MORPHOLOGY & MOLECULAR BASED IDENTIFICATION OF ORENSICALLY IMPORTANT INDIAN FLIES (DIPTERA): A REVIEW

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***Abstract*: For the correct estimation of post mortem interval (PMI), it's prerequisite to identify the insect specimens found in and around the dead bodies accurately. There are number of methods which can be used for this purpose. In the present review paper, we are presenting the review of various methods used for the species identification of flies of Indian origin.**

***Keywords*: Forensic entomology, PMI, Diptera**

INTRODUCTION

Forensic entomology has been an important investigative tool for many years, particularly through its use in court trials in providing an estimation of PMI in homicide cases. This application of entomology for investigations demands great accuracy in PMI estimations, resulting in significant research addressing this issue. The correct identification of specimens is a critical prerequisite in the estimation of PMI using insects, but this may be difficult using the traditional morphology based approach [1,2].

Catts and Goff [3] emphasized that one problem facing the entomologists is the accurate identification of the maggots collected from a corpse. All because most of the time only dead specimens or maggots collected and poorly preserved are submitted for identification. Even when the local fauna is well known, identification can be difficult, particularly of the early instar maggots. Work by Erzinclioglu [4] in England and by Liu and Greenberg [5] in US have resulted in identification keys to eggs and larvae of some forensically important species. Haskell *et al*. [6] and Hawley *et al.* [7] have described situations in which identification of the larvae of aquatic Diptera on corpse has presented problems. Aquatic flesh water and marine fauna associated with submerged and floating corpses needs more emphasis in future studies [3,6,8-13]. Recent advances that allow the DNA probe identification of insects or their isolated body fragment, either fresh or dried might be applicable to discriminating among species of maggots where morphological distinctions are lacking [3,14].

Sarcophagids adults and larvae are easily recognized at family and generic level, as all sarcophagids share common characteristics [15-18], but are morphologically remarkably similar subgenerically and inter-specically [17-19]. For some sarcophagid species it is only the adult males that can be identified with certainty by taxonomic experts [17,18]. Correctly identifying the insect specimen can be of crucial importance for a forensic entomological analysis [20]. Many young immature stages of several forensically relevant taxa are difficult to identify and would usually be reared to the adult stage for getting a safe identification [21]. Insect species have different developmental lifecycle timings and, therefore, to utilize the correct developmental information, species need to be accurately identified. This can be done based upon morphological differences [20]. However, under the far from ideal circumstances of a crime scene, the larval forms and eggs of many forensically important dipteran species are difficult to distinguish as their morphological differences may be obscured or badly preserved [22,23]. In most of the cases, the specimens found in and around the dead body are damaged and it's very difficult to identify them on the morphological methods. Furthermore, identification which is done through morphological examination of important setae (hairs) found on the body often become impossible as many of these setae break off [24].

Wallman (2001) studied the morphological characteristics of third instar larvae of common carrion-breeding *Calliphora* blow flies. He noted that the separation of the sister species within the *Calliphora stygia* and *Calliphora augur* groups morphologically remains difficult and molecular identification techniques would be useful in such circumstances.

The use of sarcophagids as PMI estimators has been greatly hampered by their highly similar morphology and inadequate documentation of their thermobiological histories [25]. To implement the use of sarcophagids for PMI estimation, a method for easy yet accurate species level identification at any life stage is required, followed by thermobiological studies [26]. A review of literature relevant to the use of biochemical techniques and scanning electron micrography for insect identification will not be out of place here.

MORPHOLOGY BASED IDENTIFICATION OF FLIES

Allozymes

Allozymes are codominant protein markers that can be visualized by appropriate staining and agarose gel electrophoresis. They are important for determining genetic relationships among species through assessment of affiliations of rare taxa and predict relative endangerment among species [27].

Allozyme markers represent genes that are expressed in the organism at the time of sampling, yielding different information about genetic variation within species than revealed by other molecular markers. Protein electrophoresis may not detect sufficient variation to answer some questions and the number of analyses that can be performed with very small insects may be limited because of inadequate amounts of proteins. Proteins are also less stable than DNA and thus may be more sensitive

to handling and storage problems [28,29]. Therefore, when estimates of total genetic variation are important, allozymes are best used in conjunction with other markers.

Cuticular Hydrocarbons Techniques

The cuticle of all the insects is covered with a thin epicuticular layer of wax. This layer consists of free lipids, a class of compounds that includes hydrocarbons, alcohols, fatty acids, waxes, acylglycerides, phospholipids and glycolipids [30]. In establishing the PMI, a forensic entomologist will try to establish the age of the oldest colonizing species; for which various methods exist [31]. One of these methods could involve the identification of hydrocarbons present on the cuticle of the insects or even on their pupae and puparia as these contain hydrocarbons as well [32]. Byrne *et al*. [33] found it unsuitable for analysis of immature samples because before analysis they should be reared to adulthood which is not possible all the time. A long standing analytical problem in cuticular hydrocarbon analysis has been the development of efficient and accurate methods for locating double bonds in alkenes and or for determining their stereochemistry [13,34].

Scanning Electron Microscopy (SEM)

Sukontason *et al.* [35] recovered the third instar of an unidentified sarcophagid fly from a mummified body of 32 years old Thai male and examined it using SEM. Although the morphological features of this larva are similar to the other sarcophagid larvae, some features including number and arrangement of papillae on the anterior spiracle, structure of spines, and size of circumspiracular tubercles at caudal segment and branching peculiarity of the posterior spiracular hairs could be helpful for species identification.

Greenberg and Singh [36] used SEM to identify calliphorid individuals, but found several confounding factors like conspecific interpopulation variability, high similarity between eggs of congeneric species and similarity between certain species of different genera. Singh *et al*. [37] also studied morphological features of larvae of the species *Parasarcophaga ruficornis* on the basis of SEM. Climate, season and weather were also cited as important factors influencing egg morphology. In cryptic species, morphological approach is difficult to handle [38].

MOLECULAR BASED IDENTIFICATION OF FLIES

i. Nuclear DNA

There are number of nuclear genes that have been used in insect systematics. Few of them are: EF-1a [39-52], PEPCK [53-56], DDC [57-60], wingless [61-65], white [66], opsin [67-72], hunchback [73], period [74] and others [52,75-77]. Nuclear genes have advantages of low level of biased base composition, slower rate of evolution than mtDNA and contain both regions having slower and rapid rates of substitution [53,76-78]. Nuclear genes have a more resolving power than mtDNA [44,56,65,66,71,78]. Because of low copy number than its mitochondrial counterparts and two or more paralogous loci that may cause problems in phylogenetic analysis [52].

ii. Nuclear Ribosomal DNA (rDNA)

Nuclear rDNA is made up of a series of tandemly repeated units, each comprising of ribosomal RNA (rRNA) genes (18S, 5.8S and 28S) separated by segments of DNA referred to as ribosomal spacer DNA [79,80]. Hairpin loops coding region demonstrate a higher rate of mutation accumulation [81]. The alteration in rates of mutation accumulation across rDNA repeating units and single rRNA genes [82], make rDNA sequence data an able molecular marker for the separation of parasitic species and even strains [83,84]. This broad utility of rDNA is because the multiple copies per genome are usually tandemly repeated and the non-coding spacers evolve faster than the coding regions [82,85]. Concerted evolution of rDNA within species has resulted in the use of the faster evolving spacers, not only for the reconstruction of phylogenies, but as diagnostic markers for differentiating species, including proximal and cryptic species [86,87].

iii. Mitochondrial DNA (mtDNA)

Mitochondrial DNA and nuclear rDNA has been widely used in insect systematics [22]. Mitochondrial DNA contains regions with a range of different rates of evolution [88], making it suitable for both phylogenetic and population genetic studies, where genes with appropriate rates of evolution can be chosen for a particular temporal scale of analysis [89,90]. It has many advantages like, lack of recombination, high copy number, easy isolation, the presence of both conserved and variable regions, and higher rates of sequence change [53,91-95]. High rate of substitution in mtDNA (five and ten times) as compare to nuclear DNA [95], making it useful for estimating relationships between recently diverged species and in population genetics [96,97].

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