Journal of Chemical Ecology, Vol. 10, No. 10, 1984

IDENTIFICATION OF TRAIL PHEROMONE OF THE ANT *Tetramorium caespitum* L. (HYMENOPTERA: MYRMICINAE)

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(Received December 1, 1983; revised February 16, 1984)

Abstract—The trail pheromone of the ant *Tetramorium caespitum* L. is a 70:30 mixture of 2,5-dimethylpyrazine and 3-ethyl-2, 5-dimethylpyrazine. The average total amount of the two pyrazines present in the poison vesicle was found to be 3.9 ng per ant, of which 2.7 ± 0.4 ng is 2,5-dimethylpyrazine and 1.15 ± 0.25 ng is 3-ethyl-2,5-dimethylpyrazine. The pyrazines constitute only 0.03% of the volume of the poison vesicle but account for the whole of the trail-following activity. A 70:30 mixture of the respective pyrazines evoked the highest activity in artificial trail-following tests.

Key Words—Ant, *Tetramorium caespitum*, trail pheromone, 2,5-dimethylpyrazine, poison gland, venom, Hymenoptera, Myrmicinae, synergism.

INTRODUCTION

A large number of ant species are known to employ trail pheromones as a means of communication, but only in a very few cases has the pheromone been chemically identified. The first trail substance to be identified was methyl 4-methylpyrrole-2-carboxylate from *Atta texana* Buckley (Tumlinson et al., 1972). The same compound was subsequently demonstrated to be active in evoking trail following in *A. cephalotes* L. (Riley et al., 1974) and *Acromyrmex octospinosus* Reich (Robinson et al., 1974).

3-Ethyl-2,5-dimethylpyrazine has been shown to be the major component of the trail pheromone of *Atta sexdens rubropilosa* Forel (Cross et al., 1979), and the same compound has since been identified as the single component of the trail pheromone of eight species of *Myrmica* (Evershed et al., 1981, 1982). In the beginning of the present study, only the aforementioned

0098-0331/84/1000-1453$03.50/0

two compounds were known as trail substances that originate from the poison glands. Faranal, a terpenoid that originates from the Dufour gland, has been identified as the major trail pheromone of *Monomorium pharaonis* L. (Ritter et al., 1977). There is a controversy about the composition of the trail pheromone of *Solenopsis invicta* Buren. Williams et al. (1981) have reported it to be (*Z*,*Z*,*Z*)-allofarnesene, while Vander Meer et al. (1981) describe it as a mixture of (*Z*,*E*)- and (*E*,*E*)-α-farnesenes and (*Z*,*Z*)- and (*Z*,*E*)-homofarnesenes. A mixture of C_6–C_{12} and C_{14}–C_{20} fatty acids are reported to be the active trail following mixtures for *Lasius fuliginosus* Latrielle (Huwyler et al., 1975) and *Pristomyrmex pungens* Mayr (Hayashi and Komae, 1977), respectively. In *Iridomyrmex humilis* Mayr (*Z*)-9-hexadecenal has been identified as one of the components of its trail pheromone (Cavill et al., 1979; Van Vorhis Key and Baker, 1982).

The above summary illustrates that the information available about the chemistry of trail pheromones is very limited. Some of the artificial trails laid with the above-mentioned single substances were not species specific, although the natural trails showed a much higher degree of species specificity. Although many trail pheromones had been recognized as multicomponent mixtures, the true quantitative and qualitative compositions of none of them were known at the beginning of this study. We have recently shown that the trail pheromone of *T. caespitum* L. contains two pyrazine compounds (Attygalle and Morgan, 1983) and give here the full identification of these substances and show how the synergistic action of these two compounds together completely accounts for the activity of the natural pheromone.

METHODS AND MATERIALS

Insect Rearing. Colonies of *T. caespitum* were collected from Heartland moor in Dorset. The ants were maintained in the laboratory at room temperature in a wooden box filled with moist soil and peat. The ants were fed on a diet of desiccated coconut, meal worm larvae (*Tenebrio molitor*), and sugar solution (10% w/v).

Preparation of Glandular Extracts for Bioassay. The ants were killed by exposing them to the cold vapor from liquid nitrogen. The poison glands and Dufour glands were separated by dissecting the ants in water. The glands were macerated with a solvent such as hexane or acetone (100 μl) and kept ice-cold for further use.

Bioassay of Trail-Following Behavior. The method of Pasteels and Verhaeghe (1974) was employed to measure the trail-following behavior of ants towards the test solutions. A circle of 5 cm radius was drawn with a lead pencil on a piece of white paper (13 × 13 cm). The circumference of the circle was marked with arcs (1 cm). The solution under investigation (usually 25–100

μl) was injected into a Standardgraph (Blundel Harling, Dorset) "S" funnel pen (0.8 mm) and a continuous streak was drawn on the circle. The solvent was allowed to evaporate for 2 min, and the paper was placed in the foraging area of the ant nest. The number of arcs run along the trail by each individual worker ant was recorded for 20 min. The median of the values thus obtained was used as a measure of activity. The activities of extracts of two poison glands and two Dufour glands were tested separately. Median values were obtained by repeating the tests three times. A blank bioassay using solvent only was always performed before a test to ensure no residual activity was present in the pen.

Thin-Layer Chromatography. An extract of two cleanly dissected poison glands was made in distilled acetone (50 μl). The extract was applied to the origin of a silica gel layer (20 cm × 5 cm × 0.3 mm) on a glass plate and developed with hexane–acetone (60:40). The solvent front was allowed to run 15 cm. The plate was air dried, and the silica was cut into ten bands (1.5 cm each). The bands were scraped separately into Pasteur pipets plugged with glass wool. Each fraction of silica was extracted with acetone (100 μl) directly into a Standardgraph pen. The trail-following activity evoked by each fraction was tested by bioassay. Blank bioassays using solvent only were performed before and in between each test to ensure no activity was present by contamination. The test was repeated in the same manner, except only the region between 4.5 and 9 cm was scraped with a small spatula and the width of each band was narrowed to 2 mm. All determinations were made in duplicate.

Similar experiments were performed to test for functional groups. Two poison glands were extracted separately in HCl in acetone (1%) and Br_2 in hexane (1%, v/v) respectively. The reaction mixtures were separated by TLC, and the bioassays were carried out as before. Samples of synthetic 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine were chromatographed under the same conditions as above. The spots were visualized under a UV lamp and the the R_f values were calculated.

Gas Chromatography. Gas chromatography (GC) was performed with a Pye 104 gas chromatograph with a flame ionization detector using a packed column of 2.75 × 4 mm, 10% PEG 20 M on Chromosorb W (100–120 mesh) at 130°C. The nitrogen carrier gas flow rate was 50 ml/min. The ionization amplifier was used at attenuation ×50. Three poison glands were cleanly dissected without the Dufour gland and the sting, blotted dry, mounted on a small piece of glass and sealed in a glass tube (25 × 1.8 mm). The contents of the tube were chromatographed via a solid injection method (Morgan and Wadhams, 1972). A Dufour gland was chromatographed under the same conditions in order to distinguish any peaks that may arise as contaminants in the poison gland GC traces.

Trapping of GC Effluent. Two poison glands were injected onto the PEG

20 M column at 130° C. The effluent was split using an all-glass splitter (Baker et al., 1976) (95:5, trap–FID) and collected in metal U-tubes (1 mm ID) cooled in a mixture of liquid nitrogen and ethyl acetate. The collection tubes were changed at 1-min intervals. The trapped material was directly washed with acetone (50 μl) into Standardgraph pens, and the activities of various fractions were bioassayed.

Gas Chromatography–Mass Spectrometry. A Pye 104 gas chromatograph linked through a glass jet separator to an AEI MS 12 mass spectrometer was used. The poison glands of 50 ants were cleanly dissected and sealed in a glass tube for solid injection (Morgan and Wadhams, 1972). The mass spectra of the two major components of the poison gland were obtained by GC-MS using a low bleed 5% SE-30 column (1.5 m × 4 mm ID) at 130° C. The mass spectrometer was operated under the following conditions. Electron energy, 70 eV; accelerating voltage, 8 kV; multiplier voltage, 3 × 10 kV; and source temperature, 140° C. Background spectra were recorded before and after the peaks of interest and subtracted from the total spectra. The mass spectra of synthetic 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, and 3-ethyl-2,5-dimethylpyrazine were obtained under the same conditions using solutions in hexane (500 ng/μl).

Quantification of Glandular Components. The poison glands of ten worker ants were chromatographed separately via the solid injection technique on the PEG 20 M column at 130° C. A computing integrator (DP 101, Spectra Physics) was employed to calculate the absolute quantities of material using a solution of 2,5-dimethylpyrazine (510 ng/μl) as an external standard.

Bioassay of Synthetic Substances. Mixtures of 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine in hexane (to give a total of 4 ng/100 μl) were made in 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10 proportions. The trail-following behavior released by each mixture (100 μl) was bioassyed. The activity of material taken from poison gland was tested for comparison. The ability of each of the following compounds to evoke trail following behavior was tested by bioassay by presenting 4 ng in hexane per 31.4-cm trail: 2-ethylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, and methyl 4-methylpyrrole-2-carboxylate. The ant species used to test the activity were *Tetramorium caespitum* L., *T. impurum* Foerster, *Myrmica rubra* L., and *M. ruginodis* Nyl.

Threshold concentrations of the two pyrazines at which the worker ants display trail-following reactions were found by presenting to them synthetic 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine separately at concentrations of 10^{-3}, 10^{-2}, 10^{-1}, 1, 10, 10^{2}, and 10^{3} ng per trail and recording activities.

To determine whether the 2,5-dimethylpyrazine and 3-ethyl-2,5-dimeth-

ylpyrazine act in synergy, bioassays were made using first the mixture at 4 ng total on a 31.4-cm trail and then 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine separately at 2.8 ng per trail and 1.2 ng per trail, respectively.

RESULTS AND DISCUSSION

Blum and Ross (1965) first reported that the trail pheromone of *T. caespitum* originates from the poison gland. As a preliminary survey, the work of Blum and Ross was repeated in the present study. Artificial trails were laid on a circular track with extracts obtained from the poison gland and the Dufour gland. The extract from the poison gland evoked high trail-following activity from the worker ants, whereas the Dufour gland extract was completely inactive. This result confirmed the observations of Blum and Ross.

The poison gland and the associated Dufour gland are attached to the sting. The poison gland contents are dispensed through the sting to lay trails as a means of communication during food gathering and change of nest sites. The two filaments of the poison-producing glands open into a spherical venom reservoir. The average diameter of the spherical reservoir was measured. The average volume was calculated (assuming it to be a sphere) to be about 14 nl.

Little or no work has been done on the chemistry of the *T. caespitum* poison gland, apart from the report by Blum and Ross (1965). They detected trace quantities of some free amino acids (aspartic acid being the major component) by paper chromatography.

The preliminary characterization of the trail pheromone was achieved by TLC. The contents of two poison glands were chromatographed on a silica gel plate. The silica gel was cut into ten bands, each representing a R_f difference of 0.1. When the bands were eluted with hexane and tested by bioassay, it was found that three bands of R_f values between 0.3 and 0.4, 0.4 and 0.5, and 0.5 and 0.6 elicited high activity. Initially, it was difficult to understand why the activity was spread over a range of R_f values. However, when the silica gel was cut into narrower bands, two regions of high activity were evident with a valley in between (Figure 1). Therefore it was possible to infer that the trail pheromone of *T. caespitum* was composed of as least two components of moderate polarity.

The chemical treatment of the glandular extracts and subsequent TLC separation and bioassay showed the trail pheromone components are basic because the activity was destroyed by acid treatment. Furthermore, the activity was unaffected by Br_2 in hexane, showing the absence of unsaturation.

GC examination of the contents of three poison glands on the PEG 20 M column showed the presence of two major components (Figure 2). Only a narrow fraction of the GC effluent containing these two components was able

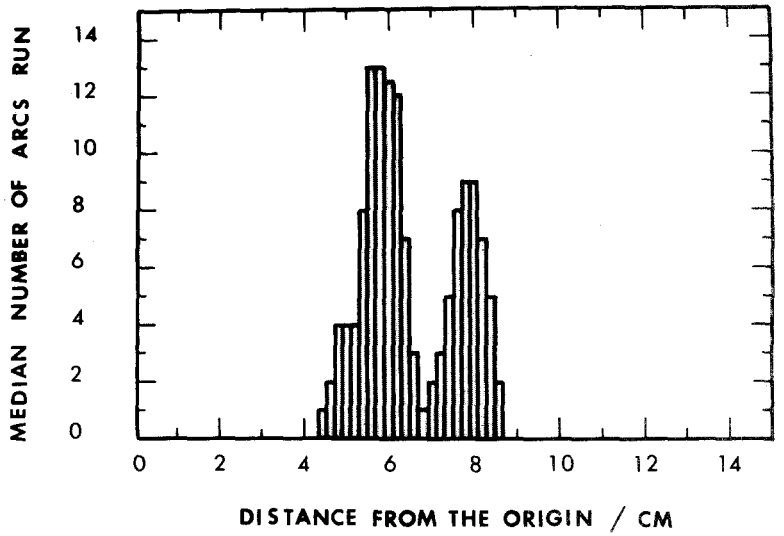


FIG. 1. TLC bioassay of poison gland contents of *Tetramorium caespitum*. An extract of two poison glands was chromatographed using hexane–acetone (60:40) as the eluent. The solvent front was allowed to run 15 cm and the silica gel was cut into narrow bands (2 mm). The trail-releasing activity of each band (stippled bars) was measured by bioassay after solvent extraction of the silica.

to evoke trail following activity when the GC effluent was trapped and bioassayed. The retention indices of the two peaks on the PEG 20 M column were 1370 and 1450, respectively. Similarly those on a SE-30 column were 859 and 1063, respectively. This indicated the compounds were moderately polar and their approximate molecular weight range to be between 100 and 150.

The identification of the two major components present in the poison gland was achieved by GC-MS. The mass spectra were obtained using 50 cleanly dissected poison vesicles sealed in a glass vial. The sample was injected by the solid injection technique on a 5% SE-30 column, and the mass spectra were recorded by GC-MS. The mass spectrometer was operated at a very high sensitivity; therefore it was necessary to record the background spectra between the peaks of interest. The mass spectra of the two major components, after the manual subtraction of the background, are given in Figure 3. The mass spectrum of the peak with lower retention time was identified as 2,5-dimethylpyrazine by comparison with published data (Deck and Chang, 1965; Stenhagen et al., 1974). Three positional isomers are possible for dimethylpyrazine; the mass spectra of synthetic samples of the three isomers were recorded under identical conditions and only the spectrum of the 2,5 isomer corresponded to that of the natural material.

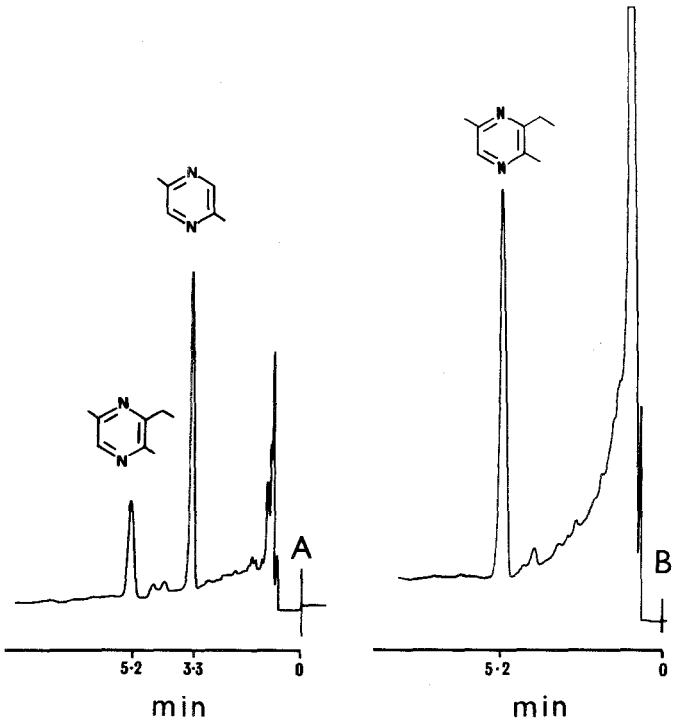


FIG. 2. Gas chromatograms of poison vesicle contents of (A) *Tetramorium caespitum* (attenuation ×50) and (B) *Myrmica ruginodis* (attenuation ×20). Three poison vesicles each were solid injected on a 2.75-m × 4-mm packed column of 10% PEG 20 M on Chromosorb W, at 130°C.

The mass spectrum of the peak with higher retention time (Figure 2) was identified as 3-ethyl-2,5-dimethylpyrazine. It was identical with that obtained under the same conditions from the synthetic material and agreed well with the published spectrum (Evershed et al., 1981; Stenhagen et al., 1974). Further confirmation of the two major components as 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine was obtained by showing they had identical retention times with those of authentic samples on three different GC phases. Furthermore, the R_f values obtained on TLC (60:40, hexane in acetone) for 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine were 0.38 and 0.52, respectively. These R_f values corresponded to the active regions shown by TLC of poison gland extracts (Figure 1).

The average total amount of the two pyrazines present in the poison vesicles of individual worker ants was quantified from the GC peak areas using a computing integrator and was found to be 3.9 ng per ant, of which

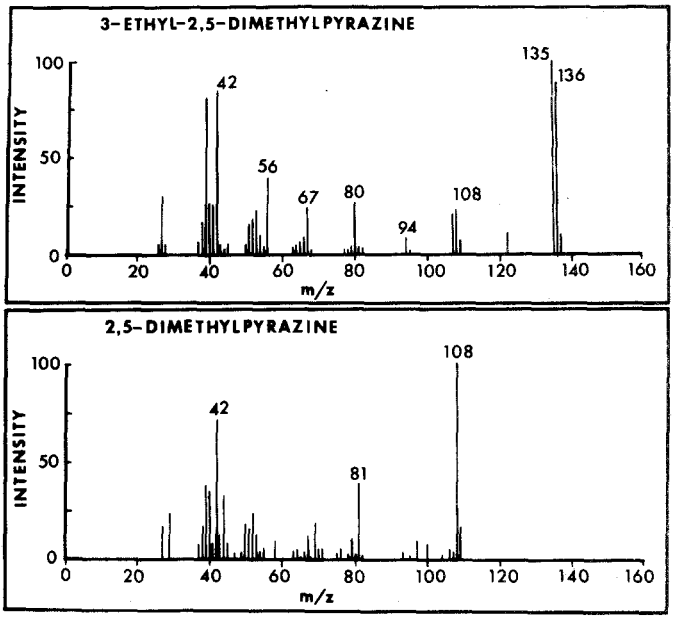


FIG. 3. Mass spectra of the pyrazines from the poison glands of *Tetramorium caespitum*.

2,5-dimethylpyrazine was 2.7 ± 0.4 ng (70 ± 4%) and 3-ethyl-2,5-dimethylpyrazine was 1.15 ± 0.25 ng (30 ± 4%).

The activities of mixtures of the two pyrazines in different proportions in releasing trail-following behavior in worker ants were tested by bioassay. A total of 4 ng of the two pyrazines was applied to the circular trail of 31.4 cm. The results are shown in Table 1. The 70:30 mixture of 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine showed the highest activity. The results given are the median values obtained from three replicate determinations. Table 1 shows that 2,5-dimethylpyrazine has twice the activity of 3-ethyl-2,5-dimethylpyrazine to evoke trail-following behavior when used in identical concentrations and conditions.

The most significant fact that can be seen from Table 1 is that the 70:30 mixture of 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine makes a synergistic mixture with highest activity. When 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine were tested separately using concentrations of 2.8 and 1.2 ng per 31.4-cm trail, the mean activities observed were 13.5 and 5.1 cm, respectively. If the activities were additive, that of the mixture would be expected to be 18.6 cm. But the 70:30 mixture demonstrated an activity of 31 cm, clearly showing that synergism operates. Furthermore, the 70:30 mixture

TABLE 1. TRAIL-FOLLOWING ACTIVITY EVOKED BY MIXTURES OF TWO PYRAZINES IN DIFFERENT PROPORTIONS COMPARED WITH THAT OF ONE POISON VESICLE[a]

Test solution	DMP-EDMP ratio[b]	Trail-following activity			
		I	II	III	Median[c]
Pyrazine mixture	10:0	14	16	15	15
	9:1	18	16	19	18
	8:2	26	24	25	25
	7:3	31	34	29	31
	6:4	28	29	26	28
	5:5	18.5	20	17	18.5
	4:6	15	17	14	15
	3:7	14	17	13	14
	2:8	10	9	9	9
	1:9	7	9	9	8
	0:10	7	9	5	7
Single poison gland		31	36	32	32
Blank (hexane)		0	0	0	0

[a]The trails were applied as hexane solutions (100 μl) on a circle of 5 cm radius marked with 1-cm arcs. The number of arcs run along the trail by each worker ant was recorded for 20 min, and the median value was determined.
[b]The total concentration of 2,5-dimethylpyrazine (DMP) + 3-ethyl-2,5-dimethylpyrazine (EDMP) was 4 ng per trail.
[c]Median values obtained from three replicate determinations.

showed no significant difference in activity from that of the trail made with a single poison vesicle. Although at least one multicomponent trail pheromone of ants has been reported (Vander Meer et al., 1981), this is the first complete identification of the composition of such a pheromone mixture.

A range of concentrations of the two pyrazines was presented separately to the ants to determine the amount of material that evokes most efficient trail-following behavior. Concentrations between 1 and 10 ng per 31.4-cm trail released highest activity (Figure 4). The ants were able to detect concentrations as low as 0.3 pg/cm but showed difficulty in following any lower concentrations. Relatively high concentrations also were less effective. When concentrations about 3 ng/cm were applied, the ants were alarmed and confused, and they exhibited a very low trail-following behavior.

A number of related compounds were tested by bioassay to obtain some information on the structural specificity of their trail-following activity. The results are summarized in Table 2. The 2,5 substitution on the pyrazine ring appears to be important because 2,3- and 2,6-dimethylpyrazines were inactive. It was interesting to find that 2,3,5-trimethylpyrazine was able to evoke weak trail-following in *T. caespitum* and the three species of *Myrmica* tested.

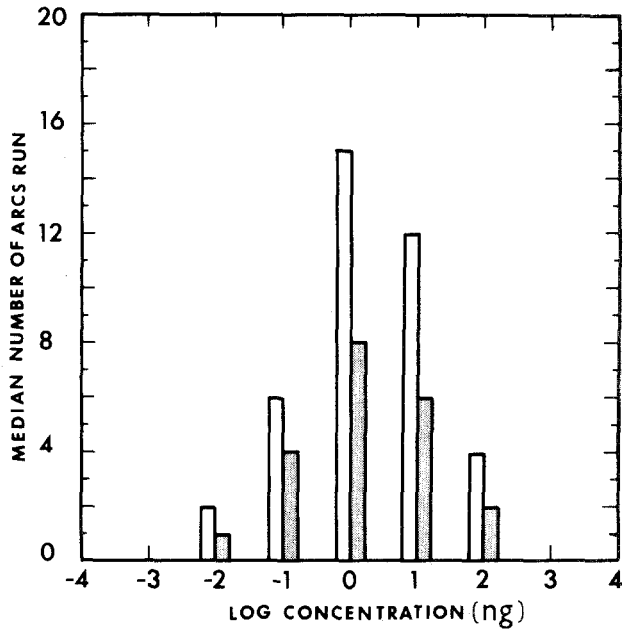


FIG. 4. Bioassay of the two pyrazines over a range of concentrations. 2,5-Dimethylpyrazine (open bars) and 3-ethyl-2,5-dimethylpyrazine (stippled bars) were applied as hexane solutions of different concentrations on a circular trail (5-cm radius). The number of arcs run along the trail by each individual worker was recorded for 20 min, and the median value was obtained.

T. impurum did not respond to any of the chemicals tested, indicating its trail pheromone to be different from any of the tested compounds.

The bioassays were always performed under identical conditions as far as possible, therefore the results obtained in any given experiment, conducted within a short period of time, were comparable. Nevertheless significant differences in absolute values can occur from time to time because the activity of ants appears to be dependent on many factors such as sunlight, temperature, humidity, time of the day, etc.

Pyrazines have been identified from a variety of sources, many of them odorous to man (Barlin, 1982). 2,5-Dimethylpyrazine has been identified as a flavor component of potato chips (Deck and Chang, 1965). It is also found in fusel oil, black tobacco, and in the smoke of nonfilter cigarettes made from these tobaccos (Barlin, 1982). 3-Ethyl-2,5-dimethylpyrazine is a component important to the aroma of baked potatoes (Buttery et al., 1973) and coffee (Goldman et al., 1967). Maga and Sizer (1973a) have published a review listing the extensive occurrence of alkylpyrazines in foods. The review published by Brophy and Cavill (1980) provides the mass spectra of a number of pyrazines.

TABLE 2. TRAIL-FOLLOWING ACTIVITY EVOKED BY VARIOUS SYNTHETIC PYRAZINES AND A PYRROLE ON SPECIES OF *Tetramorium* AND *Myrmica*[a]

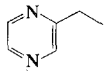
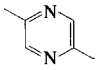
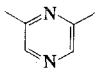
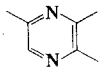
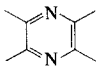
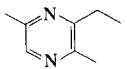
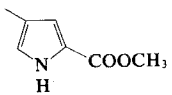
Compound	Structure	Test species				
		T. caespitum	*T. impurum*	*M. rubra*	*M. ruginodis*	*M. scabrinodis*
2-Ethylpyrazine		1	0	0	0	0
2,3-Dimethylpyrazine		0	0	0	0	0
2,5-Dimethylpyrazine		14	0	0	0	0
2,6-Dimethylpyrazine		0	0	0	0	0

TABLE 2. Continued

Compound	Structure	Test species				
		T. caespitum	*T. impurum*	*M. rubra*	*M. ruginodis*	*M. scabrinodis*
2,3,5-Trimethylpyrazine		6	0	3	4	3
2,3,5,6-Tetramethylpyrazine		0	0	0	0	0
3-Ethyl-2,5-dimethylpyrazine		8	0	10	10	12
Methyl 4-methylpyrrole-2-carboxylate		0	0	0	0	0

[a]Trails were applied as hexane solutions (4 ng/31.4 cm) on a circle of 5 cm radius marked with arcs (1 cm). The number of arcs runs along the trail by each worker ant was recorded for 20 min, and the median value was calculated.

A number of trisubstituted alkylpyrazines have been reported from the mandibular glands of some subfamilies of ants. Many other insects such as wasps (Wheeler et al., 1982), flies (Baker et al., 1982), and some beetles also have the ability to biosynthesize pyrazines.

A wide variety of alkaloids have been identified from the poison glands of ants but 3-ethyl-2,5-dimethylpyrazine was the only pyrazine that has been identified before this study. It has been shown to be present in the trail pheromone of *Atta sexdens rubropilosa* (Cross et al., 1979), *A. sexdens sexdens* (Evershed and Morgan, 1983), eight species of *Myrmica* (Evershed et al., 1981), and in small quantities in *Atta cephalotes* (Evershed and Morgan, 1983). The 2,5-dimethylpyrazine identified in *Tetramorium caespitum* is novel because it is the simplest and the only disubstituted pyrazine yet reported from ants.

Blum (1974) has postulated that the trail pheromones of myrmicine ants were originally trace constituents of the venom that were exploited as the function of the gland changed into its present form. The present study showed that the trail pheromone components of *T. caespitum* are indeed trace constituents. The pyrazines occupy only 0.03% of the volume of the poison gland. The poison reservoir contents have been reported to be rich in free amino acids (Blum and Ross, 1965), which probably can react enzymatically to form pyrazines (Maga and Sizer, 1973b). Morgan (1984) has postulated a mechanism for the biosynthesis of pyrazines from amino acid precursors.

As the same 3-ethyl-2,5-dimethylpyrazine had been identified as the trail pheromone of eight species of *Myrmica*, it was anticipated that *T. caespitum* and species of *Myrmica* may follow each other's trails. Such a transposition study has not been carried out previously for these species. Therefore cross-activity was studied with poison gland extracts of *T. caespitum* and *M. rubra*. It was found that these species indeed followed each other's trails. The results are given in Table 3. The chemical composition of the poison glands explains the observations. *T. caespitum* followed the trails of *Myrmica* moderately well

TABLE 3. TRAIL-FOLLOWING ACITIVITY EVOKED BY SINGLE POISON GLANDS OF TWO SPECIES OF MYRMICINAE SUBFAMILY[a]

Source species	Test species	
	T. caespitum	*M. ruginodis*
Tetramorium caespitum	31	14
Myrmica ruginodis	12	19

[a]The trails were applied as hexane solutions (100 μl) containing one poison gland equivalent of material, on a circle of 5 cm radius marked with 1-cm arcs. The number of arcs run along the trail by each worker ant was recorded for 20 min, and the median value was determined.

because of the presence of the 3-ethyl-2,5-dimethylpyrazine. However, the trail pheromone of *Myrmica* ants has only one component. Poison glands of *M. ruginodis* were examined carefully, and no peak corresponding to 2,5-dimethylpyrazine was found in the GC traces (Figure 2). *Myrmica* workers followed poison gland extracts of *T. caespitum* because they contained 3-ethyl-2,5-dimethylpyrazine and the effect was not marked or inhibited by the 2,5-dimethylpyrazine.

Similar observations in interspecific trail-following behavior have been reported with the leaf-cutting ants *Acromyrmex octospinosus* and *Atta sexdens* (Robinson et al., 1974). The major components of their trail pheromones are methyl 4-methylpyrrole-2-carboxylate and 3-ethyl-2,5-dimethylpyrazine respectively. However, *Acromyrmex octospinosus* will follow the trails of *Atta sexdens* because the pyrrole is also present as a trace component in the venom of the latter. But *A. sexdens* will not follow a trail of a *A. octospinosus* (Robinson et al., 1974) because the pyrazine is not present in the venom of *A. octospinosus* (Evershed and Morgan, 1983). Similarly *Atta sexdens* will not follow a trail of synthetic pyrrole (Robinson et al., 1974). On the contrary, *A. sexdens* will follow the venom of *A. texana* which contains both the pyrrole and the pyrazine (Evershed and Morgan, 1983).

Bolton (1976) has suggested on the ground of habitats and morphology that *Tetramorium* and *Myrmica* have most probably descended from the same ancestral stock. The similarities of the chemical composition of the poison glands and the trail-following behavior, as found in the present study, appear to support the postulate of Bolton, which considers the tribes Myrmicini and Tetramoriini to be closely related.

Acknowledgments—We thank J.P.J. Billen, R. Cammaerts, and P.A. Curtis for the collection; Miss V.K. Lancaster for help with the maintenance of ant colonies; Mrs. P. Flanagen for some bioassays; and Dr. R.P. Evershed for synthetic 3-ethyl-2,5-dimethylpyrazine. The British Council provided some financial assistance to A.B.A., which is gratefully acknowledged.

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