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Isolation and characterization of sex specific cuticular hydrocarbons in non-*Drosophila, Phorticella striata* (Sajjan and Krishnamurthy, 1975) (Diptera, Drosophilidae)

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ABSTRACT: Cuticular hydrocarbons are the major organic compounds synthesized by oenocytes. The CHCs act as sex pheromones, cell communicators, inhibits dissection, and chemotaxonomic cues. The CHCs were isolated from non-*Drosophila* species *Phorticella striata* (Sajjan and Krishnamurthy, 1975) (Diptera, Drosophilidae) by employing whole body extract through GCMS method. Among the identified organic compounds, a total of 38 compounds were obtained from male flies and 68 organic compounds were isolated from female flies, Majority of the CHCs identified were methyl branched alkanes, some of them were esters and alcohols. Male flies exhibit 15 specific CHCs in which tetradecane, 2, 6, 10-trimethyl was dominant with a relative abundance (15.60%) and cyclopropane tetradecanoic acid was the least found CHCs with a relative abundance (RA) of 0.75 per cent. The flies exhibit 28 female specific CHC's, among them tetradecane was dominant (r value of 19.20%) and Iodomethyl undecane was the least (RA=0.70%). Both male and female flies shared 23 CHCs, but the ratio among them showed great variance. Among the shared CHCs 3-Trifluoroacetoxy dodecane was dominant in female (RA=14.50%), the male exhibited RA of 4.66 per cent. In male flies 3-Trifluoroacetoxy pentadecane was most abundant CHC (RA=10.90%), than female (4.31%). © 2023 Association for Advancement of Entomology

KEY WORDS: Alkanes, GCMS analysis, relative abundance, specific CHCs, tetradecane

The cuticle is the integral part of the exoskeleton of members of the Insecta, Crustaceans, Arachnids, and Myriapoda of phylum Arthropoda. In insects the CHC's acts as chemical signals in inter-specific, inter-colonial, and inter-individual recognition. The role of cuticular hydrocarbons as the pheromones in *Drosophila* was studied way back in 1970s, by Ferveur (1997). The semi-chemical or chemical signal functions of CHCs are attributed to sex attractants, aphrodisiacs, defence secretions, territory marking, species and caste recognition cues, recruitment, kairomones and alarm pheromones (Howard and Blomquist, 1982; Antony *et al.,* 1985; Marisa *et al.,* 2010; Nemoto *et al.,* 1994; Kather *et al.,* 2011). The relative levels of CHCs in the *D. melanogaster* and *D. simulans* male and female flies were described by Ferveur (1997). The CHCs are chemotaxonomic tool for identification of species and show great divergence among different species (Bernier *et al.,* 1998). There are information available regarding the kinds of CHC's in the genus *Drosophila*. Our goal was

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to explore the types of CHC's in non-*Drosophila Phorticella striata* (Sajjan and Krishnamurthy, 1975) (Diptera, Drosophilidae).

Collection for CHCs profiling: *Phorticella striata* belongs to *Non-Drosophila* genera, the wild *P. striata* were collected from the Tumkur district of Karnataka and maintained in lab to obtain first generation. A total of 300 *P. striata* files were selected from F1 generation with an equal ratio of male to female flies (1:1 ratio). The adult flies with the almost same size and same growth stages were selected and anomalous flies with many variations are discarded in the collection stage only.

Extraction of CHCs using non-polar solvent (Whole body extract): The collected files were directly brought to the laboratory and immobilised using a chill treatment for 5 min at -18 °C. The 50 male and 50 female in three replicates flies were immobilized ("18°C) and then transferred to a desiccators and purged with nitrogen gas to remove surface moisture for 05min. Then the flies groups were transferred to individual 50ml rimmed glass test tubes containing 25ml pure n-hexane and vortexed for 15min to extract CHCs and care taken to keep flies intact without any physical damage or rupture. The extract was filtered off flies and extract was dried with help of nitrogen gas flow. The samples were reconstituted in the minimal amount of n-hexane and made ready for the GC-MS analysis (Antony *et al.,* 1985). Further, the analysis was carried out within 24 hours of post-collection of CHCs (Figs. 1 and 2).

Analysis of CHCs profile in male and female flies: The CHCs were analyzed using a Pekin Elmer Gas Chromatograph Clarus 680 with flame ionization detector (FID coupled with Pekin Elmer Mass Spectrometer Clarus SQ 8C) (PerkinElmer, Inc., Massachusetts, United States) for quantification. Sample of 2 ìl was injected to column Pekin Elmer: Elite-5MS column-30 m long × 0.250 mm id × 1 ìm, (60-350C) with a split mode of 10:1 under a constant Helium gas flow at the flow rate of 2ml min^{-1}.

CHCs profile of adult male and female *P. striata*: A total of 38 different CHC's molecules were observed in the adult male *P. striata* flies. The total 38 organic compounds were obtained in which 18 compounds belong to hydrocarbons and majority of them were methyl branched alkanes. Twenty of them belong to alcohols and the hydrocarbon esters. The tetradecane, 2,6,10-trimethyl were the most abundant CHCs in adult male *P. striata* with a relative abundance (RA) of about 16 per cent, followed by 3-trifluoroacetoxypentadecane and Octadecane, 6-methyl- with RA of about 11 and 10 per cent respectively. About 27 CHCs were present in the range of 5 to 1 per cent. About 7 compounds were less than 1 per cent and cyclopropane tetra decanoic acid and 3-trifluoro acetoxy tetradecane were the least present CHC's with a RA of 0.75 per cent (Table 1).

Similarly in female adult flies a total of 68 compounds were obtained. Among these compounds two compounds were inorganic compounds namely Hydroxylamine (RA 1.24%) and Silane (RA 1.08%). Among the identified 66 CHCs, the tetradecane, 3-trifluoroacetoxydodecane were the most abundant CHCs in adult male *P. striata* with a RA of about 19 and 14% respectively, followed by 3-trifluoroacetoxydodecane and 2,6,10-Trimethyl-3-oxo-12-(tetrahydropyran-2-yloxy)-dodeca-6,10-dienoic acid, methyl ester with about 10 and 8 per cent respectively (Table 2). About 51 CHCs were present in the range of 5 to 1 per cent. About 11 compounds were less than 1 per cent and 3-heptafluorobutyroxypentadecane and 4-(Prop-2-enoyloxy) tetradecane were the least present CHCs with RA of 0.70 per cent. Male and female adult flies shared 23 CHC's common to both sexes. The Octadecane, 6-methyl, 3-Trifluoroacetoxypentadecane, and 3-Trifluoroacetoxydodecane were abundantly common in both sexes with RA of more than 4 per cent in both sexes. Amount the 23 common CHCs, 13 compounds were relatively high in female flies than the male files, 4 compounds were relatively high in male than the female and 6 compounds were present almost equal ratio in both sexes. Collectively as from this study it can be observed that most of the compounds are alkanes and its derivatives. Out of total annotated CHCs observed in both male and

female flies about 45 compounds are alkanes and its derivatives with a contribution more than 60 per cent to total CHCs diversity observed in the experiments.

Jallon (1984) described 7-Tricosene, 7-pentacosene, 7,11-Pentacosadiene and 7,11-nonacosadiene as potential pheromones in *Drosophila* spp., (Z)-7-Tricosene in *D. virilis* (Oguma *et al.,* 1992). But 3-Trifluoroacetoxypentadecane, 3-Trifluoro-acetoxypentadecane and Octadecane, 6-methyl- are the three major CHCs observed in *P. striata*. Due to multivariate nature of the CHCs, smallest alteration of individual CHCs profile dramatically alter the overall composition profile of CHCs during mate selection (Sharma *et al.,* 2012). From the present study, it is evident that the cuticular profile of male and female flies of *P. striata* is unique and implies the species identification, growth stage, or age identification. The shared common CHCs can act as a species-specific biomarker for the identification of the *P. striata* flies. Some of the identified CHCs compounds were unique and are not naturally reported elsewhere in the literature. In several studies the cuticular hydrocarbons of the genera *Drosophila* exhibits all kinds of hydrocarbons viz. alkanes, alkenes and alkynes (Drijfhout *et al.,* 2010; Kather *et al.,* 2011), Since it is *non-Drosophila*, the *P. striata* exhibits only the alkanes. Methods employed in the study can pave the way for the profiling of CHCs in *P. striata* in different stages of the growth and at different ecophysiological conditions.

Table 1. Male specific CHCs and its relative abundance (RA) in adult *P. striata*

No.	CHCs - Compound name	Molecular formula	RA in %
1	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	15.60
2	Decane, 2,3,5,8-tetramethyl	$C_{14}H_{30}$	3.02
3	Dodecane, 4,6-dimethyl	$C_{14}H_{30}$	2.43
4	Hexadecane, 2,6,11,15-tetramethyl	$C_{20}H_{42}$	1.56
5	Tridecane, 4-methyl	$C_{14}H_{30}$	1.56
6	Undecane, 2-methyl	$C_{12}H_{26}$	1.56
7	Decane, 2,4,6-trimethyl-	$C_{13}H_{28}$	1.38
8	Tetradecane, 5-methyl	$C_{15}H_{32}$	1.38
9	Eicosane, 10-methyl	$C_{21}H_{44}$	1.23
10	Dodecanoic acid	$C_{12}H_{24}O_2$	1.17
11	1-Trifluorosilyltridecane	$C_{13}H_{27}F_3Si$	1.13
12	9-Octadecen-12-ynoic acid	$C_{19}H_{32}O_2$	1.13
13	3,6-Octadecadiynoic acid	$C_{18}H_{28}O_2$	0.82
14	3-Cyclopropylcarbonyloxytridecane	$C_{17}H_{32}O_2$	0.78
15	Cyclopropanetetradecanoic acid	$C_{17}H_{32}O_2$	0.75

Table 2. Female specific CHCs and its relative abundance (RA) in adult *P. striata*

No.	CHCs - Compound name	Molecular formula	RA in %
1	Tetradecane	$C_{14}H_{30}$	19.20
2	6-Dimethyl(trimethylsilyl)silyloxytetradecane	$C_{19}H_{44}OSi_2$	3.12
3	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	2.70
4	Dodecane	$C_{12}H_{26}$	2.58
5	5-Dimethyl(trimethylsilyl)silyloxytridecane	$C_{18}H_{42}OSi_2$	2.39
6	2-Bromo dodecane	$C_{12}H_{25}B$	2.18
7	2-Methyltetracosane	$C_{25}H_{52}$	2.11
8	4-trimethylsilylcyclopentane	$C_{16}H_{30}Si$	1.95
9	Tetracosane	$C_{24}H_{50}$	1.78
10	Octacosane	$C_{28}H_{28}$	1.64
11	1-Octadecanamine, N-methyl- 12. 2(1H)-	$C_{19}H_{41}N$	1.61
12	Quinoxalinone	$C_8H_6N_{2O}$	1.61
13	2H-1-benzopyran-2-amine	C_9H_9NO	1.49
14	1-Hexadecanol, 2-methyl-	$C_{16}H_{34}O$	1.34
15	Z, Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	1.34
16	2-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	1.19
17	Decane	$C_{10}H_{22}$	1.19
18	Docosane	$C_{22}H_{46}$	1.19
19	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	1.10
20	Dodecanal	$C_{12}H_{24}O$	1.05
21	Undecane	$C_{11}H_{24}$	1.05
22	3-Heptafluorobutyroxydodecane	$C_{16}H_{25}F_7O_2$	0.82
23	3-Trifluoroacetoxytridecane	$C_{15}H_{27}F_3O_2$	0.82
24	4-Trifluoroacetoxytridecane	$C_{15}H_{27}F_3O_2$	0.82
25	4-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	0.73
26	5-(Prop-2-enoyloxy) pentadecane	$C_{18}H_{34}O_2$	0.73
27	4-(Prop-2-enoyloxy) tetradecane	$C_{17}H_{32}O_2$	0.70
28	Iodomethylundecane	$C_{12}H_{12}I$	0.70

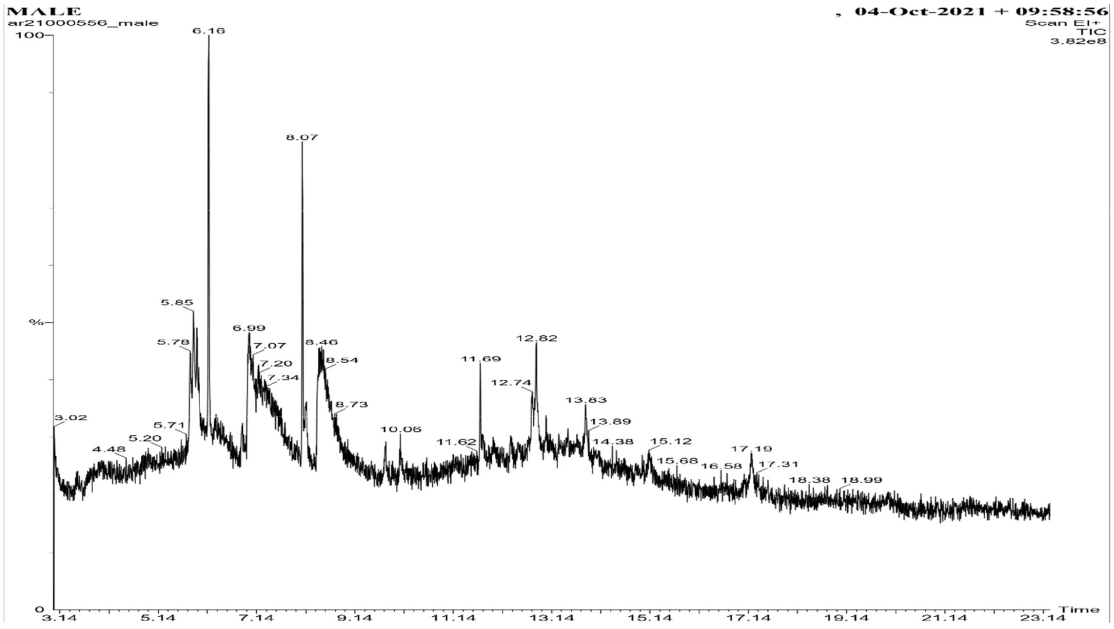

Figure 1 : The GC-MS chromatogram of the adult male *P. striata.* (X-axis- Retention time, Y axis – Relative Abundance)

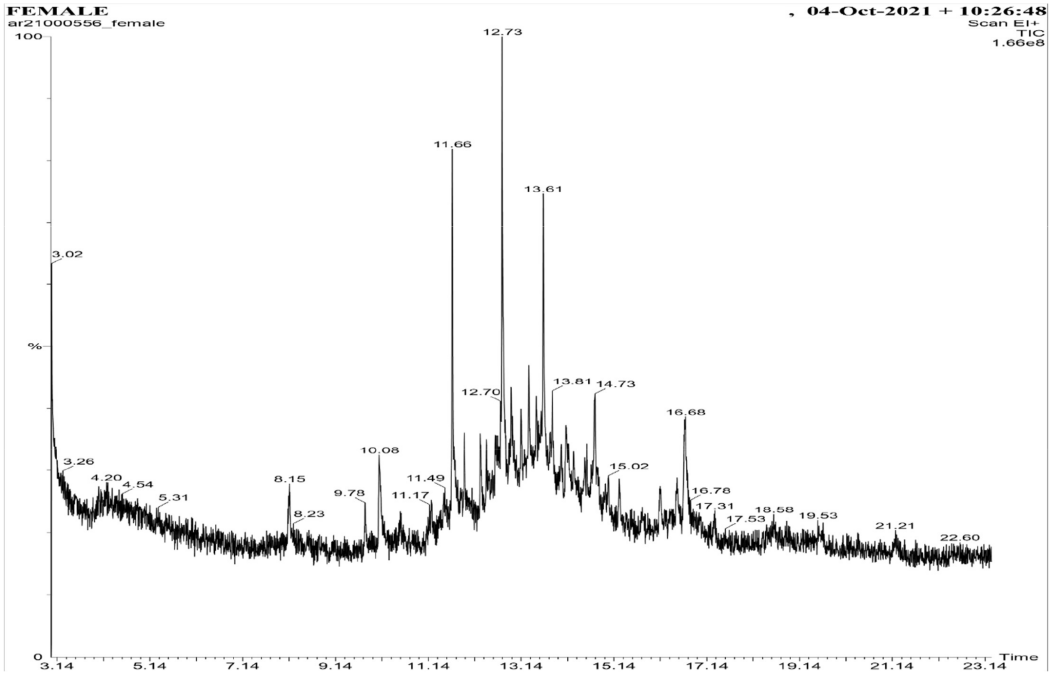

Figure 2: The GC-MS chromatogram of the adult female *P. striata.* (X-axis- Retention time, Y axis – Relative Abundance)

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