Chemical ecology of blackflies: sexual dimorphism in cuticular washes of *Wilhelmia equina* (L.) (Diptera: Simuliidae)

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A-few-hour-old imagos of blackflies *Wilhelmia equina* (L.) were washed in hexane and the washes were analysed by GC–MS methods. Sexual dimorphism both in the qualitative and quantitative composition of 55 chemicals was found. Thirty eight compounds were present in cuticular washes of one sex only, and 17 compounds were present in both sexes, but in differed amounts. Twenty seven compounds were found in males only. Those were 14 branched hydrocarbons (C_{11}–C_{19} and C_{24}–C_{36}), 2 straight hydrocarbons (undecane and dodecane), 1 cyclic saturated hydrocarbon, 2 fatty acid esters, 4 other esters, alcohol, aldehyde, nitrile, and an unknown compound. There were 11 chemicals present in females only: 8 fatty acid esters, 1 other ester, and 2 branched saturated hydrocarbons (C_{26} and C_{32}). Quantitative differences in the composition of cuticular washes were found among branched (4 compounds) and straight (4 compounds) hydrocarbons, polycyclic compounds (1 compound), fatty acids (4 compounds) and its esters (3 compounds) as well as among other esters (1 compound). The data on sexual dimorphism in the chemical composition of *W. equina* cuticula may be used in search for sex pheromones in blackflies. Peculiarities of precopulatory behaviour of blackflies are discussed in relation to the data indicating that most of the cuticular compounds that determine differences between the sexes, are of low volatility.

Key words: cuticular hydrocarbons, potential sex pheromones, precopulatory behaviour

INTRODUCTION

Chemical ecology deals with interactions between organisms and their environment by means of chemicals. Pheromones are among such chemicals. All over the order Diptera pheromone structures are known in a few dozen of species from 10 families only (Agromyzidae, Calliphoridae, Ceratopogonidae, Culicidae, Drosophilidae, Glossinidae, Muscidae, Sarcophagidae, Scaridae, Tephritidae) (Tamaki, 2001). This number is very low when compared, for example, with the order Lepidoptera, where pheromones are known in more than 400 species from 44 families (Arn and Toth, 1992). Most of dipteran pheromones were identified from cuticular surfaces.

The chemical ecology of blackflies (Simuliidae) has become of interest rather recently. Comparative analysis of cuticular chemistry in *Simulium damnosum* species complex (research at interspecific level) (Carlson et al.,, 1981) as well as search for chemicals involved in aggregation of egg-laying females (McCall, 1995; McCall, 1997a; McCall, 1994; McCall, 1997b) were carried out. However, there are no pheromone identifications in the family Simuliidae.

Wilhelmia equina (Linnaeus, 1746) are among the mammaliophylic blackfly species most abundant (Sprangauskaitė, 2000) and most important from the veterinary point of view in Lithuania. These flies can be vectors of some diseases of human and cattle. Yet there is no monitoring of the population of this economically important species. For this purpose a relevant technique should be developed. Sex and other pheromones (chemicals produced by insects for intraspecific communication) could be of use for such monitoring. Synthetic pheromones are successfully used for monitoring many lepidopteran species (Carde, Minks, 1995).

In our opinion, the precopulatory behaviour of blackflies to some extent may be influenced by sex pheromones. Search for such pheromones could be started from a comparative analysis of the chemical

composition of cuticular surfaces in the adults of blackflies of both sexes.

The aim of the present study was to compare the chemical composition of cuticular washes in *W. equina* males and females as well as to reveal qualitative and quantitative differences, if any, suitable for further search of sex pheromones in *W. equina* and other blackflies.

MATERIALS AND METHODS

Insects. Water plants with fixed blackfly pupae were collected July 26, 2000 in the Neris River in the territory of Vilnius city (Žvėrynas district), Lithuania. Most of the pupae were found fixed on *Carex* sp. plants in the littoral zone. In the laboratory, using a binocular, undamaged mature, ready for emergence (dark) pupae were separated from the collected samples by cutting off together with pieces of the plant to which they were fixed. The pupae were placed one by one into 3 ml glass-tubes to avoid emerged adult mating. To maintain moisture, pieces of damp filter-paper were placed in the test-tubes and the latter were closed with foam rubber plugs. Blackfly species and sexes were identified at pupal and imago stage, respectively. *W. equina* was identified according to Kaplich and Skulovec (Kaplich and Skupovec, 2000).

Washing. Cuticular washes of both sexes were made separately. Alive *W. equina* individuals were grouped by sex a few hours after adult emergence. Five adults of the same sex were put in 1 ml glass vials with a conical bottom. The vials with blackflies were kept in a freezer for 15 min at –10 °C. Then, 40 µl of solvent (hexane) was added into each vial and the vials were lightly shaken. Blackflies were kept in a freezer at – 2 °C and discharged from the solvent after 24 h.

Chemical analysis. Comparative chemical analysis of *W. equina* male and female cuticular washes was performed using the Finnigan SSQ 7OO GC–MS system including a Varian 3400 GC equipped with DB–wax silica capillary column (30 m, i. d. 0.25 mm, a medium polarity stationary phase thickness 0.25 µm) and a column temperature programme, which began at isothermal 60 °C for 1 minute, thereafter by 5 °C/min up to 210 °C, later was kept at 210 °C for 40 min. The splitless injector temperature was 205 °C and the splitless period 30 s. Whole of cuticular washes (five male or female equivalents) were injected at once. Helium was used as the carrier gas under 10 psi pressure.

Electron ionization mass spectra were obtained at 70 eV with the ion source at 150 °C. Data were analysed by using the X-calibre programme pack. The mass spectrum of each chemical compound, which differed in both sexes, was compared with those of library standard chemicals indicating RSI. Chemicals with the mass spectrum RSI over 900 were identified as significant.

RESULTS AND DISCUSSION

Qualitative and quantitative differences between the *W. equina* sexes were revealed by GC–MS analysis of cuticular washes (Fig. 1). Differences were determined from 55 chemical compounds (Figs. 1, 2), which were divided into 11 groups: straight hydrocarbons, branched hydrocarbons, cyclic hydrocarbons, polycyclic compounds, fatty acids, fatty acid esters, other esters, alcohols, aldehydes, nitriles and unknown chemical compounds (Table).

Qualitative differences in cuticular chemicals in both sexes of *W. equina*. Thirty-eight chemicals (69%) of the 55 compounds mentioned above were present in cuticular washes of one sex only.

Chemicals found in male cuticular washes only. Twenty seven of such chemicals were detected (Fig. 2). Most of them (14 compounds) were branched hydrocarbons with a chain length from 11 to 19 carbon atoms (Table; Fig. 2a, peaks 3–5, 8) as well as those from 24 to 36 carbon atoms (Table; Fig. 2c–e, peaks 33, 38, 41, 43, 46, 48–52). In other groups of chemicals there were less compounds specific to males: 2

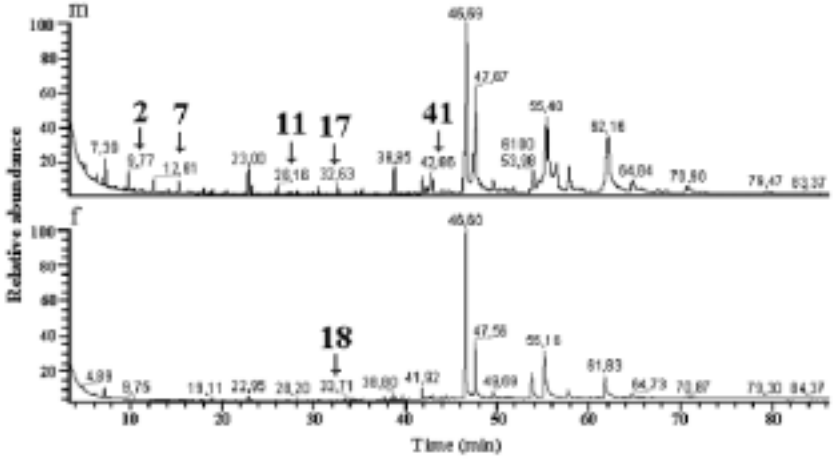

Fig. 1. Total GC–MS chromatogram of *Wilhelmia equina* cuticular washes. Arrows show the peak of chemicals; the biggest numbers in bold show quantitative and qualitative differences between males (m) and females (f); the smallest numbers below arrows – retention time of chemicals in column

Table. **Compounds defining differences in male and female cuticular washes of *Wilhelmia equina* (Linnaeus, 1746)**

Peak No.	Compounds	RSI Probablity	*Wilhelmia equina*			
			Retention time		Abundance	
			Male	Female	Male	Female
1	*2*	*3*	*4*	*5*	*6*	*7*
	Straight hydrocarbons					
1	Undecane (C_{11})	>900	7.02		+	–
2	Dodecane (C_{12})	>900	9.77		+	–
7	Tridecane (C_{13})	>900	12.61	12.59	>>	tr
9	Tetradecane (C_{14})	>900	15.39	15.36	>>	+
10	Δ-Heptadecene (C_{17})	>900	20.55	20.55	>	+
36	Heneicosane (C_{21})	877	40.53	40.46	+	tr
	Branched hydrocarbons					
3	2,6-Dimethyl-heptadecane (C_{19})	821	11.22		+	–
4	Branched (undecane or dodecane or tridecane)	app 800	11.39		+	–
5	2,9-Dimethyl-undecane (C_{13})	868	11.66		+	–
8	2-Methyl-tridecane (C_{14})	802	14.19		+	–
33	3-Ethyl-tetracosane (C_{26})	726	40.00		+	–
37	9-Octyl-docosane (C_{30})	576	41.07	41,06	>+	+
38	11-Undecyl-pentacosane (C_{36})	700	41.36		+	–
40	5-Butyl-docosane (C_{26})	715		42.70	–	+
41	3-Methyl-tricosane (C_{24})	798	42.86		+	–
42	9-Octyl-tetracosane (C_{32})	671		42.94	–	+
43	13-Undecyl-pentacosane (C_{36})	745	43.05		+	–
46	5-Butyl-docosane (C_{26})	763	44.84		+	–
48	11-Methyl-nanocosane (C_{30})	770	47.47		+	–
49	Dimethyl-(heptacosane or nanocosane) (C_{29} or C_{31})		50.41		+	–
50	2,6,10,14-Pentamethyl-2,6,10,14-eicosopentene (C_{25})	842	51.92		+	–
51	13-Methyl-hentriacontane (C_{32})	763	54.59		+	–
52	13,17-Dimethyl-hentriacontane (C_{33})	705	56.63		+	–
53	15,19-Dimethyl-unotriacontane (C_{31})	none	65.74	65.16	+	tr
54	15,19-Dimethyl-tritriacontane (C_{35})	725	67.66	66.97	+	tr
55	15,19-Dimethyl-pentatriacontane (C_{37})	730	68.57	67.88	+	tr
	Cyclic hydrocarbons					
6	4-Methylpenty-cyclohexane (C_{12})	794	12.10		+	–
	Polycyclic compounds					
12	Tetramethyltricyclo-undecanol		27.07	27.07	+	>+
	Fatty acids					
16	Octanoic acid	>900	30.54	30.54	>+	+
17	Nonanoic acid	>900	32.63	32.63	>+	+
21	Decanoic acid	841	34.63	34.64	>+	+
29	Dodecanoic acid	850	38.41	38.4	tr	+
	Fatty acid esters					
15	Hexanoic acid 2-ethyl	785	28.32		+	–
18	Methyl hexadecanoate	876	33.70	33.71	tr	+
19	Methyl 9-hexadecenoate	845		34.19	–	+
20	Ethyl hexadecanoate	812	34.42	34.41	tr	+
22	Ethyl 9-hexadecenoate	764		34.88	–	+
26	Methyl octadecanoate	753		37.45	–	+
27	Methyl 8-octadecenoate	854		37.8	–	+
28	Methyl 8-octadecenoate	766		37.93	–	+
30	Methyl 9,12-octadecadienoate	832		38.63	–	+
32	Methyl 9,12,15-Octadecatrienoate	893		39.75	–	+
35	Methyl 9,12,15-Octadecatrienoate	750		40.31	–	+
39	1-Methylbutyl hexadecanoate	702	42.47		+	–
44	Methyl 5,8,11,14,17-eicosapentanoate	848	44.60	44.59	+	>>+
	Other esters					
11	Ethanol,2-(2-butoxyetoxy)-acetate	910	26.16		+	–

Table (continued)

1	2	3	4	5	6	7
13	Tributyl phosphate	762	27.62		+	–
14	Tributyl phosphate	756	27.68		+	–
24	Diethyl phthalate	871	36.34		tr	–
31	Dimethyl phthalate	850	39.34	39,33	+	tr
45	Diisooctyl adipate	871		44.65	–	tr
	Alcohols					
34	2-methyl-hexadecanol	742	40.15		+	–
	Aldehydes					
23	2-Benzyl-octanal	736	36.20		+	–
	Nitriles					
25	Hexadecanenitrile	830	36.62		+	–
	Unknown					
47	Unknown compound		46.43		+	–

– not found, + found (more than trace); tr – trace; > more; >> much more.

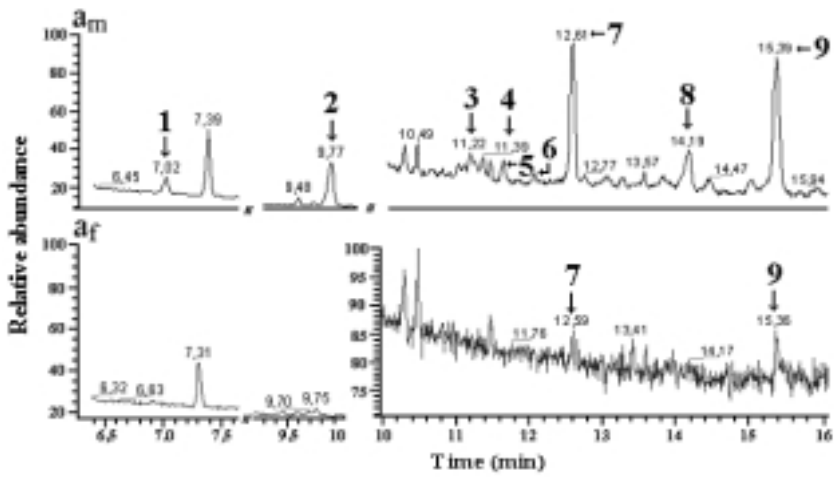

Fig. 2a. Detailed GS–MS chromatogram of *Wilhelmia equina* cuticular washes Arrows show the peak of chemicals; the biggest numbers in blod show quantitative and qualitative differences between males (m) and females (f); the smallest numbers below arrows – retention time of chemicals in column

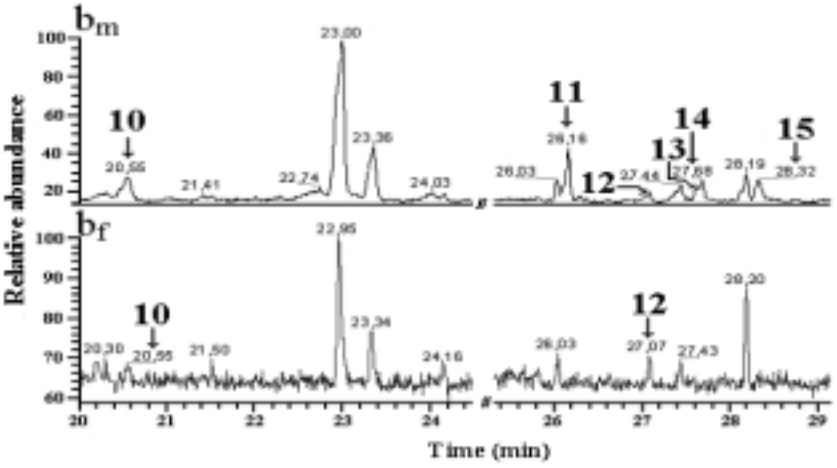

Fig. 2b. Detailed GS–MS chromatogram of *Wilhelmia equina* cuticular washes

straight saturated C_{11} and C_{12} hydrocarbons, undecane and dodecane (Table; Fig. 2a, peaks 1, 2); a cyclic hydrocarbon (Table; Fig. 2a, peak 6); 2 fatty acid esters (Table; Fig. 2b, d, peaks 15, 39); 4 other esters (Table; Fig. 2b, c, peaks 11, 13, 14, 24); an alcohol (Table; Fig. 2d, peak 34); an aldehyde (Table; Fig. 2c, peak 23); a nitrile (Table; Fig. 2c, peak 25); and an unknown chemical compound (Table; Fig. 2e, peak 47). The quantity of one of the other esters (Table; Fig. 2c, peak 24) was very low (only trace) in male cuticular washes. Although the chemical was not found in females, it can be attributed to quantitative differences between the sexes as well, because the amount found in males only was at a trace level.

Chemicals found in female cuticular washes only. There were detected 11 such chemicals (Fig. 2). Most of them (8 chemicals, or 73%) were fatty acid esters (Table; Fig. 2c, d, peaks 19, 22, 26–28, 30, 32, 35). The other chemicals were: 2 branched hydrocarbons with C_{26} and C_{32} (Table; Fig. 2d, peaks 40, 42); and 1 ester (Table; Fig. 2d, peak 45), the latter in very low quantities (only trace). Although the

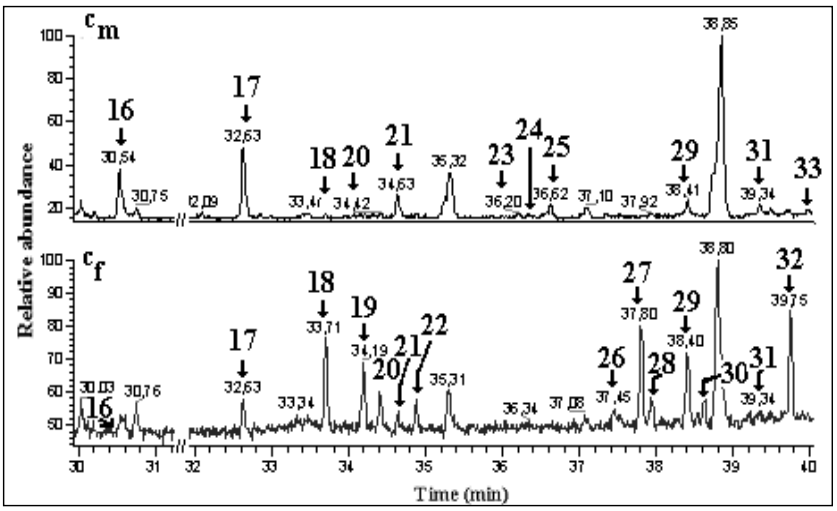

Fig. 2c. Detailed GS–MS chromatogram of *Wilhelmia equina* cuticular washes

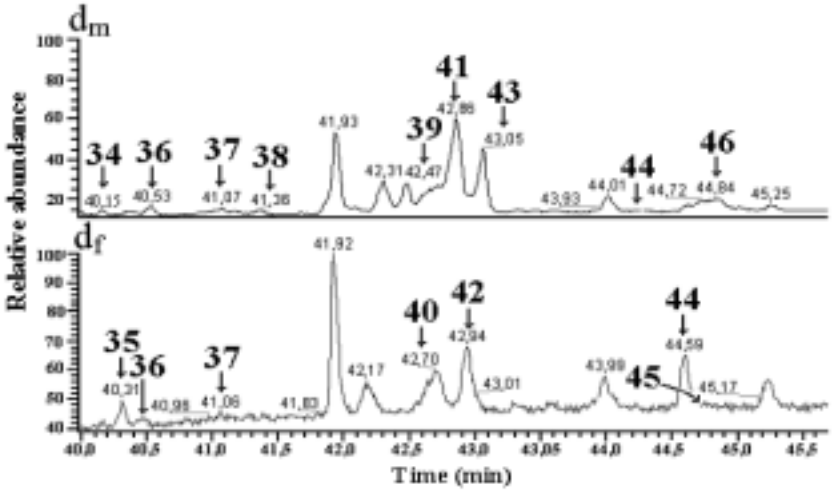

Fig. 2d. Detailed GS–MS chromatogram of *Wilhelmia equina* cuticular washes

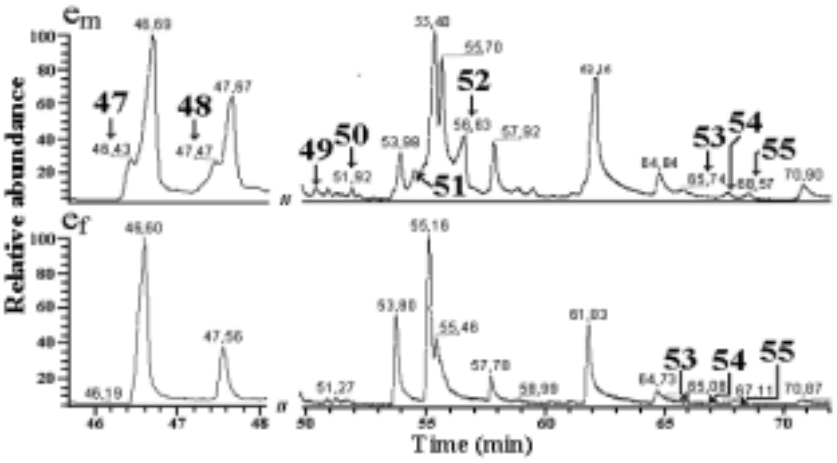

Fig. 2e. Detailed GS–MS chromatogram of *Wilhelmia equina* cuticular washes

ester was not found in males, it can be attributed to quantitative differences between the sexes as well, as it might be present below detection level in males.

Quantitative differences in cuticular chemicals in both sexes of *W. equina*. Seventeen chemicals (31%) of 55 compounds, which differ in *W. equina* males and females, were found in different quantities in cuticular washes. Twelve chemicals were more abundant in males and 5 chemicals were more abundant in females.

Chemicals with higher quantities in male cuticular washes. Among 6 chemicals found in both sexes of *W. equina,* in males they were in higher quantities (Table): 2 straight C_{14} and C_{17} hydrocarbons, namely tetradecane and heptadecene (Table; Fig. 2a, b, peaks 9, 10), 1 branched C_{30} hydrocarbon (Table; Fig. 2d, peak 37), and 3 fatty acids (Table; Fig. 2c, peaks 16, 17, 21), two being identified reliably, octanoic and nonanoic acids (peaks 16, 17).

Another 6 chemicals were found in very low amounts (traces only) in female cuticular washes (Table): 2 straight saturated hydrocarbons, tridecane (C_{13}) and heneicosane (C_{21}) (Table; Fig. 2a, d, peaks 7, 36), 3 branched hydrocarbons with 31–37 carbon atoms (Table; Fig. 2d, peaks 53–55); and 1 ester (Table; Fig. 2c, peak 31), while they were well expressed in male washes.

Chemicals with higher quantities in female cuticular washes. Five chemicals were found in both sexes of *W. equina,* in higher quantities in females than in males (Table): 1 polycyclic compound (Table; Fig. 2b, peak 12), 2 fatty acids (Table; Fig. 2c, peak 29; Fig. 2d, peak 44), and 2 fatty acid esters (Table; Fig. 2c, peaks 18, 20). In addition, two fatty acid esters and one fatty acid (peak 29) were found in male cuticular washes in trace amounts.

Comparative analysis of cuticular washes based on GC–MS data revealed a striking sexual dimorphism in *W. equina* blackflies. Both qualitative and quantitative differences in chemical composition were found. The differences were found

at least in 55 chemical compounds: 38 chemicals were present in cuticular washes of one sex only, and 17 chemicals were present in cuticular washes of both sexes, but in different quantities.

We briefly discuss these compounds below according to the groups (Table).

Straight hydrocarbons. Among 55 cuticular compounds that defined sexual dimorphism, 6 chemicals were saturated hydrocarbons (Table). Undecane and dodecane were specific only to males, while other 4 hydrocarbons, namely tridecane, tetradecane, heptadecene, and heneicosane, were found in both sexes, but in higher amounts in male cuticular washes. Tridecane and heneicosane were present in female cuticular washes in very low amounts. All discussed saturated hydrocarbons were identified reliably, with RSI probability exceeding 900 (Table).

Among straight hydrocarbons contributing to sexual dimorphism of cuticular washes, there are still no compounds known as pheromones in any Dipterans. However, some are known as pheromones in other insect orders, *e. g*., undecane in some species of Hemiptera, Hymenoptera, Orthoptera, and Coleoptera; dodecane in Hemiptera; tridecane in Hemiptera and Hymenoptera; tetradecane in Orthoptera; heneicosane in some representatives of Hemiptera and Lepidoptera (Tamaki, 2001).

Branched hydrocarbons. In the washes, the most numerous group of chemicals was the one of branched hydrocarbons, including 20 compounds (Table). Sixteen of those (14 compounds in males and 2 compounds in females) were present in cuticular washes of one sex only. Branched hydrocarbons specific to *W. equina* males contained carbon chains from 11 to 19 atoms (4 chemicals) as well as those from 24 to 36 carbon atoms (10 chemicals). Branched hydrocarbons with C_{26} and C_{32} were specific to *W. equina* females.

The group of branched hydrocarbons contains rather numerous saturated hydrocarbons, namely, methylalcanes. The latter compounds are known as pheromones or major pheromone components in many Diptera species, containing chains from C_{22} to C_{37} (Adams et al., 1995). Such chemicals are less volatile, thus act as contact substances. For example, pheromones of *Musca domestica* (L.) (Muscidae) are methylalcanes with C_{30} and act as aphrodisiacs, and arrestants when insects contact each other (Carlson et al., 1971; Uebel et al., 1978). In *Stomoxys calcitrans* (L.), the other Dipteran species from the same family, pheromone components are methylalcanes with C_{33} (Uebel et al., 1975). Shorter chain methylalcanes are known as Dipteran pheromones as well: with C_{25} and C_{27} in *Drosophila virilis* (Sturtevant) (Drosophilidae) (Oguma et al., 1992), with C_{22} and C_{23} in *Culicoides melleus* (Conquillett) (Ceratopogonidae) (preliminary data of (Linley and Carlson, 1978)). The latter family phylogenetically is closely related to blackflies (Simuliidae).

Four branched hydrocarbons were found in both sexes of *W. equina* in different quantities. Cuticular washes of males contained higher amounts of all compounds. Of those 3, namely C_{31}–$C_{37,}$ were found in female cuticular washes in trace amounts only.

Thus, within the group of branched hydrocarbons it seems highly probable to find sex pheromones of Simuliidae or their potential components, especially among the compounds of the group specific for one sex. Most of the branched hydrocarbons in *W. equina* are long-chain unvolatile compounds, thus they could act as contact substances. However, there are some exceptions, *e. g*., peaks 3–5, 8. Such chemicals are highly volatile and may act as distant substances.

Cyclic hydrocarbons and *polycyclic compounds* (two groups, see Table). Each of the two chemical groups (Table) was represented by a single compound. Cyclic hydrocarbon was found in male cuticular washes only. The polycyclic compound was registered in both sexes with higher quantities in *W. equina* females than in males (Table).

There are some data on such chemicals functioning as Dipteran pheromones in the family Tephritidae only, *e. g*., pheromone component in *Dacus oleae* (Gmelin) (Baker et al., 1980). The latter component is quite volatile (C_{11}) and may act from a distance. Chemicals of both groups are known as pheromones in some species from other orders (Coleoptera, Lepidoptera and Hymenoptera) (Tamaki, 2001).

Fatty acids. Four saturated fatty acids were found (Table). Octanoic and nonanoic acids were identified reliably with the RSI probability exceeding 900 (Table), decanoic and dodecanoic acids were identified with a slightly less RSI.

Qualitative differences between sexes were not revealed in the fatty acids group, however, quantitative differences were defined (Table). The first 3 mentioned acids prevailed in the washes of males, while dodecanoic acid in washes of females (trace amount in males only). Fatty acids are known as common resourses of energy in many organisms. However, sometimes those function as pheromones. All four fatty acids found in *W. equina* are known as pheromones in some species of Hymenoptera, and in addition octanoic acid in Hemiptera and Trichoptera (Tamaki, 2001). Besides, fatty acids may be related to pheromones indirectly as precursors of their synthesis.

Fatty acid esters. This is the second largest group of chemicals contributing to the sexual dimorphism of cuticular washes in *W. equina* blackflies. The group contains 13 compounds.

Eight chemicals from this group constitute the majority (73%) of all compounds characteristic of *W. equina* females only. In cuticular washes of males, only 2 fatty acid esters were found. Three fatty acid esters were present in both sexes with different amounts, higher in females than in males. To our knowledge, there is no fatty acid ester known to be functioning as a pheromone, at least in Diptera.

Other esters. Six esters were found in *W. equina* cuticular washes: 4 compounds in males only, 1 compound specific to female only, and 1 compound in both sexes (Table). The amount of the latter compound was higher in male cuticular washes, whereas in female cuticular washes it was found only in trace amount.

Among Dipterans, some esters function as sex pheromone synergists, *e. g*., in *Drosophilla virilis* and *D. lummei* (Bartelt et al., 1986).

Alcohols, aldehydes and *nitriles*. They were found by one compound in each group. All compounds were present in *W. equina* male cuticular washes only (Table).

It is well known that alcohols and aldehydes are pheromone components in many insect species from different orders, including Diptera (*e. g*., Tephritidae) (Tamaki, 2001).

Some aspects concerning applied the methods should be discussed also. Taking into consideration the amounts of fatty acids and their esters in cuticular washes, those could appear not only from body surfaces but from deeper layers as well. Thus, in further chemical analyses of cuticular compounds, blackflies should be washed for much shorter periods than 24 h at –2 °C.

The results obtained in the present analysis have demonstrated for the first time that there is a sexual dimorphism in the chemical composition of cuticular compounds, at least in one blackfly species. This fact supports the hypothesis of presence of sex pheromones and chemical communication in blackflies. At the same time it contradicts the generally accepted opinion that there is no necessity in chemical communication between males and females of blackflies due to a well developed vision in these insects. Sexual dimorphism in the composition of cuticular compounds in *W. equina* males and females demonstrated in this paper is important for further research in chemical ecology of blackflies, and especially in search for interactions by means of pheromones. All chemical compounds characteristic of only one sex of blackflies are among potential sex pheromone components. Which of these compounds influences the behaviour of blackflies and thus function as pheromone components should be demonstrated in further research. Comparative analysis of the chemical composition of *W. equina* male and female cuticular washes have indicated that there are quite many chemicals that might function as sex pheromone components, among them volatile compounds which could be perceived at some distance by olfaction, as well as compounds with low volatility suitable for perception by contact chemoreceptors (taste) only. Most of the candidate pheromone compounds belong to the latter group.

It is interesting to note some peculiarities in the precopulatory behaviour of *W. equina* males and females, which are characteristic of many other blackfly species as well correspond to the data obtained on the low volatility of most of cuticular compounds determining differences between the sexes. It is well known that males of *W. equina* make swarms and ready-for-mating females fly into the swarms and make couple (Crosskey, 1990). Swarming males irregularly fly up and down. Such behaviour of each male increases emission of low volatile chemicals from body surface. Aggregation of many male flies in a swarm leads to extra increase of the concentration of the chemicals they emit and thus make it possible to detect the male swarm from a longer distance by odour. So, the pattern of precopulatory behaviour in blackflies (swarming) seems to be very suitable for male/female interaction by means of sex pheromones, especially taking into consideration both the low volatility of potential pheromone compounds and the low amounts (the amount could be limited by small dimensions and small mass of blackflies, which are rather small even compared to many other insect groups) produced by a single specimen.

ACKNOWLEDGEMENTS

We express our gratitude to Prof. Anna-Karin Borg-Karlson from the Royal Institute of Technology (Sweden) for the possibility to accomplish the GS–MS analyses. The investigation was supported by the State Science and Studies Foundation of Lithuania, Project No. T-493.

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UPINIŲ MAŠALŲ (DIPTERA: SIMULIIDAE) CHEMINĖ EKOLOGIJA: *WILHELMIA EQUINA* (L.) KUTIKULĖS NUOPLOVŲ LYTINIS DIMORFIZMAS

S a n t r a u k a

Dujų chromatografijos ir masių spektroskopijos metodais atlikta *Wilhelmia equina* mašalų suaugėlių kutikulės nuoplovų cheminės sudėties analizė. Nustatytas ryškus lytinis dimorfizmas – kokybiniai ir kiekybiniai nuoplovų cheminės sudėties skirtumai. Iš skirtumus lemiančių 55 cheminių medžiagų 38 (69%) būdingos tik vienai lyčiai, o 17 (31%) – abiems lytims, tačiau skiriasi jų kiekiai. Tik patinams būdingos 27 medžiagos: 14 šakotų sočiųjų (C_{11}–C_{19} ir C_{24}–C_{36}), 2 nešakoti (undekanas ir dodekanas) ir 1 ciklinis sotusis angliavandeniliai, 2 riebalų rūgščių esteriai, 4 kiti esteriai, po 1 alkoholį, aldehidą, nitrilą ir nenustatytos sudėties cheminę medžiagą. Tik patelėms būdinga 11 medžiagų: 8 riebalų rūgščių esteriai, 1 kitas esteris ir 2 šakoti sotieji angliavandeniliai (C_{26} ir C_{32}). Kiekybiniai kutikulės cheminių medžiagų skirtumai aptikti tarp šakotų ir nešakotų angliavandenilių, policiklinių junginių, riebalų rūgščių ir jų esterių bei kitų esterių. Gauti duomenys pravers *W. equina* lytinių feromonų paieškai.

Raktažodžiai: kutikulės angliavandeniliai, potencialūs lytiniai feromonai, prieškopuliacinis elgesys