

# **Intraspecific variations of alarm pheromones between two populations of the red wood ant *Formica lugubris* Zett. (Hymenoptera, Formicidae)**

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Intraspecific variations of alarm pheromones between two populations of the red wood ant *Formica lugubris* Zett. (Hymenoptera, Formicidae)

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Comparisons between alarm pheromones of two adjacent polycalic colonies of the red wood ant *Formica lugubris* ZETT., which behaved aggressively towards each other, showed qualitative and quantitative differences. Differences were mainly related to undecane, but also to dodecane, 1-tridecene, tridecane, and nonadecanol.

When attacked, most worker ants of the genus *Formica* respond by spraying their enemy. This spray is a mixture of formic acid from the poison vesicle and the contents of Dufour's gland. BERGSTRÖM & LÖFQVIST (1975) suggested that the Dufour's gland secretion may function as an alarm-defense-recognition system.

Inter- and intraspecific aggression among ants has been reported many times in literature (BRIAN, 1965; SUDD, 1967; WILSON, 1971; BARONI-URBANI, 1979). Concerning the red wood ants of the genus *Formica*, MABELIS (1979) gives a complete review of this behavior, which he described as wood ant war. According to this author, three main conditions must be satisfied for the generation of a red wood ant war:

1. a difference in odour between populations
2. a shortage of protein-rich food and
3. a high frequency of meetings between the individuals of different nests.

After BERGSTRÖM & LÖFQVIST (1971) as well as MABELIS (1979), we anticipate that part of intraspecific aggression might be related to differences in the Dufour's gland secretions.

This kind of problem was analyzed in the case of a super-colony of *Formica lugubris* ZETT. located in the Swiss Jura mountains (CHERIX & GRIS, 1977, 1978; CHERIX, 1981). At that time the super-colony consisted of about 1200 nests distributed over 70 hectares and bounded by about 100 km of ant's roads. Moreover this super-colony was surrounded by other colonies of the same species. These colonies were smaller (3-15 nests) and behaved aggressively to each other and towards the super-colony.

We made comparisons between the Dufour's gland secretions (called «alarm pheromones») of the queens and workers of the super-colony and one of the smaller colonies, which over a period of three years, showed a very strong tendency to be aggressive towards one another (CHERIX & GRIS, 1977, 1978).

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## MATERIAL AND METHODS

### *Collection of insects*

Queens and workers were collected during spring and summer at periods of highest aggression between colonies. Individuals were first chilled at 4 °C and then their gasters were cut and placed immediately into pentane (workers = 30/2 ml; queens = 5/1 ml). The glass vials were then hermetically sealed and stored (dark, -30 °C) until analyzed.

### *Gas chromatography*

After a few attempts, it was found that the flash evaporating technique for the injection of extracts was not suitable as it gave rise to artifacts. For that reason, a direct on-column injector device was used (L.O.C., Tressel, Düsseldorf, FRG) that was connected to a gas chromatograph equipped with a flame ionization detector (Hewlett-Packard model 5840, Palo alto, U.S.A.). The separations were obtained in a WCOT glass column (20 m x 0,3 mm i. d.) coated with SP 2100 as stationary phase. The injector was kept at 50 °C as injection of the extract was done directly into the column in the oven. The flux of the He carrier was 2 ml/min at the starting oven temperature of 50 °C. The latter was maintained for 1 min., then raised at 5 °C/min. to 250 °C. The detector was kept at 275 °C and 0,7 µl was injected. The area of each peak appearing on the chromatogram was calculated by the built-in integrator.

### *Gas chromatography-mass spectrometry*

In order to establish the identity of each substance emerging from the column, a GC-MS coupled with a computer was used with the same column as for the GC analyses. The apparatus (Hewlett-Packard model 5985A) was equipped with the same injector, and repetitive scanning was recorded from m/z 40 to 500 at a rate of 1 scan per sec. The source temperature was 200 °C; the transfer-line, connecting directly the end of the column to the ion source of the mass spectrometer, was heated to 250 °C. The electron energy was 70 eV and the emission 300 µA. Identifications were based on a library search algorithm (obtained from the NIH/NBS Library) on both retention time - when available - and similarity indexes.

## RESULTS

Six and eight samples of workers and queens, respectively, were analyzed. The chromatogram of the pentane extract of Dufour's glands is representative of each kind of sample collected. The secretion contains mainly alkanes which are reported in Table 1. We identified only the substances that exceeded 1% of the sample. Although the chemistry of the different samples (workers and queens) of the same population was qualitatively very similar, there were considerable qualitative and quantitative variations between the two populations (Table 2a and 2b and Figs. 1 and 2).

In particular, undecane (1), which is a major component in Dufour's glands, seems far less important in the glands from workers of the small colony (CP1,

CP3, table 2a) and, on the other hand, is totally absent in the queens belonging to the super-colony. Other differences are less spectacular, but are still important, e. g. dodecane (2), 1-tridecene (3), tridecane (4), and nonadecanol (14).

Table 1: Identified volatile substances by GC-MS from Dufour's gland secretions which exceeded 1% of the sample.

No.	Retention time (min.)	Name	Formula	MW
1	9.4	Undecane	C <sub>11</sub> H <sub>24</sub>	156
2	11.3	Dodecane	C <sub>12</sub> H <sub>26</sub>	170
3	14.6	1-Tridecene	C <sub>13</sub> H <sub>26</sub>	182
4	14.9	Tridecane	C <sub>13</sub> H <sub>28</sub>	184
5	20.2	Pentadecane	C <sub>15</sub> H <sub>32</sub>	212
6	24.9	Heptadecane	C <sub>17</sub> H <sub>36</sub>	240
7	28.7	1-Hexadecene	C <sub>16</sub> H <sub>36</sub>	224
8	29.8	1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266
9	30.9	Hexadecanoic acid ethylester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
11	32.9	Octadecenoic acid methylester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
12	34.2	9-Octadecenoic acid ethylester	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310
13	34.5	1-Eicosene Eicosane	C <sub>20</sub> H <sub>40</sub> +C <sub>20</sub> H <sub>42</sub>	280, 282
14	34.6	Nonadecanol	C <sub>19</sub> H <sub>40</sub> O	284
17	36.3	1-Tricosene	C <sub>23</sub> H <sub>46</sub>	322
18	36.8	Tricosane	C <sub>23</sub> H <sub>48</sub>	324

Table 2a: Relative amounts (in % of the total sample) of the volatile substances from Dufour's gland of workers listed in function of their GC retention time (CP1, CP3 = samples of workers from different nests of the smaller colony). (SP2-SP5 = samples of workers from different nests of the super-colony). \* = identified substances (see Table 1).

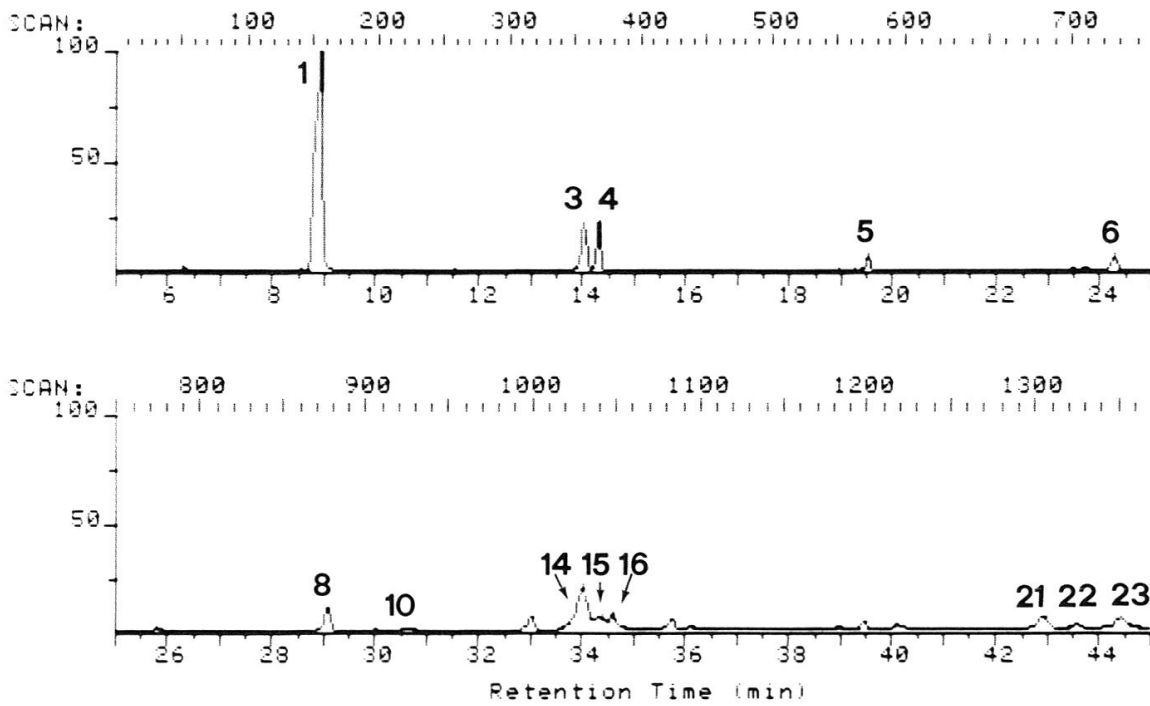
No.	Retention time (min.)	CP1 (%)	CP3 (%)	SP2 (%)	SP3 (%)	SP4 (%)	SP5 (%)
1*	9.4	11.8	21.3	41.2	33.3	45.0	34.4
3*	14.6	< 1	1.4	4.8	4.3	4.9	3.6
4*	14.9	< 1	1.2	4.8	4.5	5.4	3.6

No.	Retention time (min.)	CP1 (%)	CP3 (%)	SP2 (%)	SP3 (%)	SP4 (%)	SP5 (%)
5*	20.2	< 1	< 1	1.3	1.4	1.6	1.1
6*	24.9	< 1	< 1	1.1	1.2	1.7	1.2
7*	28.7	< 1	< 1	2.3	2.1	2.4	< 1
10	31.2	2.6	2.4	1.8	2.6	2.0	1.3
14*	34.6	28.4	15.6	10.7	17.7	13.9	5.9
15	35.0	2.9	3.1	2.4	3.4	3.1	1.4
16	35.3	-	3.3	1.6	1.8	2.2	1.8
21	43.8	3.5	6.8	2.6	2.9	2.4	5.3
22	44.5	14.4	3.5	3.6	2.7	1.4	3.4
23	45.3	8.9	6.4	8.2	7.1	3.4	7.8
24	46.4	3.6	1.2	4.7	4.5	2.5	2.5
25	47.5	1.9	2.2	4.3	4.0	1.2	2.9
26	48.4	5.5	-	2.2	2.9	-	2.2

Table 2b: Relative amounts (in % of the total sample) of the volatile substances from Dufour's gland of queens listed in function of their GC retention time (CP1, CP2 = samples of queens from different nests of the smaller colony; SP1-SP6 = samples of queens from different nests of the super-colony).  
\* = identified substances (see Table 1).

No.	Retention time (min.)	CP1 (%)	CP2 (%)	SP1 (%)	SP2 (%)	SP3 (%)	SP4 (%)	SP5 (%)	SP6 (%)
1*	9.4	6.5	15.4	-	-	-	-	-	-
2*	11.3	< 1	< 1	-	-	-	-	-	-
3*	14.6	5.0	7.3	< 1	< 1	< 1	< 1	< 1	< 1
4*	14.9	8.2	11.1	2.0	2.1	< 1	< 1	< 1	1.3
5*	20.2	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
6*	24.9	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
8*	21.8	2.2	3.5	< 1	1.4	< 1	1.8	< 1	1.2
9*	30.9	< 1	< 1	< 1	< 1	3.4	1.3	< 1	1.8
11*	32.9	-	-	< 1	-	< 1	-	< 1	1.2
12*	34.2	< 1	< 1	< 1	< 1	8.0	1.2	< 1	7.7
13*	34.5	< 1	5.2	4.5	4.5	27.2	27.2	4.5	12.9
17*	36.3	2.2	4.9	2.9	1.5	< 1	3.2	2.0	3.1
18*	36.8	< 1	< 1	3.7	< 1	< 1	2.1	2.9	3.0
19	39.7	< 1	3.8	5.2	3.6	6.9	3.3	4.2	4.4
20	40.2	< 1	3.1	2.6	2.9	4.5	3.3	6.2	3.3

## Workers SP4



## Workers CP1

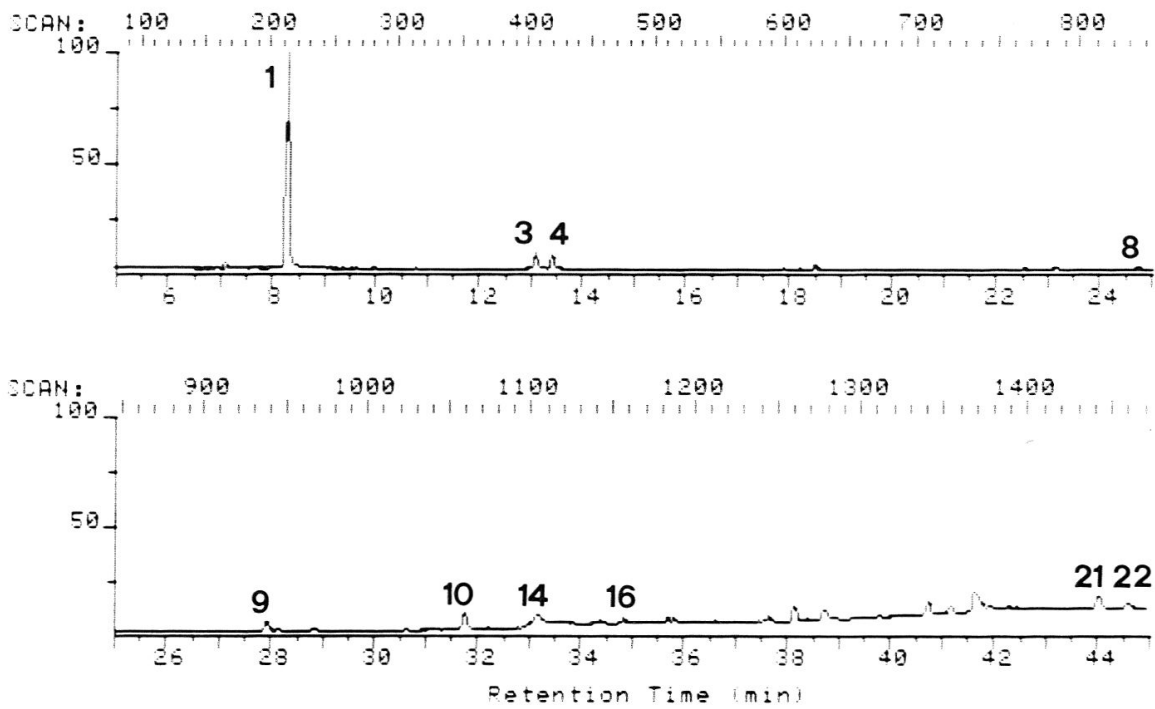
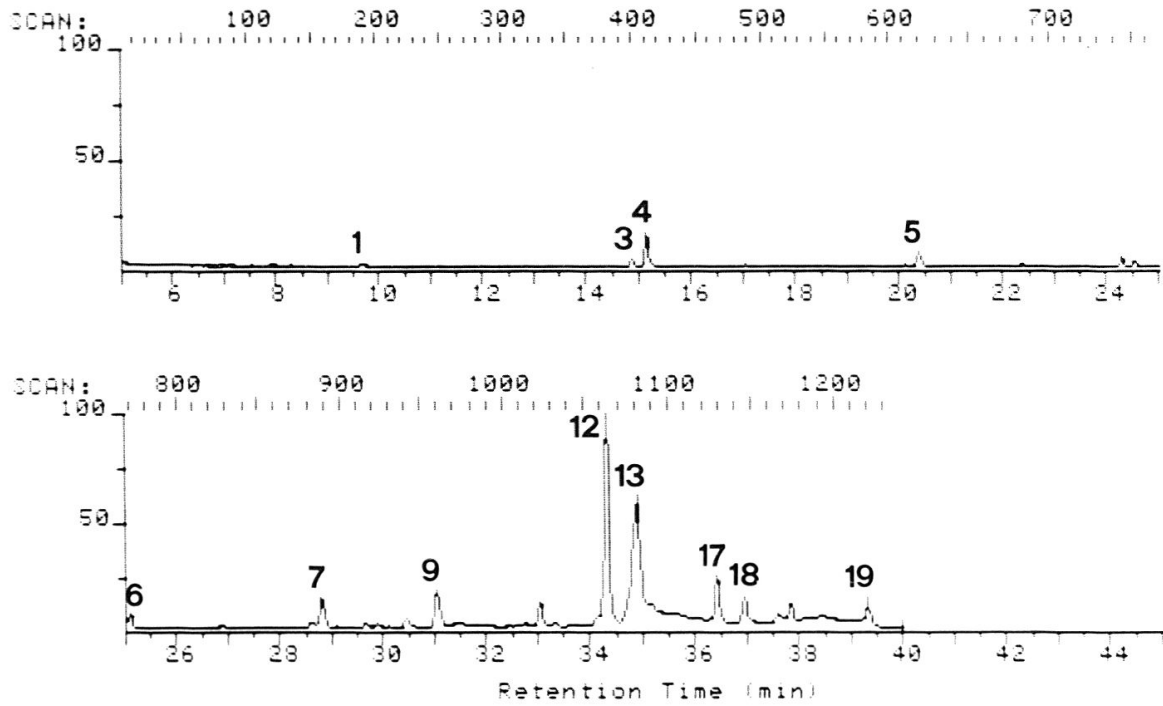


Figure 1: Chromatograms (reconstructed total ion current) of the volatile substances from Dufour's gland of workers (SP4 = super-colony; CP1 = smaller colony).

## Queens SP6



## Queens CP2

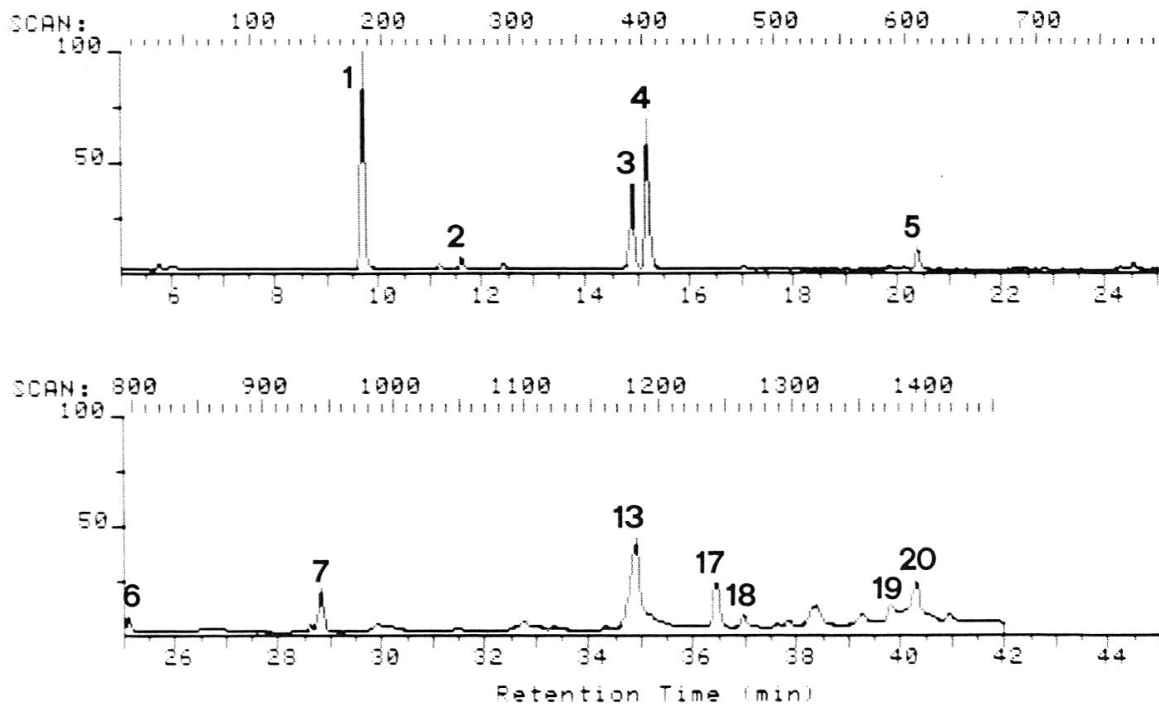


Figure 2: Chromatograms (reconstructed total ion current) of the volatile substances from Dufour's gland of queens (SP6 = super-colony; CP2 = smaller colony).

These differences cannot be attributed to different physiological states of the workers, mainly for two reasons:

1. we used the same method of sampling;
2. the high number of workers (30) in each sample.

The effect of age on glandular chemistry could not be determined because it was not possible to make accurate age determinations. Even within the same population there was some variability in the concentrations of different components, which could reflect the polymorphism occurring in the same population. Thus it seems evident that alarm pheromones in the workers and queens of the same population do not show large differences; the biggest qualitative and quantitative differences occur between the two populations.

## DISCUSSION

Previous studies of communication substances in 13 species of formicine worker ants (BERGSTRÖM & LÖFQVIST, 1968, 1970, 1972a, b, 1973) showed that the majority of the volatile substances originate from Dufour's gland. Most species have a similar homologous series of saturated aliphatic hydrocarbons ranging from nonane to nonadecane and there were the main compounds we detected. Undecane was usually the major compound, comprising 50% or more of the secretion. In workers, some of the hydrocarbons are alarm pheromones (LÖFQVIST, 1976) and defense substances (LÖFQVIST, 1977). If we follow LÖFQVIST (1976), the secretion can theoretically function as a mode of communication based on quality and quantity of each of the substances. The information content is dynamic, however, because all proportions will be changed by degrees, as the more volatile substances evaporate below the concentration that can be perceived by the animals. Coming back to the present data, it is clear that differences between Dufour's gland substances are larger between colonies than between samples from the same colony. Differences between the queens of two colonies are quite surprising: secretions of the queens of the super-colony are rather similar to those obtained by LÖFQVIST & BERGSTRÖM (1980) for *Formica polyctena* (mated queens), but secretions of the queens of the small colony are very similar to those found in virgin queens of *F. polyctena* (presence of undecane and tridecane). Perhaps the precariousness of the situation of this colony, which had to move because of the pressure of the super-colony (CHERIX & GRIS, 1977) or the presence of numerous unmated queens in the nest (see CHERIX, 1981), affected the physiology of the gland.

We noted in the introduction that, according to MABELIS (1979), three conditions had to be fulfilled to provoke an ant war. We confirmed the existence of a difference in odour between the two colonies, and there was also a shortage of protein-rich food in the study area. This was demonstrated (CHERIX, 1980, 1981; CHERIX & BOURNE, 1980) by the observation that the ants were consuming about 30% of their honey-dew producers in order to get enough protein. The frequency of meetings between the two colonies was very high, caused by their proximity. The results of this study thus support the hypothesis of MABELIS (1979).

A phenomenon that could be involved in the differences we found, has been reported by KUTTER (1967, 1977), who found that *F. lugubris* is represented by two forms: the «hairy one» (group I), present in the Jura mountains but absent in the



southern part of the Alps, and the «less hairy one» (group II), present in the Swiss and Italian Alps.

Considering the fennoscandian ant fauna, COLLINGWOOD (1979) said that the female caste of *F. lugubris* is highly variable, especially in the pilosity on the scape. In order to investigate this problem, we submitted two samples for a «blind test» to Dr. C. A. COLLINGWOOD. Each sample contained 15 queens from the super-colony and the peripheral colony respectively. His answer was: «All your queens have characteristic microsculpture i. e. crashed microdots on dorsum of gaster as in British *lugubris*. Your peripheral colony specimens have distinctly more scape hairs than those of the super-colony implying a separate genetic origin, but this is a small difference . . .» (see Figs. 3 and 4)<sup>2</sup>. We are therefore not able to say that the queens of the super-colony have to be attributed to the group II. The high variability in the species, as well as the taxonomical difficulties in this group (ROSENGREN & CHERIX, 1981) may also be reflected in the alarm pheromones as presented in this paper.

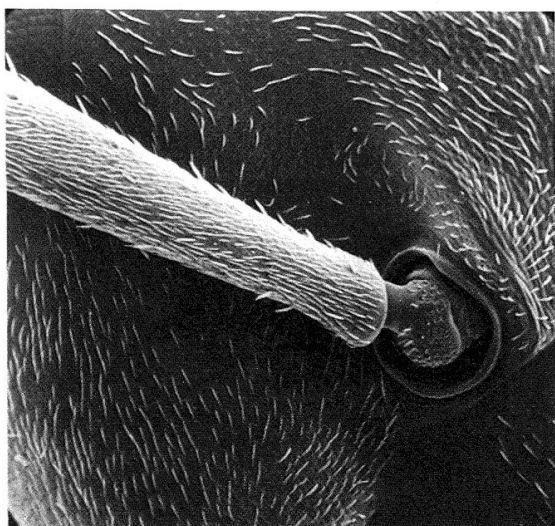


Fig. 3: Scanning electron micrograph of the scape of a queen of the peripheral colony (magnification 100 x).

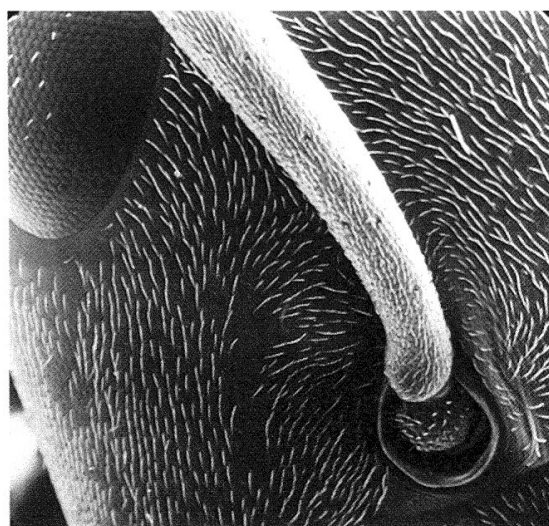


Fig. 4: Scanning electron micrograph of the scape of a queen of the super-colony (magnification 100 x).

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#### RÉSUMÉ

*Variations intraspécifiques des phéromones d'alarme entre deux populations de la fourmi rousse Formica lugubris ZETT.* La comparaison des phéromones d'alarme de la fourmi rousse *Formica lugubris* ZETT. entre deux colonies polycaliques adjacentes et agressives entre elles, montre des différences qualitatives et quantitatives. Ces différences concernent principalement l'undécane, mais aussi le dodécane, le 1-tridécène, le tridécane et le nonadécanol.

<sup>2</sup> Pictures made on a Jeol SEM 35 at the Center of Electronic Microscopy of Lausanne University.

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