Forensic Entomology in Malaysia: A Review

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ABSTRACT: Forensic entomology has been regarded as one of the most useful fields in forensic sciences especially in estimating postmortem interval. Since the last 30 years, researches in this field have evolved tremendously throughout the world, which ranged from factors influencing the development of necrophagous insects, the use of molecular-based techniques in assisting taxonomic identification of species, to the use of necrophagous insects in drawing toxicological inferences. In Malaysia, researches on species compositions and development of necrophagous insects recovered from dead bodies as well as infesting animal models decomposing in various conditions have been conducted in several states viz. Selangor, Kuala Lumpur, Penang, Kedah and Kelantan. However, such data from the other states within Peninsular Malaysia as well as from the East Malaysia i.e. Sarawak and Sabah are currently lacking. Although data on the influence of malathion and gasoline on species composition and development of necrophagous insects are available, similar studies focusing on other poisons that are used for committing suicides such as paraquat and other pesticides as well as rodenticides have not been reported, so far. In the context of taxonomic identification of species using molecular-based techniques, the use of cytochrome oxidase genes has been attempted. In addition, quantitative and qualitative determinations of poisons in necrophagous maggots for suggesting the cause of death have been reported. This paper reviews all pertinent studies conducted in the field of forensic entomology in Malaysia.

Keywords: entomotoxicology, forensic entomology, Malaysia, molecular-based techniques for taxonomic identification, postmortem interval

Introduction

It has been reported that insects are attracted to a body within minutes of death [1] attributable to various factors such as the presence of ammonia-rich compounds and amount of moisture in the decomposing body [2]. Smith (1986) divides insects that are associated with a decomposing body into four ecological categories viz. necrophagous species, predators and parasites of necrophagous species, omnivorous species and adventive species [3]. Necrophagous species that include Calliphoridae, Dermestidae and Silphidae are insects that feed on the corpse itself, responsible for majority of the biomass loss and within this category Calliphoridae are considered as the most important insects for estimating PMI [4]. Predators and parasites of necrophagous species include Silphidae, Staphylinidae and some necrophagous species that becomes predacious during the later instar stage [2, 4-5]. Omnivorous insects that include wasps, ants and some Coleoptera feed on the corpse and on its inhabitants, while the adventive insects (springtails and spiders) use the corpse as an extension of their environment and may become incidental predators [6].

Utilization of insects' developmental data, particularly Calliphoridae in providing accurate estimation of post-mortem interval (PMI) has been extensively reported in literature [7] and first popularized in Malaysia by Lee (1989) [8]. In Malaysia, *Chrysomya megacephala* (Fabricius) is the first and the most prevalent Calliphorid species recovered from corpses [9-12] and in animal carcasses [5, 13-17] followed by *Chrysomya rufifacies* (Macquart). The development of Calliphorids is influenced by variations in climatological conditions viz. temperature and rain [16], differences in sunlit and the shaded habitats [18] as well as the presence of toxic substances [4]. For estimating PMI, the accuracy in taxonomic identification is critical and in this context, molecular-based techniques for species identification of insects are gaining global popularity [7, 19]. However, several recent studies [20-21] indicated about inconsistencies in the different haplotypes among many species of flies in a worldwide scale comparison, indicating the state of inadequacy of the available information for such techniques to replace the use of traditional morphological taxonomic identification in presenting entomological evidence in the court of law. In the context of completed suicide by poisons such as malathion, the qualitative detection of such poison in necrophagous insects per se may prove useful in diagnosing the cause of death [22]. This paper is aimed at reviewing all the pertinent literatures relating to the status of forensic entomology in Malaysia.

Species Composition and Development

Researches relating to forensic entomology in Malaysia include retrospective compilation of the larval species recovered from corpses referred to major hospitals [10-12, 23] and to the Institute for Medical Research, Kuala Lumpur [9] apart from the following experimental studies described below. Lee (1989) while analyzing specimens received from various hospitals in Malaysia concluded that maggots of *C. megacephala* were predominantly found followed by *C. rufifacies* and indicated his unpublished findings that at temperature of 30±2°C and relative humidity of 85±5%, one generation time for *C. megacephala* and *C. rufifacies* were 7.15 days and 9 days, respectively [8]. Kurahashi *et al.* (1997) provided a detailed description on the morphology of *C. megacephala* adults that included fuscous (grey-black) prothoracic spiracles, orange-yellow gena and postgenal areas with pale-yellow hairs except in the area immediately around vibrissae, at least the posterior lower squama being distinctly infuscated and eye facets being conspicuously enlarged [24]. Later, Omar (2002) provided a detailed description on the morphology of the third instar larvae of *C. megacephala* that included mildly sclerotized incomplete peritremes of posterior spiracles, absence of conical tubercles on body segments, presence of dorsal arch and 'dot or club-shaped' structure in cephalopharyngeal skeleton and the anterior spiracles consisting of 11-13 papillae [25].

Lee and Marzuki (1993) used two monkey carcasses and reported the stages of development of dipterans although the season was not specified [26]. Later, Omar *et al.* (1994a) reported the developmental stages of diptera in a monkey carcass in a single occasion during October-December 1991 [5]. In another study, Omar *et al.* (1994b) used three monkey and three cat carcasses to study the behavioural patterns of dipteran larvae [27]. Ecologically, Omar *et al.* (2003) studied the synanthropic index of flies in Malaysia and identified seven species as hemisynanthropes and the highest level being *C. megacephala* [28]. Heo *et al.* (2007) used a single pig carcass for studying the species composition of adult dipterans in an oil palm plantation in Selangor [13]. Later, Heo *et al.* (2008a) decomposed one piglet carcass in a pond to study the species composition of adult dipterans [24]. Heo *et al.* (2008b) further compared the rate of decomposition and sequence of faunal succession in a partially burned pig carcass versus that of control and concluded that there were no significant differences in both parameters [15]. Six monkeys were studied in two seasons for recovering the species composition of adult dipterans and their arrivals [29].

Mahat *et al.* (2009) studied the influences of rain and malathion on *C. megacephala* using rabbit carcasses during one full year in Kelantan and indicated that a brief absence of rain during a rainy day prompted oviposition and rain (≥ 9.0 mm) on a day during pupation prolonged the period of pupation by one day [16]. They also reported that increased dosages of malathion correspondingly prolonged the pupation period and rain in the presence of malathion had a cumulative effect in prolonging the pupation period [16]. Interestingly, similar prolongation in the period of pupation of *C. megacephala* reared on liver tissues obtained from malathion-exposed rat carcasses can be made out from the results tabulated by Abd-Rashid *et al.* (2008) in their Table 2 although these authors had not reported the prolongation in the period of pupation in their text [30]. While using monkey carcasses, Rumiza *et al.* (2010) reported that the presence of gasoline and its odour on the carcass had delayed the arrival of insect to the carcasses, thereby slowing down the decomposition process in the carcass by six hours [31]. In this context, it is pertinent to quote that any factors mitigating insect development have the potential of affecting subsequent insect-based estimates of PMI [32]. Importantly, apart from the poisons mentioned above, many other substances that are available in the market such as paraquat (a quaternary ammonium herbicide), fenthion (an insecticide) and warfarin (rodenticide) can readily cause death. However, specific research investigating the possible influences of such substances on oviposition and development of necrophagous insects is lacking.

Heo *et al.* (2010) hanged a pig carcass died due to pneumonia and studied the insect succession as well as the decomposition process and reported about slower rate of decomposition observed in that study [33] when compared with their previous findings [13] in which a pig carcass placed on the ground was decomposed in the same location. Currently, Nazni *et al.* (2011) used monkey carcasses to study the differences in species composition between indoor and outdoor habitats and reported about delayed arrival of fly in carcasses decomposing in indoor habitat although composition of dipteran species remained similar with that of outdoor habitat [17]. In the context of infestation by *C. megacephala* and *C. rufifacies* in the same corpse/carcass, it has been found that *C. rufifacies* infestation invariably succeeds that by *C. megacephala* [13, 16, 27] and maggots of *C. rufifacies* develop predatory and cannibalistic behaviours during its third instar stage [5, 27, 29]. It has been reported that the predatory larvae of blowflies such as *C. rufifacies* prefer to predate larvae that are smaller than the predator since larger prey has better defense response [34]. As had been observed by earlier researchers [5, 27], the third instar larvae of *C. megacephala* on the carcass were found away from the masses of the third instar larvae of *C. rufifacies,* lessening predation. In this context it is pertinent to mention that during the presence of the third instar larvae of *C. rufifacies* in the carcasses, the earlier batches of *C. megacephala* were already in their wandering and prepupal stages thus were away from being in contact with them. In Malaysia, predatory behaviour of third instar larvae of other necrophagous species such as *Chrysomya villeneuvi* Patton and *Ophyra spinigera* (Stein) has also been observed in the field [5, 27]. Since the infestation of other necrophagous species such as *Chrysomya nigripes* Aubertin, *Chrysomya chani* Kurahashi and Sarcophagidae occurs following that of *C. megacephala* [5, 9]; the applied values of utilizing these species for estimating PMI in Malaysia are limited. A study on the nocturnal behaviour of necrophagous dipterans conducted in Kelantan revealed that oviposition was delayed and limited at night [35]. The authors (Pritam & Jayaprakash, 2009) stressed that such delayed and limited dipteran nocturnal oviposition to be forensically insignificant in estimating PMI since due to their larger size, it is the diurnally oviposited maggots that would be considered when estimating postmortem interval, according to customary in forensic practice [35]. Interestingly, although Sarawak and Sabah are geographically separated by the South China Sea from the Peninsular Malaysia and perceived to have a more diverse ecology, empirical data on species composition and duration of development of necrophagous species infesting animal models as well as dead bodies from those states have never been reported in literature so far.

Molecular-based Taxonomic Identification

In the context of using the molecular based techniques for identifying necrophagous larvae, Tan *et al.* (2009) reported for the first time in Malaysia the complete DNA sequence of CO1 and CO11 genes in *C. megacephala* (from six localities) and *C. rufifacies* (from three localities), indicating 0.26% and 0.17% intraspecific variations for both species, respectively [36]. Later, Tan *et al.* (2010) reported the complete DNA sequence of Cytochrome oxidase 1 (CO1) and II (CO11) genes in the different species of Sarcophagidae (Flesh flies) and concluded that the DNA sequence can facilitate and complement the morphology-based species identification [37].

Use of Necrophagous Insects for Suggesting the Cause of Death

Abd-Rashid *et al.* (2008) studied the concentration of malathion in larvae of *C.*

megacephala reared on malathion-exposed dissected liver of rats and reported that no correlation was found between the concentrations of malathion in larvae with that found in liver [30]. While studying the accumulation of malathion residues within the bodies of *C. megacephala* feeding on whole rabbits that had died of malathion poisoning and how these compared to the concentrations of malathion recovered from the rabbits' bodies, Mahat *et al.* (2012) concluded that it would not be reliable to suggest the formulation of mathematical algorithms for relating the concentration of malathion found in the different stages of development of *C. megacephala* with those found in the visceral organs [22]. However, the authors emphasized that unlike heroin and morphine, malathion is not a drug of abuse; instead, it is a common poison used for committing suicide and hence its qualitative detection per se can enable inferring cause of death [22].

Conclusion and recommendations

Review of all the pertinent articles published in the area of forensic entomology in Malaysia reveals that, its potential in assisting crime investigations especially those pertain to estimation of PMI during the later stages of decomposition proves to be useful. Poisons and/or drugs being reported to influence the development of necrophagous insects and since there are numerous poisons that can readily cause death available in the market, it is found pertinent to investigate the role of such poisons and/or drugs on the different species of necrophagous insects for providing empirical data for estimating PMI. Erroneous species identification resulting from similarity in the general appearances of the closely related species especially during larval and pupal stages is another prevailing problem in applying entomological evidence; hence, molecular techniques have been suggested. However, inconsistencies observed in the different haplotypes used for such purpose among the different species of flies across the different biogeoclimatic regions indicate that much data need to be gathered before molecular techniques can be accepted as a means for taxonomic identification, replacing the traditional morphology-based taxonomic identification. Previous studies indicated that reliable quantitative inference could not be made in relating the concentrations of drugs and/or poisons found in larvae with that in visceral organs or body tissues. However, in cases involving poisons that can readily cause death such as malathion and paraquat, qualitative detection of such poisons in necrophagous insects recovered from the decomposing body *per se* may prove useful in inferring the cause of death. This is because, in contrast to heroin and morphine, such poisons have never been abused, thus their presence in necrophagous insects feeding on dead bodies would definitely indicate unnatural deaths. Despite the fact that researches and applications of entomological evidence in Malaysia are rapidly gaining popularity, such researches and applications are limited to several states within Peninsular Malaysia. Being geographically separated over a great distance by the South China Sea, data relating to the species compositions and development of necrophagous insects in Sarawak and Sabah remain lacking, providing enormous avenues for research in this specialized field in Malaysia.

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