Table 1. Analytical characteristics of HAs extracted from soils belonging to three different Great Soil Groups and of the proposed HA structure

	Udic Boroll	Haplaquod	Haplaquoll	Proposed[a]
C [%]	56.4	58.2	54.2	61.8
H [%]	5.5	5.4	6.0	5.9
N [%]	4.1	3.1	6.0	2.5
S [%]	1.1	0.7	0.9	–
O [%]	32.9	32.6	32.9	29.8
Total acidity [meq/g]	6.6	5.7	6.4	5.8
CO_2H [meq/g]	4.5	3.2	3.5	4.4
Phenolic OH [meq/g]	2.1	2.5	2.9	1.4
Alcoholic OH [meq/g]	2.8	3.2	3.0	1.4
OCH_3[meq/g]	0.3	0.4	0.4	0.3

[a]MW = 6651 Da

years, and exhaustive consultations on the voluminous literature on this subject. More detailed experimental support for the proposed structure will be published elsewhere.

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Identification of the Sex Pheromone of an Ant, *Formica lugubris* (Hymenoptera, Formicidae)

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Pheromones play a cardinal role in mediating intraspecific communication in animals [1], especially in insects where they are involved in a number of processes including attraction of the sexes and social interactions. Although sex pheromones have been identified in a variety of insect species which include a number of Hymenoptera [2], none has yet been identified in ants (Formicidae). This is in contrast to the dozens of pheromones serving a social function, e.g., trail and alarm pheromones, that have been identified from among this group [3]. Evidence has been previously obtained in several species that females attract males using sex pheromones [4], but characterization of the active components has so far proven elusive. We have identified what appears to be the major and two minor components of the sex pheromone of the red wood ant, *Formica lugubris* Zett., by testing in a natural field situation the principal volatile compounds emitted by sexually attractive females collected from the field. The major component, undecane, also acts as an alarm pheromone in workers of this and other species. Thus, undecane serves different functions in the two castes of *F. lugubris*.

The ants used in this study belong to a supercolony of *F. lugubris* located in the Swiss Jura [5]. Winged males and females (sexuals) fly from their nests to open places in the forest and mate on the ground [6]. Male ants can be seen to assemble at well-defined locations on meadows where workers are infrequently encountered and to compete for females when these arrive. Opportunities to collect mature sexuals to obtain putative sex pheromones and to conduct assays of the candidate compounds occur only when sexuals are present at the mating stations in sufficient numbers. Such opportunities are strictly limited to a few days only during the 2 to 3 weeks in June when mating flights normally occur. Thus, the chemical studies were conducted on material collected in June, 1988, most of the assays were conducted from June 14 to 16, 1989, and the remainder of the assays on June 16 and 17, 1990, after a protracted period of inclement weather contracted usable mating flights into only 2 days.

Virgin alate queens were collected as they arrived at a mating station and samples of 5–20 were confined in 10-ml glass containers. After these captured alates ceased to exhibit any signs of alarm, a stream of air was drawn through the containers at a rate of 25 ml/min for periods of 5–20 min. Volatile compounds emitted by the alates were adsorbed on a commercial 1.5-mg charcoal filter (Carlo Erba, Hofheim, Germany). A total of three samples were treated in this manner, and in a further sample five males were added to the container to ascertain whether their presence would stimulate the virgin queens to release sex pheromones, or to release them in greater quantity. These males were collected at the mating station at the same time as the alate queens.

Undecane and tridecane were obtained from commercial sources (Merck, Darmstadt, Germany, 99.5 % purity). The synthesis of Z-4-tridecene followed the alkyne approach: 1-decyne was deprotonated with buthyl lithium in THF at 78 °C and coupled with 1-bromopropane. After work up, the resulting 4-tridecyne was submitted to cis-hydrogenation by using a nickel catalyst in the presence of ethylenediamine according to the method in [7].

In the three samples from virgin queens only, three major components were found to be consistently present, undecane, tridecane, and tridecene in a mean ratio of 100:5.23:4.25. The

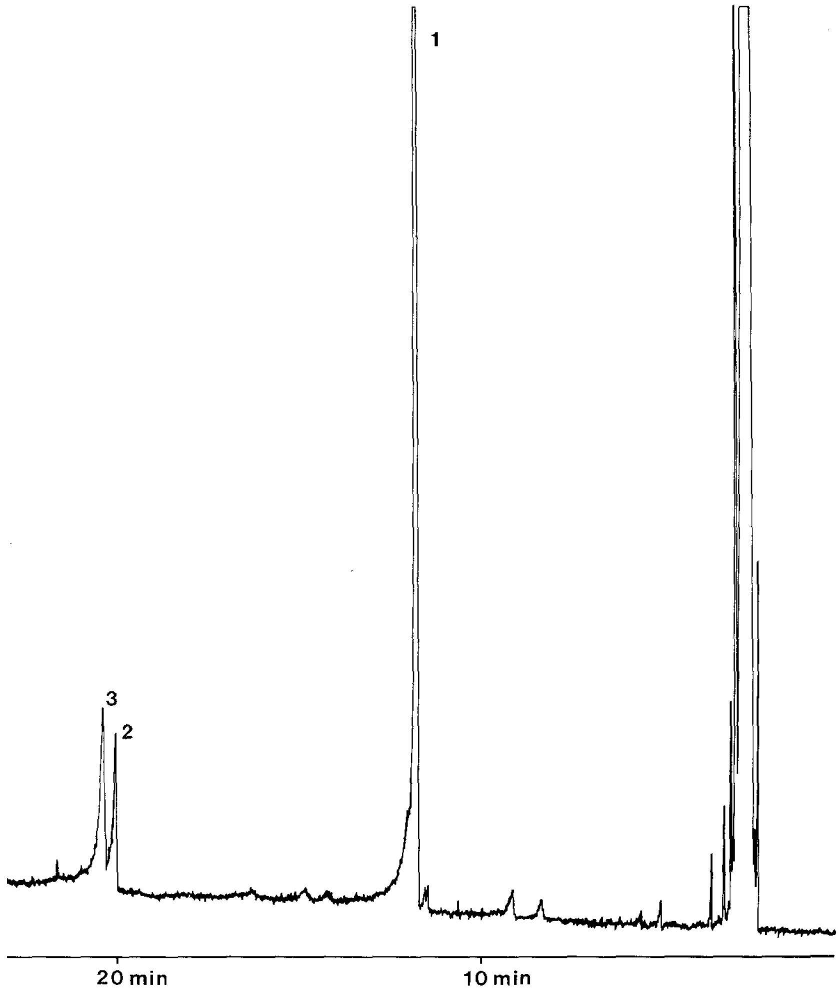

Fig. 1. Chromatogram of the adsorbed compounds of five virgin female alates and five males (June 22, 1988); *1* undecane, *2* Z-4-tridecane, *3* tridecane. Double-bond location in the tridecene was carried out by mass spectrometric investigation of its adduct with dimethyl disulfide according to the method in [9]. The mass spectrum of the pooled samples unambiguously demonstrated the presence of a 4-tridecene. Synthetic Z-4-tridecene (see test materials) proved to coelute with the natural product, whereas the retention time of E-4-tridecene was found to be different

Table 1. Relative proportions of the three volatile components released by virgin female alates of *Formica lugubris*. The compounds adsorbed on the charcoal filters were eluted with 30–50 μl of carbon disulfide; the solutions were concentrated to 10–20 μl in a microtube [8] and submitted to GC and GC-MS. GC analyses were carried out on a fused silica column (30 m long, 0.25 mm i.d.) with DB-Wax or CP-Wax 58 CP as stationary phases and a temperature program of 60–240 °C at a rate of 3 °C/min. GC-MS was performed on a VG 250 S mass spectrometer linked to a Hewlett-Packard 5895 gas chromatograph. Quantitative analysis of the natural compounds in the carbon disulfide extracts was carried out on fused silica column (30 m long, 0.25 mm i.d.) with a temperature program of 60–240 °C at a rate of 3 °C/min and CP-Sil 8 as stationary phase. A Shimadzu C-RIB Chrompac was used as the integrator

		N		Composition [%]		
Sample #	Date (1988)	♀	♂	Undecane	Z-4-Tridecene	Tridecane
1	19 June	10	0	87.0	4.3	5.4
2	21 June	11	0	89.3	3.7	4.0
3	22 June	5	5	92.5	3.5	4.0
4	24 June	10	0	92.6	3.1	3.3
	vs			Means: 90.4	3.7	4.2

sample in which males were present with the females showed a similar ratio. Results are summarized in Table 1 and the chromatogram of sample 4 is shown in Fig. 1. These substances are also found in virgin queens of the closely related species *F. polyctena*, where they are produced in the Dufour's gland [10]. To determine whether this gland served as the source of these volatiles in *F. lugubris*, the Dufour's gland was removed by dissection from 35 females collected from the mating stations and their contents assayed for the presence of the compounds. Undecane, tridecane, and tridecene were found to comprise over 75% of the glandular contents. The latter two compounds were present in considerably higher ratios in the gland than in the volatile secretion (undecane:tridecane:tridecene = 100:76.8:52.5). In both *F. lugubris* and *F. polyctena* these substances are among the major components of a considerably more complex glandular exudate [10, 11].

In order to evaluate the relative efficacy of the candidate compounds we assayed these in equivalent amounts in 1 µl/test during the 1989 season. This series of tests also included a mixture containing 1 µl of each compound. Male ants were attracted to this mixture at a highly significant rate when compared with an untreated control (Table 2). In fact, while 100 males landed in the squares containing the mixture during ten replications of this experiment, only six landed in the control squares. Undecane alone was also highly active, since it attracted 60 males in six replications and the control seven only, whereas both tridecane and *Z*-4-tridecene were substantially less active when tested alone (Table 2). In pairwise comparisons between the three compounds, undecane was significantly more attractive than either tridecane or *Z*-4-tridecene, whereas these last two were not significantly different from each other, and a mixture of all three was not more attractive than undecane alone.

These results suggested that undecane might provide all of the attraction required of the sex pheromone. However, it remained possible that a more natural mixture of the three compounds might be more attractive. Therefore, during the 1990 season, we conducted an additional assay of a mixture containing the proportions suggested by the results given in Table 1, i.e., 92% undecane, 4% tridecane, and 4% *Z*-4-tridecene. The assay consisted of 20 tests of 1 µl of the mixture against 1 µl of undecane; the result was nonsignificant. The mean number of landings/min for the mixture was 8.16 (SD = 6.77) and for undecane 6.8 (SD = 8.2) (t_{19} = 1.23; NS; square-root transformation). This result further suggests that undecane may be the "real" sex pheromone of *F. lugubris*, in which case both tridecane and *Z*-4-tridecene may exhibit minor activity in this context solely by virtue of a degree of similarity to undecane in their molecular structure. They could be functionless by-products within the Dufour's gland secretion, but since they did in fact occur in the air drawn over virgin females, they may equally well represent true components of the sex pheromone (see below).

The results show unequivocally that in a natural setting, i.e., a field location at which *F. lugubris* males and females are mating at the time the tests are conducted, undecane is highly attractive to males actively searching for virgin females, and that both tridecane and *Z*-4-tridecene are also active to a lesser degree. To the extent that the compounds competed successfully with on-site females, it would appear that they are indeed components of the sex pheromone of *F. lugubris* females. However, since we do not know the rate at which the compounds are naturally released, the decision to employ 1 µl quantities in our tests was arbitrary. This problem is a formidable hurdle in pheromone research, because many factors may influence the rate of pher-

Table 2. Pairwise comparisons of male attraction among the components of the sex pheromone of *Formica lugubris*. Un: undecane; Ta: tridecane; Te: *Z*-4-tridecene; M: a mixture of undecane, tridecane, and tridecene; C: untreated control. All compounds were tested at the rate of 1 µl/replication, while the mixture contained 1 µl of each. To assay the candidate compounds, small areas (50 × 50 cm) were demarcated by squares of plastic-covered wire placed on the ground approximately 1 m apart. A small piece of cotton on which a test compound was dispersed was then placed on a toothpick at the center of one square and either a blank control (untreated cotton), or a different compound, was placed on a similar toothpick at the center of the other square. Two observers, one to a square, then counted the numbers of males that landed in each square. As soon as ten males had landed in one of the squares, or after 15 min, whichever occurred first, the test was terminated. The number of males that had landed in each square and the duration of the test to the nearest minute were then recorded. All tests were conducted in different locations to avoid any bias that might occur in the numbers of males present at a location as a result of a test just completed. Each of the pairwise comparisons made constituted a separate experiment and all replications of each experiment were completed in as short a time as possible to minimize variations in the conditions under which they were conducted. The strict limits on times suitable for carrying out field tests precluded an experimental design that would permit multiple comparisons to be made within a single experiment. Thus, results, in the form of the mean number of landings per minute, were evaluated by means of paired tests using Student's *t* statistic on the square-root transformed data

Experiment	*N*	Mean landings/min (SD)			*P*
M vs C	10	2.27 (1.50)	vs	0.17 (0.24)	<0.0001
Un vs C	6	6.39 (2.87)	vs	0.72 (0.84)	<0.001
Ta vs C	6	1.03 (0.77)	vs	0.19 (0.32)	<0.04
Te vs C	6	3.87 (3.31)	vs	0.99 (0.95)	<0.01
Un vs Ta	6	5.00 (2.58)	vs	0.89 (0.94)	<0.001
Un vs Te	6	6.41 (4.12)	vs	0.39 (0.56)	<0.01
Te vs Ta	10	1.21 (1.50)	vs	0.66 (0.97)	>0.2
Un vs M	10	2.85 (1.43)	vs	2.08 (0.97)	>0.3

omone release by individual females. It seems most unlikely that it would be constant, even in the same individual and it is well beyond our current capability to measure it in a natural situation. However, we were able to carry out two tests of undecane alone at a concentration of only 0.01 μl in 1 μl of pentane against a blank control before flight activity ceased in 1990 and even at these very low concentrations it remained highly attractive to males (mean no. landings/min: 9.95 and 3.78 for undecane and control, respectively).

All three compounds tested occur in Dufour's gland secretions of *F. lugubris* workers [11], and these compounds are evidently common among ants. For example, two or more of them have been found in the workers of several species of *Formica* [12, 13] and other formicines, including *Lasius* [14, 15], *Acanthomyops* [16], *Camponotus* [17], *Anoplolepis* [18], *Oecophylla* [19], and *Polyrhachis* [20], as well as the myrmicines, *Tetramorium*, *Crematogaster*, and *Pheidole* [20]. For further references on their occurrence in formicine ants, as well as among other ants and arthropods, see [21]. In most cases, including *Formica rufa* [22], they have been shown to function as alarm pheromones, but according to [15, 16], undecane also acts as a spreading agent for formic acid in *Acanthomyops* and *Lasius*, thereby enhancing its efficacy as a defensive secretion. We have obtained data indicating that undecane also elicits alarm behavior in *F. lugubris* workers (Table 3).

Few studies appear to have investigated the possible occurrence of the compounds in female and male sexuals, although several authors reported finding undecane in the winged females of *Formica rufa* [23] and *F. polyctena* [10, 13]. Löfqvist and Bergström [10] even suspected that undecane serves as a sex pheromone in the latter species but were unable to confirm this in laboratory tests. On the other hand, they found that this compound made up 74% of the Dufour's gland secretion in virgin females compared with < 1% in mated queens. This difference provides additional evidence that undecane does indeed function as a sex pheromone in some species of *Formica*. Simple hydrocarbons have previously been found to be biologically active components of the sex pheromones of other insects, e.g., the tenebrionid beetle, *Tribolium confusum* [24], and the sciarid fly, *Lycoriella mali* [26].

It will no doubt seem very surprising to many chemical ecologists and others, as indeed it did to us initially, that undecane, tridecane, and 4-tridecene could be constituents of the sex pheromone of an ant. One question that may be asked is how the same compounds could evolve as both alarm pheromones and sex pheromones. In the insects many pheromones are known to serve multiple functions (for examples, see [21]) and comparative studies among the Formicinae may provide evidence as to which of the two functions of undecane and its associated compounds was evolutionarily primary. In some instances differences in concentration appear to be responsible for the different functional responses of the same compound, e.g., trail following and sexual attraction in the termite *Trinervitermes bettonianus* [26]. Although differences in absolute concentration and/or relative amounts of minor components may play some role in the ability of *F. lugubris* males to distinguish between the alarm signal of workers and the sex pheromone of female sexuals, they are probably not of great significance; the sex pheromone is emitted only at mating places, distinct open areas where males actively patrol for females and where, except for the occasional forager, workers are absent. Therefore, the different contexts and physical locations in which the signals are employed are likely the key to the two functional responses. A second related question concerns how the gene complex responsible for the synthesis and release of the Dufour's gland contents is regulated in a caste-specific manner; synthesis occurs at different points during the life stages of queens and workers, occurring for only a short period early in life just before mating in the queen caste, and release by the two castes is triggered by different stimuli. A third question concerns how reproductive isolation is achieved among the many species of sympatric formicines that probably produce these seemingly ubiquitous compounds. Perhaps the proportions of the active components are species-specific, or perhaps there are additional minor (or major) components that remain to be identified that confer specificity. Or again, there may be temporal differences in mating flights, or sexuals may cue in to different features of the environment that cause them to assemble in different localities. In fact, only sexuals of one other ant species, the myrmicine *Manica rubida*, have been seen at the mating places during the 3 years of our studies. Future research will focus on the above questions.

Table 3. Elicitation of alarm behavior in *Formica lugubris* workers in response to undecane. Workers, collected from the *F. lugubris* supercolony on March 18, 1991, were tested in groups of 20 nest mates. Tests were conducted in a 22-cm diam. circular arena with a 4 × 4 × 4 cm inverted cardboard carton placed in the center. After an habituation period of at least 3 min, a small cotton wad containing 5 μl of undecane or with nothing added (control) was introduced on top of the carton. Two measures of alarm response were recorded. The fastrunning phase of alarm behavior was measured by counting the number of ants crossing the carton during the 3 min following introduction of the cotton. Aggressivity was measured by counting the number of ants on the carton exhibiting a threat display with open mandibles and/or directly attacking the cotton. The total number of these acts occurring during the 3 min following introduction of the cotton was recorded. Each group of ants ($N = 20$, one group from each of 20 different nests) was tested twice, once in the presence of undecane and once in the presence of the untreated cotton. The order of the tests was alternated so that 10 groups were tested first in the presence of undecane and 10 groups were tested first with the untreated cotton. Shown are means ± SD. Comparisons were made by way of paired t-tests on the square-root transformed data

No. ants traversing carton		No. aggressive acts	
Undecane	Control	Undecane	Control
35.6 ± 8.5	24.3 ± 8.0	3.1 ± 2.1	1.6 ± 1.3
$P < 0.001$		$P < 0.005$	

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Male and Female Sex Pheromones Produced by *Acarus immobilis* Griffiths (Acaridae: Acarina)

Chemical Ecology of Astigmatid Mites XXXIV [1]

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The presence of male and female sex pheromones (M and F pheromones) has been postulated in a nonparthenogenetic bisexual astigmatid mite based on behavioral observation [2], and has been biologically demonstrated in the flour mite *Acarus siro* [3]. Our studies enabled the identification of the chemical nature of F pheromones responsible for male excitation in two species; β-acaridial [2(*E*)-(4-methyl-3-pentenylidene)-butanedial] as the pheromone of *Caloglyphus polyphyllae* [4] and 2-hydroxy-6-methyl-benzaldehyde (2,6-HMBD) as the pheromone of *Aleuroglyphus ovatus* [5], while no chemicals responsible for female attraction (M pheromone) have ever been reported.

The aim of the experiments reported here was to identify both sex pheromones from the same acarid mite, *Acarus immobilis* Griffiths, and to analyze various aspects relating to the pheromone communication system of astigmatid mites in general.

The mite *A. immobilis*, which is an economically important graminivorous pest, feeds directly on the commodities which they infest and may cause considerable damage [6]. The species were raised by using a mixture of dried yeast and wheat flour (1:1) at 25 °C and 80 % RH. The F-pheromone activities, such as sexual arousal and homosexual mounting attempts, were reproducible among 20–25 males isolated and conditioned in a small glass tube [5] by feeding them moist food for 3 days, if the active compound(s) was placed on a small glass rod (1 mm o.d. × 10 cm). On the other hand, the M-pheromone activity was expressed as the attraction and aggregation of similarly conditioned females to the tip of the glass rod, on which the active compound(s) was loaded. The activity of the candidate material was evaluated based on

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