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Puparial case hydrocarbons of *Chrysomya megacephala* as an indicator of the postmortem interval

Guang H. Zhu [a,*], Xiao H. Xu [a], Xiao J. Yu [a], Yuan Zhang [b], Jiang F. Wang [c]

[a] *Department of Forensic Medicine, Shantou University Medical College, Shantou 515031, China*
[b] *Central Labaratory, Shantou University Medical College, Shantou 515031, China*
[c] *Department of Forensic Medicine, Guangdong Police College, Guangzhou 510230, China*

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Abstract

The puparial case is one of the most common 'stages' of necrophagous flies encountered in crime investigations of highly decomposed corpses. If methods for determining the weathering time of these puparial cases are developed, it is possible that the postmortem interval (PMI) could be estimated accordingly. Gas chromatography coupled with mass spectrometry (GC–MS) was used to determine the changes with the weathering time in cuticular hydrocarbons of the puparial cases of *Chrysomya megacephala* in the laboratory. The results have shown that cuticular hydrocarbons of the puparial cases were a mixture of *n*-alkanes, methyl-branched alkanes, and dimethyl-branched alkanes. The carbon chain length ranged from C21 to C35, and the hydrocarbon composition showed significant regular changes with the weathering time. For the even numbered *n*-alkanes with low molecular weight, namely *n*-C22, *n*-C24 and *n*-C26, the abundance increased significantly with the weathering time. For *n*-C26, in particular, a linear increase in abundance with the weathering time was observed. In addition, for most of the other low molecular weight hydrocarbons (*n*-C26 or below), the abundance decreased significantly with the weathering time. It is concluded that, cuticular hydrocarbon is a potential indicator of the weathering time in *C. megacephala*, and possibly in other necrophagous flies, and might further be used to determine the PMI.

Keywords: Cuticular hydrocarbon; Weathering time; Puparial case; Postmortem interval; *Chrysomya megacephala*

1. Introduction

Necrophagous flies, particularly calliphorids, are recognized as the first wave of the faunal succession on human cadavers [1–5]. They are therefore the primary and most accurate forensic indicators of the postmortem interval (PMI).

So far, a number of experimental investigations have been carried out to determine the PMI based on the eggs, larvae, pupae and young adults of flies [6–14], but the potential of the puparial cases for determination of the PMI has not yet been fully realized. We suggest that, if methods for determining the weathering time of the puparial cases are developed, it is possible that the postmortem interval (PMI) could be estimated accordingly.

The outer surface of all insects is covered with a layer of species-specific cuticular lipids, which are frequently composed of a complex mixture of hydrocarbons and function primarily to limit water loss and to serve as pheromones or kairomones [15–22]. Cuticular hydrocarbons of insects are usually comprised of *n*-alkanes, alkenes, terminally and internally branched monomethyl alkanes and polymethyl alkanes [15–22]. These hydrocarbons have different boiling points and different volatility. Considering the differences in volatility and other properties, we predicted that, for puparial cases found at a murder scene, the composition of cuticular hydrocarbons would vary with the weathering time and therefore have the potential for determining the weathering time of the puparial cases and furthermore the PMI.

This report describes the changes that the weathering time had on the cuticular hydrocarbons of the puparial cases of *Chrysomya megacephala* in the laboratory. This work was conducted in order to provide a basis for development of a new method for determining the PMI based on cuticular hydrocarbons of necrophagous fly puparial cases.

* Corresponding author. Tel.: +86 754 8900433; fax: +86 754 8900837.
E-mail address: ghzhu@126.com (G.H. Zhu).

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2. Materials and methods

2.1. Insect materials

The puparial cases of *C. megacephala* were obtained from a colony of insects, which developed from wild eggs collected in the city of Shantou, Guangdong Province, China in November of 2003. The larvae were fed with fresh pork in a 35 cm × 45 cm plastic pan kept in a bigger plastic box containing a layer of sawdust to provide a dry place for pupation of the flies. The puparial cases were collected daily from the first emergence of the adults and kept at −30 °C until use. The corresponding adults were collected and identified to ensure that the insects were *C. megacephala*.

2.2. Collection of samples

The puparial cases were placed in 35 cm × 45 cm stainless steel pans held in an incubator at 25 ± 1 °C with a photoperiod of 14:10 h (L:D). To eliminate hydrocarbons volatilized into the incubator, 350 g of activated granulated carbon held in a 35 cm × 45 cm stainless steel pan was placed in the incubator and was replaced with new carbon every 10 d. The puparial cases were sampled and kept at −30 °C for analysis every 5 d in the first 20 d, and every 10 d from day 30 to 90.

2.3. Chemical analysis

For puparial cases in each group, 32 of them were cleaned with a small brush in ultrapure water, blotted dry with filter paper and divided into 4 groups equally. Cuticular hydrocarbons were extracted by immersing puparial cases of each group in glass tubes containing 500 μl redistilled hexane at room temperature for 20 min. The extracts were concentrated to dryness under a stream of nitrogen and redissolved in 10 μl redistilled hexane for GC and GC–MS analysis.

Quantitative analysis of cuticular hydrocarbons was carried out using a Hewlett Packard 5890 instrument fitted with a 25 m Ultra-2 capillary column (0.32 mm i.d.) and a flame ionization detector (FID). Two microliters of the sample was injected on the column using the splitless injection mode. Nitrogen was used as the carrier gas with the column head pressure of 5.0 psi. Injector and detector temperatures were 250 and 300 °C, respectively. The oven temperature was programmed from 55 °C (2 min) to 230 °C at 25 °C/min, and then from 230 to 320 °C (20 min) at 3 °C/min. The chromatograms were integrated using the HW-2000 Chromatograph Station Software (Ltd. of Qianpu Software, China).

GC–MS was used to identify the chromatographic peaks. It was performed on an Agilent 6890N gas chromatograph connected to an Agilent 5973 mass selective detector. The GC fitted with a 30 m HP-5 capillary column (0.25 mm i.d.). Ultrapure helium was used as the carrier gas with the column head pressure of 11.3 psi. Injector and detector temperatures were 250 and 300 °C, respectively. Oven temperature was programmed from 55 °C (2 min) to 200 °C at 30 °C/min, and then from 200 to 310 °C (30 min) at 3 °C/min. Mass spectra were obtained at 70 eV and the GC/MSD interface temperature was set at 280 °C. The identification of cuticular hydrocarbons was based upon EI mass spectra and literature data [21,23,24].

2.4. Statistical analysis

The abundance of each peak was expressed as a percentage of the total peak areas of all components in the sample. The data were analyzed using SPSS 11.0 Statistical Package for windows. Univariate linear regression was performed to find the relationship between the weathering time and the abundance of different hydrocarbons peaks. *P*-values of *t*-test less than 0.05 (two-sided) were considered statistically significant. The figures were plotted with Microsoft Excel 2001 software.

3. Results

The typical gas chromatographic profile of hydrocarbons extracted from the puparial cases of *C. megacephala* is shown in Fig. 1. The cuticular hydrocarbons of the puparial cases of *C. megacephala* were found by GC–MS analysis to be a mixture of *n*-alkanes, methyl-branched alkanes, and dimethyl-branched alkanes with the carbon chain length of C21–C35. The straight chain hydrocarbons accounted for 51.30% of the total hydrocarbon in the unweathered puparial cases. Odd-chain components prevailed with *n*-C29, which accounted for 23.49% of the total hydrocarbons, followed by *n*-C27, *n*-C31 and *n*-C25; smaller amounts of even-numbered *n*-alkanes, *n*-C22 through *n*-C30, were also detected. Methyl-branched alkanes were also the majority of the cuticular hydrocarbons with those identified accounting for 26.70% of the total hydrocarbons. Dimethyl-branched alkanes prevailed with 13,17-dimethyl-C31 accounting for 3.18% of the total hydrocarbons (Table 1).

Univariate regressions for time versus the abundance of each peak revealed rich time-dependent changes, of which there were two traits. The first was that the abundance of low molecular weight hydrocarbons decreased with time with the exception of the even-numbered *n*-alkanes. Of the 15 chromatographic peaks eluted first (*n*-C26 or below), all of the other peaks had significant ($P < 0.01$) negative relationships with the weathering time except for *n*-C22, *n*-C24, *n*-C26,

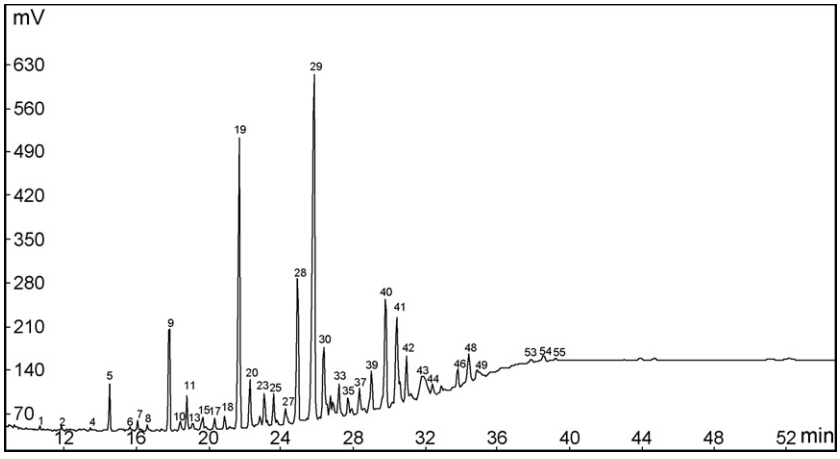

Fig. 1. The gas chromatogram of cuticular hydrocarbons derived from puparial cases of *Chrysomya megacephala*. Numbers identify peaks listed in Table 1.