Ontogenetic Patterns in Amounts and Proportions of Dufour's Gland Volatile Secretions in Virgin and Nesting Queens of *Lasioglossum malachurum* (Hymenoptera: Halictidae)

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Z. Naturforsch. **45c,** 709–714 (1990); received February 28/April 11, 1990

Lasioglossum malachurum, Sweat Bee, Dufour's Gland Volatiles, Ontogenetic Patterns of Odorous Compounds, Pheromones

In the primitively eusocial sweat bee, *Lasioglossum malachurum,* 66 volatile compounds could be identified from queen Dufour's gland secretions. The patterns found in gynes and in old nesting queens differed in the absolute amounts of extractable volatiles as well as in the relative proportions. 3-Methyl-3-butenyl octadecanoate is the main component in gynes, while 18-octadecanolide, 20-eicosanolide and 22-docosanolide largely dominate the bouquets of old queens. The probable roles of some specific compounds in pheromonal communication of mates and in nest recognition are discussed. Correlations with the volume of the fat body and the vitellogenic status of the ovary are described.

Introduction

Age-dependent variations in the compositions of exocrine secretions of insects have been investigated predominantly in Coleoptera, Lepidoptera and Diptera [1], including the production of sex pheromones [2, 3]. In many species females show qualitative and quantitative postmating changes in the composition of volatile compounds [4]. There are only a few data on social insects [5, 6] and almost none on eusocial bees [7]. Queens of the stingless bee, *Scaptotrigona postica,* are attractive for drones only during a distinct age [8], corresponding to specific patterns of cephalic volatiles [9]. Concerning Dufour's gland secretions, similar differences exist between virgin and mated females of the communally nesting bee, *Andrena ferox,* [10]. In the primitively eusocial sweat bee, *Lasioglossum malachurum,* young queens, collected in the field, are highly attractive to males due to a female produced sex pheromone [11], while mated queens were found to be much less attractive [12]. With respect to relative proportions and absolute amounts of hydrocarbons and lactones, there are significant differences between attractive and unattractive queens [12]. The objective of our present study was the investigation of the chemical background of individual variations in the attractiveness of different queens. We assumed that the attractive queens are young and unmated, while the unattractive ones are older, already mated, and usually in the nesting phase. We therefore analyzed and compared the patterns of volatile constituents of gynes and old nesting queens.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/0600–0709 $ 01.30/0

Materials and Methods

Sample collection

L. malachurum queens were collected from a large nesting aggregation in the vicinity of Tübingen. To obtain gynes, nests were excavated in July, 1988, and female pupae as well as old larvae were carefully placed in clean glass vials and transported to the laboratory. After emerging, the gynes were fed for 3–7 days with a mixture of honey and pollen until they were killed by freezing. All these young queens were found to be attractive to males.

In addition, nesting queens were collected during May to Aug., 1989, in cooled, clean glass vials, transferred to the laboratory, freezed and stored at −20 °C. Individual Dufour's glands were extracted with pentane (Merck, Uvasol). For quantitative analyses, *n*-octacosane was added as an internal standard. Furthermore, the size of the fat body of individuals was recorded as being small, intermediate or large, and the status of the ovaries was checked. The following three stages were distinguished: small = all follicels beyond the vitellogenic phase; enlarged = vitellogenic follicel growth initiated; large = ovarioles containing eggs.

Chemical analysis

A Carlo Erba Fractovap 2450 gas chromatograph was used, equipped with a FS-SE 54 capillary column (50 m × 0.25 mm id.), operating at 120 °C as a starting temperature, followed by programming to 280 °C at 4 °C/min. Response factors were determined by single level calibration. Mass spectra (70 eV) were obtained with a VG 70/250 SE instrument, connected to a HP 5890 gas chromatograph, operated under the above GC-conditions. NMR-spectra of synthesized reference samples were run on a Bruker WM-250 instrument.

Statistics

Relative proportions and total contents of single Dufour's gland compounds were tested for statistical significance of differences between young and old queens by the Mann-Whitney U-Test [13]. The Pearson product-moment correlation coefficient was calculated for relations between the size of the fat body, ovarian status and the absolute amounts of different gland compounds. Computations were performed using SPSS/PC + [13].

Results

Overall, 66 volatile compounds could be identified (Fig. 1, Table I), which gives a much more complete picture of the complex volatile secretions as compared to earlier investigations [14]. Gynes and nesting queens showed striking differences in the relative proportions as well as in the absolute amounts of the Dufour's gland secretions. In young queens, isopentenyl esters of saturated and unsaturated fatty acids containing 18, 20 and 22 carbon atoms, respectively, represent the main compounds which are almost completely absent in old, nesting queens (Fig. 1, 2). In contrast, in nesting queens saturated and unsaturated lactones are dominating, while in gynes these compounds occur in small amounts only.

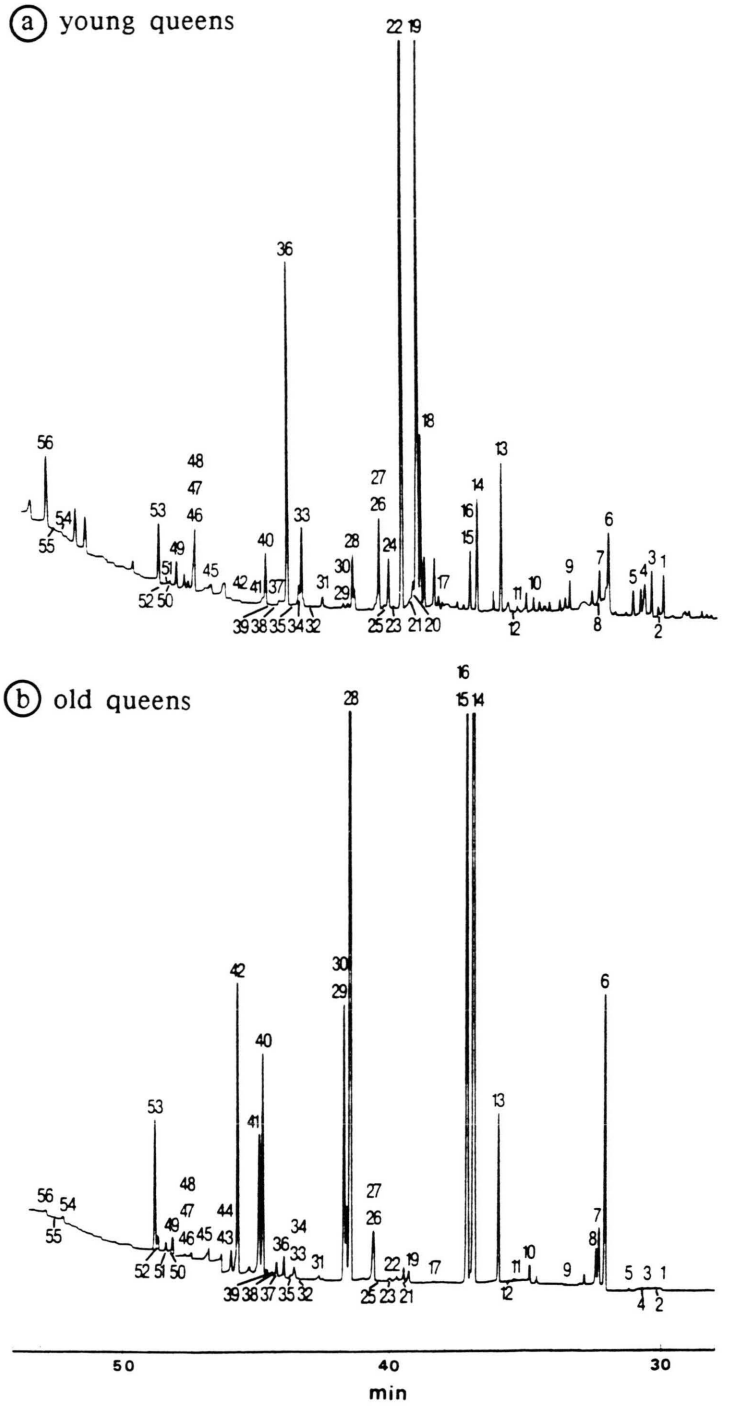
With almost all of the identified compounds the absolute amounts were found to be higher in nesting than in young queens (Table I). A gyne's gland contained approximately 2.3 µg of volatiles, while 309 µg could be extracted from nesting queens. These striking differences in contents and also in relative proportions are statistically significant according to the Mann-Whitney U-Test (Table I, $P < 0.05$; Fig. 2).

There is a negative correlation between the size of the fat body and the vitellogenic growth of the follicels ($r = -0.795$, $P < 0.001$). In young queens the fat body is large and the ovaries are small, whereas in nesting queens it is just the opposite. The total amounts of volatiles are negatively correlated with the size of the fat body ($r = -0.677$, $P < 0.001$) and positively with the vitellogenic status of the ovary ($r = 0.672$, $P < 0.001$).

Double bond positions of alkenes can be unambiguously determined by the DMDS-method [15]. Similarly, location of double bonds in unsaturated macrocyclic lactones was shown to be possible: During fragmentation of the respective DMDS-derivative, a McLafferty rearrangement at the ester side yields an open chain which is cleaved at the geminal methylthio group, producing two key ions. Determination of the geometry of the double bonds in the natural hydrocarbons and lactones was carried out by coinjection on the basis of authentic reference samples under conditions which separated the *E,Z*-isomers. Details will be published elsewhere.

Discussion

In the primitively eusocial bee, *Lasioglossum malachurum* (Hymenoptera: Halictidae), individual odor patterns play an important role in mating biology [11, 12] and colonial life [16, 17]. GC-analyses revealed patterns of volatile compounds specific for young unmated gynes and old nesting queens, respectively (Fig. 1). In extracts of young queens isopentenyl esters of saturated and



1. 3-Methyl-3-butenyl hexadecanoate
2. 18-Octadecanolide (branched)
3. (Z)-9-Heneicosene
4. (Z)-7-Heneicosene
5. Heneicosane
6. 18-Octadecanolide
7. 18-Octadec-(Z)-9-enolide
8. 18-Octadec-(Z)-11-enolide
9. Ethyl octadecanoate
10. 20-Eicosanolide (branched)
11. (Z)-9-Tricosene
12. (Z)-7-Tricosene
13. Tricosane
14. 20-Eicosanolide
15. 20-Eicos-(Z)-11-enolide
16. 20-Eicos-(Z)-13-enolide
17. Ethyl eicosanoate
18. 3-Methyl-3-butenyl octadecadienoate
19. 3-Methyl-3-butenyl octadecenoate
20. 3-Methyl-3-butenyl octadecatrienoate
21. 20-Docosanolide (branched)
22. 3-Methyl-3-butenyl octadecanoate
23. (Z)-9-Pentacosene
24. (Z)-7-Pentacosene
25. 3-Methyl-2-butenyl octadecanoate
26. Pentacosane
27. Unknown (M=326)
28. 22-Docosanolide
29. 22-Docos-(Z)-13-enolide
30. 22-Docos-(Z)-15-enolide
31. Ethyl docosanoate
32. 3-Methyl-3-butenyl eicosadienoate
33. 3-Methyl-3-butenyl eicosenoate
34. 3-Methyl-3-butenyl eicosatrienoate
35. 24-Tetracosanolide (branched)
36. 3-Methyl-3-butenyl eicosanoate
37. (Z)-9-Heptacosene
38. (Z)-7-Heptacosene
39. 3-Methyl-2-butenyl eicosanoate
40. Heptacosane
41. Unknown (M=354)
42. 24-Tetracosanolide
43. 24-Tetracos-(Z)-15-enolide
44. 24-Tetracos-(Z)-17-enolide
45. Ethyl tetracosanoate
46. 3-Methyl-3-butenyl docosadienoate
47. 3-Methyl-3-butenyl docosenoate
48. 3-Methyl-3-butenyl docosatrienoate
49. 3-Methyl-3-butenyl docosanoate
50. (Z)-9-Nonacosene
51. (Z)-7-Nonacosene
52. 3-Methyl-2-butenyl docosanoate
53. Nonacosane
54. 3-Methyl-3-butenyl tetracosanoate
55. 3-Methyl-2-butenyl tetracosanoate
56. Hentriacontane

Fig. 1. Gas chromatograms of Dufour's gland extracts of a) young virgin, and b) old nesting *L. malachurum* queens. Except of the above listed compounds, some even-numbered (C_{16}-C_{30}) and odd-numbered (C_{11}, C_{15}, C_{17}, C_{19}) saturated hydrocarbons, which were not always present, could be identified. In gynes (a) some isopentenyl esters (18, 19, 22, 36) are predominant, whereas in nesting queens (b) several macrocyclic lactones (6, 14, 15, 16, 28, 29, 30) are present in high concentrations.

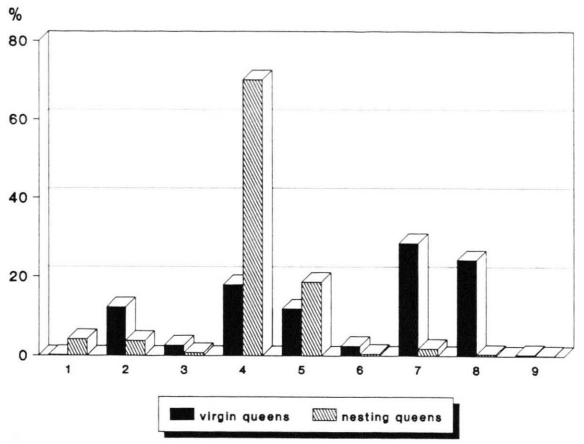


Fig. 2. Comparison of the relative amounts of yet unknown compounds (1), alkanes (2), alkenes (3), saturated macrocyclic lactones (4), unsaturated macrocyclic lactones (5), branched lactones (6), isopentenyl esters of saturated (7), and unsaturated fatty acids (8) and ethyl esters of Dufour's gland extracts in young virgin and old nesting *L. malachurum* queens. In all groups of compounds except of the ethyl esters there were significant differences ($P < 0.001$, Mann-Whitney U-Test).

unsaturated fatty acids and hydrocarbons are dominating, whereas macrocyclic lactones represent the main components in old queens (Fig. 2). In addition, the absolute content of the Dufour's glands was found to differ significantly. Old nesting queens showed total amounts of volatiles, predominantly lactones, 100 times higher, as compared to young and attractive queens.

In previous experiments males were found to discriminate between queens encountered before or yet unknown, and in addition between those of different colonies and populations [11]. Since alien queens are always prefered, sexual selection by male choice evidently has to be interpreted as part of an outbreeding mating strategy. They recognize and approach receptive gynes and reject mated queens [12]. Young but mated gynes, old nesting queens and foraging workers are not attractive to the males (Ayasse, unpubl.). Dead young unmated queens, recently killed by freezing, whole body extracts and cuticular washings of such females

Table I. Relative amounts and total Dufour's gland contents (ng) of young ($n = 18$) and nesting ($n = 17$) *L. malachurum* queens ($\bar{x}$ = mean; S_E = standard error of the mean).

Compound	% Total Extract				Amount/Gland [ng]			
	young		old		young		old	
	$\bar{x}$	S_E	$\bar{x}$	S_E	$\bar{x}$	S_E	$\bar{x}$	S_E
Hydrocarbons								
Undecane	0.59	0.19	0.09	0.04	8.16	1.66	131.35	62.49
Pentadecane	t		t		t		t	
Hexadecane	t		t		t		t	
Heptadecane	0.28	0.03	t		5.11	0.89	1.39	1.39
Octadecane	t		t		t		t	
Nonadecane	0.30	0.05	t		4.72	0.89	4.09	2.40
Eicosane	t		t		t		t	
Heneicosane	0.53	0.10	0.05	0.01	8.44	1.10	190.35	53.88
Docosane	t		t		t		t	
Tricosane	2.06	0.24	1.52*	0.10	39.13	5.76	4695.50	790.25
Tetracosane	t		t		t		t	
Pentacosane	1.60	0.16	0.29	0.05	33.65	6.68	725.39	155.98
Hexacosane	t		t		t		t	
Heptacosane	0.98	0.11	0.84*	0.13	16.37	1.64	2400.63	544.76
Nonacosane	1.55	0.34	0.74*	0.11	23.15	2.52	2210.78	442.61
Triacontane	2.36	0.61	0.10	0.03	29.36	4.05	228.55	112.91
Hentriacontane	1.99	0.44	0.12	0.03	21.14	4.21	254.42	49.34
(*Z*)-9-Heneicosene	1.18	0.15	t		19.61	1.52	6.79	3.56
(*Z*)-7-Heneicosene	0.28	0.08	0.02*	0.00	5.13	1.52	53.84	14.27
(*Z*)-9-Tricosene	0.22	0.05	0.04	0.01	5.70	0.89	136.87	32.92
(*Z*)-7-Tricosene	0.38	0.10	0.03	0.01	8.10	1.26	79.34	18.97
(*Z*)-9-Pentacosene	0.16	0.06	0.17	0.02	2.19	0.60	519.27	108.06
(*Z*)-7-Pentacosene	0.09	0.04	0.05	0.01	1.74	0.55	175.60	66.57
(*Z*)-9-Heptacosene	0.20	0.07	0.20*	0.02	4.91	1.07	571.14	109.65
(*Z*)-7-Heptacosene	0.04	0.04	0.03	0.00	0.27	0.27	96.92	19.31
(*Z*)-9-Nonacosene	0.07	0.05	0.20	0.02	0.65	0.45	709.14	151.83

Compound	% Total Extract				Amount/Gland [ng]			
	young		old		young		old	
	$\bar{x}$	S_E	$\bar{x}$	S_E	$\bar{x}$	S_E	$\bar{x}$	S_E
Lactones								
18-Octadecanolide	7.06	0.77	16.67	2.64	127.21	37.69	58849.24	14903.17
Octadecanolide (branched)	1.21	0.31	0.20	0.02	20.75	3.04	572.01	100.77
18-Octadec-(*Z*)-9-enolide 18-Octadec-(*Z*)-11-enolide	3.98	0.73	3.53*	0.53	81.89	17.44	12808.11	3451.20
20-Eicosanolide	8.98	1.53	25.02	0.52	230.33	48.02	77125.72	12740.75
Eicosanolide (branched)	1.20	0.18	0.25	0.02	27.99	5.45	773.78	127.33
20-Eicos-(*Z*)-11-enolide 20-Eicos-(*Z*)-13-enolide	4.94	0.52	8.71	0.37	143.66	28.24	27867.67	5179.72
22-Docosanolide	1.72	0.27	24.19	1.84	41.85	7.80	67663.14	10495.82
22-Docos-(*Z*)-13-enolide 22-Docos-(*Z*)-15-enolide	1.85	0.18	5.55	0.22	53.81	10.88	16404.27	2535.04
24-Tetracosanolide	0.11	0.06	4.18	0.52	1.63	0.78	10977.22	1965.41
24-Tetracos-(*Z*)-15-enolide 24-Tetracos-(*Z*)-17-enolide	1.01	0.16	0.78*	0.26	1.63	0.78	2502.36	1059.64
Isopentenyl esters								
3-Methyl-3-butenyl hexadecanoate	t		t		t		t	
3-Methyl-3-butenyl octadecanoate	12.29	1.51	0.14	0.02	276.62	53.78	452.44*	82.61
3-Methyl-2-butenyl octadecanoate	0.12	0.04	0.06*	0.01	2.12	0.54	174.30	47.79
3-Methyl-3-butenyl eicosanoate	11.26	1.41	0.49	0.09	232.79	48.02	1344.14	277.59
3-Methyl-2-butenyl eicosanoate	t		0.08	0.01	t		257.84	63.16
3-Methyl-3-butenyl docosanoate	0.61	0.10	0.29	0.07	13.93	3.50	685.21	162.89
3-Methyl-2-butenyl docosanoate	0.08	0.08	t*		1.76	1.65	t*	
3-Methyl-3-butenyl tetracosanoate	4.06	1.48	0.72	0.17	50.36	11.59	1399.54	323.76
3-Methyl-3-butenyl octadecadienoate	11.75	1.74	0.01	0.01	350.37	88.29	8.28	3.58
3-Methyl-3-butenyl octadecenoate	5.86	1.29	0.01	0.01	158.07	36.78	24.75	14.62
3-Methyl-3-butenyl octadecatrienoate	1.95	0.55	0.14	0.01	25.25	7.44	434.06	74.39
3-Methyl-3-butenyl eicosadienoate	0.02	0.02	0.03	0.00	0.12	0.12	81.85	14.55
3-Methyl-3-butenyl eicosenoate	3.30	0.36	0.17	0.02	88.09	18.66	512.31	100.64
3-Methyl-3-butenyl eicosatrienoate	1.34	0.28	0.04	0.01	43.84	11.11	131.51	23.59
Ethyl esters								
Ethyl octadecanoate	0.11	0.04	0.02*	0.00	1.04	0.44	51.65	9.84
Ethyl eicosanoate	0.11	0.03	0.02*	0.00	1.81	0.91	53.37	10.75
Ethyl docosanoate	0.12	0.06	0.06	0.01	0.92	0.48	161.22	33.29
Ethyl tetracosanoate	t		t		t		1.03*	1.03
Unknown compounds								
Unknown (M = 326)	0.09	0.08	1.72	0.26	3.46	1.82	6028.88	1431.07
Unknown (M = 354)	0.02	0.02	2.44	0.07	0.40	0.40	7412.21	1184.80

* Compounds, in which the relative proportions or the total amounts are not significantly different at $P < 0.05$ (U-Test).
t trace amounts < 0.01% resp. < 0.1 ng only.

elicited male mating behavior. In field biotests, preextracted dead females which were free of any volatiles were found to be completely unattractive to the males. However, after impregnating with extracts or the washings mentioned above, these dummies were pounced by the males. The determination of the active principle in the extracts, *i.e.* the identification of compounds which actually represent components of the female sex pheromone, is presently under investigation. The isopentenyl esters, which seem to constitute a specific signal of gynes, may be reasonable candidates. Such esters have been reported as volatile components of the Dufour's glands of Halictinae [14, 18] and Nomiinae [19]. However, no biological function was reported up to now. In *L. zephyum*, a mixture of the macrocyclic lactones may play a role in the attraction of males [20]. Furthermore, these

compounds seem to be important in kin and nest recognition in *L. zephyrum* [21, 22] and likewise in *L. malachurum* [11].

The significance of macrocyclic lactones in brood cell lining and impregnation of the nest entrance construction has been discussed several times [7]. In *L. malachurum* only old nesting queens with large ovaries contained large amounts of volatiles (predominantly saturated macrocyclic lactones). If not used in odor communication, these compounds should, for economic reasons, not be produced before the queen starts nesting.

In typical nesting habitats of *L. malachurum* thousands of nests are spread over a relatively small area. Therefore, a highly developed nest orientation behavior is presumably required. The observation that guarding bees mark the nest entrances with Dufour's gland volatiles suggests olfactory cues to be involved in nest allocation. This presumption was confirmed by behavioral tests in the field [16]. Females returning to their nests were able to discriminate between their own nest entrance and foreign ones even when the close range visual orientation was experimentally altered. The main odor compounds extractable by pentane from of nest entrances were found to be alkanes and alkenes (Ayasse, unpubl.). However, the role of these volatiles in nest recognition is still unknown. Further investigations are presently under way.

Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft (En 89/11, Fr 507/8) is gratefully acknowledged. We also like to thank our colleague Jan Tengö, Uppsala University, for helpful suggestions.

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