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CHARACTERIZATION OF DOMINANT CUTICULAR HYDROCARBONS IN INVERSION AND INVERSION-FREE STRAINS OF *DROSOPHILA ANANASSAE* (DOLESCHALL)

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ABSTRACT

Cuticular hydrocarbons (CHCs) of *Drosophila ananassae* (Doleschall) was characterized and identified using gas chromatography and mass spectrometry (GC-MS) analysis. A high % of methyl-branched alkanes were identified in all inversion (2LA, 3LA and 2LA+3LA) and inversion-free strains followed by linear alkanes and alkenes. The present work unfolds the significant patterns of variations in the isomeric forms of methylated alkanes between the inversion and inversion free strains (F-46.6; df-3, p 0.005), and non-significant between the sex (F-2.14; df-1, p 0.2394). But in linear alkanes shows significant variation between the inversion strains (F-30.49; df-3, p 0.009) and between the male and female (F-115.45; df-1, *p* 0.001) was observed. In particular there is a significant correlation between the chromosomal inversion and synthesis of CHCs in *D. ananassae*. Unique blend of CHCs in *Drosophila* performs dual role as desiccation resistance and act as chemical signalling molecule. Linear alkanes are majorly involved in desiccation resistance but in methyl- branched CHCs length variation is a key determinant of desiccation resistance. Presence of longer methyl- branched alkanes and higher desiccation resistance, shorter the carbon chain length act as a signalling molecules. The current study revealed the influence of chromosomal inversion on the cuticular hydrocarbon profile in *D. ananassae*.

Key words: *Drosophila ananassae,* alkenes, CHCs, chromosomal inversion, chemical cues, desiccation resistance, GC-MS, inversion free, linear alkanes, methylated alkanes, length variation, carbon chain length

In insects specialized oenocyte cells synthesize the epicuticular lipids. The primary constituents of epicuticular lipids are hydrocarbons which transported through lipophorin proteins coats on the outer epiculticular waxy layer known as cuticular hydrocarbons (CHCs) (Lockey, 1988). CHCs profile displays a wide range of complex mixtures of straight-chain alkanes, branched alkanes with one or more methyl group (methyl-branched alkanes) and unsaturated alkenes. There are additional constituents such as alkyl esters, fatty acids, long-chain alcohols, glycerides, sterols, ketones, and aldehydes are also present in insects. In *Drosophila* and other insects the chain length range in between 21 to 50 carbons and linear -alkanes are one of the major abundant CHCs class followed by methyl-branched alkanes (Blomquist, G. J., and Ginzel, M. D., 2021; Holze, et al., 2021). CHCs are species-specific and serve multiple functions. The primary role of CHCs is protecting the insects from environmental stress and also evolved secondary functions as chemical signals (Hatano et al., 2019; Pardy et al., 2019). n-alkanes are majorly involved in desiccation resistance increasing the chain length strengthens van der Waals forces with high melting points leads to stability. Methyl-branched alkanes are second significant with constituents of the insects CHCs profile, with methyl groups on the 2-, 3-, 4-, and 5-position.

In *Drosophila* the use of methyl-branched alkanes for modulating beyond water proofing have evolved with functions as chemical cues. Moreover, a methyl branch can reduce the melting point because these branched compounds do not pack as tightly as n-alkanes. The addition of double bonds and methyl branches leads to increased structural complexity and encodes diverse signal information like colony and nest mate recognition, act as sex pheromones, mate choice, as antiaphrodisiacs and kairomones (Wilkes, 2020; Wang et al., 2022). While the CHCs involved in chemical communications are more diverse and have the potential for high information content, but their low melting points reduce their water proofing potential. The current research work focused on the presence of selective class of hydrocarbons like linear alkanes and methylated alkanes, which are predominating types of hydrocarbons, showing the significant variations in inversions and inversion-free strains of *Drosophila ananassae* (Doleschall).

MATERIALS AND METHODS

For the study, flies were collected from outbreed population of Mysuru city, Karnataka, India (Hegde et al., 1999). The male flies were used for species identification (Sturtevant, 1926) and female flies were used to establish the fly stocks. *D. ananassae* is a cosmopolitan and domestic species and a member of melanogaster group. About 20 females of *D. ananassae* were individually placed in a vial for the establishment of the iso-female line. After the appearance of third instar larvae, 8 to 10 larvae were collected from each vial and identify the different inversion in polytene chromosome (Ashburner, 1989). When all eight larvae carried inversion loop in left arm of chromosome 2 (2LA), left arm of chromosome 3 (3LA) and inversion in both the left arm of chromosome 2 and 3 (2LA+3LA) are individually designated as particular inversion carrying strains. Absence of inversion loop is designated as inversion free strains. These strains were separately maintained up to twelve generations to establish the inversion strains and inversion-free strains of *D. ananassae* (Jayaramu, 2009).

Male and female flies were separated immediately after eclosion to avoid random mating. After six days 50 virgin male and female flies of 2LA, 3LA, 2LA +3LA, and inversion-free strains were immobilised (-18°C) and transferred to a desiccator and purged with nitrogen gas to remove surface moisture for 5 min. Then each strain of flies was transferred separately in to 50 ml rimmed glass test tube containing 25 ml pure n-hexane and kept for about 15 min. The extract was filtered off flies and then dried with the help of nitrogen gas flow. The samples were reconstituted in a minimal amount of n-hexane and made ready for the GC-MS analysis. The extracted CHCs were analyzed using Pekin Elmer Gas Chromatograph Clarus 680 with flame ionization detector (FID coupled with Pekin Elmer Mass Spectrometer Clarus SQ 8C) (Perkin Elmer, Inc., Massachusetts, United States) for quantification. 2 µl Sample was injected to column Pekin Elmer: Elite-5MS

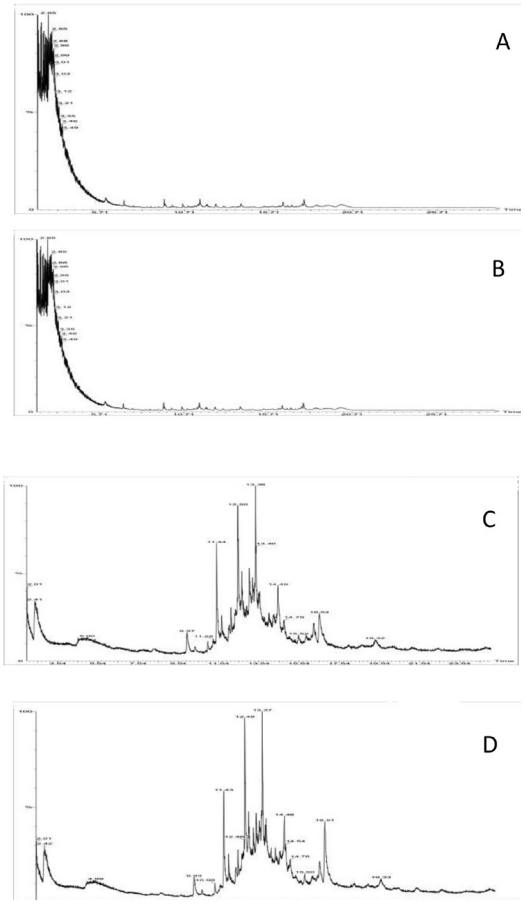

Fig. 1. GC- MS Chromatogram of 5-6 days old male whole body extraction in hexane: A-Inversion free strain; B-2LA; C-3LA; D-2LA+3LA

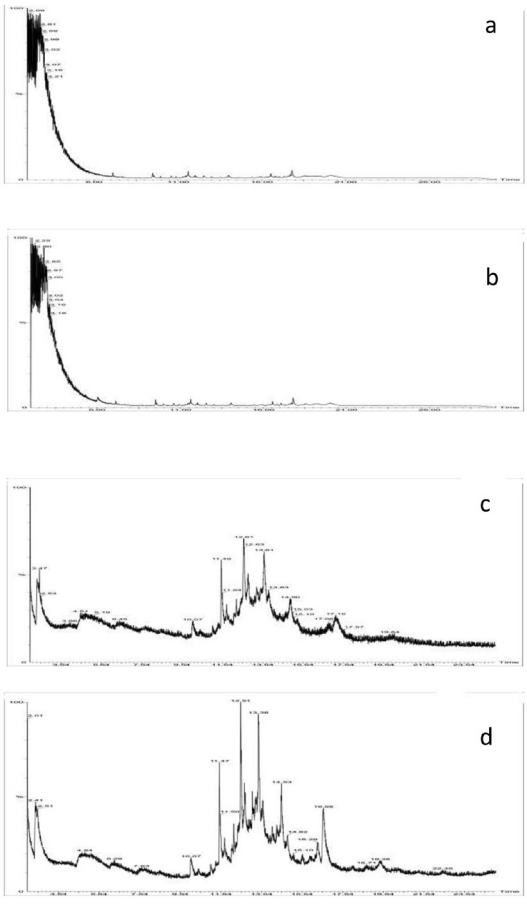

Fig. 2. GC- MS Chromatogram of 5-6 days old female whole body extraction in hexane: a-Inversion free strain; b-2LA; c-3LA; d-2LA+3LA

Table 1. N-alkanes, mono, di, tri, and tetra-methylated alkanes in inversion and inversion-free strains of male and female *D. ananassae*

Types of alknaes	Male				Female			
	IF	2LA	3LA	2LA+3LA	IF	2LA	3LA	2LA+3LA
n-alkanes	n-C16 n-C17 n-C27	n-C14 n-C16 n-C17 n-C20 n-C21 n-C27 n-C28	n-C16 n-C17 n-C19 n-C20 n-C21 n-C27 n-C28	n-C16 n-C17 n-C19 n-C20 n-C21 n-C27 n-C31	n-C27	n-C16 n-C19 n-C27 n-C28	n-C13 n-C16 n-C19 n-C27	n-C20 n-C27 n-C31 n-C44
Mono methylated alkanes	3 Me,C12 2 Me, C18 2Me, C19 2Me,C20 4Me, C22	2Me, C13 2Me, C11 2 Me, C18 2Me, C19 2Me,C20 2Me, C28 6Me, C18	2Me, C12 2Me, C15 2 Me, C18 6Me, C18 2Me, C19 2Me,C20 2Me,C24 2Me, C26 2Me, C28	2Me, C11 2Me, C12 4 Me,C13 2 Me, C18 6Me, C18 2Me, C19 2Me,C20 2Me, C26 2Me, C28	2Me, C13 2 Me, C18 2Me, C15 2Me,C20	2 Me, C18 2Me,C20 2Me, C19 2Me, C28	2Me, C11 2Me, C12 2 Me, C18 6Me, C18 2Me, C19 2Me,C20 2Me, C26	2Me, C13 2 Me, C18 6Me, C18 2Me, C19 10Me,C19 2Me,C20 2Me,C24 2Me, C26 2Me, C28
Di methylated alkanes	2,9 Di-Me,C11	3,8Di-Me,C11	3,7 Di-Me,C10 3,8Di-Me,C11	2,10 Di-Me,C11 4,6 Di-Me,C12 2,6 Di-Me,C17	2,3 Di-Me, C5	2,2 Di-Me C8	2,9 Di-Me,C10 3,8Di-Me,C11	2,6 Di-Me,C17
Tri methylated alkanes	2,6,8 Tri-Me,C10 2,6,10 Tri-Me,C14	2,6,11 Tri-Me,C12 2,6,10 Tri-Me,C12 2,6,10 Tri-Me,C14	2,6,11 Tri-Me,C12 2,6,10 Tri-Me,C12 2,6,10 Tri-Me,C14	2,4,6 Tri-Me,C8 2,6,10 Tri-Me,C12 2,6,10 Tri-Me,C14 3,2,24 Tri-Me C40	2,2,3 Tri-Me,C9 2,6,10 Tri-Me,C14	2,2,9 Tri-Me,C10 2,2,6 Tri-Me,C10 2,2,3 Tri-Me,C10 2,6,10 Tri-Me,C14 2,6,11,Tri-Me C12	2,4,6 Tri-Me,C10 2,6,10 Tri-Me,C12 2,6,10 Tri-Me,C14 2,2,7 Tri-Me,C10	2,4,6 Tri-Me,C10 2,6,11 Tri-Me,C12 2,6,10 Tri-Me,C14 2,6,10 Tri-Me,C12 3,2,24 Tri-Me C40
Tetramethylated alkanes	2,6,11,15 Tetra Me-C16 2,6,10,15 Tetra Me-C17 2,6,10,14 Tetra Me-C17	2,3,5,8 Tetra Me-C10 2,6,11,15 Tetra Me-C16 2,6,10,14 Tetra Me-C16 2,6,10,15 Tetra Me-C17 2,6,10,14 Tetra Me-C17	2,3,5,8 Tetra Me-C10 2,6,11,15 Tetra Me-C16 2,6,10,14 Tetra Me-C16 2,6,10,15 Tetra Me-C17 2,6,10,14 Tetra Me-C17	2,3,5,8 Tetra Me-C10 2,6,11,15 Tetra Me-C16 2,6,10,14 Tetra Me-C16 2,6,10,15 Tetra Me-C17 2,6,10,14 Tetra Me-C17	2,3,5,8 Tetra Me-C10 2,6,11,15 Tetra Me-C16 2,6,10,15 Tetra Me-C17	2,3,5,8 Tetra Me-C10 2,6,11,15 Tetra Me-C16 2,6,10,15 Tetra Me-C17	2,3,5,8 Tetra Me-C10 2,6,11,15 Tetra Me-C16 2,6,10,15 Tetra Me-C17 2,6,10,14 Tetra Me-C17	2,3,5,8 Tetra Me-C10 2,6,11,15 Tetra Me-C16 2,6,10,15 Tetra Me-C17 2,2,7,7 Tetr-Me, C8 2,2,3,4 Tetra-Me,C5 2,6,10,14 Tetra Me-C17 2,6,10,14 Tetra Me-C16

column-30 m long x 0.250 mm id x 1 µm. (60-35°C) with a split mode of 10:1 under a constant Helium gas flow at flow rate of 2 ml/ min. The GC operating conditions were as follows: Initial temperature was 80°C and initial hold time of 2.00 min with ramp rate of 10.0/ min to 150°C and hold time of 1.00 min., and second rap rate setup of 15.0/ min to 150°C and hold time of 10.00 min. The MS instrument was operated with inlet temperature of 250°C and ion source temperature of 230°C with operating voltage of 70eV. The reference library employed for the identification of CHCs compounds was Wiley 7th/ NIST 05.

RESULTS AND DISCUSSION

In inversion and inversion free strains of *D. ananassae* four major classes of CHCs were isolated and comprises of n-alkanes, methylated-alkanes, haloalkanes, and alkenes in both the sexes (Table 1). Present study emphasizes on the two predominant groups of alkanes namely methyl branched alkanes (mb-alkanes) and linear alkanes (n-alkanes). In many *Drosophila* species the linear n-alkanes are large proportion of total hydrocarbons in the wax layer. The CHCs found in the melanogaster subgroup range from 23 to 29 carbons in chain lengths. But in *D. mojavensis* (Etges et al., 2001) revealed that the most abundant hydrocarbons with chain lengths between 28 and 40 carbons. The linear n-alkanes are closely packed and highly hydrophobic and when longer the chain length, increasing the melting temperatures, and potentially better barriers against water loss (Wang et al., 2022). But in *D. ananassae* all inversion strains carbon chain length in n-alkanes ranges from 14 to 31 in males and 13 to 44 in females. The longest n-alkane is tetratetracontane (n-C_{44}) present in 3LA and 2LA+ 3LA inversion strains of female. *D. ananassae* exhibits a sexual dimorphism in desiccation resistance, with females showing greater resistance than males.

The mono-, di-, tri-, and tetra- methylated-alkanes are present in both male and female flies. The mono-methylated alkanes occur in different isomeric forms but 2-Me-alkanes are present as predominating type in both the sexes of *D. ananassae.* In *D. melanogaster* and *D. virilis* have high proportions of 2-Me-alkanes are characteristic of the *Drosophilidae* (Jackson and Bartelt, 1986). In insects shorter Me-alkanes such as 2MeC26 and 2MeC28 are used as signalling molecules. In *D. serrata*, male having higher amounts of 2MeC26 leads to higher mating success (Chung et al., 2014). The present study ascertains the 3, 2, 24 tri-Me-alkanes of C_{40} are the longest carbon containing methylated hydrocarbons in 2LA+3LA double inversion strains (Table 1); *D. mojavensis* and *D. arizonae*, with higher quantities of 2MeC30 and an even longer mbCHC, 2MeC32, are observed. Significant correlation is observed with longer mbCHCs and higher desiccation resistance in the *Drosophila* genus (Wang et al., 2022). The unique blends of methylated alkanes in *Drosophila* has dual role as desiccation resistance and as a chemical signalling molecule. In the present study the variations in the class of CHCs in inversion and inversion free strains of *D. ananassae* is subjected to two-way ANOVA using statistical software SPSS. The results showed that the significant variations in the number of n-alkanes (F-46.70; df-3, p 0 .005) and Me-alkanes (F-30.46; df-3, p 0.009) between the inversion and inversion- free strains. But in case of sex, n-alkanes shows significant variation (F-115.45; df-1, p 0.001) and non-significant variation was observed in methylated-alkanes (F-2.14; df-1, p 0.2394). The present work reveals the interrelationship between the chromosomal inversions on the synthesis of CHCs blend in *D. ananassae* (Fig. 1, 2).

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Kavitha R S is the author and did complete research work under the guidance of Prof. Jayaramu S C.

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No conflict of interest.

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