Atypical Secretions of the Male Cephalic Labial Glands in Bumblebees: The Case of *Bombus* (*Rhodobombus*) *mesomelas* GERSTAECKER (Hymenoptera, Apidae)

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In bumblebees, the male secretion of the cephalic labial gland is species-specific. It is highly involved in the nuptial behavior, acting as a sexual attracting pheromone. Therefore, it is also used to accurately identify the species. In contrast to this common scheme, the secretions of *Bombus mesomelas* are found to be strongly reduced and do not include the most volatile compounds that are present in the secretions of all the other studied bumblebee species. These secretions correspond to cuticular hydrocarbons that can be found in all bumblebee species. This was also the case for another bumblebee species from the same *Rhodobombus* subgenus: *Bombus pomorum*. This atypical composition of the male cephalic labial gland secretions seems to indicate that, at least for these two species of *Rhodobombus*, these secretions are not used to attract virgin females from a long distance, as it is the case for all the other bumblebee species studied.

1. Introduction. – It is not always possible to identify all the bumblebee species accurately based on their morphological characters or color features. On the contrary, the species-specific character of the male cephalic labial gland secretions has been confirmed for over 30 species in Europe [1][2] and in North America [3]. This character is thus well-suited for a species definition based on the *Paterson*'s species-recognition concept [4]. Bumblebee species with identical combinations of sexual pheromones and nuptial behavior may be regarded as conspecific.

The prenuptial behavior of male bumblebees includes the olfactory marking of chosen objects in the landscape by the cephalic labial gland secretions to attract conspecific females. The prenuptial marking behavior has been observed in most West-Palaearctic species [5], in many species in North America [6], and Himalaya [7]. The use of chemical marking during prenuptial behavior may, therefore, be regarded as general among male bumblebees.

The cephalic labial gland secretions of male bumblebees can be long-chain (C_{12}–C_{20}) aliphatic alcohols, aldehydes, and methyl or ethyl esters of fatty acids, and/or acyclic mono-, sesqui-, and diterpenic alcohols or aldehydes. Aliphatic compounds usually contain one to three C=C bounds. Until now, only one bumblebee species seems to be excluded from that common scheme: *Bombus* (*Rhodobombus*) *pomorum* (PANZER 1805). *Valterová et al.* [8] reported on the very limited and most unusual composition of its cephalic labial secretions for a bumblebee species-specific pheromone. The gland contains mostly aliphatic hydrocarbons, no terpenes, and, with one exception (hexadecanol in traces), no compound with an oxygenated functional group. All the most volatile and species-specific compounds of other bumblebee species are absent. Moreover, in *Bombus pomorum*, no marking behavior has been observed [9].

On the other hand, the composition of bumblebee male cephalic labial gland secretions seems to share traits between species from the same genera [2]. For instance, ethyl dodecanoate is present in all the studied species of the subgenus *Bombus sensu stricto*, and only in those species. Both studied species of the subgenus *Cullumanobombus* (*B. cullumanus* and *B. semenoviellus*) share the same main compounds: (all-*E*)-geranylgeranyl acetate [10]. The same main compound is also in common in the two studied species of the subgenus *Sibiricobombus* (*B. niveatus* and *B. sulfureus*) [11].

The morphology and histology of the cephalic labial glands of *Bombus* (*Rhodobombus*) *mesomelas* has been shown to be much smaller than in other bumblebee species, and it may even be non-functional [12]. Our present research aims at identifying the chemical composition of the cephalic labial gland secretions of *Bombus* (*Rhodobombus*) *mesomelas* GERSTAECKER 1869, a species that is closely related to the above mentioned *B. pomorum*.

2. Results and Discussion. – The cephalic labial gland secretions of *B. mesomelas* males were *ca.* 100–1000 times less concentrated than those obtained from wild males of *B. terrestris* (estimated from total-ion current in GC/MS under equal conditions; *Fig.*). Mostly, hydrocarbons were present in the gland extracts (86% in average; *Table 1*). Variability in the quantification of individual samples was due to their low concentration, which made it impossible to integrate minor or trace compounds.

As it has been shown for *B. pomorum* [8], the secretions of the cephalic labial gland of the *B. mesomelas* males do not contain the volatile compounds that are usually met with in males of other bumblebees [1][2]. In the latter, major compounds are always either terpenes or aliphatic alcohols, aldehydes or esters. The only exceptions are *Bombus* (*Megabombus*) *hortorum* (L.) and *B.* (*Megabombus*) *consobrinus* DAHLBOM: Their main component is an aliphatic hydrocarbon (nonadecene). However, beside this compound, both species secrete large amounts of geranylgeranial and hexadecenol [13].

The main components in the two *Rhodobombus* species are aliphatic hydrocarbons and wax esters (*Table 2*). However, they are secreted by *B. pomorum* and *B. mesomelas* in very small quantities (*Fig.*). In other bumblebee subgenera, hydrocarbons make up 3 to 15% of the total volume of the secretion. These substances are also the same as those detected on the cuticle of a great many bumblebee species and are very similar to the secretion of *Dufour*'s gland in bumblebee workers [14]. Besides, in *B. pomorum*, 13 from the 20 compounds detected in the cephalic labial gland secretions are just aliphatic hydrocarbons that are also present on the cuticle of the same species [8].

In spite of their very weak concentrations, the secretions of *B. mesomelas* and *B. pomorum* remain different and may be species-specific. Both species present original compounds: hexadecenol and hydrocarbons with two C=C bonds in *B. pomorum*; esters in *B. mesomelas*.

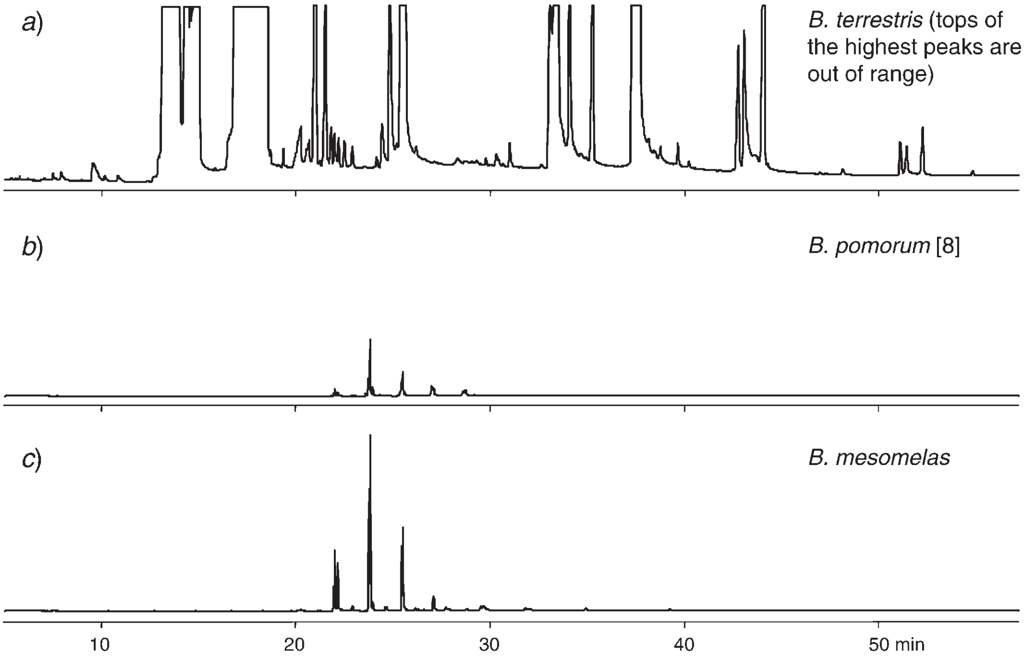

Figure. *Chromatograms of the male cephalic labial gland secretions of* a) Bombus (Bombus) terrestris, b) Bombus (Rhodobombus) pomorum, *and* c) Bombus (Rhodobombus) mesomelas, *represented on the same scale*

All the *B. mesomelas* males examined here have been collected during their nuptial flight. If their cephalic labial-gland secretions are in any way related to the sexual attraction, they should have been active at this time. However, the aliphatic hydrocarbons found in their secretions are usually not considered to be attractive for bumblebee virgin females [15]. Their presence in the extract of the cephalic labial glands of *B. pomorum* and *B. mesomelas* as in the other bumblebee species could result from their presence in the hemolymph, as it is the case in moths and flies [16]. In this case, the aliphatic hydrocarbons could be not the result of a secretion but simple residues of hemolymph. This is coherent with the observations of *Terzo et al.* [12], who showed that the histology of the cephalic labial glands of *B. mesomelas* is best explained by a very weak or maybe null secretion activity.

Of course, one cannot exclude that those hydrocarbons are nevertheless used as a pheromone. They could be implicated in intraspecific communication, including premating behavior. Differences of cephalic labial gland secretions between *B. mesomelas* and *B. pomorum* do exist (*Table 2*), and could be recognized by the females as species-specific. But hydrocarbons are usually acting at very short distance or by contact [17]. Attracting females from far away with a so small amount of such compounds seems to be unlikely in bumblebees.

Table 1. *Composition of the Male Cephalic Labial Gland Secretions in* Bombus mesomelas. Relative proportions [%] of compounds in order of their retention times (t_R).

Compounds	t_R [min]	Relative proportions [%]								
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Mean	Median	Standard deviation
Tricos-9-ene	18.65	0.83	0.37	–	–	–	–	0.20	0.00	0.27
Tricos-7-ene	18.70	3.72	2.54	0.45	0.66	1.15	1.66	1.70	1.41	0.95
Tricosane	18.85	6.79	23.38	24.88	26.83	2.69	12.31	16.15	17.84	8.88
Tetracos-9-ene	19.50	0.25	–	–	–	–	–	0.04	0.00	0.07
Tetracos-7-ene	19.57	0.69	–	–	–	–	–	0.11	0.00	0.19
Tetracosane	19.72	0.18	0.47	0.90	1.20	–	–	0.46	0.33	0.40
Pentacos-9-ene	20.22	8.04	2.87	0.42	–	–	2.85	2.36	1.64	2.22
Pentacos-7-ene	20.28	20.79	13.55	6.81	6.57	1.48	10.36	9.93	8.58	4.97
Pentacosane	20.52	1.97	13.95	24.85	24.55	6.44	15.07	14.47	14.51	7.02
Hexacos-9-ene	21.13	0.29	–	–	–	–	–	0.05	0.00	0.08
Hexacos-7-ene	21.18	0.68	–	–	–	–	–	0.11	0.00	0.19
Hexacosane	21.32	0.16	0.57	0.51	0.82	1.43	0.73	0.70	0.65	0.29
Heptacos-9-ene	21.82	5.16	2.72	0.84	0.96	3.05	2.92	2.61	2.82	1.14
Heptacos-7-ene	21.89	10.53	7.35	2.75	3.58	3.47	6.20	5.65	4.89	2.38
Heptacosane	22.05	1.35	9.76	19.48	17.47	1.08	14.93	10.68	12.34	6.62
Octacosene isomer 1[a])	22.62	0.32	–	–	–	–	–	0.05	0.00	0.09
Octacosene isomer 2[a])	22.67	0.43	0.50	–	–	1.06	–	0.33	0.22	0.33
Octacosane	22.72	0.45	1.01	1.08	0.83	–	1.40	0.79	0.92	0.38
Nonacos-9-ene	23.35	1.68	0.61	–	–	0.82	1.52	0.77	0.72	0.57
Nonacos-7-ene	23.42	2.12	1.91	1.45	0.97	1.11	2.32	1.65	1.68	0.47
Nonacosane	23.54	0.60	2.91	8.46	8.76	0.34	6.50	4.59	4.70	3.31
Triacontene	24.02	1.19	1.32	1.10	0.60	4.02	1.79	1.67	1.25	0.82
Hentriacont-9-ene	24.80	0.53	0.90	3.73	2.91	0.51	1.42	1.67	1.16	1.10
Octadec-11-enyl dodecanoate	25.35	1.66	1.78	–	–	13.01	2.98	3.24	1.72	3.26
Octadec-11-enyl tetradecanoate	26.74	6.23	4.09	2.31	3.28	21.46	5.14	7.09	4.62	4.79
Octadec-11-enyl hexadecenoate	28.25	10.51	4.27	–	–	28.51	9.92	8.87	7.09	7.44
Octadec-11-enyl octadecenoate	30.20	10.22	3.17	–	–	8.38	–	3.63	1.59	3.78
Icosyl octadecenoate	32.80	2.63	–	–	–	–	–	0.44	0.00	0.73

[a]) Due to the too small amount of octacosene isomers, the position of the C=C bond could not be determined.

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Table 2. *Comparison of the Compositions of the Male Cephalic Labial Gland Secretions in* Bombus mesomelas, B. pomorum [8], *and on the cuticle of* B. pomorum

Compounds	Cephalic labial gland		Cuticle
	B. mesomelas Present work	*B. pomorum* [8]	*B. pomorum* [8]
Hexadecanol		X	
Tricosenes	X	X	X
Tricosane	X	X	X
Tetracosenes	X	X	
Tetracosane	X		X
Pentacosadienes		X	X
Pentacosenes	X	X	X
Pentacosane	X	X	X
Hexacosenes	X	X	
Hexacosane	X		X
Heptacosadienes		X	X
Heptacosenes	X	X	X
Heptacosane	X	X	X
Octacosadienes		X	
Octacosenes	X	X	
Octacosane	X		
Nonacosadienes		X	X
Nonacosenes	X	X	X
Nonacosane	X	X	X
Triacontadienes		X	
Triacontenes	X	X	
Hentriacontadienes		X	X
Hentriacontenes	X	X	X
Hentriacontane			X
Octadecenyl dodecanoate	X		
Octadecenyl tetradecanoate	X		
Octadecenyl hexadecanoate	X		
Octadecenyl octadecanoate	X		
Icosyl octadecanoate	X		

Experimental Part

Six males of *B.* (*Rhodobombus*) *mesomelas mesomelas* were collected in Cerdagne (France, Pyrénées-Orientales) in Dorres (WGS: 42°29′N 1°56′E; 1710 m), Err (WGS: 42°26’N 2°02’E; 1790 m), and Eyne (WGS: 42°28′N 2°06′E; 2050 m), from August 29th to 30th 2001. The males were killed just before dissection, by a brief deep-freezing. Both parts of their labial cephalic glands were taken to a glass vial, with 200 μl of hexane and kept for 24 h at 20°, then at −30° until analysis.

The composition of the extracts was determined with a gas chromatograph coupled to a quadrupole mass spectrometer *Fisons MD800: DB-5ms* non-polar column (5% phenyl(methyl)polysiloxane stationary phase; 30 m column length; 0.25 mm inner diameter; 0.25 μm film thickness); initial temp. held 2 min at 70°, then programmed to 320° at 10°/min and held 30 min at 320°; temp. of the injector: 220°; carrier gas: He (1 ml/min); injection mode: ‘splitless’; electron ionization: ‘full scan (30–600)’; injection of 1 μl of the extract; chemical ionization measured on an ion-trap instrument (*Varian Saturn 2000*) with MeCN as a reagent gas [18] was used for the determination of the C=C bond positions.

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