individuals (shown for six mated alphas in Figure 3). Intraindividual variation was lower than interindividual variation for most major peaks, except those in the higher range of the chromatographic profiles. This indicates that our measurements are reliable to group individuals according to the similarity of their profiles.

After assigning individual workers to four arbitrary groups (based on age

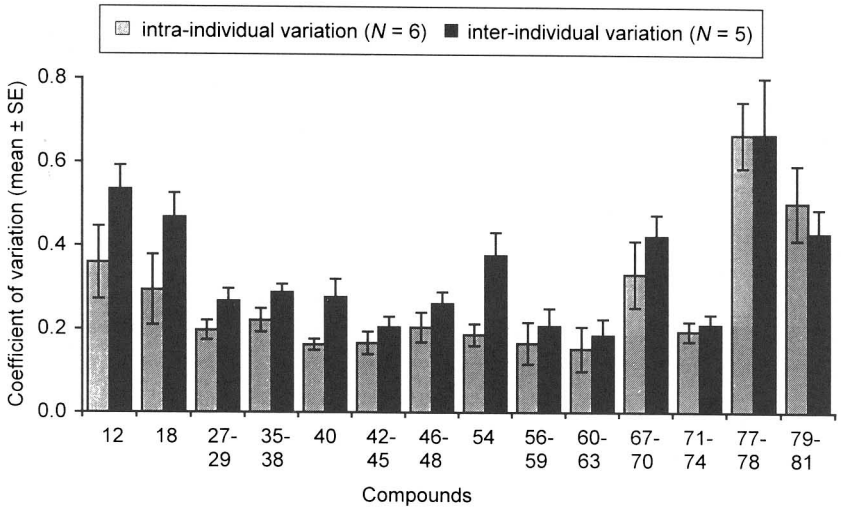

FIG. 3. Repeatability of SPME measurements for each of the 14 major peaks. A coefficient of intraindividual variation was computed with five repeated measurements for each of six alphas. A coefficient of interindividual variation was computed using one of the measurements for each alphas, and this was repeated five times.

and dominance characteristics), we used a discriminant analysis to compare their cuticular hydrocarbon profiles (based on the areas of 14 major peaks). Our biological groupings were retained (Figure 4), revealing a link with cuticular differences. Three groups were clearly separated: alphas (both virgin and mated), young sterile workers, and older sterile workers. The factor structure coefficients indicated that 9-C_{31} contributes most to the discrimination between these groups along factor 1 (74% of the variance). The ANOVA confirmed that alphas yield higher percentages of 9-C_{31} than subordinate workers (Table 3). Although the discriminant analysis did not separate mated alphas from virgin alphas (Figure 4), the ANOVA found that mated alphas have higher percentages of 9-C_{31}. Peak 54 (9-tritriacontene) discriminated both alphas and young sterile workers from older workers along factor 1, and young sterile workers from alphas along factor 2 (20% of the variance) (Figure 4). Further evidence that this compound is not characteristic of alphas is its higher relative proportion in young sterile workers (Table 3). When the discriminant analysis was repeated without 9-C_{31} and 9-tritriacontene, the worker groups were no longer separated (Figure 5), indicating that no other compounds can discriminate alphas from sterile workers.

SPME of alpha workers revealed that the proportion of 9-C_{31} does not differ between head, petiole, and abdomen (6.6 $\pm$ 0.5, 7.1 $\pm$ 0.6, and 7.8 $\pm$ 0.7%, respectively; $N = 12$, ANOVA: $P = 0.205$). However, all compounds were present in higher quantities on the abdomen than on the head and the petiole (704 $\pm$ 88, 367 $\pm$ 60, and 380 $\pm$ 39 ng, respectively; ANOVA, post-hoc comparison: Scheffé test: $P < 0.01$). 9-C_{31} was not found in the Dufour's gland of five alpha workers (identified products included saturated, mono- and diunsaturated hydrocarbons from n-C_{14} to n-C_{23}), whereas in two of these workers, pentane washes of the cuticle yielded 9.3 and 9.8% of 9-C_{31}.

Comparison of SPME ($N = 11$) and pentane extractions ($N = 4$) of the reference mixture indicated that pentane gives the closest profiles, while SPME preferentially extracted some shorter-chain hydrocarbons (43% vs 26% for n-C_{23} with SPME and pentane, respectively) and underextracted longer-chain hydrocarbons (11, 13, 4, and 3% vs. 16, 24, 8, and 5% for n-C_{30}, n-C_{32}, n-C_{34}, and n-C_{36} with SPME and pentane, respectively). The difference was significant at $P < 0.05$ for n-C_{36} and $P < 0.01$ for the other compounds (Mann-Whitney U test). In contrast, SPME ($N = 26$) and pentane extraction ($N = 3$) of alpha workers revealed that SPME underestimated shorter-chain compounds (No. 12, 18, 27–29, 40) and overestimated longer-chain compounds (No. 54, 56–59, 60–63, 67–70, and 71–74). Extraction of older sterile workers by SPME ($N = 36$) and pentane ($N = 7$) showed similar tendencies, but the percentages of each peak did not differ significantly (Mann-Whitney U test: $P > 0.05$). Both SPME and pentane extracted approximately seven times more 9-C_{31} on alphas than on sterile workers. SPME measured 53 $\pm$ 6 vs. 7 $\pm$ 1 ng for alphas

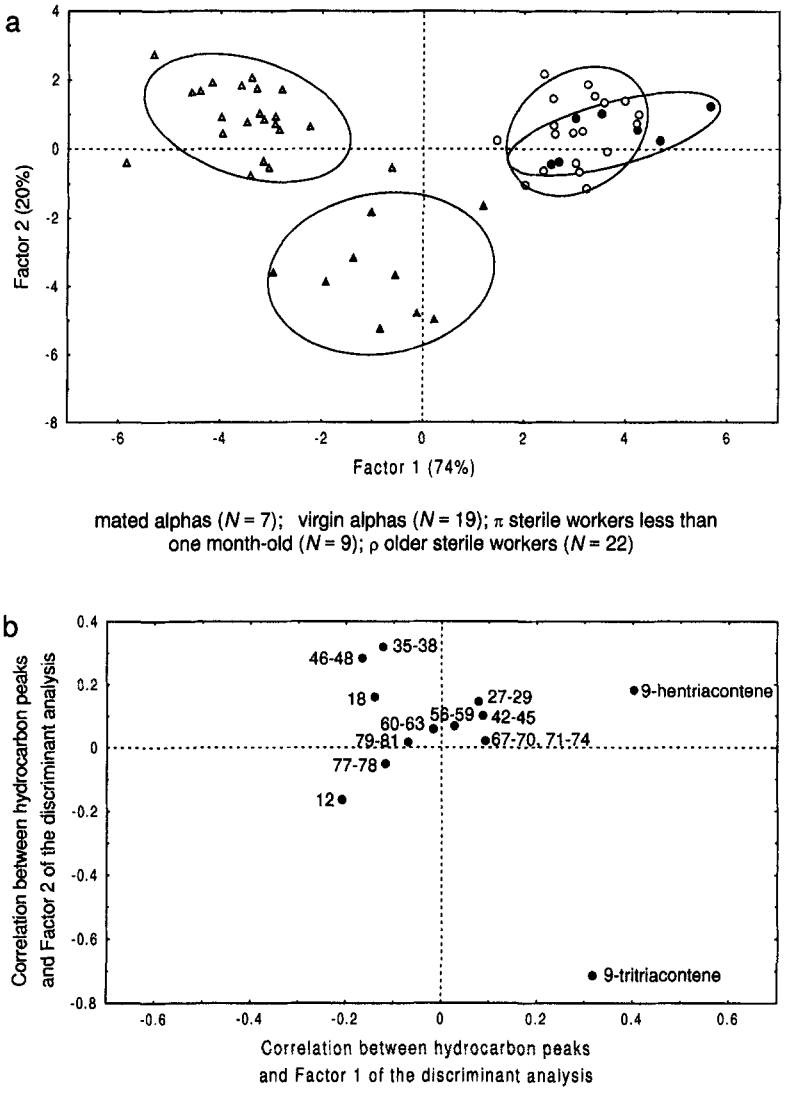

FIG. 4. Discriminant analysis of 57 workers assigned to four functional groups. The areas of 14 major peaks were compared (after standardization). Each data point represents the mean measure for one individual in one group (two workers switched groups). Envelopes represent the 95% confidence ellipses. Factor structure coefficients are indicated in lower graph.

TABLE 3. MEAN PERCENTAGES OF 14 MAJOR PEAKS (±SE) OBTAINED BY 233 SPME-GC OF 73 WORKERS[a]

Compound number	Mated alphas (N = 7)	Virgin alphas (N = 19)	Young sterile workers (N = 11)	Older sterile workers (N = 36)
12	2.9 ± 0.4	3.1 ± 0.2	3.5 ± 0.7	3.6 ± 0.3
18	3.4 ± 0.5	4.4 ± 0.3	3.3 ± 0.6	4.2 ± 0.3
27-29	10.0 ± 0.8ab	11.7 ± 0.7a	7.0 ± 1.0b	10.5 ± 0.7ab
35-38	2.0 ± 0.1ab	2.3 ± 0.1a	1.5 ± 0.2b	2.4 ± 0.1a
40	9.7 ± 0.9a	7.3 ± 0.7b	1.7 ± 0.3c	0.8 ± 0.1c
42-45	13.9 ± 0.7ab	15.2 ± 0.7ab	11.0 ± 1.2b	17.2 ± 0.8a
46-48	3.0 ± 0.2ab	3.1 ± 0.2a	1.9 ± 0.3b	3.0 ± 0.2ab
54	3.1 ± 0.3ac	2.3 ± 0.2a	5.0 ± 0.9c	0.4 ± 0.1b
56-59	7.9 ± 0.2	7.5 ± 0.2	6.9 ± 0.7	8.3 ± 0.4
60-63	6.3 ± 0.4	6.3 ± 0.2	6.2 ± 0.6	7.4 ± 0.2
67-70	19.0 ± 2.3	18.4 ± 2.0	28.4 ± 3.8	23.0 ± 2.1
71-74	13.0 ± 1.2ab	12.4 ± 0.7a	16.1 ± 1.1b	12.2 ± 0.4a
77-78	1.8 ± 0.2	1.8 ± 0.2	2.6 ± 1.1	2.8 ± 0.5
79-81	4.0 ± 0.4	4.1 ± 0.2	4.8 ± 1.3	4.2 ± 0.4
Total	100	100	100	100

[a]The peaks are constituted by 39 compounds identified in Table 2. The percentage of each peak was compared between the groups of workers with an ANOVA (post-hoc comparison: Tukey HSD test for unequal sample size). Categories differing at $P < 0.05$ are indicated with different letters. Two workers switched groups between successive measurements.

and sterile workers, respectively (mean ± SE, i.e., 7.6 times more on alphas), while pentane extracted 33 ± 11 vs. 5 ± 2 μm on alphas and sterile workers, respectively (6.6 times more on alphas).

DISCUSSION

Gas chromatography and mass spectrometry of long-chain hydrocarbons on the cuticle clearly demonstrate that the alpha workers of *D. quadriceps* differ from their sterile nestmates by the relative proportion of 9-hentriacontene. This difference was not affected by colony membership since it was found for all alphas. Discriminant analysis revealed that 9-tritriacontene is also important to discriminate between groups. It differed significantly between young workers and virgin alphas, and its proportion was lowest in old sterile workers, suggesting that it is characteristic of the cuticle of recently eclosed workers.

The alpha ant lays most or all the eggs in *D. quadriceps* and is the only

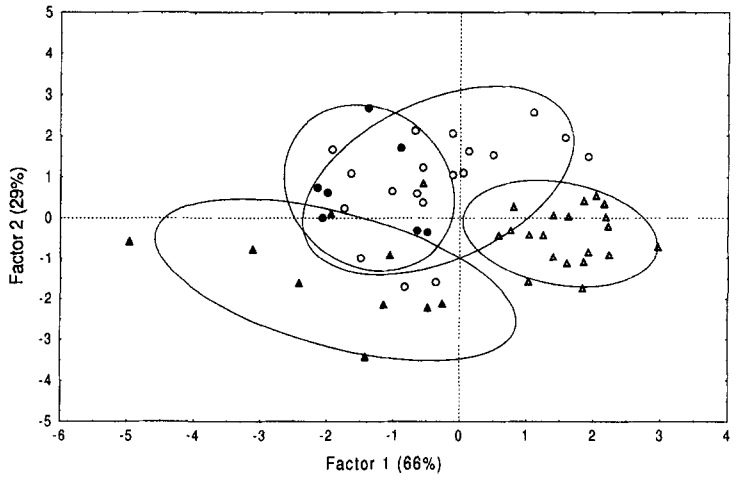

FIG. 5. Same discriminant analysis after removing 9-hentriacontene and 9-tritriacontene. Areas were restandardized with the geometric mean of the 12 remaining peaks. The groups are no longer separated.

individual with fully developed ovaries. Although one to three high-ranking workers sometimes lay a few eggs, their ovaries are much less developed (Monnin and Peeters, 1997). The correlations presented here strongly suggest that 9-C_{31} levels are related to ovarian activity. Virgin and mated alphas had significantly different levels, which may be associated with a small difference in ovarian activity due to insemination. Cuticular hydrocarbons have been shown to transmit information used in colonial or kin recognition in a number of ants, bees, and wasps (Bonavita-Cougourdan et al., 1987; Espelie et al., 1994; Arnold et al., 1996; Bagnères et al., 1996). We think this function may also be important in *D. quadriceps*, and thus the differences in long-chain hydrocarbons described in our study are probably superimposed onto intercolony differences.

Repeated SPME-GC measurements of the same individuals demonstrated a low variability, and thus this extraction technique can produce reliable data about individuals. Although SPME slightly altered the profile of a reference mixture, its results are consistent when extracting both this mixture as well as cuticular hydrocarbons from live ants, thus allowing the comparison of individuals. SPME presents several advantages over classical solvent extraction. It is easy to use and, most of all, it is not destructive and allows the study of valuable individuals without sacrificing them. Thus, it also permits studying of the tem-

poral dynamics of an hydrocarbon profile. Since it is solventless, it does not extract compounds from inside the insect, unlike whole-body solvent washes. Furthermore, SPME permits extraction of a precise part of the body. Given the large size of *D. quadriceps* workers, a sufficient quantity of cuticular hydrocarbons could be extracted that allowed GC analysis, and even GC-MS. This may be more difficult with smaller insects, although sensitivity is increased since no solvent is used. Nevertheless, SPME has the disadvantage that samples cannot be stored and must be analyzed immediately.

Long-chain hydrocarbons were more abundant on the abdomen than on the head and petiole, suggesting that they are synthesized in this part of the body. 9-C_{31} was not found in the Dufour's glands of two alphas, whereas it had been measured on their cuticle. Since the antennae of subordinates are rubbed against the membranes between abdominal segments V and VII, which lie over the pygidial gland in *D. quadriceps* (J. Billen, personal communication), this gland needs to be considered as a possible source of 9-C_{31}. In *D. australis* the pygidial gland is composed of secretory cells connected to a small invagination of the intersegmental membrane, which forms a tiny reservoir (Billen et al., 1995). We initially dissected the pygidial glands of alphas and sterile workers and extracted them in solvent, but failed to obtain resolution of any peaks. Comparative insect physiology hints that 9-C_{31} and other long-chain hydrocarbons are more likely to be produced by the oenocytes, which lie just underneath the intersegmental membranes of the abdomen. Epidermal oenocytes are located at the base of the epidermis, usually against the basal lamina, and are generally involved in the biosynthesis of cuticular hydrocarbons. Indeed, oenocytes may be considered to be true exocrine cells, because their secretory products are transported to the cuticle (Noirot and Quennedey, 1991). In honeybees, the oenocytes secrete wax, which is carried to the outside via microtubules (Hepburn, 1986). In the desert locust, oenocytes synthesize cuticular lipids (Diehl, 1975), and in *Culicoides nubeculosus* (Diptera), they are involved in the production of sex pheromones present on the cuticle (Ismail and Zachary, 1984). A relationship between ovarian maturation and sexual attractiveness (mediated by cuticular hydrocarbons) has been shown in *Calliphora vomitoria* (Diptera) (Trabalon et al., 1990). Whatever their precise glandular origin in *D. quadriceps*, long-chain hydrocarbons seem to diffuse over the rest of the body surface. Rubbing the antennae of subordinates on the alpha's abdomen may be an efficient means to transfer nonvolatile cuticular hydrocarbons (the abdomen yields the highest amount of hydrocarbons).

Dinoponera australis is another species in which only the alpha worker can mate and has active ovaries (Paiva and Brandão, 1995). After studying a single colony, Oldham et al. (1994) reported that the alpha differed from sterile workers in her mandibular gland secretions (aldehydes and pyrazines). However this difference concerns the total amount of secretion (one gamergate was extracted

once in solvent). Oldham et al. (1994) suggested that this difference is linked either with reproduction or with foraging. The latter seems better supported by the limited evidence, since the mandibular gland of one forager contained the highest amount of secretion, while the gland of the gamergate contained the lowest.

Most polistine wasps lack a morphologically specialized reproductive caste, and sterility is regulated through aggressive interactions—only the alpha lays eggs, as in many queenless ants (Reeve, 1991; Röseler, 1991a). In *P. dominulus*, the alpha was found to differ in the relative proportions of several hydrocarbons (C_{31}–C_{35}) relative to her sterile subordinates (Bonavita-Cougourdan et al., 1991). The characteristic cuticular hydrocarbon profile of the alpha ant was not colony-specific, and the alpha differed more from her subordinates than these differed from foreign workers. Several cuticular compounds identified in *P. dominulus* were the same as in *D. quadriceps*.

In bumblebees (*Bombus hypnorum*), reproductive division of labor is also regulated by a dominance hierarchy (which can be affected by size differences among the monomorphic adult females) (Röseler, 1991b). The alpha individual lays most of the eggs, although high-ranking subordinates also oviposit. Pentane rinses of the cuticle yielded a variety of long-chain alkanes, alkenes, and alkadienes (Ayasse et al., 1995). Alphas had higher amounts of (*Z*)-11-pentacosene, (*Z*)-7-pentacosene, methyltricosene, methylpentacosene, heptacosadiene, octacosadiene, and tricontadiene relative to egg-laying subordinates, which themselves yielded higher amounts than sterile subordinates. These differences were found in all colonies, and the alphas differed more from their nestmate workers than the latter differed from foreign workers (Ayasse et al., 1995).

The queenless ant *D. quadriceps*, the wasp *P. dominulus*, and the bee *B. hypnorum* share the characteristic that reproductive differentiation occurs in the adult stage (unlike social insects with dimorphic queens and workers). Dominance interactions regulate ovarian activity, and there is accumulating evidence that differences in cuticular hydrocarbons characterize fertile and sterile individuals. Other studies have shown that either dominance rank or ovarian development is recognized by means other than physical aggression alone (West-Eberhard, 1977; Downing and Jeanne, 1985). Similarly in honeybees, orphaned workers with active ovaries are discriminated against (Visscher and Dukas, 1995). Unlike the more complex differences in hydrocarbon bouquets described in *P. dominulus* and *B. hypnorum*, the olfactory signal of fertile workers in *D. quadriceps* appears to differ by one hydrocarbon only, revealing it to be an ideal species to investigate status recognition. Bioassays are now needed to test the activity of 9-hentriacontene. The nondestructive nature of SPME has also enabled us to study the temporal characteristics of the induction of 9-C_{31} in workers that accede to the alpha rank (Peeters et al., unpublished data).

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