

THE DUFOUR GLAND SUBSTANCES OF THE WORKERS OF *FORMICA FUSCA* AND *FORMICA LEMANI* (HYMENOPTERA: FORMICIDAE)

MAHMOUD FADL ALI*, ATHULA B. ATTYGALLE†, E. DAVID MORGAN*‡ and
JOHAN P. J. BILLEN§

*Chemistry Department, Keele University, Keele, Staffs ST5 5BG, UK (Tel. (0782) 621111) †Institute for Organic Chemistry, University of Erlangen-Nürnberg, D-8520 Erlangen, FRG and §Department SBM, Limburgs Universitair Centrum, B-3610 Diepenbeek, Belgium

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Abstract—1. The Dufour gland secretions of *Formica fusca* consist mainly of saturated straight and branched chain hydrocarbons (C₉–C₁₉), one unsaturated hydrocarbon (C₁₃) and two sesquiterpenoids, farnesene and homofarnesene.

2. In *F. lemni*, the Dufour gland contains branched, saturated and unsaturated hydrocarbons (C₉–C₁₉) and two farnesenes.

3. The two species were distinguished chiefly by the presence of a relatively large proportion of farnesene in *F. fusca*, with very little homofarnesene and by contrast, little farnesene but much more homofarnesene in *F. lemni*.

4. The contents of the Dufour gland can be used as a chemotaxonomic clue to distinguish between the species.

INTRODUCTION

The accessory gland of the poison apparatus, found in all aculeate Hymenoptera, first described by Dufour (1841) and generally known by his name, is part of the exocrine system of this insect order. It has been described as having an opening into the duct of the poison gland (Forel, 1878), which in formicine ants is believed to result in a simultaneous secretion of both glands (Hermann and Blum, 1968; Regnier and Wilson, 1968). More recent morphological studies on ants, however, indicate that the Dufour gland duct has a separate opening and its own muscular control mechanism for regulating the discharge of secretion, both in the stinging Myrmicinae, as in the stingless Formicinae (Billen, 1986). The contents of the Dufour glands of ants, where studied, have generally been found to be either mixtures of hydrocarbons or, in formicines, mixtures of hydrocarbons and long chain aliphatic compounds, such as alcohols, ketones or esters (Blum and Hermann, 1978).

Maschwitz (1964) showed that the secretion from this gland in *Formica polyctena*, *F. fusca* and *F. cinerea* releases alarm behaviour. A number of authors have supported the idea that undecane, one of the principle substances found in formicine Dufour glands, acts as an alarm pheromone (Regnier and Wilson, 1968, 1969; Bergström and Löfqvist, 1970, 1972a,b; Ayre and Blum, 1971; Löfqvist, 1976). Another view is that the hydrocarbons and the acetates acted as wetting agents for the formic acid released from the poison gland (Löfqvist, 1977).

In all cases so far studied, the mixture of substances in the Dufour gland has been found to be species specific. The primary purpose of this specificity remains obscure, although it is easy to see its usefulness

in chemical communication. The chemical composition of Dufour glands of a number of *Formica* species have already been studied, including *F. fusca* but not *F. lemni*. Bergström and Löfqvist (1968) in examination of four *Formica* species from southern Sweden, found that *F. fusca* contained a relatively simple mixture of linear alkanes plus a small quantity of sesquiterpene farnesene.

Our present interest in *F. fusca* and *F. lemni* was prompted by the close taxonomic relation between the two species. We were asked whether they were distinguishable by the chemical composition of the Dufour gland secretion. We have found that *F. lemni* and *F. fusca* are easily distinguishable chemically, and we have confirmed the results on *F. fusca*, obtained by Bergström and Löfqvist (1968), by examination of samples from Belgium and England and identified several further minor components.

MATERIALS AND METHODS

Maintenance of the colonies

The ants were reared in artificial nests made from plastic bottles, partially filled with moistened plaster of Paris. Each bottle was placed in a plastic bowl to serve as the foraging area. The inner vertical walls of the bowl were covered with polytetrafluoroethylene paste to prevent the ants from escaping. The ants were kept in the laboratory on a diet of sugar solution and larvae or pupae of dipterous flies.

For chemical analysis, the Dufour glands were isolated by momentarily immersing individual ants in liquid nitrogen, then dissecting out the Dufour gland in distilled water using fine tweezers under a binocular microscope. Excess water was removed by touching with a fragment of filter paper and the Dufour glands were attached to a fragment of glass and placed individually in short glass capillaries, sealed at one end, and the other end was then sealed in a flame.

‡Author to whom all correspondence should be addressed.

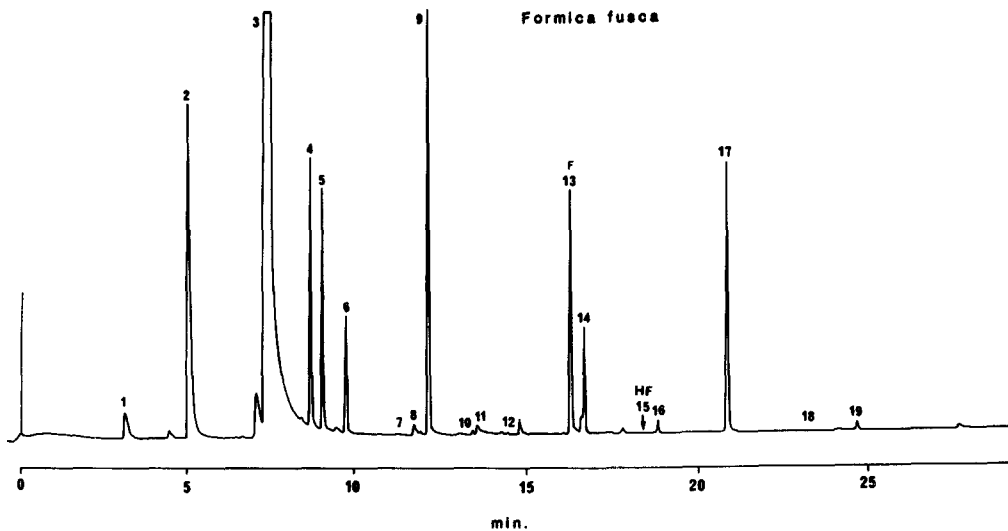


Fig. 1. Gas chromatogram of a single Dufour gland from a worker of *F. fusca*, analysed on an OV-1 capillary column, conditions as in the text.

Gas chromatography

A Carlo Erba gas chromatograph with a flame ionization detector and a Shimadzu CR3-A computer integrator was used for analysis and quantifications. A Dufour gland was introduced in a sealed capillary into the heated injector (145°C) via a solid sampler (Morgan and Wadhams, 1972a). For analysis, a fused silica capillary column (25 m × 0.4 mm) coated with OV-1 silicone was used, with helium at 2 ml/min as a carrier gas. The temperature programme was from 60 to 280°C at 6°C/min and then 10 min at 280°C.

Linked gas chromatography-mass spectrometry

Mass spectrometry was performed on a Finnigan 3200E quadrupole spectrometer with a Finnigan 6000 Data System. A fused silica column (Chrompak CP-19, 38 m × 0.22 mm) was directly coupled to the mass spectrometer. Helium was used as the carrier gas at a flow rate of 1 ml/min. Spectra at 79 eV EI were recorded at a rate of 2 sec/scan.

RESULTS

In *Formica fusca*, the Dufour gland secretion consists mainly of saturated straight chain hydrocarbons (Fig. 1). In addition to the saturated hydrocarbons we found only one compound with one double bond represented by tridecene (0.80%). Two sesquiterpenoid compounds, farnesene (1.97%) and homofarnesene, which appeared as a trace, were present. The saturated chain hydrocarbons range from C₉ to C₁₉ with undecane the most abundant hydrocarbon (75% of the total) followed by tridecane (8.3%). The branched chain alkanes contain a methyl group in 3-, 4- or 5-position with 3-methylundecane the most abundant. Satisfactory mass spectra were obtained for all the compounds listed in Table 1. No oxygenated compounds (alcohols, ketones or esters), as found in some other formicine species, were present in detectable quantities.

Two samples of *F. fusca*, both collected in summer 1985, were examined, one in midsummer from Belgium and one in late summer from England. Both showed the same compounds and, within the vari-

ations shown by individual workers, in the same proportions. The Belgian sample had less material, on average, in the glands (i.e. $2.17 \pm 1.64 \mu\text{g}$) compared with the English sample ($4.9 \mu\text{g}$). This difference is not considered significant; it may reflect the age of the workers, the season, state of nutrition, or confinement during transport of the Belgian colony.

In *Formica lemni*, the Dufour gland appeared to be filled with a larger number of homologous branched, saturated and unsaturated hydrocarbons ranging from C₉ to C₂₂, and two farnesenes (Fig. 2). Undecane was again the major component (57%) followed by tridecane (15.2%). Of the branched chain hydrocarbons, 3-methylundecane was again the major one (3.47%). Farnesene and homofarnesene were both present (0.64% and 2.3%, respectively) but in quite different proportions from that in *F. fusca*.

Table 1. Mean values for the composition of the contents of the Dufour gland of *Formica fusca* workers, determined by gas chromatography and mass spectrometry, on individual samples

Number	Compound	% ± SD	Mean quantity ($\mu\text{g} \pm \text{SD}$)
1	<i>n</i> -Nonane	0.31 ± 0.32	0.02 ± 0.02
2	<i>n</i> -Decane	2.40 ± 1.44	0.13 ± 0.11
3	<i>n</i> -Undecane	75.0 ± 11.1	3.39 ± 1.36
4	5-Methylundecane	1.78 ± 1.18	0.11 ± 0.11
5	3-Methylundecane	1.96 ± 1.64	0.12 ± 0.15
6	<i>n</i> -Dodecane	1.74 ± 1.91	0.12 ± 0.19
7	4-Methyldodecane	t	t
8	Tridecene	0.80 ± 0.59	0.05 ± 0.05
9	<i>n</i> -Tridecane	8.28 ± 4.60	0.44 ± 0.42
10	5-Methyltridecane	t	t
11	3-Methyltridecane	0.58 ± 0.54	0.03 ± 0.02
12	<i>n</i> -Tetradecane	t	t
13	Farnesene	1.97 ± 0.93	0.11 ± 0.08
14	<i>n</i> -Pentadecane	1.92 ± 1.06	0.11 ± 0.09
15	Homofarnesene	t	t
16	<i>n</i> -Hexadecane	t	t
17	<i>n</i> -Heptadecane	2.13 ± 1.14	0.11 ± 0.09
18	<i>n</i> -Octadecane	t	t
19	<i>n</i> -Nonadecane	t	t
Total			4.83 ± 2.47

t = trace, <20 ng.

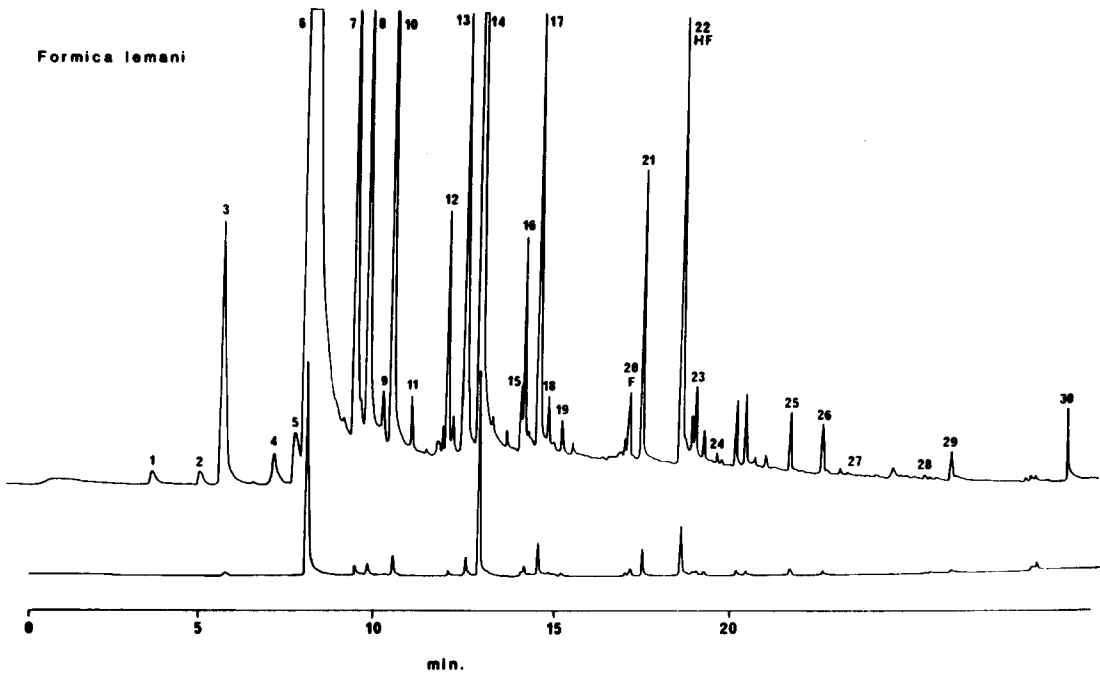


Fig. 2. Gas chromatogram of a single Dufour gland from a worker of *F. lemami*, analysed on an OV-1 capillary column. The trace is shown at two sensitivities to show the overall proportions and also the minor components. Conditions are as given in the text.

The mean values in terms of micrograms per individual worker and percentage composition are listed in Table 2. In all species we find the amount of secretion varies considerably from one individual worker to another, as indicated by the large standard deviations, therefore the mean percentage values are arrived at by calculating the percent composition for each individual worker and averaging these values.

Two samples of *F. lemami* were examined. The above results are from a colony collected at Skipton in Yorkshire in 1985. Two colonies collected at Mow Cop in Cheshire in June 1986 had smaller individuals and less material on average in the Dufour gland. Examination of a few individuals from both Mow Cop nests showed no consistent difference between them, therefore 10 samples were taken from the larger colony and the mean values calculated. The total amount of hydrocarbons was $3.3 \pm 2.2 \mu\text{g}$ per individual. Undecane represented a slightly larger proportion at $68.9 \pm 9.8\%$ with tridecane second at $11.1 \pm 3.0\%$. The amounts of farnesene (0.24%) and homofarnesene (0.88%) were smaller but homofarnesene was the greater of the two. Otherwise, the compositions were very similar and nowhere were they significantly different.

DISCUSSION

In an earlier study, we compared the two morphologically similar *Formica* species *F. rufibarbis* Fabr. and *F. cunicularia* Latr. by means of their Dufour gland contents (Billen *et al.*, 1983). We found they were easily distinguished, for although both contained linear hydrocarbons, dominated by undecane, *F. rufibarbis* contained decyl, undecyl and

dodecyl acetates as well, while in *F. cunicularia*, only the saturated straight chain alkanes were found.

The Dufour gland secretions of *Formica* species contain a wide variety of substances ranging among alkanes, methylbranched alkanes, alkenes, ketones,

Table 2. Mean value of the composition of the contents of the Dufour gland of *Formica lemami* workers determined by gas chromatography and mass spectrometry on 10 individuals

Number	Compound	% \pm SD	Mean quantity ($\mu\text{g} \pm$ SD)
1	<i>n</i> -Nonane	0.18 \pm 0.21	0.009 \pm 0.009
2	3-Methylnonane	t	t
3	<i>n</i> -Decane	2.52 \pm 0.95	0.16 \pm 0.13
4	4-Methyldecane	t	t
5	Undecene	t	t
6	<i>n</i> -Undecane	57.0 \pm 6.67	2.87 \pm 1.29
7	5-Methylundecane	3.12 \pm 0.95	0.18 \pm 0.13
8	3-Methylundecane	3.47 \pm 1.16	0.20 \pm 0.15
9	Dodecene	0.66 \pm 0.33	0.04 \pm 0.03
10	<i>n</i> -Dodecane	4.44 \pm 1.37	0.25 \pm 0.19
11	3,7-Dimethylundecane	0.14 \pm 0.25	0.008 \pm 0.01
12	4-Methyldodecane	0.63 \pm 0.40	0.04 \pm 0.03
13	Tridecene	2.45 \pm 0.64	0.14 \pm 0.11
14	<i>n</i> -Tridecane	15.2 \pm 2.91	0.80 \pm 0.50
15	7-Methyltridecane	t	t
16	5-Methyltridecane	0.57 \pm 0.23	0.04 \pm 0.03
17	3-Methyltridecane	2.23 \pm 0.55	0.12 \pm 0.10
18	Tetradecene	0.63 \pm 0.94	0.05 \pm 0.12
19	<i>n</i> -Tetradecane	0.42 \pm 0.20	0.03 \pm 0.02
20	Farnesene	0.64 \pm 0.33	0.04 \pm 0.03
21	<i>n</i> -Pentadecane	1.91 \pm 0.59	0.11 \pm 0.06
22	Homofarnesene	2.30 \pm 1.28	0.14 \pm 0.10
23	5-Methylpentadecane	t	t
24	<i>n</i> -Hexadecane	t	t
25	<i>n</i> -Heptadecane	0.45 \pm 0.19	0.03 \pm 0.01
26	7-Methylheptadecane	0.31 \pm 0.16	0.03 \pm 0.03
27	<i>n</i> -Octadecane	t	t
28	<i>n</i> -Nonadecane	t	t
29	9-Methylnonadecane	0.17 \pm 0.12	0.01 \pm 0.009
30	11-Methylheneicosane	t	t
Total			5.27 \pm 2.86

t = trace, < 10 ng.

alcohols, aldehydes, acetates, formates, farnesene, all-*trans*-geranylgeraniol and all-*trans*-geranylgeranyl acetate.

In *F. sanguinea*, the secretions from the Dufour gland contain 11 compounds consisting of a mixture of alkanes, acetates, alcohols and one farnesene with *n*-undecane, decyl acetate and dodecyl acetate as major compounds (Bergström and Löfqvist, 1968). *Polyergus rufescens* contains only three compounds, *n*-undecane, *n*-heptadecane and farnesene (Bergström and Löfqvist, 1968) with the latter compound as the major one, providing an exception to the general rule that undecane is the most abundant compound in formicine species. In *Formica rufibarbis*, the slave ant of both *Formica sanguinea* and *Polyergus rufescens*, the gland contains straight chain saturated hydrocarbons, acetates and one ketone represented by 2-tridecanone; heptadecene is the only unsaturated compound. Undecane, *n*-tridecane and decyl acetate are the major compounds (Bergström and Löfqvist, 1968). They found that the other slave species, *Formica fusca*, contains 10 compounds including eight alkanes with one branched compound (C₁₂), with undecane and tridecane as the major compounds; tridecene and farnesene were also identified (Bergström and Löfqvist, 1968). Bergström and Löfqvist (1973) found a greater similarity between the three species *F. nigricans*, *F. rufa* and *F. polyctena*, which all contain alkanes, branched alkanes, with a methyl group in the 3rd and 5th positions, but 3-methylnonane, 4-methyldodecane and 5-methylpentadecane were only present in *F. nigricans*. Alkenes with one or two double bonds, saturated and unsaturated acetates, all-*trans*-geranylgeraniol and all-*trans*-geranylgeranyl acetate were present in all three species; two unsaturated alcohols, tetradecenol and hexadecenol were present in small amount in both *F. nigricans* and *F. polyctena*. In a recent re-examination of hexane extracts of *F. rufa* abdomens (Francke *et al.*, 1985) additional compounds were identified, some of which, present in more than traces, namely nonanal, undecene, tridecene and hexadecyl formate, arise from the Dufour gland. They did not find the geranylgeraniol and geranylgeranyl acetate described by Bergström and Löfqvist (1973). Indeed, traces of a number of formates were found by Francke *et al.* (1985) which had not been found before in Dufour glands.

We have been asked if this chemical test of Dufour gland contents would distinguish between the morphologically similar pair *F. fusca* and *F. lemni*. We find here that such a difference is indeed apparent. Both species contain only linear alkanes, a little linear alkenes and branched alkanes (Table 1 and 2), plus the sesquiterpenoids farnesene and homofarnesene (Morgan and Wadhams, 1972b; Attygalle and Morgan, 1982). The two species are complimentary in this respect. Farnesene is more abundant in *F. fusca* and homofarnesene is only present as a trace component. In *F. lemni* there is only a trace of farnesene while homofarnesene is more abundant. In addition, there is a rather richer mixture of hydrocarbons in *F. lemni* (at least 30 identified components while *F. fusca* has 19).

The analysis of *F. fusca* glands from colonies from England and Belgium were qualitatively the same,

and agreed with that recorded by Bergström and Löfqvist (1968) from the Island of Oland in Sweden (though they did not identify the isomers of farnesene and homofarnesene). This would suggest that this species is homogeneous at least over this range of territory. Other work (cf. Ali *et al.*, 1987) with colonies of *Tetramorium caespitum* from widely distributed habitats suggests that a species can remain homogeneous for this character over wide areas. Two samples of *F. lemni* from different counties of England showed a similar homogeneity.

The homofarnesene found in *F. fusca* is the same (Z, E)- α -farnesene as was found in *Myrmica* ants (Attygalle and Morgan, 1984), although there was a little (E, E)- α -farnesene in addition. This was only apparent in the mass spectrum, and it is not known whether it occurs naturally or is produced by thermal isomerism during vaporization in the gas chromatograph. Similarly, the homofarnesene found in *F. lemni* is the same compound [i.e. (Z, E)-7-ethyl-3,11-dimethyldodeca-1,3,6,10-tetralene] as that in *Myrmica* species (Attygalle and Morgan, 1984) by mass spectrometry and comparison of g.c. retention times. Recently we have found different farnesene and homofarnesene isomers in the myrmicine *Harpagoxenus sublaevis* (Ollett *et al.*, 1986).

Whereas the methyl branches tend to be near the end of the chain in the lower-mass branched alkanes, we were surprised to find in *F. lemni* 5-methylpentadecane, 7-methylheptadecane, 9-methylnonadecane and 11-methylheneicosane forming a prominent series in the high mass region, with 11-methylheneicosane standing along unaccompanied by other C₂₁ or C₂₂ hydrocarbons (Fig. 2). 13-Methyltricosane was identified in the GC-MS experiments though it was visible in the chromatograms of only a few individual Dufour glands. The series could arise biosynthetically by chain extension of 2-methyldodecanoic acid before decarboxylation. The relatively large amount of *n*-dodecane may be formed from the same postulated 2-methyldodecanoic acid by decarboxylation rather than from an unlikely tridecanoic acid. Tetradecane, by contrast, is present only in trace quantities.

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