Comparison of Dufour Gland Secretions of Two Species of *Leptothorax* Ants (Hymenoptera: Formicidae)

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Z. Naturforsch. **42c,** 955–960 (1987); received February 10/March 19, 1987

Leptothorax, Harpagoxenus, Dufour Gland, Hydrocarbons, Sesquiterpenes

The Dufour glands of *Leptothorax acervorum* and *L. nylanderi* both contain species-specific mixtures of hydrocarbons, dominated by C_{17} hydrocarbons in *L. acervorum* and C_{15} hydrocarbons in *L. nylanderi*. Both species contain the sesquiterpenoid tetramorene-2, but neither contain (*E*)-β-farnesene, which has earlier been found in the glands of *Harpagoxenus sublaevis* which raids *Leptothorax* nests and enslaves its workers. The contents of the glands of workers and queens of *L. acervorum* were very similar.

Leptothorax is a widely distributed genus of myrmicine ant, described by Donisthorpe [1] as being agile and robust, but of timid and adaptable temperament, not at all war-like. *L. acervorum* Fabr. is found throughout Britain and the whole of Europe except the extreme south. *L. nylanderi* Först. is found throughout central and southern Europe and occasionally in southern England. *L. acervorum* is preyed upon in different ways by two related species, *Harpagoxenus sublaevis* (Nyl.) and *Leptothorax kutteri* (Buschinger). *H. sublaevis* workers raid nests of *L. acervorum,* carrying off the pupae, which emerge as adults in the *H. sublaevis* nest, where they perform all the duties of food gathering, brood rearing and nest maintenance for their *H. sublaevis* mistresses [2–4]. On the other hand, *L. kutteri* queens enter the nest of *L. acervorum* singly, overcome the natural reaction of the *L. acervorum* workers to eject or kill them, and settle there to lay their eggs to be nurtured and reared by the *L. acervorum* workers [5].

It is reported that *H. sublaevis* workers smear the captured brood of *L. acervorum* with their Dufour gland secretion, making the brood unattractive to the *Leptothorax* defenders [6, 7]. The "propaganda substance" that the workerless *L. kutteri* use to disrupt the defense of *L. acervorum* workers, causing them to attack each other, and permitting the entry of the *L. kutteri* queens, is also located in the Dufour gland [8].

We have recently analyzed the contents of the very large Dufour glands of *H. sublaevis* [9]. We found the expected mixture of hydrocarbons but also (*E*)-β-farnesene, a compound not previously found in ants, but commonly encountered as an alarm pheromone of aphids [10]. The farnesene is accompanied by smaller amounts of a homologue with one more carbon atom [9]. We have now examined the much smaller Dufour glands in its potential slave *L. acervorum* and its close relative *L. nylanderi* to see if a comparison of their compositions shed light on the dulotic relationship between *H. sublaevis* and *L. acervorum.*

Materials and Methods

Maintenance of colonies

L. acervorum from Sweden and *L. nylanderi* from Belgium were reared in small artificial nests made with a cardboard separation between two glass microscope slides. The nests were placed in plastic bowls to serve as foraging areas. The inner vertical walls of the bowls were covered with polytetrafluoroethylene paste (I.C.I. Fluon) to prevent the ants escaping. The ants were fed with water, sugar solution and at least once a week the larvae of mealworms.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0700–0955 $ 01.30/0

Sample preparation

Live ants were momentarily dipped in the cold vapour above liquid nitrogen, and the Dufour gland plus poison reservoir dissected out in distilled water under a binocular microscope. The glandular apparatus was supported on a fragment of glass, dried by touching with a fragment of tissue paper and sealed in a glass capillary, which was introduced into the gas chromatograph by the solid sampling technique of Morgan and Wadhams [11], whereby the sample was heated to 140 °C in the injection port before crushing the glass.

Gas chromatography

A Fractovap gas chromatograph (Carlo Erba) equipped with a flame ionization detector and Chromatopac C-R3A recorder-integrator (Shimadzu) were used for analysis and quantification. Separation was performed on a fused silica capillary column (25 m × 0.4 mm) coated with OV-1 silicone with helium carrier gas (2 ml min^{-1}) and temperature programmed from 120 °C at 3 °C min^{-1} to 300 °C.

Quantification

A standard solution of hydrocarbon (200 ng μl^{-1} of tridecane, pentadecane and heptadecane and 113 ng μl^{-1} of nonadecane) in hexane was injected (1 µl) several times onto the gas chromatograph and the peak areas averaged. The detector response was calculated from the mean peak area per ng and used to calculate the amount of each substance in the gland. From this, percentage values for each gland were calculated, and then the mean amount and mean percentage values, together with their standard deviations, for ten replicates of each type of gland analyzed.

Gas chromatography – mass spectrometry (GC-MS)

Mass spectrometry was performed on a 3200E quadrupole spectrometer (Finnigan) with a 6000 Data system (Finnigan). A fused silica column (CP-19, 38 m × 0.22 mm, Chrompac) was directly coupled to the mass spectrometer. Helium was used as carrier gas at a flow rate of 1 ml min^{-1}. Spectra were recorded at 70 eV (electron impact) at a rate of 2 sec $scan^{-1}$. A sealed capillary containing two glands was used with the solid sampling system in a split-splitless injector. The split valve was opened after 1 min, the GC was left at room temperature for 4 min and then programmed to 280 °C at 6 °C min^{-1}.

Trapping and ozonolysis

Heptadecene was collected from the Dufour glands of 8 workers of *L. acervorum* using an effluent splitter and trapping device [12]. Heptadecene was trapped in a glass capillary (5 cm × 0.5 mm i.d.) cooled with liquid nitrogen [13]. The glass tube containing the heptadecene was broken into three parts and the pieces placed in a larger capillary sealed at one end. Ozonized oxygen was passed into the capillary for 10 sec, then the other end was sealed, and the capillary inserted into the injection port of the gas chromatograph with the same solid sampler [11], heated for 5 min to 200 °C and crushed.

Results

The Dufour gland of *L. acervorum* workers is filled almost entirely with linear hydrocarbons from C_{15} to C_{19} with the addition of one sesquiterpenoid substance (Fig. 1). 8-Heptadecene is the major substance, first found in *Myrmica rubra* [14] but frequently in other ants. The position of the double bond was located by micro-ozonolysis, which yielded a mixture of *n*-octanal and *n*-nonanal. The linear C_{17} hydrocarbons, *n*-heptadecane, 8-heptadecene and an incompletely characterized heptadecadiene together made-up over 90% of the total contents. The mean amounts of the substances found from ten replicate determinations are given in Table I. The same mixture was found in mature queens of *L. acervorum*, though their glands were rather bigger (Table I). The amount of material in the Dufour gland can vary considerably from one individual ant to another, and the mean value for one nest can be different from that of another, the difference observed here is, however, real because the queens and workers came from the same nests.

Three new homologous sesquiterpenoid compounds were found in *Tetramorium caespitum* and *T. impurum* [15] and there referred to only by their molecular masses (M^+ 236, 250 and 264). We have now found the homologue with M^+ 250 in both queens and workers of *L. acervorum*, though only in trace quantities. The substance is readily identified by its mass spectrum (Fig. 2), but its structure, which cannot be deduced from the mass spectrum alone,

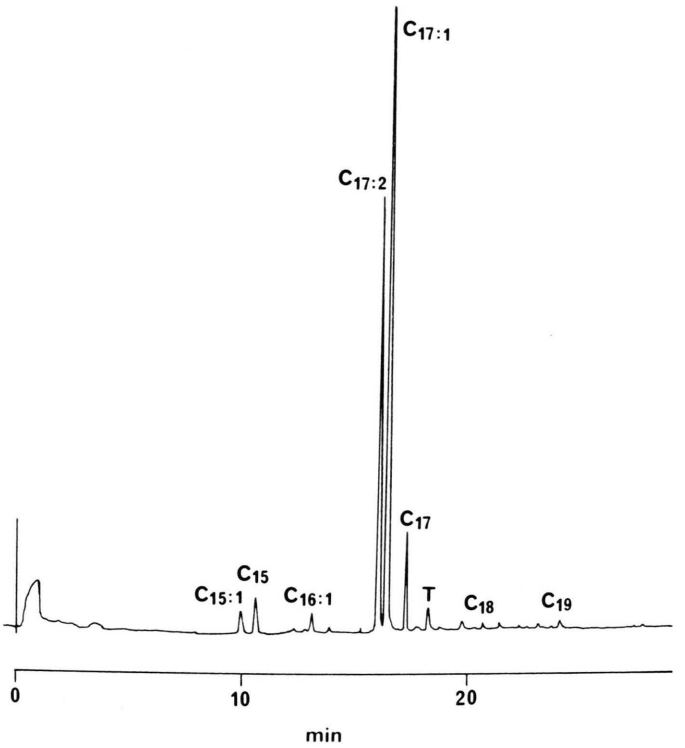

Fig. 1. An example of a gas chromatogram of a single Dufour gland of a worker of *L. acervorum*. C_{15}:1 indicates pentadecene, etc. T indicates the sesquiterpenoid substance tetramorene-2. The chromatogram obtained from queen Dufour glands was very similar. Chromatographic conditions are as described in the text.

Table I. Chemical composition of the contents of the Dufour gland of workers and mature queens of *Leptothorax acervorum* determined by gas chromatography and mass spectrometry. Results are the mean of ten determinations calculated as described in Materials and Methods.

Compound	Queens		Workers	
	Mean quantity ng ± SD	Fraction of total % ± SD	Mean quantity ng ± SD	Fraction of total % ± SD
Pentadecene	4.21 ± 2.12	2.6 ± 0.5	3.0 ± 2.0	3.0 ± 1.2
n-Pentadecane	5.21 ± 2.02	3.6 ± 1.4	3.0 ± 2.0	3.9 ± 2.0
Hexadecene	1.77 ± 1.13	0.95 ± 0.5	0.6 ± 0.8	0.5 ± 0.7
n-Hexadecane	t	t	t	t
Heptadecadiene	28.9 ± 12.5	18.2 ± 2.9	20 ± 10	19.0 ± 4.6
8-Heptadecene	113 ± 47.6	71.1 ± 3.0	60 ± 20	68.6 ± 3.7
n-Heptadecane	4.86 ± 2.24	3.2 ± 0.9	4 ± 1	4.3 ± 0.5
Tetramorene-2	t	t	t	t
Octadecene	t	t	t	t
n-Octadecane	t	t	t	t
n-Nonadecane	t	t	t	t
Total	159 ± 66		91 ± 36	

t, trace detected, < 0.2 ng.

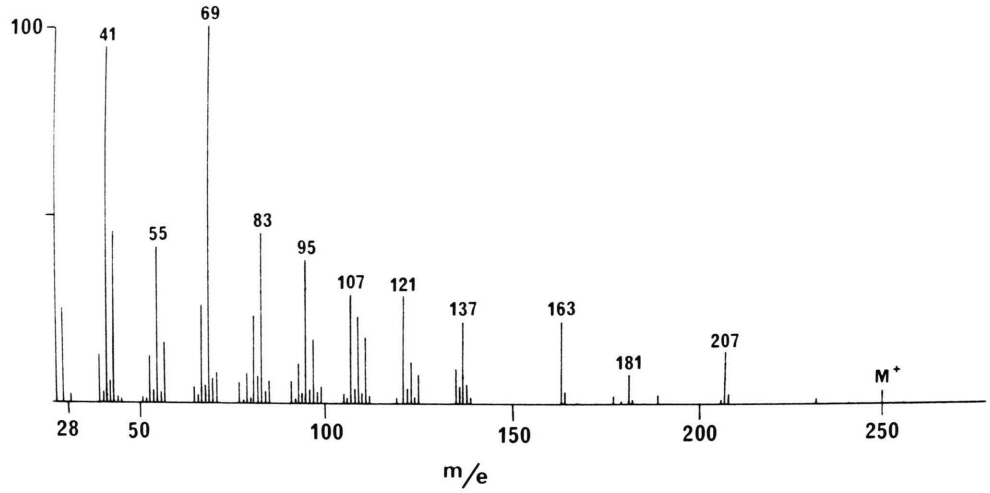

Fig. 2. Mass spectrum of tetramorene-2, a sesquiterpenoid found in the Dufour glands of *Tetramorium caespitum, T. impurum, L. acervorum* and *L. nylanderi.*

remains to be studied. For ease of description, it is now called tetramorene-2 (1- and 3- are reserved for the lower and higher homologues).

There was no evidence of any (*E*)-β-farnesene in this species. Selective scanning of the GC-MS experiment at high sensitivity for *m/z* 204, the molecular ion of the farnesenes, showed none present. This technique would have detected as little as 0.2 ng.

The Dufour glands of workers of *L. nylanderi* contained a simpler mixture of linear hydrocarbons with pentadecane the major component (Fig. 3). The glands of this species were extremely small and contained a mean of only 20 ng per worker (Table II). The second most abundant compound is the tetramorene-2 found also in traces in *L. acervorum.* No (*E*)-β-farnesene was detected.

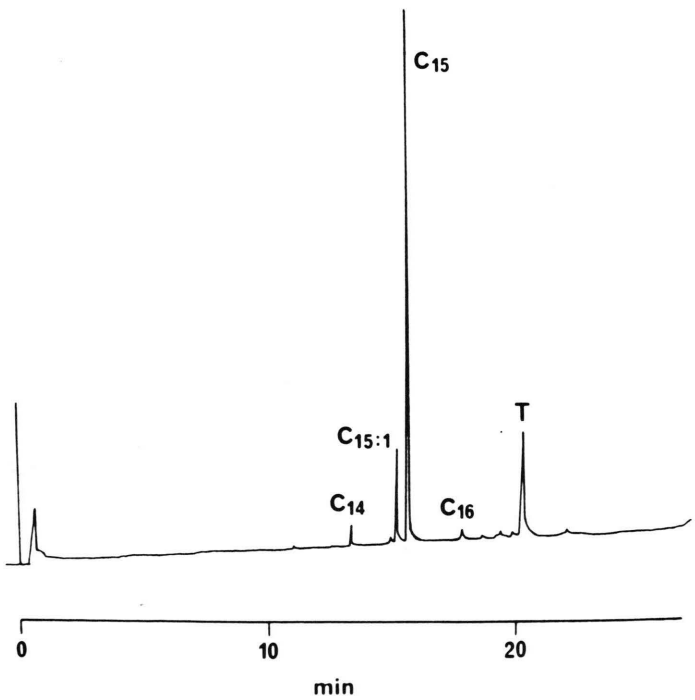

Fig. 3. A gas chromatogram of the single Dufour gland of a worker of *L. nylanderi.* T indicates tetramorene-2. Conditions are the same as in Fig. 1 and are described in the text.

Table II. Chemical composition of the content of the Dufour gland of workers of *Leptothorax nylanderi* determined by gas chromatography and mass spectrometry (means of ten determinations).

Compound	Mean quantity ng ± SD	Fraction of total % ± SD
n-Tetradecane	t	t
Pentadecene	3 ± 2	13.9 ± 7.0
n-Pentadecane	10 ± 7	57.1 ± 13.3
n-Hexadecane	0.1 ± 0.2	0.3 ± 0.6
Heptadecene	0.04 ± 0.1[a]	0.2 ± 0.8
n-Heptadecane	0.1 ± 0.3	0.2 ± 0.7
Tetramorene-2	6 ± 8	26.0 ± 15.4
n-Octadecane	0.7 ± 2	1.5 ± 2.7
Total	21 ± 20	

[a] Present in only one worker analyzed.
t, trace.

Discussion

There are several points to observe here. First, the mixture of hydrocarbons is different between *L. acervorum* and *L. nylanderi*. *L. acervorum* has a relatively simple mixture of linear alkanes and alkenes with one alkadiene, with over 90% of the total consisting of C_{17} compounds. *L. nylanderi* has a still simpler pattern with C_{15} compounds (70%) predominant. The hypothesis that the Dufour gland substances form a species-specific mixture, already substantiated in many species is further confirmed by this example.

Secondly, both species contain the same sesquiterpenoid compound, tetramorene-2 present as a trace (< 0.1% of the total) in *L. acervorum* and the second most abundant component in *L. nylanderi*. This substance, still of unknown structure is also present in the Dufour glands of *Tetramorium caespitum* and *T. impurum*, there accompanied by two homologues tetramorene-1 in *T. caespitum* and tetramorene-3 in *T. impurum*. One or more of such sesquiterpenoid compounds, frequently one of the isomers of farnesene or a C_{16}, C_{17} or C_{18} homologue, have been found in all but one of the myrmicine Dufour glands we have studied (ten species of *Myrmica*, three of *Atta*, two of *Tetramorium*, and one each of *Acromyrmex*, *Harpagoxenus*, *Monomorium*, *Pogonomyrmex* and *Solenopsis*, and now two *Leptothorax*). The only exception has been *T. semilaeve* which has very small glands and now deserves re-examination at greater sensitivity.

Thirdly, the (*E*)-β-farnesene and its C_{16} homologue found in *H. sublaevis* [9] are not present in either species of *Leptothorax*. The possibility is strengthened that this substance is responsible for the disruptive effect caused by raiding *H. sublaevis* workers in the *L. acervorum* nest. However, preliminary attempts to identify the same substance in *L. kutteri* queens have been unsuccessful.

Fourthly, the mixture of hydrocarbons in *H. sublaevis* [9] is close in composition to that found in *L. acervorum*, but quite unlike that in *L. nylanderi*. In both *H. sublaevis* and *L. acervorum* heptadecene is the major substance, with heptadecadiene second. The chief differences are (1) there is no detectable (*E*)-β-farnesene in either *Leptothorax* species. (2) There are small amounts of C_{19}–C_{23} hydrocarbons in *H. sublaevis* not present in *L. acervorum*. These would render the *Harpagoxenus* secretion somewhat less volatile than that of *Leptothorax*. (3) Tetramorene-2 has not been found in *H. sublaevis*. Regnier and Wilson [16] have described the importance of Dufour gland secretions in slave raiding in formicines and Bergström and Löfqvist [17] found similarities between the Dufour gland secretions of slave and slave-making species in several examples among formicine ants.

Finally, no essential composition difference was found between mated queens and workers of *L. acervorum*. We have recently reviewed a comparison between queen and worker Dufour gland contents in a description of small differences found in *Camponotus* aethiops [18].

Acknowledgement

We thank N. R. Franks for the supply of *L. acervorum* and for helpful discussions. J. P. J. B. thanks the Belgian National Fund for Scientific Research for the award of a senior research assistantship; A. B. A., the Alexander von Humboldt Stiftung for the award of a fellowship, and E. D. M., the Royal Society for a grant for the purchase of gas chromatography equipment.

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