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THE CHEMICAL SECRETIONS OF *Myrmica specioides* Bondroit AND *Myrmica gallieni* Bondroit (MYRMICINAE)

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Summary: The rare myrmicine ants *Myrmica specioides* and *M. gallieni* have been examined chemically for the first time. The Dufour glands of both species contain homofarnesene, bishomofarnesene and farnesene as the major components. Previous studies of the Dufour gland secretions of *Myrmica* species have shown them to contain species-specific mixtures of hydrocarbons. The genus can be divided into 2 groups depending on whether linear hydrocarbons ('R group') or sesquiterpenes ('S group') predominate. *M. specioides* and *M. gallieni* hence belong to the 'S group'. The composition of the mandibular gland contents also varies with species. The secretions of both *M. specioides* and *M. gallieni* are similar to those of the other *Myrmica* previously investigated. 3-Octanone, 3-octanol and 3-decanone are the major components in a mixture of alcohols and ketones. These two species, like the nine *Myrmica* species already investigated, use 2,5-dimethyl-3-ethylpyrazine as their trail pheromone.

Key words: Ant, *Myrmica specioides, Myrmica gallieni,* Dufour gland, mandibular gland, trail pheromone.

Les sécrétions chimiques de *Myrmica specioides* Bondroit et *Myrmica gallieni* Bondroit (Myrmicinae)

Résumé: Les fourmis rares de la famille Myrmicine, *Myrmica specioides* et *Myrmica gallieni* out été étudiées du point de vue chimique pour la première fois. Les principaux constituants produits par les glandes de Dufour de ces deux espèces sont l'homofarnésène, le bishomofarnésène et le farnésène. Des études précédentes portant sur les sécrétions de la glande de Dufour du genre *Myrmica* ont montré qu'elles contenaient des mélanges d'hydrocarbures particuliers à chaque espèce. Le genre peut être divisé en deux groupes selon la prédominance des hydrocarbures linéaires ('groupe R') ou des sesquiterpènes ('groupe S'). *M. specioides* et *M. gallieni* appartiennent donc au 'groupe S'. La composition du contenu de la glande mandibulaire varie aussi selon l'espèce. Les sécrétions de *M. specioides* et *M. gallieni* sont semblables à l'autre *Myrmica* précédemment étudiée. 3-Octanone, 3-octanol et 3-décanone sont les principaux constituants d'un mélange d'alcools et de cétones. Ces deux espèces, tout comme dans le cas des neuf espèces de *Myrmica* précédemment étudiées, sécrètent une phéromone de piste composée de 2,5-dimethyl-3-ethylpyrazine.

Mots-clés: Fourmis, *Myrmica specioides, Myrmica gallieni*, glande de Dufour, glande mandibulaire, phéromone de piste.

INTRODUCTION

Species of the common ant genus *Myrmica* have been the subject of a number of studies of their pheromone chemistry and behaviour. In particular, the contents of the mandibular gland (CAMMAERTS et al., 1982; 1983), the poison gland (EVERSHED et al., 1982) and the Dufour gland (ATTYGALLE et al., 1983) have been reported for eight European species. The Dufour gland contents of a further three species have also recently been studied (ALI, 1987). CREWE and BLUM (1970) have also reported the mandibular gland contents of six North American species. As well as their ethological significance, the secretions are of chemotaxonomic use, since species-characteristic mixtures have been found.

M. specioides Bondr. and *M. gallieni* Bondr. are uncommon European species, although their presence may be overlooked by confusion in the female castes with *M. scabrinodis* and *M. sulcinodis*, respectively (COLLINGWOOD, 1979). The three major exocrine secretions of *M. gallieni* and *M. specioides* are described here to provide further knowledge on the systematic relationships between species of *Myrmica*.

MATERIALS AND METHODS

One colony each of *Myrmica specioides* and *M. gallieni* were collected in Switzerland in October 1987, and a further three of *M. gallieni* in July 1988. They were transported to Keele soon after collection, where they were kept in artificial nests. The heads, poison glands and Dufour glands were dissected from freshly killed workers and sealed individually in soft-glass capillaries (2 mm x 2 cm). The glands were analysed by gas chromatography-mass spectrometry on a Hewlett Packard 5890 gas chromatograph and 5970B Mass Selective Detector with HP59970C ChemStation software. The glands sealed in the capillary tubes were introduced directly onto the gas chromatograph by the solid-sampling method of MORGAN and WADHAMS (1972), without the intervention of solvent. The injection port temperature was 140°C. The mandibular glands (heads) were analysed using a fused silica capillary column (12 m x 0.2 mm) coated with HP-1 of 0.33 μm film thickness. The oven temperature was 30°C for 2 minutes then increased at 4°C min^{-1}. For the poison and Dufour glands, a fused silica capillary column (15 m x 0.32 mm) coated with 0.25 μm of SE-54 was used, with a temperature programme of 30°C for 2 minutes then 6°C min^{-1}. The carrier gas was helium at 1 ml min^{-1}. The mass spectrometer was set to monitor m/z 35 to 350 under 'Autotune' conditions. Quantification was achieved by external standards.

Trail tests were carried out using circular trails, after the method of PASTEELS and VERHAEGE (1974). A hexane solution of 3-ethyl-2,5-dimethyl-pyrazine was used, prepared in this laboratory by a new method (E.D. Morgan and D.G. Ollett, unpublished).

RESULTS

The total amount of material in each gland was found to vary between individuals, as has been found for all other species. However, the relative proportions of the components of each gland of each species did not vary much. No significant differences in composition were found between the four different nests of *M. gallieni* for either gland.

The mandibular gland secretions of both species contain 3-alkanones and 3-alkanols, as has been found in other *Myrmica* species. However, the mean proportions of these compounds are quite different. For *M. gallieni* (Fig. 1a, Table 1) the two major components, 3-octanone (72%) and 3-octanol (20%) represent 92% of the total secretion. The major component of *M. specioides* (Fig.

Table I - Chemical composition of the mandibular glands of Myrmica gallieni *and* M. specioides.

Table I - Composition chimique de la glande mandibulaire de Myrmica gallieni *et* M. specioides.

		Myrmica gallieni				*Myrmica specioides*			
	Compound	%	SD	ng/ant	SD	%	SD	ng/ant	SD
1	3-Hexanone					0.4	0.2	4	3
2	3-Hexanol	0.4	0.3	5	4	1.5	0.7	17	10
3	3-Heptanone	1.1	0.4	15	6	0.8	0.2	9	4
4	3-Heptanol	0.9	0.3	13	6	0.8	0.2	8	5
5	3-Octanone	72.2	4.2	988	270	46.8	5.1	490	172
6	3-Octanol	19.8	3.9	278	93	16.8	1.7	174	62
7	6-Methyl-3-octanone	1.0	0.3	14	7	6.2	1.9	65	21
8	6-Methyl-3-octanol	0.1	0.1	1	1	0.5	0.1	5	2
9	3-Nonanone	1.6	0.3	24	11	1.8	0.1	19	4
10	3-Decanone	2.6	1.6	37	22	20.3	3.3	215	81
11	3-Decanol	0.1	0.1	1	1	0.7	0.2	7	3
12	6-Methyl-3-decanone					0.8	0.4	9	6
13	8-Methyl-3-decanone					3.5	1.0	39	18
14	3-Undecanone					0.1	0.1	1	1
15	3-Dodecanone					0.5	0.3	4	3
	Total			1323				1066	

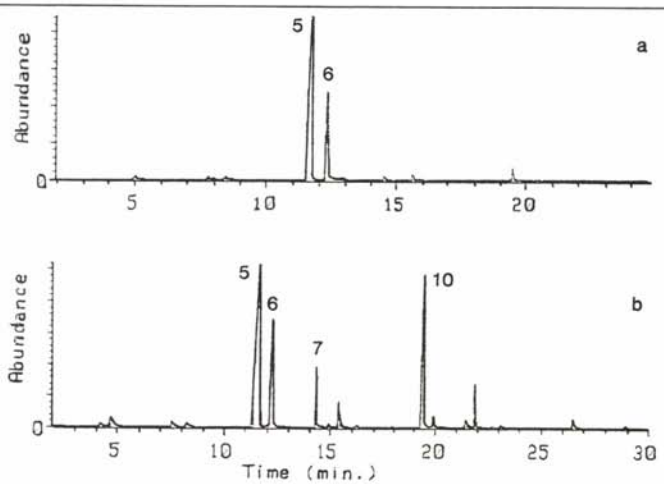

Fig. 1. – Gas chromatograms of the volatiles of an individual mandibular gland of (a) M. gallieni *and (b)* M. specioides. *Peak numbers refer to compounds listed in Table 1.*

Fig. 1. – Chromatogramme en phase gazeuse des sécrétions d'une glande mandibulaire de (a) M. gallieni *et (b)* M. specioides. *Légende, voir Table 1.*

Table 2 - Chemical composition of the Dufour glands of Myrmica gallieni *and* M. specioides.

Table 2 - Composition chimique de la glande de Dufour de Myrmica gallieni *et* M. specioides.

		Myrmica gallieni				*Myrmica specioides*			
	Compound	%	SD	ng/ant	SD	%	SD	ng/ant	SD
1	Pentadecadiene	1.4	0.6	25	14	1.8	0.4	16	8
2	Pentadecene	1.7	0.5	30	13	3.6	0.5	33	13
3	Farnesene	5.8	2.7	103	51	23.9	9.5	218	125
4	Pentadecane	1.8	0.8	31	18	1.8	0.7	17	9
5	Homofarnesene	33.2	8.0	598	180	43.7	4.3	386	87
6	Hexadecene	0.6	0.3	9	5				
7	Unknown*	1.3	0.2	23	6				
8	Hexadecane	0.4	0.1	6	2	0.2	0.2	2	2
9	Bishomofarnesene	40.1	8.5	718	218	20.7	10.0	188	128
10	Heptadecadiene	0.6	0.2	10	4	0.1	0.1	1	1
11	Heptadecene	4.2	1.6	77	31	0.3	0.2	3	2
12	Heptadecane	5.3	2.7	95	47	1.5	0.1	12	4
13	Trishomofarnesene	0.7	0.5	12	10				
14	Tetramorene-2	1.2	0.6	19	11				
	Total			1756				876	

*Probably an isomer of bishomofarnesene

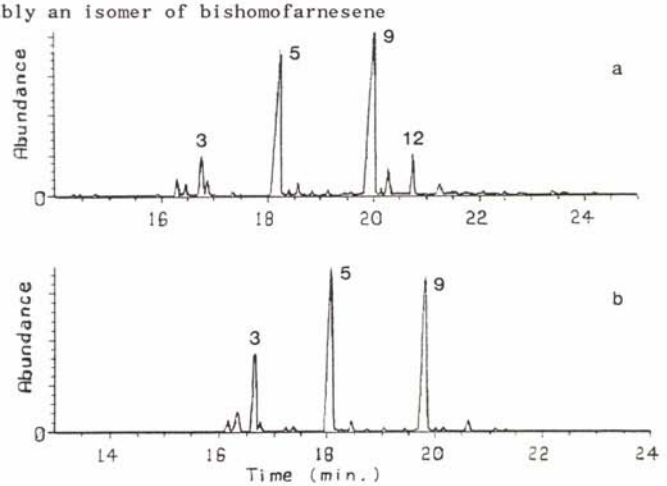

Fig. 2. – Gas chromatograms of the volatiles of an individual Dufour gland of (a) M. gallieni *and (b)* M. specioides. *Peak numbers refer to compounds listed in Table 2.*

Fig. 2. – Chromatogramme en phase gazeuse des sécrétions d'une glande de Dufour de (a) M. gallieni *et (b)* M. specioides. *Légende, voir Table 2.*

1b, Table 1) is again 3-octanone, but the second largest is 3-decanone, with 3-octanol third.

The poison glands of both species contain 3-ethyl-2,5-dimethylpyrazine (EDMP) and both species followed artificial trails of synthetic EDMP.

The Dufour glands of both species contain typical mixtures of mainly sesquiterpenoids and some linear hydrocarbons, but again with differing proportions. *M. gallieni* has a greater proportion of higher molecular weight substances, with (Z,E)-α-bishomofarnesene the major component (Fig. 2a, Table 2). *M. gallieni* also contains tetramorene-2, a sesquiterpenoid, related to dihydrofarnesal (Jackson, unpublished), found in four myrmicine species (ALI, et al., 1987) which has not previously been identified in *Myrmica*. On average, 16% of the secretion of *M. gallieni* is linear hydrocarbons. *M. specioides* has a similar mixture with (Z,E)-α-homofarnesene as the major component and with 10% linear hydrocarbons (Fig. 2b, Table 2).

DISCUSSION

Myrmica mandibular glands have been found to contain homologous series of 3-alkanols and 3-alkanones in the C_5-C_{10} chain length range, with quantitative differences in the composition between species (CAMMAERTS et al., 1982; 1983). In all species studied this mixture serves as an attractant pheromone. *M. gallieni* and *M. specioides* secrete similar mixtures, but again of different composition. Of the ten European species now investigated, *M. specioides* is unique in having 3-decanone as the second largest component. *M. schencki*, which has 3-decanol as the second largest component (CAMMAERTS et al., 1982), is the only other species not to have 3-octanone and 3-octanol as the two major components. However, for *M. gallieni*, these two C_8 compounds are 92% of the secretion. This species has the largest proportion of 3-octanone (72%) of the 10 species, the species with the next largest being *M. sulcinodis* (63%, CAMMAERTS et al., 1983) and *M. sabuleti* (62%, CAMMAERTS et al., 1981a).

Like the other European species of *Myrmica, M. gallieni* and *M. specioides* both use 3-ethyl-2,5-dimethylpyrazine from their poison glands as their trail pheromone. The use of EDMP as a trail pheromone component has now been found to be widespread among myrmicine ants, including *Manica rubida* (ATTYGALLE et al., 1986), *Atta sexdens* (EVERSHED AND MORGAN, 1983), *Tetramorium caespitum* (ATTYGALLE and MORGAN, 1983), *Pheidole pallidula* (ALI et al., 1988) and *Messor bouvieri* (JACKSON et al., 1989).

For all species of *Myrmica* studied, the Dufour gland has been found to contain a mixture of hydrocarbons characteristic of that species. A simple scheme has been devised (ATTYGALLE et al., 1983) in which *Myrmica* species are divided into two groups, the 'R group', typified by *M. rubra* and *M. ruginodis*, whose Dufour glands are predominantly filled with linear hydrocarbons, and the 'S group', for example *M. scabrinodis*, whose Dufour glands contain chiefly farnesene homologues. Thus, *M. gallieni* and *M. specioides*, with Dufour glands rich in sesquiterpenes, both belong to the 'S group'. However, this scheme hides the differences in individual compounds, which also separate the species. It is not surprising that species can be distinguished by the composition of this secretion, because it has been shown for eight *Myrmica* species previously studied (ATTYGALLE et al., 1983, and references therein) that the Dufour gland secretion is used to mark foraging areas, and that ants of each species can distinguish territories marked with their own, or an alien secretion.

M. specioides and *M. scabrinodis* are morphologically closely similar, but chemically they show two distinct differences. The proportion of 3-decanone in the mandibular gland of *M. specioides* (20%) is significantly larger than in *M. scabrinodis* (12%, CAMMAERTS et al., 1978), and trishomofarnesene is present in

the Dufour glands of *M. scabrinodis* (2%, CAMMAERTS et al., 1981b) but not in *M. specioides*. *M. gallieni* is close to *M. sulcinodis* morphologically. Chemically they are distinguished by a much lower proportion of linear hydrocarbons (16%) in the Dufour gland of *M. gallieni* than in *M. sulcinodis* (38%, CAMMAERTS et al., 1981b).

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