

The Role of Cuticular Hydrocarbons in Forensic Entomology: A Mini Review

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ABSTRACT

The cuticular hydrocarbons (CHCs) are expressed on insect cuticle and provide protection from desiccation and abrasion. The CHC profiles are species-specific and vary in each insect. Forensic entomologists have utilized varying expressions of CHCs to determine the age as well as to delimit insects. This review focuses on the role of CHCs in species identification and aging in the field of forensic entomology.

KEYWORDS: cuticular hydrocarbons, forensic entomology, blow flies, age determination, species delimitation, PMI calculations

INTRODUCTION

The cuticular hydrocarbons (CHCs) are expressed on insect cuticle along with other compounds such as esters, alcohol, and free fatty acids [1]. The waxy layer protects insect from desiccation, abrasion, and for chemical communication [2]. The CHC compounds occur in all life stages of insects and are biologically stable [1]. Their synthesis is genetically controlled but is influenced by diet, sex, developmental stages, or temperature [1,3,4,5]. The CHC layers are overly complex and are composed of 100 different compounds in a single insect species [6]. The insect hydrocarbons are long linear molecules with varying chain lengths. Generally, it is a mixture of saturated compounds such as n-alkanes, mono-, di- or tri-methyl alkanes (with one to three methyl groups) or unsaturated compounds like alkenes, alkadienes and more rarely methyl-branched alkenes [7]. It has been observed that environment and internal factors shape the CHC profiles of insects. The insects are plastic towards their CHC profiles with changes in environment [7,8]. To prevent desiccation, insects should possess long chain alkenes or n-alkanes compounds but on the other hand, for communication,

insects require the CHCs to be transferable so that these could be easily taken up by other insects in the vicinity [3]. Thus, insect CHC profile is constrained by these conflicting requirements. To maintain homogenous CHCs, the insect must trade-off high viscosity for waterproofing against low viscosity for substance diffusion [7,9].

The CHC biosynthesis is co-opted from fatty acid synthesis pathway in insects. Cytosolic fatty acid synthase and microsomal fatty acid synthase are both responsible for the synthesis of different cuticular hydrocarbons. All the CHCs share a common biochemical pathway but evolutionary changes in the genes in these pathways are responsible for diverse CHC profiles found in insects [3]. Moreover, diet also affects the CHC blends in insects. It has been observed that different food sources provide different amino acids as precursors for the formation of different fatty acids and CHCs [10].

Cuticular hydrocarbons (CHCs), apart from preventing insect from desiccation and mate recognition, are responsible for adaptation and speciation [3]. It has been proposed by scientists that

traits that help in ecological adaptation of species as well as in sexual selection could lead to speciation [3,11]. There is enough species-level evidence in the case of blow flies and other insect groups to prove that CHCs are the magic traits which play an important role in their survival, reproduction, and speciation (i.e., divergent evolution) [3,11].

Forensic entomologists use developmental stages of necrophagous insects such as blow flies or flesh flies to decipher the post-mortem interval (PMI) of a corpse. A plethora of methods exist to calculate the post-mortem interval with the help of forensically important insects [12]. One such method is to study the CHC profile of insects across different developmental stages and to compare these findings with the other necrophagous insect species [13]. As the cuticular hydrocarbon profiles are not static and change with each development stage (i.e., from egg to adult), therefore, scientists are attempting to develop a model that could be used in estimating the age of larvae or pupae and thus increasing the accuracy of PMI calculations. This review focuses on the role of CHCs in species delimitation and aging in the field of forensic entomology.

CHCs in Species Delimitation

Taxonomic characters fulfil a set of criteria that makes them good indicators of reproductive isolation [14], and the species specific CHCs fits this description. Thus far, thousands of CHC profiles have been generated but no two profiles have been found to be similar [15]. It is believed that the composition of CHC compounds vary quantitatively as well as qualitatively [15]. The presence or absence of CHC compounds is related to the presence, absence, or kinetics of the biosynthetic enzymes [16]. Taking cue from the above findings, Roux et al., [17] analysed the CHC profiles of three species of blow flies (Calliphoridae) namely *Calliphora vomitoria* (Linnaeus), *Calliphora vicina* (Robineau-Desvoidy) and *Protophormia terraenovae* (Robineau-Desvoidy) during ontogeny (egg to eight-day old adult) using Gas Chromatography. A total of 83 compounds were discriminated from 745 specimens, of which some were widespread in all the species while few were species-specific. They observed quantitative

changes in the CHCs between the wild type and the laboratory strain of *C. vicina*, but not in the quality of the compounds. Ye et al., [18] analysed CHC profiles extracted from exuviae of six necrophagous fly species namely, *Aldrichina grahami* (Aldrich), *Chrysomya megacephala* (Fabricius), *Lucilia sericata* (Meigen), *Achoetandrus* (= *Chrysomya*) *rufifacies* (Macquart), *Boettcherisca peregrina* (Robineau-Desvoidy), and *Parasarcophaga crassipalpis* (Macquart). Gas chromatography-mass spectrometry (GCMS) technique was used to generate CHC profiles. The discriminant data analysis revealed variations in eight spectral peaks for compounds tricosane, 9-,11-,13-methyl-pentacosane; 11,12-,9,13-dimethyl-heptacosane; octocosane; 7,11-dimethyl-nonacosane, 3-methyl-nonacosane, 2-methyl-hentriacontane and one unidentified compound, for the six fly species taken for analysis. Hence, distinct GC patterns in CHC composition and discriminant analysis resulted in correct species identification.

Sexual dimorphism at the level of CHCs has been studied in different families of order Diptera, but their role in mate recognition and as sexual attractant is a matter of debate. Three species of forensically important blow flies were tested for sex specific CHCs as sexual attractants namely *Cochliomyia hominivorax* (Coquerel) [19], *Phormia regina* Meigen [20] and *Chrysomya varipes* (Macquart) [21] and results showed that CHCs do not appear essential for sexual attraction. Later, Butterworth et al., [11] studied the CHC profiles of 10 species of the genus *Chrysomya* and investigated the intra and inter-specific variation in the CHC profiles of these species. The team observed sex-specific and species-specific variation in the CHC profiles of the studied species. As mating is controlled by males in blow flies so it becomes imperative for males to identify their conspecific during mid-flight. Thus, to avoid the cost of hybrid mating, males need to differentiate between the odour of conspecific from that of heterospecific female [11]. On the other hand, conspecific females need to develop unique CHC profiles so that these could be easily identified by the conspecific males. They suggested that CHCs have diverged in a punctuated and non-phylogenetic manner in blow flies due to saltational changes during speciation events. It has been suggested that unlike

gradualism, saltational changes have been seen in organisms which are undergoing adaptive radiations. In case of blow flies, CHCs have diversified rapidly and thus have played important role in the evolution of this group. It is likely that ecological and sexual selection must have shaped the diversification of CHCs. Therefore, species-specific, sex-specific hydrocarbon compounds are one of the best tools to delimit species boundaries and help forensic entomologist to solve criminal cases.

Age Estimation of Blow Flies using CHCs

At a typical crime scene, forensic entomologists generally encounter immature stages (egg, larvae, or pupae) to estimate minimum Post-Mortem Interval (mPMI) of a body [12,22]. For this, the investigator compares the developmental stages with the known life history pattern of the collected specimen and makes estimates regarding the age range of the insect. The data obtained is used as a surrogate for mPMI estimations [12]. But these estimations based on life history patterns have their own limitations. There are some gaps in the life stages, for instance, the post-feeding stage of the fly larvae or the pre-pupal stage, which could not be resolved based on morphological changes. The post-feeding stage accounts for half the larval stage [22]. Moreover, there is dearth of literature on the identification keys to the immature stages of forensically important insects. In such scenario, CHC profiles of all the stages in an insect's development could come in handy. Zhu et al., [22], who employed GC-MS, worked out the CHC composition in 2.5 to 8 days old larvae of *Chrysomya rufifacies*. The team observed that short chain hydrocarbons dominated the CHC profiles of younger larvae (3 days or less) whereas the long chain hydrocarbons, required for waterproofing, dominated the profiles of older larvae (4 to 8 days). On the same lines, Moore et al., [23] examined the hydrocarbon profiles of the larvae of forensically important blow fly *L. sericata*. The focus of this study was to analyse the variations in non-polar hydrocarbon compounds among different larval stages. They noticed that with age the hydrocarbon profile of larvae changes from short chain hydrocarbons to long chained hydrocarbons that have higher melting points and thus protect the insect from desiccation. On the

other hand, the methyl-branched alkanes and alkenes required for flexibility of the cuticle, have lower melting points. Therefore, hydrocarbon composition of immature larvae (up to 5 days old) comprises of 31% branched and 19% alkenes compared to older larvae which possess only 4% branched alkanes and 8% alkenes. Building on the previous work, Moore et al., [24] extracted the cuticular hydrocarbons of the first instar of three forensically relevant blow flies i.e., *L. sericata*, *C. vicina* and *C. vomitoria*. It was observed that the three species exhibited a similar mixture of n-alkanes, alkenes and methyl branched hydrocarbons but varied quantitatively in the three species. The 1st instars of the three species could be easily distinguished based on CHCs as the qualitative differences in both n-alkanes and methyl branched compounds formed three distinct clusters in Principal Component Analysis (PCA) corresponding to the blow fly species. Similarly, Xu et al., [25] used GC-MS technique to characterize age dependant quantitative changes in the cuticular hydrocarbons of *A. grahmi* larvae. The CHC obtained were a mixture of n-alkanes, methyl branched alkanes, dimethyl branched alkanes, and few alkenes (C21-C31). It was observed that the relative abundance of low molecular weight hydrocarbons (less than C25, including rich pentacosenes and methyl alkanes) predominated in the 3-day old larvae but decreased with age. During later developmental stages an increase in high molecular weight hydrocarbons was noticed which could be positively correlated with improving waterproofing capability of the pupal stages to protect insects against dehydration. The authors pointed out that unlike larval stages, post-feeding, and pupal stages are in direct contact with the environment and to prevent desiccation require varied proportion of different classes of hydrocarbons. These variations in profiles could be used in calculating the age of immature stages. On the same lines, Moore et al., [26] calculated the larval age of two forensically important blow flies *C. vicina* and *C. vomitoria* by observing change in their cuticular hydrocarbon profiles. The change was observed from one day old larvae until pupation under lab conditions. GC-MS was used to obtain chemical profiles of the immature stages and the data set obtained was then analysed statistically as well as with ANN (artificial neural network) approach for pattern recognition and

clustering. The principal component analysis (PCA) and artificial neural networks approach showed test accuracy of 89% (*C. vicina*) and 87% (*C. vomitoria*) for calculating the age of immature stages and hence, emphasized on the role of CHCs in age estimation in insects.

Apart from larval stages, other important stages of forensic value are the pupal stage and empty pupal cases, which are encountered on highly decomposed corpses. Zhu et al., [27] and Zhu et al., [28] studied the impact of weathering on the CHC composition of pupal cases in blow fly *Ch. megacephala*. The authors concluded that the CHC profiles weathered over time. Of 106 CHCs obtained during the 90 days period, approximately 104 decreased in abundance with age. Weathering rate of CHCs depend significantly on the chemical nature and molecular weight of the compound. It was observed that the n-alkanes were the most stable and had the highest residual ratio (RR, negatively correlated with weathering rate) of 31.05%; whereas mono-methyl alkanes had RR of 6.50%. Moreover, weathering of puparial hydrocarbons is influenced by environmental factors such as temperature, humidity, food type, and nutritional status. Bosorang et al., [29] studied the change in the CHC profiles of pupae of *Ch. megacephala* with age. The analysis was conducted on the first five days of pupal development and observed strong correlation to blow fly ages, which were then utilized to create a prediction equation for the pupal age estimation. The age dependant model (prediction of equation) revealed that estimated age of pupae correlated significantly with the chronological age of the samples of *Ch. megacephala*. One of the CHCs, n-Pentacosane (n-C25), was found to decrease with increasing age in *Aedes aegypti* (Diptera: Culicidae) [30] and *Musca domestica* L. (Diptera: Muscidae) [31] and was used to predict age in the above-mentioned species. Moore et al., [32] determined age differences in the empty pupal cases in *C. vicina* and *L. sericata* with the help of cuticular hydrocarbons over a period of nine months. A list of specific hydrocarbon compounds was assembled which contributed immensely to calculating pupal age. Paula et al., [33] examined the chemical composition of 50 puparia of *Ch. megacephala* from different cycles of oviposition using GC-MS. A total of 60 compounds of varying chain

lengths (C18-C34) were obtained of which 38 were common in all three generations of flies. An interesting trend was observed in the chemical composition as well as in the number of compounds from first generation to the third generation. The number of chemical compounds decreased with increase in generations, and this could be attributed to inbreeding, which led to low genetic variability causing homogeneity of cuticular profile of puparia along the generations [33]. Nevertheless, the authors demonstrated the feasibility of separating puparia from different generations on the basis of their CHC profiles and could be used in cases where due to low temperature, the process of decomposition is slow and therefore, blow flies may colonize the carcass multiple times [33].

Bernhardt et al., [34] analysed the fate of n-Pentacosane (n-C25) in two forensically important species (*L. sericata* and *C. vicina*) and their role in age estimations of the adult blow flies. They observed an increase in n-Pentacosane with increase in adult age for the two species. A linear correlation was observed in both the sexes in case of *C. vicina* but females of *L. sericata* produced higher amounts of n-C25 as compared to their male counterparts. The increase in n-C25 could be attributed to their role in waterproofing and in maintaining the water balance in an insect. Moreover, the sex specific quantitative difference in *L. sericata* points towards sexual communication in this species. Pechal et al., [12] investigated the variability of CHC profiles with age (post-emergence) among females of two blow fly species (*Co. macellaria* and *Ch. rufifacies*). A total of 37 compounds were detected from the adults of *Co. macellaria* and 35 from *Ch. rufifacies* aged 1 to 30 days post-emergence. The profiles of the two species were unique and shifted with age. The CHC profiles obtained can be considered as fingerprint chemical profiles and could be used in age estimation as well as in species delimitation. The age estimation of the collected data from the crime scene is of utmost importance for a forensic entomologist and the potential usefulness of this technique should be used for calculating the age of adult insects. Braga et al., [35] quantified CHC profiles of 1 to 5 days old males and females of *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae). The quantitative differences in the relative abundance of CHC with age were observed in

both sexes. For both sexes, some compounds were age exclusive, but this phenomenon was more pronounced in the case of males (n-C29 in females and n-C31 in males).

Chemical communication is the oldest form of communication in living organisms. Insects communicate via pheromones or kairomones which are volatile in nature and are perceived by other insects in the vicinity. Cuticular hydrocarbons, apart from preventing desiccation, also help insects in mating and species recognition [6]. Adaptations to different environments have shaped the composition of CHCs in different insects. Insects can detect small differences in the CHC compound structure with olfactory receptors present on their sensilla. Their olfactory systems are well developed and can differentiate between compounds of the same chain length that vary in position of their double bond, methyl groups, chirality etc [6, 36]. Studies on solitary insects as well as on social insects have demonstrated the role of CHCs in ecological divergence and sexual selection [6,37,38]. Because of dual role of CHCs in insects, scientists have zeroed in on compounds which help in waterproofing as well as in ecological adaptation and sexual selection. It has been established that olefinic and branched hydrocarbons which not only prevents desiccation but also play an important role in sexual communication. These compounds show varied structural diversity across different species. The olefins and branched hydrocarbons also show variations in abundance among sexes of the same species [3].

CONCLUSION

The high CHC diversity in insects is presumed to be the basis of evolution of communication system in insects which when acted upon by natural selection led to speciation. Therefore, it is a potential tool which could be used by a forensic entomologist to delimit species and to calculate age of the immature stages generally encountered on a corpse.

Conflict of Interest

Author declare none.

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