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DUFOUR GLAND CONTENTS OF ANTS OF THE *Cataglyphis bicolor* GROUP

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Abstract—The species of desert-dwelling ants of the *Cataglyphis bicolor* (Hymenoptera: Formicidae) group are difficult to distinguish by morphological features. Analysis of the secretion from the Dufour glands of workers of a number of colonies was undertaken to see if it provided a clear test of species. Linked 6c-ms showed in all samples straight and branched-chain alkanes, linear alkenes, ketones, aldehydes, acetates, and a group of C_{22} to C_{28} esters not previously identified in this genus. Contents of the Dufour glands of *C. savignyi* from Tunisia and Egypt were similar, and comprised straight and branched-chain alkanes, alkenes and small amounts of esters. *C. bicolor* from Tunisia contained compounds similar to *C. savignyi* but was distinguished from the latter by larger amounts of the esters. The major compound in the glands of *C. viaticus* was tridecane, in contrast to the pentadecane of other species. It also contained a branched alkane, 3-methyltridecane as a major component. Branched-chain esters and a wide variety of acetates were also found in this species. *C. diehlii* had a limited range of compounds, with branched alkanes almost completely absent and high proportions of pentadecene and dodecyl acetate. *C. bombycinus*, a sympatric species, but recognized as not belonging to the *bicolor* group by its different mandibular gland substances, was notable in having butanoate esters in its Dufour glands. Despite these differences among species, both the great variability of individuals from a single colony and the among between conspecific colonies make species diagnosis from a few individuals difficult, in contrast with postpharyngeal glands, which, as recently reported, give a clearer indication of species.

Key Words—Hymenoptera, Formicidae, Formicinae, exocrine secretion, hydrocarbons, esters, species specificity, species diagnosis.

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INTRODUCTION

The ant genus *Cataglyphis* includes more than one hundred named taxa, with others yet undescribed, and is distributed in the Old World from Mauritania to the Gobi Desert. Almost all species thrive in open habitats, including deserts and dry salt plains. Because of their thermophilic and desert-dwelling behavior, they have been the subject of intensive research in neuroethology (Wehner, 1994) and physiology (Lighton and Wehner, 1993). Much of this work was carried out on "*Cataglyphis bicolor*", but during the course of this work it became apparent that this "*C. bicolor*" was either one species which varied slightly in external appearance from place to place or it was a group of closely similar species, and these were tentatively referred to as forms a, b, and c in Wehner et al., (1983).

Cataglyphis are unique among formicine ants in that the morphology of the male genitalia is diverse, making this genus easily distinguishable from others. However, anatomical similarities make the classification difficult at the species level. Because the morphological characters are lacking, neither can the species be diagnosed sufficiently nor can the phylogenetic relationships among species be easily inferred (Agosti, 1990). The work of Santschi (1929), who spent four decades in Tunisia concentrating on *Cataglyphis*, left a tangle of specific and subspecific names and synonyms. The work of Agosti (1990) supported by chemical studies of exocrine secretions of the groups (Keegans et al., 1992) and isozyme and *m* DNA analyses (Ready and Agosti, unpublished) have made possible the identification of some species of the group.

The Dufour gland is present in all females and workers of aculeate Hymenoptera, and although it has been shown to have a function (presumably secondary) in some individual species or genera, it has no known common function. Nevertheless, its contents have been found to be species-specific in many studies where closely related species have been compared. Usually the gland contains a mixture of linear hydrocarbons, terpenes, or oxygenated alkyl compounds.

Some chemotaxonomic studies have already been carried out on the Dufour gland contents of *Cataglyphis* species. The Dufour glands of *C. savignyi* from Egypt were shown to contain straight chain saturated and unsaturated hydrocarbons from C_{11} to C_{23}, together with some branched hydrocarbons with methyl groups at 3-, 5-, and 7-position. Oxygenated compounds (e.g., 2-ketones, 1-alcohols and branched chain aldehydes) were identified in trace quantities (Ali et al., 1988). Hefetz and Lenoir (1992) analysed Dufour glands of six *Cataglyphis* species from Israel, Tunisia, Spain, and the South of France. The major common feature was saturated hydrocarbons ranging from C_{11} to C_{19}. Minor amounts of corresponding alkenes and some oxygenated components in trace amounts were also observed. The main differences among species were expressed in the identity of the major components and the relative amounts of all components present in the secretion.

Our work, previously described for 10 species of *Cataglyphis* collected in Syria and Tunisia, showed that those species could be distinguished by their Dufour gland contents (Keegans et al., 1992). Five belonging to the bicolor group were similar. *C. niger* and *C. nodus* had pentadecane, tridecane, heptadecane, tetradecane, and tridecane in decreasing amounts as the major hydrocarbons. However, *C. niger* had equal quantities of 2-pentadecanone and 2-heptadecanone, whereas *C. nodus* had more 2-pentadecanone than 2-heptadecanone. The two types from Tunisia, called at that time *C. bicolor*-1 and *C. bicolor*-2, were similar to *C. niger* and *C. nodus* but also showed some differences. *C. bicolor*-1 had more tridecane (the major compound) and dodecanol than the other species but less pentadecanone than *C. niger* or *C. nodus*. The most obvious difference in *C. bicolor*-2 was the low amount of oxygenated compounds in its gland. *C. isis*, also from the bicolor group, was distinguished from the other four by the large amounts of dodecanol and tridecane it contained (Keegans et al., 1992).

We also discovered that the *bicolor* group has a distinctive feature in that the species examined all contain 2-methyl-1-hexanol, a new substance to exocrine secretions, as the major substance in their mandibular glands (Agosti et al., 1996). This substance was not found in any species outside the *bicolor* group.

For the present work, colonies of the *C. bicolor* group were collected at various sites in Tunisia and Egypt, identified only by a code (which referred to some morphological character, Table 1) or by the name of the place of collection. Samples of Dufour glands were taken from workers for chemical analysis. The code names were united with the new species names (*bicolor*, *diehlii*, *savignyi* and *viaticus*) derived from morphological examination of the male genitalia, after the analyses were finished. We describe the chemical examination and statistical analysis of the results of the analysis of Dufour glands here as they relate to these species and one more sympatric species, *C. bombycinus*, which was different and belongs to another *Cataglyphis* group.

MATERIALS AND METHODS

The ants were collected at sites in Tunisia and Egypt by D. Agosti during 1992. For location, see Wehner et al., (1994). The nests were brought live to Zürich where the workers were dissected. The Dufour glands were sealed in glass capillaries for transport to Keele as described by Morgan (1990). They were analyzed there by linked gas-chromatography-mass spectrometry (GC-MS), by the method of Morgan and Wadhams (1972).

The GC-MS was performed with a Hewlett-Packard 5890 gas chromatograph linked to a 5970B Mass Selective Detector, set to monitor m/z 35–350. The system was controlled by an HP Series 300 computer with HP 59970C ChemStation software. A fused silica capillary column (12 m $\times$ 0.32 mm; 0.25 μm) of 5%

TABLE 1. COLLECTION AREAS OF THE *Cataglyphis bicolor* GROUP SAMPLES IN NORTH AFRICA

Sample name*	Collection area†	Species	Country
bdw t1 10a	Transect 1-location 10	*savignyi*	Tunisia
bdw t1 10b	Transect 1-location 10	*savignyi*	Tunisia
bdw t1 10c	Transect 1-location 10	*savignyi*	Tunisia
bdw t3 1a	Transect 3-location 1	*savignyi*	Tunisia
bdw t3 2a	Transect 3-location 2	*savignyi*	Tunisia
bdw t3 2b	Transect 3-location 2	*savignyi*	Tunisia
bdb t3 1b	Transect 3-location 1	*bicolor*	Tunisia
bdb t3 2c	Transect 3-location 2	*bicolor*	Tunisia
bdb t3 2e	Transect 3-location 2	*bicolor*	Tunisia
bdb t3 3d	Transect 3-location 3	*bicolor*	Tunisia
bdb t3 6a	Transect 3-location 6	*bicolor*	Tunisia
bdb t3 7c	Transect 3-location 7	*bicolor*	Tunisia
bb t3 6a	Transect 3-location 6	*viaticus*	Tunisia
bb t3 7d	Transect 3-location 7	*viaticus*	Tunisia
bb t3 8c	Transect 3-location 8	*viaticus*	Tunisia
bb t3 10a	Transect 3-location 10	*viaticus*	Tunisia
Gafsa-bom	Gafsa	*bombycinus*	Tunisia
Kebili-bom	Kebili	*bombycinus*	Tunisia
Diehlii	Metlaoui	*diehlii*	Tunisia
Siwa 3	Siwa	*savignyi*	Egypt
Siwa 6	Siwa	*savignyi*	Egypt
Gizeh 3 31	Gizeh	*savignyi*	Egypt
Luxor	Luxor	*savignyi*	Egypt
Marsah Matruh	Marsah Matruh	*savignyi*	Egypt
Dakhla	Dakhla	*savignyi*	Egypt

*Samples were assigned to groups by the following characters: bdw, Large workers with head and alitrunk dark red to black, legs concolor to black; bdb, Alitrunk and dorsum of petiole with few white or yellowish, short erect hairs, which are less than half the length of the black, erect hairs on the occiput; and bb, Large workers with head and alitrunk bright red, legs sometimes slightly darker.

†For map, see Figure 1, also Wehner et al. (1994) or Agosti et al. (1996).

phenyl −95% methylsiloxane (non-polar phase) (SGE Milton Keynes) was used for chromatography. The samples were heated in the injector at 200°C for 3 min before crushing the glass capillary. The column was initially at 30°C for 2 min, then heated at 8°C min^{-1} to 250°C, and held at that temperature, to give a total run time of 40 min. The split valve was closed for the injection and opened 30 sec after injection.

Identification of the compounds was made with the aid of The Wiley- NIST mass spectral library (1994 version), and collections of spectra made in the laboratory. Final identification was made, wherever possible, by comparing retention times and mass spectra with commercially available materials (linear alkanes,

2-alcohols, and 2-alkanones) or in the cases of 2-tridecyl acetate, dodecyl decanoate, dodecyl tetradecanoate, and butyl oleate they were synthesized in the laboratory. Esters were prepared on a small scale by the method of Attygalle et al., (1987). For quantitative analysis, peak areas for each component in the chromatogram were determined by computer integration, and the percentage of each substance in the gland was calculated. For each compound, average percentage value and standard deviation were calculated for each colony and for each group of colonies identified by the same code. In the *t* test, data transformed by arcsin $[\sqrt{(\%/100)}]$ were used (Sokal and Rohlf, 1995).

RESULTS

Since the work was begun blind, a few mandibular glands of workers from each of the 25 colonies provided were examined first by GC-MS. In 23 out of 25 colonies, samples all contained 2-methyl-1-hexanol (Agosti et al., 1996), and these colonies were all placed in the *bicolor* group based on this evidence. Only the Gafsa and Kebili samples did not contain 2-methylhexanol; they both contained citronellol and citral (c.f. Keegans et al., 1992), and these colonies were assigned to the *bombycinus* group.

Altogether, 7057 chromatographic peaks from Dufour glands were identified and quantified for 195 workers from 25 colonies. For each of these colonies, the mean values of the area for each peak were calculated together with their standard deviations.

Table 1 gives the code names of the samples, the number of specimens analyzed per colony, their place of origin, and the species names assigned. The location of the sample sites are given in Figure 1.

C. savignyi, Tunisia (bdw)

Five colonies were analyzed. In each specimen but 3, the major compounds, beginning with the one in greatest quantity were pentadecane, tridecane, and undecane (Table 2). The substances found at highest retention times (peak numbers 84, 93, 94, 96–99, and 105 in Table 2) corresponded to long-chain esters. Some samples from locations t1 10b and t1 10c also contained some linear and branched ketones, mainly 2-pentadecanone and 2-heptadecanone, which in other samples were present in tiny amounts or were undetectable.

C. savignyi, Egypt

In general, the Dufour gland contents of the colonies collected in Egypt were similar to those of the same species samples collected in Tunisia. For example,

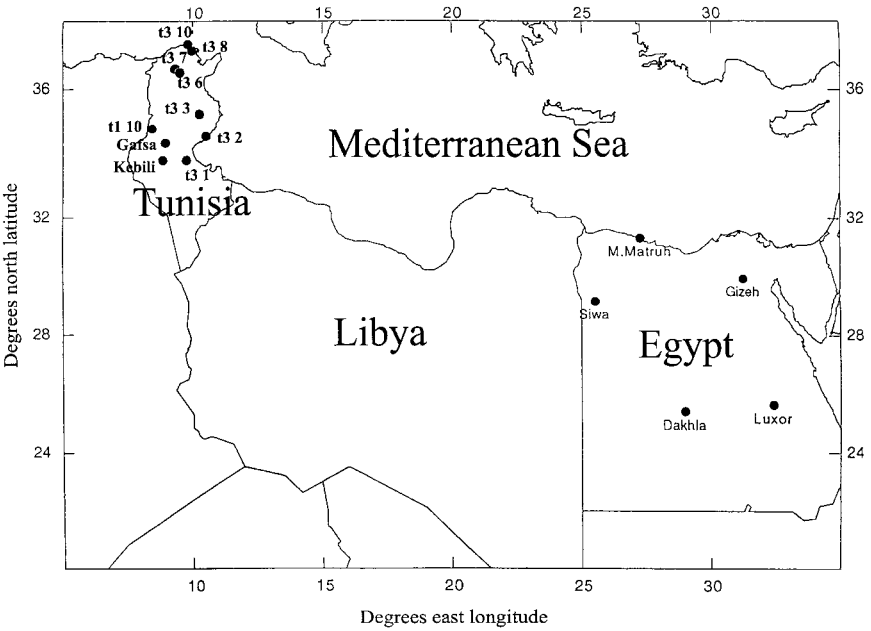

FIG. 1. The collection sites of nests of *Cataglyphis* ants in Tunisia and Egypt. The locations in Tunisia are explained in the text.

in all the Egyptian specimens, the order of the major compounds was pentadecane, tridecane, and undecane as in the Tunisian samples. However, undecane and tridecane were present in a significantly higher amount in the Tunisian samples (t test on the transformed data, respectively $t = 4.11$, $P < 0.001$ and $t = 4.44$, $P < 0.001$), while there was proportionally more of tetradecane and heptadecane in the Egyptian samples ($t = 7.55$, $P < 0.001$ and $t = 2.01$, $P = 0.048$, respectively). Moreover, in the Egyptian samples, the average percentage of branched hydrocarbons is almost twice the value found in the Tunisian specimens (13.5 and 7.4, respectively; t test on the transformed data $t = 4$, $P < 0.001$). No relevant differences were noted between the two samples for the non-hydrocarbon components.

C. bicolor (bdb)

The immediate difference noted in the bdb samples, collected across transect 3 in Tunisia, was their higher proportion of esters compared with the *savignyi* samples. All the esters identified were also found in the *savignyi* samples (Table 2), with the exception of those corresponding to peak numbers 85, 86, 102, and 103.

TABLE 2. AVERAGE PERCENTAGES ± STANDARD DEVIATION OF THE COMPOUNDS FOUND IN THE SIX *Cataglyphis* SPECIES ANALYZED*

Peak number	Compound name	*C. savignyi* Tunisia (bdw)	*C. savignyi* Egypt	*C. bicolor* Tunisia (bdb)	*C. viaticus* Tunisia (bb)	*C.diehlii* Tunisia	*C. bombycinus* Tunisia
1	Undecene	*t*	*t*	—	—	—	—
2	Undecane	**11.7 ± 6.5**	**6.6 ± 2.3**	**11.9 ± 4.7**	**5.6 ± 2.1**	3.7 ± 3.1	0.7 ± 0.4
3	5-Methylundecane	0.2 ± 0.2	0.6 ± 0.3	*0.1 ± 0.1*	*0.1 ± 0.1*	—	
4	3-Methylundecane	1.7 ± 1.0	1.4 ± 0.7	*0.1 ± 0.1*	0.4 ± 0.3	—	
5	Dodecane	1.2 ± 0.6	1.5 ± 0.3	1.5 ± 0.3	2.1 ± 0.4	—	0.2 ± 0.1
6	3-Methyldodecane	—	0.1 ± 0.1	—	0.2 ± 0.1	—	
7	Tridecene	*0.3 ± 0.4*[1]	*0.2 ± 0.1*[1]	0.1 ± 0.1	0.3 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
8	Tridecane	**23.1 ± 6.4**	**17.1 ± 4.0**	**22.5 ± 6.2**	**32.7 ± 4.5**	**45.1 ± 23.5**	**13 ± 0.4**
9	7-Methylridecane	—	*0.1 ± 0.2*	—	*t*	—	—
10	5-Methyltridecane	0.6 ± 0.2	*0.7 ± 0.7*	—	0.5 ± 0.2	—	*t*
11	3-Methyltridecane	2.0 ± 0.6	3.2 ± 1.5	1.5 ± 0.4	**7.0 ± 1.1**	0.1 ± 0.1	0.8 ± 0.4
12	Tetradecene	*0.1 ± 0.1*	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2[1]	—	—
13	Tetradecane	3.1 ± 0.7	**5.3 ± 1.1**	3.5 ± 0.5	2.7 ± 0.6	0.1 ± 0.1	2.3 ± 0.4
14	3,11-Dimethyltridecane	—	*0.1 ± 0.1*	—	0.2 ± 0.1	—	—
15	5-Methyltetradecane	*t*	*0.1 ± 0.1*	—	—	—	—
16	4-Methyltetradecane	—	*0.1 ± 0.1*	—	0.2 ± 0.1	—	—
17	3-Methyltetradecane	—	*0.1 ± 0.1*	—	*0.1 ± 0.1*	—	*0.1 ± 0.1*
18	Pentadecadiene	—	—	—	*0.1 ± 0.1*	—	—
19	Pentadecene	0.8 ± 0.8[1]	0.4 ± 0.4	0.7 ± 0.8[1]	2.1 ± 1.7[1]	**17.6 ± 12.7**	0.5 ± 0.4
20	Pentadecane	**38.7 ± 11.7**	**37.1 ± 6.6**	**36.0 ± 7.3**	**20.2 ± 3.3**	**7.9 ± 7.0**	**44.3 ± 0.4**
21	2-Tridecanol	—	—	—	*0.1 ± 0.1*	—	—
22	4,11-Dimethyltetradecane	—	—	—	0.5 ± 0.3	—	—
23	Unidentified	—	—	—	*t*	—	—
24	7-Methylpentadecane	0.6 ± 0.5	*0.8 ± 0.8*	*t*	*0.1 ± 0.1*	—	*0.1 ± 0.1*
25	5-Methylpentadecane	1.5 ± 0.7	2.9 ± 1.6	1.7 ± 1.1	2.3 ± 1.3	—	2.1 ± 0.7

TABLE 2. CONTINUED

Peak number	Compound name	*C. savignyi* Tunisia (bdw)	*C. savignyi* Egypt	*C. bicolor* Tunisia (bdb)	*C. viaticus* Tunisia (bb)	*C.diehlii* Tunisia	*C. bombycinus* Tunisia
26	3-Methyl-2-tridecanone	—	—	—	—	—	0.2 ± 0.1
27	3-Methylpentadecane	0.7 ± 0.4	3.3 ± 2.0	*0.4 ± 0.3*	1.3 ± 0.6	—	4.5 ± 2.3
28	Hexadecene	0.1 ± 0.2	0.3 ± 0.3	0.1 ± 0.1[1]	0.2 ± 0.1	0.5 ± 1.0	0.2 ± 0.1
29	Hexadecane	*0.8 ± 0.6*	0.9 ± 0.9	0.5 ± 0.4	*0.4 ± 0.2*	0.2 ± 0.2	2.4 ± 0.9
30	3-Tetradecanone	—	—	—	*0.1 ± 0.2*	—	—
31	2-Tetradecanone	*t*	*0.1 ± 0.1*	*t*	0.3 ± 0.1	—	0.2 ± 0.1
32	2-Tetradecanol	—	*t*	*t*	*t*	—	—
33	Dodecyl acetate	—	—	*t*	*0.2 ± 0.2*	**15.0 ± 12.8**	1.7 ± 0.5
34	3-Methyldodecyl acetate	—	—	—	—	0.2 ± 0.3	—
35	7-Methylhexadecane	—	*t*	—	—	—	—
36	5- Methylhexadecane	—	*t*	—	—	—	—
37	3-Methylhexadecane	—	*t*	—	—	—	—
38	x-Methyl-2-tetradecanone	—	*t*	—	—	—	—
39	3-Methyl-2-tetradecanone	—	—	—	—	—	*0.1 ± 0.1*
40	2-Tridecyl acetate	—	—	—	*0.2 ± 0.2*	—	—
41	10-Methyldodecyl acetate	—	—	—	*0.4 ± 0.5*	—	—
42	Heptadecadiene	*t*	*0.1 ± 0.1*	—	—	—	0.5 ± 0.2
43	2-Methyltetradecanal	*t*	*0.2 ± 0.2*	*0.3 ± 0.4*	0.7 ± 0.2	—	—
44	Heptadecene	*0.2 ± 0.3*	*t*	*t*	*0.2 ± 0.3*	2.1 ± 2.4	0.9 ± 0.3
45	Heptadecane	**5.4 ± 4.8**	**7.6 ± 3.4**	1.6 ± 1.5	0.4 ± 0.1	1.4 ± 0.9	**15.8 ± 1.0**
46	Dodecyl propionate	—	—	—	—	—	*0.6 ± 0.8*
47	2-Pentadecanone	*0.4 ± 0.4*	*0.4 ± 0.7*	*0.2 ± 0.2*	1.0 ± 0.5	—	*4.1 ± 5.7*
48	Tridecyl acetate	—	—	—	*t*	1.0 ± 1.0	*0.1 ± 0.1*
49	7-Methylheptadecane	*t*	*0.2 ± 0.2*	—	—	—	*0.1 ± 0.1*
50	5-Methylheptadecane	*t*	*0.2 ± 0.2*	—	—	—	0.2 ± 0.1

51	Unidentified	—	—	*t*	—	—	—
52	Dodecyl isobutyrate	—	—	—	—	—	*0.4 ± 0.5*
53	2-Methylpentadecanal	—	—	—	*0.1 ± 0.1*	—	—
54	3-Methyl-2-pentadecanone	*t*	*t*	—	—	—	1.0 ± 0.2
55	3-Methylheptadecane	*t*	*0.1 ± 0.1*	—	—	—	0.4 ± 0.2
56	x-Methyl-2-pentadecanone	*t*	*t*	—	*0.1 ± 0.1*	—	*0.4 ± 0.5*
57	Octadecane	—	*0.2 ± 0.2*	—	—	—	*0.1 ± 0.1*
58	3-Hexadecanone	*t*	*0.1 ± 0.1*	—	0.3 ± 0.2	—	0.4 ± 0.3
59	2-Hexadecanone	*t*	*0.1 ± 0.1*	*t*	—	—	*0.1 ± 0.1*
60	2-Hexadecanol	—	*t*	—	—	—	—
61	Dodecyl butyrate	—	—	—	—	—	0.2 ± 0.2
62	Tridecyl propionate	—	—	—	—	—	*0.1 ± 0.1*
63	Tetradecyl acetate	—	—	—	*0.1 ± 0.3*	2.6 ± 3.5	0.4 ± 0.3
64	Tridecyl isobutyrate	—	—	—	—	—	*0.1 ± 0.1*
65	12-Methyltetradecyl acetate	—	—	—	*0.2 ± 0.2*	—	—
66	Nonadecadiene	—	*t*	—	—	—	—
67	Nonadecene	*0.1 ± 0.2*	*0.1 ± 0.1*	—	—	—	—
68	2-Methylhexadecanal	*t*	*0.1 ± 0.1*	*0.1 ± 0.1*	*0.1 ± 0.1*	—	—
69	Nonadecane	*0.4 ± 0.4*	*1.7 ± 1.7*	*0.1 ± 0.1*	—	—	0.2 ± 0.1
70	Tetradecyl propionate	—	—	—	—	—	*0.1 ± 0.1*
71	2-Heptadecanone	*0.7 ± 1.2*	*1.9 ± 1.5*	*0.1 ± 0.2*	—	—	*0.4 ± 0.5*
72	Pentadecyl acetate	—	—	—	—	—	0.2 ± 0.2
73	Tetradecyl isobutyrate	—	—	—	—	—	*0.1 ± 0.1*
74	Hexadecyl acetate	—	—	—	—	—	*0.1 ± 0.1*
75	3-Methylnonadecane	—	*t*	—	—	—	—
76	x-Methyl-2-heptadecanone	*t*	*0.1 ± 0.1*	—	—	—	—
77	3-Octadecanone	—	*t*	—	—	—	—
78	2-Octadecanone	—	*t*	—	—	—	—
79	2-Methylhexyl hexadecanoate	—	—	—	—	0.1 ± 0.2[3]	—
80	2-Methylhexyl 12-methylhexadecanoate	—	—	—	—	0.1 ± 0.4[3]	—

TABLE 2. CONTINUED

Peak number	Compound name	*C. savignyi* Tunisia (bdw)	*C. savignyi* Egypt	*C. bicolor* Tunisia (bdb)	*C. viaticus* Tunisia (bb)	*C.diehlii* Tunisia	*C. bombycinus* Tunisia
81	2-Methylhexyl 10-methylhexadecanoate	—	—	—	—	0.1 ± 0.2[3]	—
82	2-Methylhexyl heptadecanoate	—	—	—	—	0.1 ± 0.2[3]	—
83	2-Methylhexyl 13-methylheptadecanoate	—	—	—	—	0.1 ± 0.2[3]	—
84	Dodecyl decanoate	*0.1 ± 0.1*	—	2.0 ± 0.7	1.0 ± 0.8	—	—
85	Mixture of isomeric C_{23} esters	—	—	0.4 ± 0.2	—	—	—
86	Mixture of dodecyl undecanoate & undecyl dodecanoate	—	—	*0.2 ± 0.1*	—	—	—
87	2-Methylhexyl octadecenoate	—	—	—	—	0.2 ± 0.6[3]	—
88	2-Methylhexyl octadecanoate	—	—	—	—	0.1 ± 0.2[3]	—
89	Dodecyl x-methyldecanoate	*t*	—	—	2.6 ± 1.3	—	—
90	Mixture of branched C_{23} esters	—	—	—	1.1 ± 0.7	—	—
91	Mixture of branched undecyl dodecanoate & branched dodecyl undecanoate	—	—	—	0.5 ± 0.4	—	—
92	Mixture of branched C_{24} esters	—	—	—	0.5 ± 0.4	—	—
93	Dodecyl dodecenoate	*0.1 ± 0.2*	—	—	—	—	—
94	Dodecyl dodecanoate	*0.5 ± 0.5*	*0.2 ± 0.4*	4.1 ± 1.5	2.3 ±1.4	0.2 ± 0.5	—
95	13-Methylheptacosane	—	—	—	—	0.2 ± 0.2	—

96	Dodecyl x-methyldodecanoate	*0.4 ± 0.3*	*0.1 ± 0.1*	0.7 ± 0.3	2.0 ± 0.9	—	—
97	Dodecyl 6-methyldodecanoate	*0.2 ± 0.2*	*t*	0.8 ± 0.3	*0.9 ± 0.7*	—	—
98	Dodecyl 10-methyldodecanoate	*0.3 ± 0.3*	*0.1 ± 0.1*	1.3 ± 0.6	*1.8 ± 1.4*	—	—
99	Mixture of branched dodecyl tridecanoate & branched tridecyl dodecanoate	*0.1 ± 0.2*	—	—	—	—	—
100	Mixture of branched C_{25} and C_{26} esters	—	—	—	*1.6 ± 1.2*	—	—
101	Mixture of C_{25} esters	—	*0.1 ± 0.1*	—	—	—	—
102	Mixture of dodecyl tridecanoate & tridecyl dodecanoate	—	—	*0.3 ± 0.3*	—	—	—
103	Mixture of isomeric C_{25} esters	—	—	*0.1 ± 0.2*	—	—	—
104	Dodecyl tetradecenoate	*0.3 ± 0.5*	—	0.1 ± 0.2		—	—
105	Dodecyl tetradecanoate	*1.5 ± 1.7*	*1.2 ± 1.7*	5.4 ± 2.2	1.0 ± 0.4	0.3 ± 0.6	—
106	Mixture of C_{26} esters	—	*0.2 ± 0.4*	—		—	—
107	Mixture of branched C_{26} and C_{27} esters	—	—	—	*0.3 ± 0.6*	—	—
108	Mixture of branched dodecyl pentadecanoate & branched tridecyl tetradecanoate	*0.6 ± 0.6*	*0.3 ± 0.4*	—	0.3 ± 0.1	—	—

TABLE 2. CONTINUED

Peak number	Compound name	*C. savignyi* Tunisia (bdw)	*C. savignyi* Egypt	*C. bicolor* Tunisia (bdb)	*C. viaticus* Tunisia (bb)	*C.diehlii* Tunisia	*C. bombycinus* Tunisia
109	Dodecyl pentadecanoate	*0.1 ± 0.2*	*0.2 ± 0.3*	0.4 ± 0.3[2]	*0.1 ± 0.1*	—	—
110	Mixture of C_{27} esters	—	*t*	—	—	—	—
111	Dodecyl hexadecanoate	*0.4 ± 0.6*	1.0 ± 1.0	*0.9 ± 0.7*	—	0.8 ± 1.6	—
112	Dodecyl heptadecanoate	—	*0.3 ± 0.3*	—	—	—	—

*Average values higher than 5.0% are written **bold**; when value is written in *Italic* the compound was not found in all the colonies (this does not apply to *C. diehlii* for which only one colony was available); when the value is underlined not all the individuals belonging to the same colony contained the compound in detectable amounts.
[1]Present as two isomers in some specimens.
[2]The peak also contains tridecyl tetradecanoate.
[3]Only present in one of the 9 specimens analysed (the same specimen for all the compounds indicated with this number.

However, some of the esters were present in much higher proportion, as dodecyl tetradecanoate (peak 104, 5.4%), dodecyl dodecanoate (peak 94, 4.1%), dodecyl decanoate (peak 84, 2.0%) and dodecyl 10-methyldodecanoate (peak 98, 1.3%) ($8.02 \leq t \leq 13.78$, $P < 0.001$). These esters were almost constantly present among all the samples analyzed (only one specimen out of 47 lacked both dodecyl dodecanoate and dodecyl tetradecanoate, one lacked decyl decanoate, and four lacked dodecyl 10-methyldodecanoate). Among the colonies, the average total proportion of esters varied from 4% to 22%. Despite this variation, the total percentage of esters in *C. bicolor* was higher than in the Tunisian *C. savignyi* (averages respectively, 16.08% and 4.68%; *t* test on the transformed data $t = 8.39$, $P < 0.001$).

With regard to the hydrocarbons, the average values were close among the Tunisian *savignyi* and the *bicolor* samples, with the exception of heptadecane which was in a lower amount in *bicolor* ($t = 5.70$, $P < 0.001$). By comparing the sympatric bdw and bdb samples collected on location 2 on transect 3, the same differences as reported above were noted.

C. viaticus (bb)

Samples given the code name bb contained the same hydrocarbons and esters as found in the other groups already described, but the percentage values were obviously different from the others (Table 2). First, all but 3 samples contained tridecane as the major component and pentadecane as second. Secondly, they all had a branched alkane, 3-methyltridecane, as an important component (*t* test for the above compounds against Tunisian *savignyi* and *bicolor*: $3.5 \leq t \leq 17.3$, $0.001 \leq P < 0.001$). Moreover, undecane and heptadecane were in lower amounts than in the Tunisian *savignyi* and in the *bicolor* samples ($5.30 \leq t \leq 9.6$; $P < 0.001$).

The amount of esters ranged among the colonies from 10 to 24%. However, compared to the *bicolor* samples, instead of dodecyl esters of tetradecanoic, dodecanoic, and decanoic acids dominating the esters (all in a significantly lower amount in the *viaticus* samples $3.57 \leq t \leq 9.93$, $P < 0.001$), there was a more complex mixture including more branched esters. Thus, the pattern of esters in the chromatogram looked quite different. The branched esters corresponding to the peak numbers 90–92 and 100, which all together gave 3.7% of the total, were only found in this species, while dodecyl x-methyldecanoate, corresponding to peak 89, whose average amount was 2.6%, was present only in traces in the Tunisian *savignyi* and in the *bicolor* samples. All these compounds were present in all the *viaticus* colonies analyzed. Four acetates were found (peak numbers 40, 41, 63, and 65), which were not found in the other samples, but their presence was variable both among colonies and among individuals belonging to the same colony (Table 2).

C. diehlii

One group of specimens, all belonging to the same colony, labelled diehlii from Methaoui in Tunisia was different from the others containing a simpler mixture of substances (Table 2). There was low consistency of composition in these nine specimens, as reflected in the larger standard deviation in Table 2, compared to all the other samples. As in the bb samples, the major component was tridecane ($t = 1.70$, n.s. in the comparison with the bb samples; $2.86 \leq t \leq 3.79$, $0.021 \leq P \leq 0.005$ in the comparison with bdb, bdw and Egyptian *savignyi*), but the amount of pentadecane and undecane was lower than in all the other samples analysed (respectively, $3.48 \leq t \leq 7.44$, $0.004 \leq P < 0.001$; $1.97 \leq t \leq 5.56$, $0.073 \leq P < 0.001$ in comparison with bdw, Egyptian *savignyi*, bdb and bb). Moreover a higher percentage of pentadecene was present (comparison with bb samples, $t = 2.96$, $P = 0.018$), despite the amount being variable among specimens. The unresolved peak of pentadecene and pentadecane was integrated by plotting single ion chromatograms for m/z values 210 and 212, respectively. Dodecyl acetate, only found in traces in the other samples, was present in a high average amount (15%), although it was absent in 2 out of the 9 analyzed glands. Tetradecyl acetate was also present in a high average amount (2.6%), but was missing in four out of the nine samples.

It is noteworthy that we found esters with a common ion at m/z 98 in their mass spectra in one specimen of this group. We determined these to be 2-methylhexyl esters of fatty acids. The esters with branched-chain acid portions gave clear strong ions at the branching points. For example, 2-methylhexyl 12-methylhexadecanoate had a weak molecular ion (M^+ 368, intensity 2%), and ions at m/z 311 (M-C_4H_7, 2%), 271 ($RCO_2H_2^+$, 20%), 270 (RCO_2H, 16%), 253 (RCO^+, 18%), 213 ($RCO_2H-C_4H_7$, 19%), 98 ($C_7H_{14}^+$ from the 2-methylhexanol, 53%) and 57 (C_4H_7, from both acid and alcohol parts, base peak). In 2-methylhexyl 13-methylheptadecanoate, the lower mass ions were unchanged and the high mass ions were shifted to 382 (M^+), 325, 285, 284, 267, and 227, respectively. Since all members of the *bicolor* group have the necessary enzymes to produce 2-methylhexanol in their mandibular glands, it is interesting to see this substance bound as esters in their Dufour glands. If similar esters are present in the other *bicolor* species, they are below our limit of detection.

C. bombycinus

The absence of 2-methyl-1-hexanol and the presence of citronellol and citral in the mandibular glands of the samples Gafsa bom and Kebili bom drew attention to these samples as not belonging to the group we had come to recognize as *bicolor*. As for most of the *bicolor* group, the major hydrocarbon was pentadecane (Table 2), but its average percentage was higher than in all the other samples analyzed

($2.22 \leq t \leq 11.82$, $0.032 \leq P < 0.001$). Tridecane was in a lower percentage amount than in all the other samples ($3.27 \leq t \leq 8.85$, $0.002 \leq P < 0.001$), and undecane was present in a low amount. Heptadecane was in a higher proportion than in all the *bicolor* samples ($7.43 \leq t \leq 32.18$, $P < 0.001$). Dodecyl acetate was found in about half of the samples, sometimes in high amounts; however, its proportion was lower than in the *diehlii* specimens ($t = 2.96$, $P = 0.016$). All together, the low standard deviation for the *bombycinus* indicated their homogeneity (Table 2), but the Gafsa sample lacked the traces of propionate esters present in the Kebili colony. The absence of further branching in peaks 26, 39, and 54, 3-methyl-2-tridecanone, 3-methyl-2-tetradecanone, and 3-methyl-2-pentadecanone (Table 2) cannot be excluded with as great a certainty as for the branched esters of *C. diehlii* above, and synthetic samples were not available. *C. bombycinus* is placed in the *bombycinus* group (Wehner et al., 1994).

DISCUSSION

Chemotaxonomy, or the use of chemical composition of an animal or plant, together with other criteria, to define a species has been in use for considerable time in particular cases. From a number of studies made of the secretion of the Dufour glands of ants (c.f. Morgan, 1992), it would appear that this secretion is relatively constant in composition and unique to each species. Because of the difficulty in diagnosing species in the large *Cataglyphis* genus, a chemotaxonomic study of exocrine secretions was undertaken (Keegans et al., 1992; Agosti et al., 1996). The group of large desert-dwelling *Cataglyphis* ants of Tunisia has been refined into five species (Wehner et al., 1994), four in the *bicolor* group (with 2-methyl-1-hexanol as their characteristic mandibular gland substance) and one in the *bombycinus* group (with citronellol and citral in the mandibular glands). Among the *bicolor* group species, *C. savignyi*, *C. bicolor*, and *C. viaticus* were quite similar for the composition of the Dufour's gland secretion, while *C. diehlii* was markedly differed from these three species showing (i) a much less complicated mixture and (ii) a high concentration of dodecyl acetate, a compound only found in low concentrations in some specimens of *C. viaticus* and in *C. bicolor*, and (iii) the presence of the 2-methylhexyl esters of long chain fatty acids, unique among the five species analyzed. Thus, the Dufour gland chemistry appears to be a clear diagnostic character for *C. diehlii*, while based on the postpharyngeal gland secretion, *C. diehlii* is not readily distinguishable from *C. bicolor*. Although less evident, differences between C. *savignyi*, *C. bicolor*, and *C. viaticus* were found in the concentration of the major constituents. As might be expected for insects so geographically separated, the glands of *C. savignyi* from Egypt and Tunisia were chemically differentiated, and since this difference is of an extent comparable to that observed between species, the chemistry of the Dufour gland does not appear

to be a reliable character for species identification if based on the description of the composition of allopatric populations. We have found for these closely related species the contents of the postpharyngeal gland (Oldham et al., 1999) to be a better separator than the Dufour gland contents.

In contrast to the differences in the mandibular glands between the *bicolor* group and *C. bombycinus*, the Dufour gland chemistry did not differ remarkably between them. However, the two groups were differentiated by the lack of the high molecular weight constituents, mainly long chain fatty acid esters, in *C. bombycinus*. It can be seen from Table 2 that in all the species, several of the minor components were absent in some of the analyzed specimens and sometimes in all the specimens sampled from the same colony. Although this sort of variability is generally not discussed in papers describing the composition of exocrine volatile secretion in insects, we know from direct experience it is a common situation. Moreover, even considerable deviations from the average values of concentration for several of the compounds are common. In this sense, we believe that the composition of just one volatile exocrine secretion is rarely a reliable and readily usable taxonomic character, but may prove to be useful for species determination if considered together with other chemotaxonomic and anatomical characters.

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