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CHEMOTAXONOMIC STUDY OF UNDESCRIBED SPECIES[1] OF *Myrmica* ANT FROM IDAHO

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Abstract—An undescribed species of *Myrmica* collected in Idaho has been shown to have the same substances in its mandibular glands (3-octanol and 3-octanone and related 3-alkanols and 3-alkanones) and in its Dufour gland (linear alkanes, alkenes, and farnesene isomers and homologs) as previously examined European species of *Myrmica*. The poison gland contains the trail pheromone 3-ethyl-2,5-dimethylpyrazine, common to all *Myrmica* species studied so far. The Dufour gland contains large amounts of bishomofarnesene, which easily distinguishes it from some 13 other *Myrmica* already known.

Key Words—Ant, *Myrmica*, Hymenoptera, Formicidae, exocrine secretions, mandibular gland, Dufour gland, trail pheromone, 3-ethyl-2,5-dimethylpyrazine, bishomofarnesene.

INTRODUCTION

Yensen et al. (1977), in their checklist of Idaho ants, list 12 species of *Myrmica*. Recently, an undescribed species was discovered in the Idaho National Envi-

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[1] Species description to appear elsewhere.

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ronmental Research Park, which is located in the desert area of southeastern Idaho. André Francoeur has recognized this as a new species in the course of revision of the Nearctic species and will describe it in the near future. The species is not confined to Idaho; it is a western species found infrequently in warm habitats (Francoeur, personal communication). In order to define this new species more fully, a chemotaxonomic study has been made of its exocrine secretions. There is an increasing awareness that such characteristics can be a valuable addition to the use of morphological characters in the diagnosis of a species. Numerous studies of ants have shown that in all cases examined, the Dufour gland in particular contains a species-specific mixture of substances, dominated by oily hydrocarbons.

Crewe and Blum (1970a,b) carried out the earliest studies on the mandibular gland secretions of *Myrmica* species and showed that 3-octanol and 3-octanone are the principal constituents in each one, although the proportions varied. Subsequently a systematic study was carried out on the eight species of *Myrmica* commonly found in Great Britain (Cammaerts et al., 1982; Attygalle et al., 1983a), and it was further extended to five other European species (Jackson et al., 1989, and unpublished). In each case we have found a species-specific mixture of alcohols and ketones, usually dominated by 3-octanol and 3-octanone. Similarly, we have studied the Dufour gland contents of the eight species of British *Myrmica* (Cammaerts et al., 1983; Attygalle et al., 1983b) and the five European species (Jackson et al., 1989, and unpublished) and have found a mixture of alkanes, alkenes, and (*Z*,*E*)-α-farnesene and it homologs with one, two, or three extra carbon atoms (Attygalle and Morgan, 1982). Whether the linear or terpenoid hydrocarbons are dominant in the gland varies widely with species (Attygalle et al., 1983b).

Ant trail pheromones commonly are shared by more than one species (Attygalle and Morgan, 1985). Our investigation of the trail pheromone of *Myrmica* (Evershed et al., 1981, 1982; Jackson et al., 1989, and unpublished), has shown that the same single substance 3-ethyl-2,5-dimethylpyrazine (EDMP), present in the poison gland as a trace component, provides the trail pheromone in each of 12 species examined.

We show here that this undescribed species from Idaho, while fitting perfectly within the pattern found for its European cousins, has a specific composition of secretion in its mandibular and Dufour glands that should distinguish it from other species of *Myrmica*. It shares the same trail pheromone as all the other *Myrmica* species.

METHODS AND MATERIALS

A colony of this *Myrmica* sp. collected at the Idaho National Environmental Research Park (WHC #8919, 30-IX-1989) was sent live to Keele, where it was maintained in an artificial nest until analysis could be carried out. A colony

of *Myrmica rubra*, collected near Keele was maintained in a similar artificial nest and used in the trail pheromone tests.

For analysis of the mandibular gland substances, whole worker heads were sealed in soft glass capillaries (2 × 20 mm). For the analysis of the Dufour glands, whole poison apparatuses (sting lance, Dufour gland, poison filaments, and reservoir) were similarly sealed in soft glass capillaries. These capillaries were introduced into the gas chromatograph as described by Morgan and Wadhams (1972) and there heated and crushed to produce the chromatogram.

The samples were analyzed by GC-MS, on a Hewlett Packard 5890 gas chromatograph and 5970B mass selective detector with HP59970C chemstation software. A fused silica capillary column (12 m × 0.2 mm) coated with immobilized dimethylsiloxane of 0.33 μm film thickness was used. The injection port temperature was 140°C, and the capillary tubes were equilibrated in the solid injector for 2 min. The oven temperature was initially set at 30°C for two minutes and then increased at 8°C/min. The flow rate of the helium carrier gas was 1 ml/min. The mass selective detector was set to detect ions with *m/z* 35–350. Calibration was achieved by injecting external standards of C_{15} to C_{18} hydrocarbons for Dufour gland substances 3-octanone, 3-octanol, 3-nonanone, and 3-nonanol for mandibular gland substances, and EDMP for the trail pheromone.

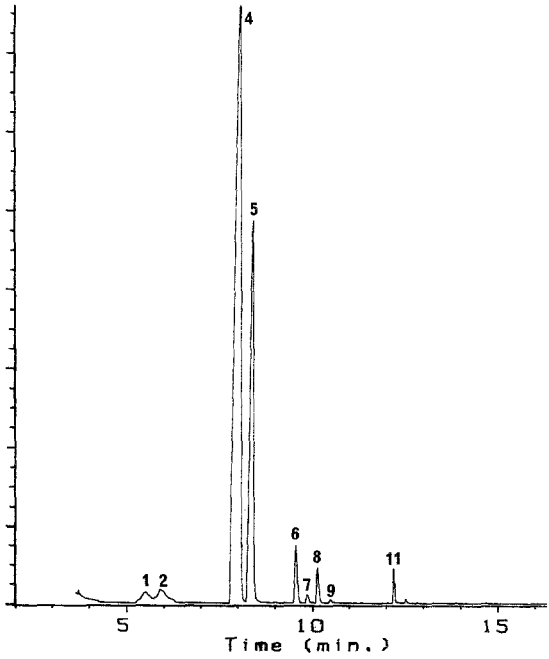

FIG. 1. Gas chromatogram of the volatiles from the mandibular glands of a single worker of *Myrmica* sp. (undescribed). Peak numbers refer to compounds listed in Table 1.

These substances also were used for comparison of retention times and mass spectra in identification of the various substances. Farnesenes were identified by comparison with retention times and mass spectra of those identified in other *Myrmica* species (cf., Attygalle and Morgan, 1982).

The method of Pasteels and Verhaege (1974), using circular trails, was used for determination of the activity of the trail pheromone and for cross-activity testing between *Myrmica rubra* and *Myrmica* sp. Solutions of extracts were prepared in hexane (100 μl) applied to the circumference of a circle (r = 5 cm) drawn on paper, with 1-cm arcs marked off on the circumference, and after allowing 2 min for the hexane to evaporate, the paper was presented to foraging workers. The number of 1-cm arcs for which each worker followed the circular trail before deviating from it were recorded. The median value of 30 such observations was calculated for each test. Hexane solvent alone was used for control tests. Synthetic 3-ethyl-2,5-dimethylpyrazine was tested similarly.

Voucher specimens are deposited in the collection of College of Idaho

TABLE 1. AMOUNTS AND PERCENTAGES OF VARIOUS SUBSTANCES FOUND IN MANDIBULAR GLANDS OF WORKERS OF UNDESCRIBED SPECIES OF *Myrmica* FROM IDAHO[a]

	Compound	Amount ± SD (ng/ant)	Percentage (% ± SD)
1	3-Heptanone	35 ± 23	1.9 ± 0.4
2	3-Heptanol	39 ± 43	1.8 ± 0.7
3	Unknown	1 ± 2	t[b]
4	3-Octanone	1186 ± 788	65.0 ± 11.0
5	3-Octanol	456 ± 322	25.6 ± 10.4
6	6-Methyl-3-octanone	33 ± 19	1.8 ± 0.7
7	6-Methyl-3-octanol	8 ± 5	0.5 ± 0.2
8	3-Nonanone	32 ± 24	1.7 ± 0.4
9	3-Nonanol	5 ± 3	0.3 ± 0.2
10	Unknown	1 ± 1	t
11	3-Decanone	20 ± 12	1.2 ± 0.4
12	3-Decanol	3 ± 2	0.2 ±
13	Unknown	1 ± 1	0.1 ±
14	3-Undecanone	1	0.1 ±
15	3-Undecanol	1 ± 1	t
	Total	1820	

[a] Results are the means (with sample standard deviations) of analysis of 10 individuals.
[b] t = less than 0.1%.

Museum of National History (CIDA), André Francoeur, and the William F. Barr Entomological Museum, University of Idaho (UICM).

RESULTS

The mandibular glands of this undescribed species contain chiefly 3-octanone, with less of 3-octanol and traces of other related alcohols and ketones (Figure 1). The mean amount of each substance and the mean percent that each substance represents of the total, plus their sample standard deviations are given in Table 1. As found elsewhere, the amounts in the gland vary widely, but the composition remains within a fairly narrow band of values.

The Dufour gland contained very little linear hydrocarbons but was dominated by a large amount of (*Z*,*E*)-α-bishomofarnesene (Figure 2). The amounts and percentages of the major components are given in Table 2.

The presence of EDMP was evident in the chromatogram obtained from the poison apparatus (Figure 2). The amount was similar to that found in *M.*

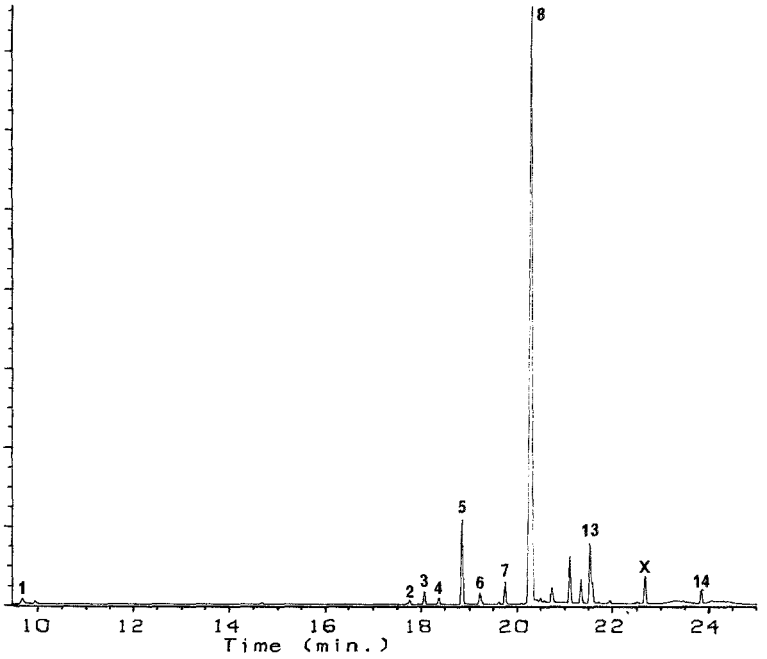

FIG. 2. Gas chromatogram of the poison apparatus of a single worker of *Myrmica* sp. (undescribed). Peak numbers refer to compounds listed in Table 2; X is isopropyl myristate, a contaminant.

TABLE 2. AMOUNTS OF 3-ETHYL-2,5-DIMETHYLPYRAZINE (EDMP) IN POISON RESERVOIR AND AMOUNTS AND PERCENTAGES OF VARIOUS SUBSTANCES FOUND IN DUFOUR GLAND OF WORKERS OF UNDESCRIBED SPECIES OF *Myrmica* FROM IDAHO[a]

	Compound	Amount (ng/ant ± SD)	Percentage (% ± SD)
1	EDMP (from poison gland)[b]	4.9 ± 5.4	
2	*(Z,E)*-α-Farnesene	5.5 ± 6.2	3.1 ± 5.0
3	Pentadecane	10.4 ± 14.8	1.9 ± 2.8
4	Homofarnesene isomer 1	6.0 ± 11.8	0.5 ± 0.3
5	*(Z,E)*-α-Homofarnesene	60.6 ± 100	7.4 ± 3.6
6	Homofarnesene isomer 2	13.3 ± 24.5	1.2 ± 0.4
7	Bishomofarnesene isomer 1	23.4 ± 47.8	2.0 ± 1.0
8	*(Z,E)*-α-Bishomofarnesene	514 ± 633	67.4 ± 7.6
9	Bishomofarnesene isomer 2	1.4 ± 2.3	0.2 ± 0.3
10	Heptadecene	6.3 ± 6.8	1.2 ± 1.0
11	Heptadecane	29.7 ± 66.7	5.5 ± 1.4
12	Trishomofarnesene isomer 1	30.4 ± 61.5	2.3 ± 1.3
13	Trishomofarnesene 2	67.8 ± 121	6.0 ± 2.9
14	Nonadecane	11.6 ± 14.5	1.4 ± 0.7
	Total	780	

[a] Results are the means (with sample standard deviations) of 10 determinations on individual workers.

[b] The whole poison apparatus was used, consisting of poison glands, poison reservoir, and Dufour gland. The protein venom of the poison reservoir is not seen in the chromatogram, and the EDMP is the only substance not coming from the Dufour gland.

rubra (Evershed et al., 1981). All control experiments gave zero median values, and all test results were significantly different ($P < 0.001$) from controls using the Mann-Whitney-Wilcoxon test (Siegel and Castellan, 1988). The results of the activity tests are shown in Table 3.

DISCUSSION

The results obtained here are conveniently compared with those recently obtained for *M. gallieni* and *M. specioides*, collected in Switzerland (Jackson et al., 1989) and examined under almost identical conditions. The mandibular gland contents of *Myrmica* sp. are superficially very similar to that of *M. gallieni*, with octanone and octanol comprising over 80% of the total contents, but there are minor differences, such as the absence of undecanone and undecanol in *M. gallieni*. The size of the gland is somewhat larger here (1.8 μg secretion)

TABLE 3. RESULTS OF TRAIL-FOLLOWING TESTS ACCORDING TO METHOD OF PASTEELS AND VERHAEGHE (1974) ON CIRCULAR TRAIL (RADIUS 5 cm)[a]

Source of material for tests	Species	
	M. rubra	*Myrmica* sp. (undescribed)
Myrmica sp. (undescribed), 1 gaster extracted in 100 μl hexane	7	4
Myrmica rubra, 1 gaster extracted in 100 μl hexane	7	4
EDMP		
1 ng in 100 μl hexane	2	3
7 ng in 100 μl hexane	[b]	6

[a]Results are expressed as the median number of 1-cm arcs on the circumference followed on the trail by 30 worker ants. Hexane controls in the same test gave 0 cm.
[b]Not tested.

than in either *M. gallieni* (1.3 μg) or *M. specioides* (1.1 μg). The unknown compounds are trace substances from the head. They have been recognized in other chromatograms where large amounts of insect tissue have been injected. The sample standard deviations of amount are very large because the quantity of substance in an individual gland varies widely; for example, there was 3.0 μg of 3-octanone in the largest gland, and 0.2 μg in the smallest.

The Dufour gland is rather distinct from the European species in having bishomofarnesene representing 70% of the total contents. It also is unusual in having small amounts of other isomers of homofarnesene, bishomofarnesene, and trishomofarnesene. If these have been present in other species, the amounts have been too small to detect. Comparatively, the Dufour gland is smaller in this species (contains 0.8 μg) compared to *M. rubra*, *M. gallieni* (1.8 μg), or *M. specioides* (0.9 μg). Large variations in the amount per gland were again observed.

The quantity of EDMP found in the poison glands (4.9 ng) is comparable with what has been found in other *Myrmica* species, and the trail tests (Table 3) show that the trail-following ability of this species is similar to, or slightly less than, that of *M. rubra*. Moreover, *M. rubra* readily followed artificial trails made with extracts of the gaster of this species and vice versa. It can be seen from Table 3 that the activity of a single gland and the equivalent amount of pure EDMP are comparable.

Myrmica sp. clearly fits into the pattern of trail pheromone and exocrine secretions of its geographically distant cousins from Europe.

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