Forensic Science International 259 (2016) e37–e47

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Preliminary Communication

Effect of age on cuticular hydrocarbon profiles in adult *Chrysomya putoria* (Diptera: Calliphoridae)

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ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 9 October 2015
Accepted 12 November 2015
Available online 19 December 2015

Keywords:
Cuticular hydrocarbons
Calliphoridae
GC–MS
Adults
Age
Sex

ABSTRACT

A species-specific complex mixture of highly stable cuticular hydrocarbons (CHCs) covers the external surface of all insects. Components can be readily analyzed by gas chromatography coupled to mass spectrometry (GC–MS) to obtain a cuticular hydrocarbon profile, which may be used as an additional tool for the taxonomic differentiation of insect species and also for the determination of the age and sex of adult and immature forms. We used GC–MS to identify and quantify the CHCs of female and male *Chrysomya putoria* (Wiedemann, 1818) (Diptera: Calliphoridae) from one to five days old. CHCs ranged from C21 to C35 for females and from C21 to C37 in males. Major compounds were the same for both sexes and were 2-MeC28, C29:1, *n*-C29, 15-,13-MeC29, 2-MeC30, C31:1, *n*-C31 and 15-,13-MeC31. The relative abundance of each component, however, varied with age. Cluster Analysis using Bray–Curtis measure for abundance showed that cuticular hydrocarbon profiles are a strong and useful tool for the determination of age in adult *C. putoria*.

1. Introduction

All insects are covered by a layer of species-specific cuticular lipid that often consists of a complex mixture of hydrocarbons (CHCs) whose main roles are to limit water loss, and in many species, to function in chemical communication. The cuticular hydrocarbons include *n*-alkanes, terminally and internally branched mono and multimethyl alkanes, and alkenes [1–10].

Although molecular techniques can be used for the separation of species, DNA and proteins are usually decomposed in insects used for forensic analysis, and are often not useful [11]. CHCs have been used for species differentiation of some insects including parasitic wasps [12], phlebotomines [13,14], anophelines [15,16], culicids [17], triatomines [18,19] and the forensically important Diptera Calliphoridae [20–27] and Sarcophagidae [28].

A number of studies show that the CHC profiles vary according to the age and sex of the insects [17,21,23,24,27,29–43]. These differences may be related to female attractiveness to males and play key roles in reproduction in some species [29,31].

There are relatively few papers on hydrocarbon profiles for necrophagous flies (Diptera Calliphoridae and Sarcophagidae) [20–28]. These insects, including *Chrysomya putoria*, are pioneers in cadaver colonization and play an important role in decomposition. The determination of the late post mortem interval (PMI) is based on the age of larvae and adults and on species succession [30].

The aim of this study was to determine if GC–MS analyses of CHCs could be used to identify the sex and determine the age of one to five day old females and males of the Calliphoridae *C. putoria*.

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http://dx.doi.org/10.1016/j.forsciint.2015.11.006
0379-0738/

2. Methodology

2.1. Collection and maintenance of the insects

Colonies of *C. putoria* were established and maintained in the Laboratory of Medical and Forensic Entomology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil. The insects were placed in cubic cages (30 cm × 30 cm × 30 cm) made of a wooden frame closed with nylon fabric. One of the sides was closed with a sleeve-like fabric to facilitate changes of water and food and to avoid the escape of the flies during these proceedings. The eggs were transferred to a new

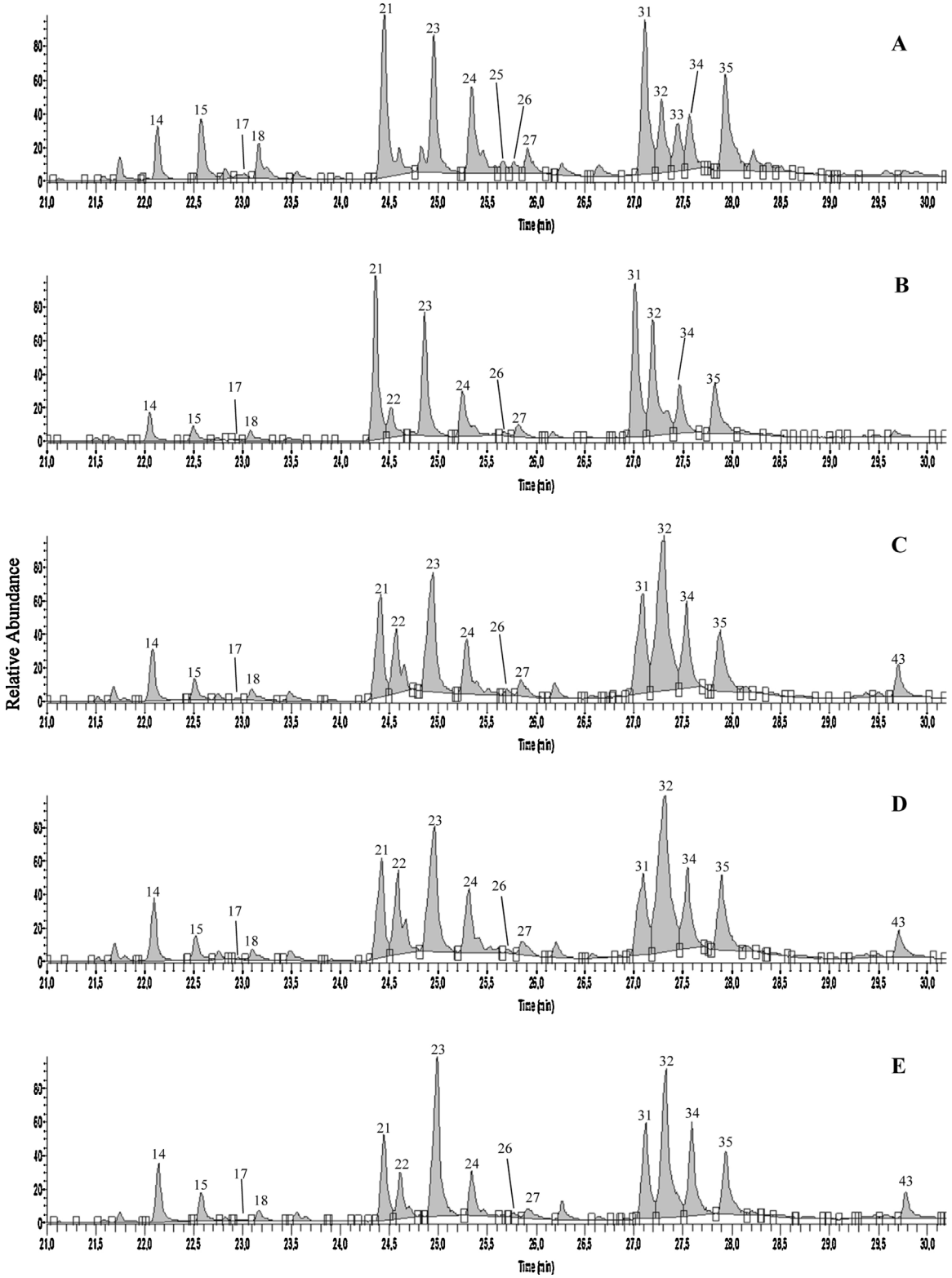

Fig. 1. Comparison of the cuticular hydrocarbon gas chromatogram profiles (HCs) from one day old (A), two days old (B), three days old (C), four days old (D) and five days old (E) females of *Chrysomya putoria*. The numbers over the peaks indicate the corresponding peaks in Table 1. Gray shading indicates the area of each peak.