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REVIEW ARTICLE

Review of some recent techniques of age determination of blow flies having forensic implications

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Abstract Forensic entomology can aid death investigation by using predictable developmental changes to estimate the age of flies associated with a body. Forensic entomologists use size and developmental stages to estimate blowfly age, and from those, a Postmortem Interval. Calliphorids are very interesting in forensic sciences from an applied point of view, because they provide relevant evidence for estimating the Postmortem Interval. Since such estimates are generally accurate but often lack precision, particularly in the older developmental stages, so there is a need of some alternative aging methods. The range of techniques available for age grading of adult insects is reviewed, with particular emphasis on species of medical importance. The techniques described include pteridine fluorescence analysis, internal morphological analysis, cuticular hydrocarbon analysis, gene expression analysis, cuticular banding pattern analysis, volatile organic compounds analysis released by larvae and pupae.

Contents

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1. Introduction

The utilization of insects and other arthropods as forensic evidence in both civil and criminal investigation is known as Forensic entomology.[1] Insects found on human remains are useful in estimating a Postmortem Interval (PMI) during death investigation. Accurate methods for determining the age of adult insects in field are of considerable importance, the age of immature stages of insects found on the dead body can provide evidence for the estimation of minimum PMI ranging from one day to more than one month. They are therefore the primary and most accurate forensic indicators for time of death (Postmortem Interval, PMI). Under field conditions however, when unidentified larvae are collected from a body, reliable age determination is challenged by the fact that the larvae of different species look extremely similar but grow to different body lengths, and that temperature has a variable effect on development of some of the most common blow flies. Age determination of postfeeding larvae (the period between the cessation of feeding by third- instar larvae and the onset of pupation) is still problematic, with no satisfactory age indicator found in previous morphological studies.[2]

However, determining age of pupae is more difficult compared to the age of larvae due to lack of morphological changes; weight, length and even the color of the pupal case does not change at all after the first hour of pupation. Metamorphosis is characterized by developmental process such as cellular proliferation, tissue remodeling, cell migration and programmed cell death. Many genes are involved in these actions and changes in their gene expression function as a modern tool in age determination. Age estimation methods have most frequently focused on the larval stage, with little published data for the age estimation of pupae.

Majority of age grading techniques are based on predictable changes in the reproductive system including the accumulation of follicular relics[3] and appearance of ovarian tracheoles.[4] Other methods rely on somatic changes, such as deposition of growth bands in cuticle,[5,6] the size of larval or adult fat bodies,[7] and the accumulation of fluorescent pigments in the eyes,[8,4] The following age grading techniques were reviewed in this article:

1. Pteridine fluorescence analysis: Chronological age is calculated using the known relationship between age and pteridine fluorescence.
2. Internal morphological analysis: Provide additional internal development information to that of external morphological analysis, allowing a more accurate age and thus PMI to be estimated.
3. Cuticular hydrocarbon analysis: Hydrocarbon profile differs at distinguishable ages, and this provides a reliable method that complements the current methods used for aging the immature life stages of the blowfly life cycle.
4. Gene expression analysis: Potentially serve as a molecular tool to mirror the aging process of a pupae. Genetic assessment of blowfly age is more pronounced during the third instar and pupal stages.
5. Cuticular banding pattern analysis: Used to give an accurate estimation of the chronological age of young insects of both sexes, and may be an appropriate technique for use in studies where field material needs to be stored.
6. Volatile organic compounds analysis: Increase the accuracy of the estimated PMI, through improved estimation of the age of blow flies present on the cadaver.

2. Pteridine fluorescence analysis

Pteridine fluorescence analysis method, based on the level of fluorescent pigments, i.e. pteridines, which are degradation products of purine metabolism and accumulate over time in the compound eyes, have the advantage not only of being applicable to both male and female insects but also of easily being assayable. Pteridine content in both laboratory and field captured flies is typically a level of magnitude higher than the minimally detectable level and can be used to predict individual age in the laboratory population with high certainty. Laboratory studies of individuals of known age indicate that while pteridine levels increase linearly with age, they also increase in a linear manner with rearing temperature and ambient light levels, but are independent of sex.

Insect pteridine has been discussed in detail by Ziegler and Harmson[9] and Pfleiderer.[10] A number of biochemical techniques have also been used to predict the age of individual insects. Pteridines, a group of fluorescent chemicals derived from a pyrimidine-pyrazine ring structure[9] increase with chronological age in populations of various dipteran taxa populations reared under laboratory conditions. After Mail et al.[8] firstly develop the pteridine aging method for determining the age of *Stomoxy calcitrans* adults, this method has been widely employed to determine the adult age of many vector and medicinal insects such as *Glossina morsitans morsitans*,[11] *S. calcitrans*,[12] *Glossina pallidipes*,[13] *Cochliomyia hominivorax*,[14] *Chrysomya bezziana*,[15] *Lucilia sericata*,[16] *Bactrocera cucurbitae*,[17] *Musca domestica*,[18] *Musca autumnalis*,[4] *Chrysomya megacephala*,[19] *Boettcherisca peregrine*.[20] Zhu et al.[19] studied the relationship between head pteridine fluorescence (HPF) levels and age in adult females and males of a common necrophagous fly, *C. megacephala*. The effects of temperature and fly sex on the relationship were also studied by pteridine fluorescence spectrometry. Factors affecting HPF levels in flies were found to include fly age, temperature and sex, among which the fly age was the most dominate one. HPF level (P) in adult flies grew with an increase of the adult chronological age (d). A significant linear relationship was found between them. The linear regression equation is: