

Potential Use of Cuticular Hydrocarbons in Estimating the Age of Blowfly Pupae *Chrysomya megacephala* (Diptera: Calliphoridae)

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ABSTRACT

Gas chromatography coupled with mass spectrometry (GC–MS) was used to determine the weathering time in cuticular hydrocarbon of pupae *Chrysomya megacephala* in sheltered condition. The results have shown that cuticular hydrocarbons (CHC) of the pupae were a mixture of *n*-alkanes, methyl-branched alkanes, and dimethyl-branched alkanes, with carbon chain length ranging from C₁₉ to C₃₉. The study presents the significant correlation between the changes pattern in relative abundance of several CHC and development phase in pupae. Further analysis with multiple linear regression indicated that several CHC compounds showed strong correlation to blowfly pupae age, which were then utilized to create a prediction equation for the age estimation. Finally, the application of the age-dependent model had revealed that estimated age correlated significantly with chronological age of samples *C. megacephala*, $y = 0.97x + 0.092$, $R^2 = 0.9698$. The study concluded that, CHC have a potential to estimate age of immature *C. megacephala*, and possibly in other flies species, and might further be used to determine the PMI.

Keywords: *Chrysomya megacephala*, blowfly pupae, cuticular hydrocarbons, gas chromatography-mass spectrometry

INTRODUCTION

Calliphorids are often used to estimate the post-mortem interval (PMI) on human remains as they are recognized as the first wave of the faunal succession (Amendt *et al.*, 2004). However, forensic investigators always encounter serious difficulties when estimating the time of death of human remains that are in an advanced state of decomposition. Usually, age of fly larvae estimated by rearing them into adult stage and PMI is calculated based on the total development time (Gennard, 2007). However, the age of the fly inside the puparium is more difficult to be estimated. For example, *Chrysomya megacephala* undergo pupation for about 75-90 hours and it could lead to error in the estimation of up to 2-3 days if PMI estimation involving pupae developmental stage (Salleh *et al.*, 2009). Therefore, the use of cuticular hydrocarbon profile analysis may resolved the issue highlighted by estimating the age of the insect specimens directly.

The variation in proportions of several cuticular hydrocarbon compounds at different stages of development have been reported (Blomquist *et al.*, 1999; Brown *et al.*, 2000; Nelson & Charlet, 2003; Hugo *et al.*, 2006). Their differences can also be detected

throughout their developmental cycle (Juarez & Fernandez, 2007). Qualitatively, the cuticular hydrocarbon patterns are species-specific, and at the same time changes in the composition of cuticular hydrocarbons during development have been observed. Buckner *et al.* (1999) reported that the composition of cuticular waxes from different development stages is concurrent with the development process of an individual. It therefore showed that the changes in profile of cuticular hydrocarbons were due to the ecdysis and development stages.

The quantitative variations on the profile of cuticular hydrocarbons at different stages of development provides more argument about the availability of cuticular hydrocarbons as an alternative biological marker for species recognition. On the other hand, inconsistencies in the distribution of cuticular hydrocarbons in insects give the impression that there is a correlation between hydrocarbon production and development of insect cuticle. If the pattern changes occurred in cuticular hydrocarbon composition is consistent at different development stages, the changes in cuticular hydrocarbon composition would be correlated to the age of the insect.