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THE TRAIL PHEROMONE OF THE ANT MANICA RUBIDA (Hymenoptera, Formicidae)

by

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LA PHÉROMONE DE PISTE CHEZ LA FOURMI MANICA RUBIDA (Hymenoptera: Formicidae)

Résumé: La glande à poison des ouvrières de Manica rubida contient la phéromone de piste. Par une combinaison de méthodes microchimiques et expériments éthologiques, nous avons identifié la substance active comme étant le 3-éthyl-2,5-diméthylpyrazine, donc la même substance utilisée par huit espèces de Myrmica et trois d'Atta. La quantité moyenne par ouvrière est environ 7 ng. La substance 2,5-diméthylpyrazine est également présente mais ne montre aucune activité phéromonale.

Mots-clés: *Phéromone de piste, fourmi, Manica rubida, gland à poison, pyrazine, Myrmicine.*

Summary: The poison gland of the workers of Manica rubida contains a trail pheromone which has been identified by micro-chemical methods and trail-following experiments to be 3-ethyl-2,5-dimethylpyrazine, the same substance as used by at least eight species of Myrmica and three species of Atta. There is on average 7 ng of the pheromone per individual worker's gland. The substance 2,5-dimethylpyrazine is also present in the gland but has no pheromonal function.

Key words: *Trail pheromone, ant, Manica rubida, poison gland, pyrazine, Myrmicine.*

INTRODUCTION

Manica rubida is the only European member of this widely distributed North American genus of primitive Myrmicine ants. Goetsch (1934) showed that *M. rubida* laid odour trails and Blum (1974) showed further that the trails were laid from the poison gland of three North American *Manica* species. Blum found a complex pattern in transposition studies with artificial trails made from the glands of *Manica* and other species. Our information on the specificity of odour trails, or the lack of it, is at present fragmentary, but it is known that many ant species share the same trail pheromones and will follow artificial trails made from the glands of other species. The subject has been reviewed recently (Attygalle and Morgan, 1984a).

Cammaerts and Cammaerts (1985) have now described the food-gathering behaviour of *M. rubida*. They found that workers do not recruit congeners to food gathering, although they lay odour trails. The Cammaerts found a large nest of *M. rubida* in central France in 1983, and through their good offices, part of this colony was made available to us for trail pheromone studies.

We describe here our investigations which show that *M. rubida* follows trails by the odour of a single substance in its poison gland. It possesses the same substances as *Tetramorium caespitum*, but responds to them differently.

MATERIALS AND METHODS

The techniques used throughout are the same as those described in detail by Attygalle and Morgan (1984b).

Maintenance of Colonies The ants were kept in an artificial nest of glass and moistened plaster of Paris, and fed on dilute sugar solution, mealworm and dipteran larvae. Their foraging area was inside a large plastic bowl, with its walls coated with polytetrafluoroethylene to prevent the ants escaping.

Gas chromatography Ants were killed immediately before use by exposure to the cold vapour from liquid nitrogen. Single poison glands were cleanly dissected from the gaster, so as to avoid contamination from the much more abundant substances of the Dufour gland. The poison gland was sealed in a short piece of glass capillary, and introduced onto the gas chromatography column by our usual technique of solid sampling (Morgan and Wadhams, 1972). The

conditions used were a 1.5m x 4mm column of 10% PEG 20M on Chromosorb W with nitrogen at 50 ml min^{-1}, isothermal at 128 ^{o}C and a 2.75m x 4mm column of 10% PEGA with nitrogen at 50 ml min^{-1} and isothermal at 135 ^{o}C. Fractions were collected from the gas chromatograph for bioassay by attaching an effluent splitter which diverted 95% of the material to the outside of the chromatograph for collection in metal U-tubes cooled in liquid nitrogen and ethyl acetate. The trapped material was washed from the tubes with acetone (50 $\mu\ell$) and used in the bioassay.

Quantification of glandular components The poison glands of 10 workers were chromatographed singly, using the injection technique outlined above. A computing integrator (DP101 Spectra Physics) was attached to measure peak areas and to calculate the absolute quantities of material in each gland, using a solution of 3-ethyl-2,5--dimethylpyrazine as an external standard.

Bioassay for trail-following behaviour Whole poison glands crushed in acetone or hexane (100 $\mu\ell$) or acetone solutions obtained by trapping from the gas chromatograph were assayed by the method of Pasteels and Verhaeghe (1974). The solution was placed on the circumference of a circle or radius 5 cm on paper using a Standardgraph pen (0.8 mm Blundell Harling, Dorset). The solvent was allowed to evaporate (2 min) and the paper then placed in the foraging area of the colony. The number of arcs of 1 cm on the circumference, which each individual worker ran along the trail, when it reached the circle was recorded for a total of 20 min observation. The median of the number of arcs followed was used as a measure of activity for each test and mean values of the medians were obtained by repeating each test three times. A blank bioassay using solvent alone was performed before each test to ensure that there was no residual activity in the pen.

RESULTS

Workers of *Manica rubida* followed artificial trails made from their poison glands but not from their Dufour glands. When poison glands were subjected to gas chromatography and the whole of the volatile material collected in a single fraction, this was equally active, therefore it was concluded the pheromone was volatile and stable to chromatography. Further glands were then chromatographed and collected in fractions. All fractions were inactive in bioassay (Table I) except that collected between 4.0 and 7.0 min after crushing the

capillary and releasing the substances on a PEG20M (polyethylene glycol) phase (Fig. 1) and that between 5 and 7 min on a PEGA column.

Table I. Trail-following activity of fractions collected from gas chromatography of Manica rubida poison glands.

A. Using a column of PEG 20M. One poison gland was chromatographed, and the material from each fraction was collected in a cooled metal U-tube, and washed from there with 100 µl of acetone, which was placed on a circle of radius 5 cm drawn on paper. The workers were allowed access to the paper after allowing 2min for the acetone to evaporate.

Time of elution of fraction (min)	No. of ants reaching circle in 20 min.	Median No. of 1 cm arcs run per insect	Trail following activity
0-2	20	0	-
2-4	22	0	-
4-7	25	*	++
7-9	25	0	-
9-21	29	0	-

*each individual ant continued to circle 3 or 4 times

B. Using a column of PEGA. Conditions otherwise as in A, above.

Time of elution of fraction (min)	No. of ants reaching circle in.20 min.	Median No. of 1 cm arcs run per insect	Trail-following activity
0-2	21	0	-
2-5	17	0	-
5-7	13	23	++
7-9	22	0	-
9-20	23	0	-

The peak observed in this region corresponded in retention time on both columns to 3-ethyl-2,5-dimethylpyrazine, which had already been identified in several other species (Attygalle and Morgan, 1984a). Trails made with synthetic ethyldimethylpyrazine (at 5 ng/100 µℓ per trail) were equally active for M. rubida workers (Table II).

The only other peak visible in the gas chromatogram, originating from the poison gland was a smaller peak corresponding in retention time on both columns to 2,5-dimethylpyrazine (Fig. 1). This peak was inactive when trapped and assayed, though it was active for Tetramorium caespitum workers for whom this substance is the chief component of the trail pheromone (Attygalle and Morgan, 1984b). Likewise, synthetic dimethylpyrazine was inactive for M. rubida.

When the amount of ethyldimethylpyrazine in M. rubida workers was quantified by determining ten replicates of individual glands, a mean value of 7 ng was obtained. The amount of dimethylpyrazine was not quantified, and the ratio of ethyldimethylpyrazine to dimethylpyrazine varied from one worker to another, but from peak areas was on average 5:1, so that a mean of approximately 1 to 2 ng of dimethylpyrazine was present in each gland.

Table II. Activity of gland extracts and synthetic compounds in artificial trail-following experiments according to the method of Pasteels and Verhaeghe (1974). The number of ants (N) reaching the circular trail during a 20 minute period and the median number of 1 cm arcs which they then walked along the trail (m) were recorded.

Test substance	N	m	activity
Hexane blank*	30	0	-
Acetone blank*	35	0	-
1 Poison gland of M. rubida	40	7	+
1 " " " " "	34	5	+
5 ng of 2,5-dimethylpyrazine	23	0	-
5 ng "	41	0	-
5 ng of 3-ethyl-2,5-dimethylpyrazine	42	9	+
5 ng "	40	6	+
5 ng of 2,5-dimethylpyrazine (as above, except using T. caespitum workers)	38	1	+
5 ng "	32	2	+

* These are two of many such tests giving the same result.
All tests were made with a M. rubida colony, except the last two, which used a T. caespitum nest.

DISCUSSION

Although M. rubida workers do not recruit congeners to food gathering, yet they have a clearly developed ability to follow odour trails. The trail pheromone, like that studied by Blum in North American Manica species, is found in the poison gland. The only two volatile substances found in the poison gland in any quantity (i.e. more than 5×10^{-10}g) are 2,5-dimethylpyrazine and 3-ethyl-2,5-

-dimethylpyrazine, and only the latter displays activity. We have recently identified these two substances as together forming the trail pheromone of another Myrmicine, _Tetramorium caespitum_, (Attygalle and Morgan, 1983; 1984b).

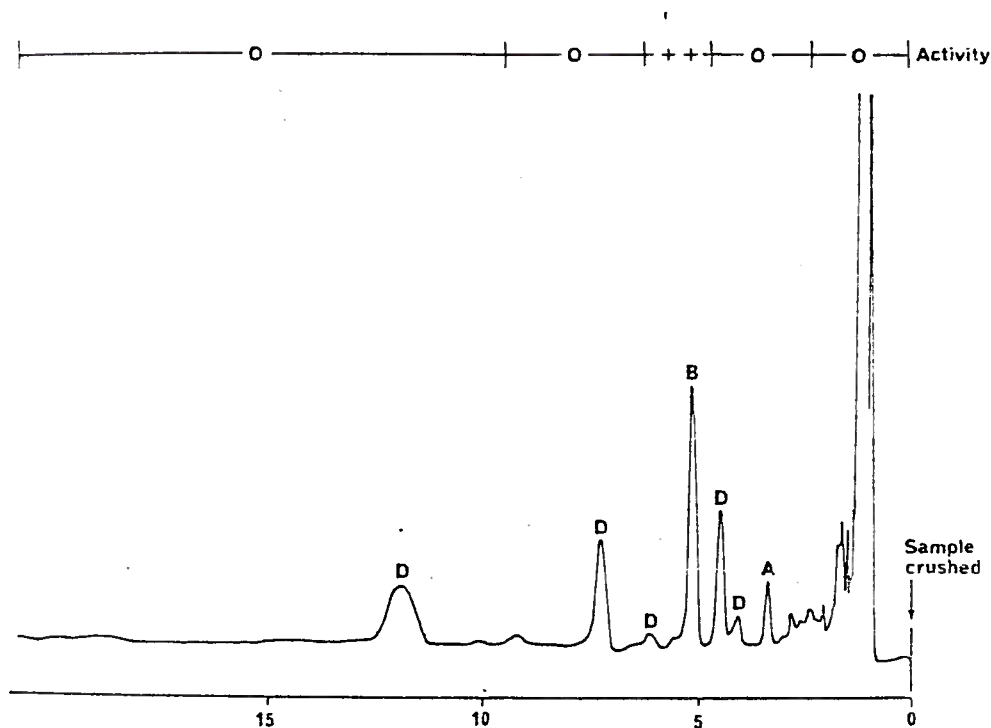

Fig. 1.
Gas chromatograph trace obtained by chromatography without solvent (solid injection) of one poison gland of _M. rubida_ on a PEG20M column at 128 °C. The effluent was split between detector and collector trap and the trapped material bioassayed. At the top of the figure are indicated the trapped fractions, and their activity in the trail-following test. O, no trail following; ++, highly active fraction in trail test; A, 2,5-dimethylpyrazine; B, 3-ethyl-2,5-dimethylpyrazine; D, traces of contaminating substances from the Dufour gland.

In _T. caespitum_ the compounds are in a 7:3 ratio and this mixture is the most active at a concentration of 1 ng per trail. In _M. rubida_ the ratio is 1:5 and only the ethyldimethylpyrazine is active. Neither the dimethylpyrazine from the gland nor synthetic material showed any activity, yet _T. caespitum_ responded to the same solutions. Without electrophysiological studies, we cannot know if _M. rubida_ can detect dimethylpyrazine, but certainly they do not respond to it by any

obvious behavioural change.

The active ethyldimethylpyrazine represents a fraction of a percent of the total poison gland volume, it is therefore only a trace component of the venom.

In the eight species of Myrmica we have examined, ethyldimethylpyrazine alone is present and completely accounts for the trail-following activity (Evershed et al., 1981; 1982). In Atta species, either methyl 4-methylpyrrole-2-carboxylate (in A. texana and A. cephalotes) or ethyldimethylpyrazine (in Atta sexdens) can be the trail pheromone, though we have demonstrated that both substances are present in A. cephalotes and A. sexdens (Evershed and Morgan, 1983). In T. caespitum the pheromone is a synergistic mixture of both ethyldimethylpyrazine and dimethylpyrazine (Attygalle and Morgan, 1984b). Among this group of poison-gland derived trail pheromones we therefore have three known substances one or more of which can be active. It may be noted that we have failed to detect the pyrrole substance in Myrmica, Manica or Tetramorium thus far.

Much further work may be necessary to understand the rational behind the adoption and use of these pyrazines by different species as trail pheromones.

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