

THE CHEMICAL COMPOSITION
OF THE DUFOUR GLAND CONTENTS OF WORKERS
OF THE ANT *FORMICA CUNICULARIA*
A TEST FOR RECOGNITION OF THE SPECIES

by

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Key words : Ants, Dufour gland, *Formica cunicularia*, *Formica rufibarbis*, Hydrocarbons, Chemical taxonomy.

SUMMARY

The Dufour glands of workers of *Formica cunicularia* contain, on average, 8.4 μ g of a relatively simple mixture of linear hydrocarbons. *n*-Undecane (6.7 μ g, 80 % of total) is the dominant component. None of the oxygenated compounds observed in a number of other Formicinae were detected in this species. *F. cunicularia* LATREILLE and *F. rufibarbis* FABRICIUS, two taxonomically very similar species can be distinguished very easily by the chemical differences in their Dufour gland compounds.

INTRODUCTION

Slavery in ants has been reported for at least eight genera (BUSCHINGER *et al.*, 1980). In the Formicinae, *Formica sanguinea* LATREILLE and *Polyergus rufescens* (NYLANDER) raid nests of species of the *Formica fusca* LINNAEUS group including *F. cunicularia* LATREILLE and *F. rufibarbis* FABRICIUS. Both *F. cunicularia* and *F. rufibarbis* have a very similar distribution and somewhat similar habitats in Europe and occasionally local populations or individual workers with a similar colour pattern may be hard to distinguish at some sites. Following the separation clues in taxonomic handbooks, *F. rufibarbis* should display a higher degree of pubescence (*rufus* + *barba*, Latin : bearing red hairs), while in *F. cunicularia* the gaster should be more greyish (*cuniculus*, Latin : rabbit) (KUTTER, 1977; VAN BOVEN, 1977; COLLINGWOOD, 1979).

From recent studies of the glandular secretions of a broad sample of ant species in many laboratories (for a review, see BLUM and HERMANN, 1978) it is apparent

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that many of these secretions have a species-specific composition (BERGSTRÖM and LÖFQVIST, 1968, 1970, 1972, 1973; LONGHURST *et al.*, 1980; CAMMAERTS *et al.*, 1981a, b; EVERSHED and MORGAN, 1981). In particular, the Dufour gland, which is found in close association with the sting of females and worker castes of all Hymenoptera, has been investigated chemically in a large number of species of Formicidae, and often consists of mixtures of simple aliphatic and terpenoid compounds.

In this paper we report the results of a chemical study of the volatile constituents of the Dufour gland of *F. cunicularia*. The chemistry of the gland of this species is compared with that of *F. rufibarbis* (BERGSTRÖM and LÖFQVIST, 1968). The chemical differences are much more distinct between the two species than the taxonomic differences and provide a simple way to distinguish them.

MATERIALS AND METHODS

Source of insect material

Workers of *F. cunicularia* were collected in the nature reserve « Het LEUDAL » near Roermond in the Netherlands. The taxonomic determination of this species was confirmed by ecological research in this area by one of us, extending over 40 years, taking into account the evolution of the local distribution pattern of this species and also by comparison with previously collected samples of both *F. cunicularia* and *F. rufibarbis*.

Sampling preparation

Workers were killed by momentary immersion in liquid nitrogen taking care not to alarm them beforehand. The Dufour gland was removed by gently pulling off the abdominal tip. The whole gland, free of haemolymph and supported on a fragment of glass, was sealed in a small soda glass vial and introduced into the gas chromatograph using a solid sampling technique (MORGAN and WADHAMS, 1972).

Gas chromatography (GC)

A Pye 104 series gas chromatograph fitted with flame ionisation detectors was employed throughout these investigations. Two types of GC column were used :

- 1) 10 % PEGA coated on Diatomite M (100-120 mesh, acid washed and treated with hexamethyldisilazane AW-HMDS) packed in a 3 m × 4 mm internal diameter glass column.
- 2) 5 % OV-101 silicone coated on chromosorb W (100-120 mesh, AW-HMDS) packed in a 1.5 m × 4 mm internal diameter glass column.

The GC oven temperature was programmed, except when comparing retention times or plotting log of retention time (t_R) against the number of carbon atoms per molecule. Under these circumstances isothermal analyses were performed.

Quantification of the glandular components was performed by comparing the areas of the GC peaks obtained from the analysis of single glands with those of known amounts of a standard hydrocarbon.

Gas Chromatography — Mass Spectrometry (GC-MS)

GC-MS studies were performed on a Pye 104 series GC with FID linked to an AEI MS12 mass spectrometer through a jet separator. The spectra were obtained

through the solid injection of 3 worker Dufour glands onto the PEGA column (1). The helium carrier gas flow rate was maintained at 15 ml/min while the GC oven temperature was programmed from 80-160° C at 5°/min and then held at 160° C. Identifications were made by interpretation of the fragmentation pattern and by comparison with reference spectra.

RESULTS

The Dufour gland secretion of *F. cunicularia* workers is composed largely of linear alkyl hydrocarbons in the C₉-C₁₈ chain length range. Figure 1 shows a GC profile typical of that obtained through the solid injection of a single Dufour gland.

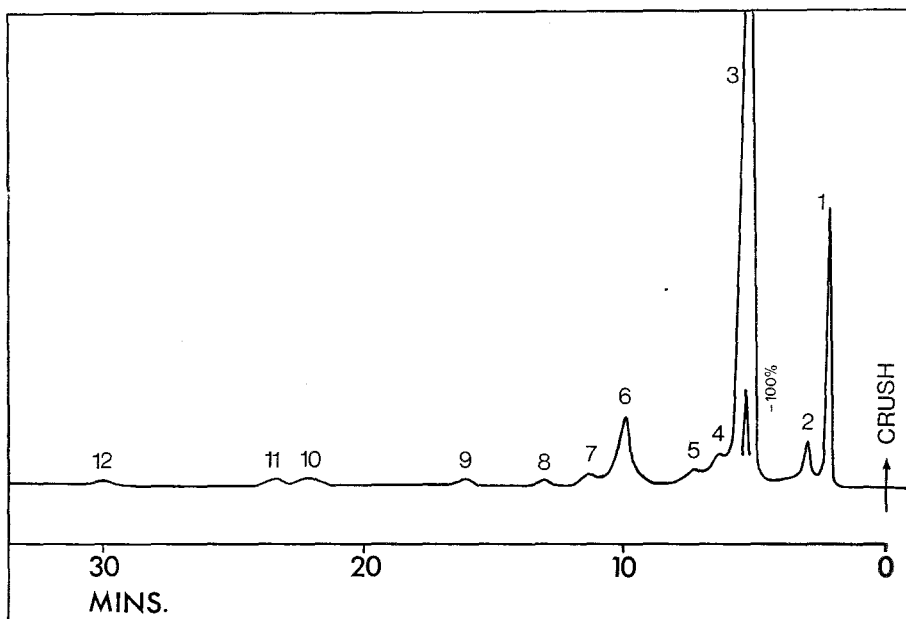


Fig. 1. — Gas chromatography trace of the contents of a single Dufour gland from a worker of *Formica cunicularia*. The gland was sealed in a glass capillary, which was heated in the chromatograph and then crushed, at time 0. For identification of all the numbered components, see Table 1. Compound 1 is nonane and 3 is undecane. Chromatographic conditions : temperature programme from 95° C to 170° C at 4° C/min, on a 10 % PEGA column (column 1) with nitrogen flow of 60 ml/min.

Components 1, 2, 3, 5, 6, 8, 9, 10 and 12 were found to correspond exactly in retention time (t_R) on polar and non-polar GC phases to C₉-C₁₈ *n*-alkanes.

Plots of $\log t_R$ against the number of carbon atoms in the chain on both GC phases suggested that the remaining components, 5, 7 and 11 are the simple alkenes, undecene, tridecene and heptadecene.

The identities of all except the four minor hydrocarbon components were confirmed by linked GC-MS. The alkenes belong to the linear series, but the position of the double bond was not determined. By comparison with the corresponding fatty acids and the substances found in Dufour glands of other ants, they are probably *cis*-5-tridecene, *cis*-8-heptadecene and 1- or 3-undecene.

The quantity of these substances contained in the gland was calculated from ten replicate analyses of single worker's Dufour glands. These results are summarised in Table I together with the analytical evidence for identification. Clearly, the predominant glandular component is *n*-undecane (6.7 μ g, 80 %), the next most abundant alkane is *n*-nonane (0.85 μ g, 10 %) while the most abundant alkene is tridecene (50 ng, 0.6 %).

TABLE I
*Composition of the contents of the Dufour gland
of Formica cunicularia, determined by gas chromatography*

Number in Fig. 1	Compound	% \pm SD	Mean Quantity (ng \pm SD) (n = 10)	Evidence (*)
1	<i>n</i> -Nonane	10.13 \pm 6.04	850 \pm 580	MS, GC
2	<i>n</i> -Decane	1.75 \pm 0.74	150 \pm 90	MS, GC
3	<i>n</i> -Undecane	79.46 \pm 4.74	6730 \pm 2410	MS, GC
4	Undecene	0.43 \pm 0.15	40 \pm 20	GC
5	<i>n</i> -Dodecane	0.27 \pm 0.09	20 \pm 10	MS, GC
6	<i>n</i> -Tridecane	5.51 \pm 2.66	430 \pm 200	MS, GC
7	Tridecene	0.59 \pm 0.27	50 \pm 30	MS, GC
8	<i>n</i> -Tetradecane	trace (+)	trace (+)	GC
9	<i>n</i> -Pentadecane	1.27 \pm 0.62	60 \pm 60	MS, GC
10	<i>n</i> -Heptadecane	0.47 \pm 0.17	40 \pm 30	MS, GC
11	Heptadecene	trace (+)	trace (+)	GC
12	<i>n</i> -Octadecane	trace (+)	trace (+)	GC
	Total		8430 \pm 2920	

(*) MS, identity confirmed by mass spectrometry.

GC, indicates that the substance has the same retention time as standard samples on both polar and non-polar GC phases. Position of the double bonds not determined.

(+) Trace, less than 10 ng (*i.e.* < 0.1 %) per ant.

The standard deviations have been calculated for both the percentages and absolute quantities of each component for ten workers. The values given in Table I reveal that while the variation in the absolute amounts of the individual components is quite large, the overall composition from worker to worker is fairly constant especially with respect to the major components.

The ten workers examined during the course of this study were found to contain on average 8.43 μ g of hydrocarbons, with some of the workers containing over 10 μ g of material.

At no time in these analyses were any oxygenated compounds observed. Analyses were performed specifically to look for decyl, undecyl and dodecyl acetates, which are present in *F. rufibarbis* (BERGSTRÖM and LÖFQVIST, 1968) but none were

detected. Fig. 1 can be conveniently compared with Fig. 6 in BERGSTRÖM and LÖFQVIST (*op. cit.*),

DISCUSSION

The Dufour gland in formicine ants is known to produce a wide variety of simple organic compounds. In the wood ants (subgenus *Formica*) over 30 substances were found in the worker's Dufour gland while in *Camponotus* more than 40 compounds were found (BERGSTRÖM and LÖFQVIST, 1972, 1973). *Lasius* workers, on the other hand, have been found to contain less than 15 compounds (BERGSTRÖM and LÖFQVIST, 1970). The slave-making species *Formica sanguinea* and *Polyergus rufescens* and the slaves *Formica fusca* and *F. rufibarbis* contain only 15 compounds (BERGSTRÖM and LÖFQVIST, 1968).

While some of the above species produce complex mixtures of substances including hydrocarbons, alcohols, ketones and acetates the focus of the present study, *F. cunicularia*, has been found to produce a relatively simple mixture of *n*-alkanes and alkenes.

Although the general pattern of the Dufour gland substances can be considered as species-specific, the differences in the compositions between closely related species are often restricted to subtle quantitative variations.

Thus colonies of *F. cunicularia* in Belgium, Denmark and South-east England often have workers with a distinctly reddish alitrunk and some in addition may have one or more pairs of bristles on the promesonotal dorsum. By contrast *F. rufibarbis* may sometimes have colonies of quite dark workers and in some individuals, irrespective of colour, the characteristic bristles on the pronotum may be reduced or abraded while in others the diagnostic setae on the dorsal surface of the scale may also be hard to find. The reddish *cunicularia* were formerly called *F. glebaria* var. *rubescens* FOREL, which together with the greyish *F. glebaria* NYLANDER constitute the present *F. cunicularia* LATREILLE.

It is the experience of one of us that both *F. rufibarbis* and *F. cunicularia* used to occur together at the collection site of « het LEUDAL », but today *F. rufibarbis* has been completely displaced, so no comparison could be made for the two species from the same area.

In an earlier investigation of the odour similarities between *Formica* ants and their slaves, BERGSTRÖM and LÖFQVIST (1968) showed the Dufour gland secretion of *F. rufibarbis* to be composed mainly of C₉-C₁₈ hydrocarbons. However, significant amounts of oxygenated components were also present, including *n*-decyl acetate, *n*-undecyl acetate, *n*-dodecyl acetate and 2-tridecanone.

In contrast to the aforementioned indefinite morphological features, the chemical analyses of the Dufour gland contents of the two species reveal a distinct species-specific pattern showing considerable differences. The most conspicuous feature in *F. cunicularia* is the complete absence of the less volatile decyl, undecyl and dodecyl acetates, whereas in *F. rufibarbis* these compounds are significant constituents of the Dufour gland. Moreover, *F. cunicularia* contains no tridecanone but has a tridecene peak, while in *F. rufibarbis* 2-tridecanone was identified but no tridecene was found (BERGSTRÖM and LÖFQVIST, 1968).

The volatile compounds stored in the glands, such as the mandibular and Dufour glands of ants, frequently have a pheromonal function (for a review, see PARRY and MORGAN, 1979). No ethological studies have yet been made on the Dufour gland

secretion of *F. cunicularia*. The hydrocarbons probably participate with the formic acid from the venom gland, in the alarm-defence system of the species, frequently suggested for others of the Formicinae (WILSON and REGNIER, 1971). The Dufour gland secretion (hydrocarbons and alkyl acetates) of slave-making ants such as *F. sanguinea*, may be discharged during slaving raids to panic and disorganize the potential slaves (REGNIER and WILSON, 1971). For this language to be effective, the chemical language of both slaves and slave-makers must incorporate common substances, and their concentration in the blend should also be critical for the ethological response they provoke. The presence of *n*-alkyl acetates in the Dufour gland of *F. fusca* and *F. rufibarbis* is compatible with this idea (BERGSTRÖM and LÖFQVIST, 1968), but these acetates are absent from *F. cunicularia*, so we must tentatively conclude that *F. cunicularia* are responding to the hydrocarbon part of the slave-makers secretion.

It is also suggested that the Dufour gland secretion acts as a wetting and spreading agent for the formic acid from the venom gland, aiding its penetration through the cuticle of arthropod prey. In this sense, therefore, the Dufour gland secretion has no pheromonal function (LÖFQVIST, 1977). This question is best resolved by careful observational experiments with the secretion and its pure components.

Regardless of these questions, the GC analysis of the Dufour secretion of *F. cunicularia* offers a clear chemical taxonomic test to distinguish it from its close relative *F. rufibarbis*.

This analysis has been carried out on only one colony of *F. cunicularia*, but from several years work with *Myrmica* ants at Keele, we have found that colonies of a given species, taken at different times and in different areas, and even from the British Isles and adjacent continent contain the same substances and (within certain limits) in the same proportions. We (and we hope, others) will check these results as and when further colonies of *F. rufibarbis* and *F. cunicularia* become available.

ACKNOWLEDGEMENTS

J. P. J. BILLEN thanks the Belgian National Fund for Scientific Research for the award of a research assistantship and R. P. EVERSLED thanks the S.R.C. and Rothamsted Experimental Station for support through a CASE award. We also thank C. A. COLLINGWOOD for helpful criticisms and for reading the manuscript.

REFERENCES

- BERGSTRÖM, G. and J. LÖFQVIST (1968) — Odour Similarities between the Slave-Keeping Ants *Formica sanguinea* and *Polyergus rufescens* and their slaves *Formica fusca* and *Formica rufibarbis*. *J. Insect. Physiol.*, **14**, 995-1011.
- BERGSTRÖM, G. and J. LÖFQVIST (1970) — Chemical Basis for Odour Communication in Four Species of *Lasius* Ants. *J. Insect. Physiol.*, **16**, 2353-2375.
- BERGSTRÖM, G. and J. LÖFQVIST (1972) — Similarities between the Dufour gland secretions of the Ants *Camponotus ligniperda* (Latr.) and *Camponotus herculeanus* (L.) (Hym.). *Ent. Scand.*, **3**, 225-238.
- BERGSTRÖM, G. and J. LÖFQVIST (1973) — Chemical Congruence of the Complex Odoriferous Secretions from Dufour's Gland in Three Species of Ants of the Genus *Formica*. *J. Insect Physiol.*, **19**, 877-907.
- BLUM, M. S. and H. R. HERMANN (1978) — Venom and venom apparatuses of the Formicidae: Myrmecinae, Ponerinae, Dorylinae, Pseudomyrmecinae, Myrmicinae and

- Formicinae. In *Arthropod Venoms*, S. BETTINI (Ed.), *Handb. Exp. Pharm.*, **48**, Springer, Berlin, 801-894.
- BUSCHINGER, A., W. EHRHARDT and U. WINTER (1980) — The Organization of Slave Raids in Dulotic Ants — A Comparative Study (Hymenoptera : Formicidae). *Z. Tierpsychol.*, **53**, 245-264.
- CAMMAERTS, M. C., R. P. EVERSLED and E. D. MORGAN (1981a) — Comparative Study of the Dufour Gland Secretions of Workers of Four Species of *Myrmica* Ants. *J. Insect Physiol.*, **27**, 59-65.
- CAMMAERTS, M. C., R. P. EVERSLED and E. D. MORGAN (1981b) — Comparative Study of the Mandibular Gland Secretion of Four Species of *Myrmica* Ants. *J. Insect Physiol.*, **27**, 225-231.
- COLLINGWOOD, C. A. (1979) — The Formicidae (Hymenoptera) of Fennoscandia and Denmark. *Fauna et entomologica scandinavica*, **8**, 1-174.
- EVERSLED, R. P. and E. D. MORGAN (1981) — Chemical Investigations of Dufour Gland contents of Attine ants. *Insect Biochem.*, **11**, 343-351.
- KUTTER, H. (1977) — Hymenoptera Formicidae. *Insecta Helvetica*, **6**, 1-298.
- LÖFQVIST, J. (1977) — Toxic properties of the chemical defence systems in the competitive ants *Formica rufa* and *F. sanguinea*. *Oikos*, **28**, 137-151.
- LONGHURST, C., R. BAKER and P. E. HOWSE (1980) — A Comparative Analysis of Mandibular Gland Secretions in the Ant Tribe Tetramoriini. *Insect Biochem.*, **10**, 107-112.
- MORGAN, E. D. and L. J. WADHAMS (1972) — Gas Chromatography of Volatile Compounds in Small Samples of Biological Material. *J. Chromatogr. Sci.*, **10**, 528-529.
- PARRY, K. and E. D. MORGAN (1979) — Pheromones of Ants : A Review. *Physiol. Entomol.*, **4**, 161-189.
- REGNIER, F. E. and E. O. WILSON (1971) — Chemical Communication and « Propaganda » in Slave-Maker Ants. *Science*, **172**, 267-269.
- VAN BOVEN, J. K. A. (1977) — De Mierenfauna van België (Hymenoptera : Formicidae). *Acta Zool. Pathol. Antwerp*, **67**, 1-191.
- WILSON, E. O. and F. E. REGNIER (1971) — The Evolution of the Alarm-Defence System in the Formicine Ants. *Amer. Nat.*, **105**, 279-289.