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Electrophoretic Studies in Indian Calliphorids

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Abstract: The predominantly occurring Calliphoridae include many species of veterinary, forensic and medical importance. Although diagnostic morphological characters are present for taxonomic identification of all described species of Calliphoridae but these morphological characters are complicated because there exists great similarity among these flies. The purpose of accurate identification in larval stages or in incomplete specimens of flies is not possible on the basis of morphological characters only. Therefore, different molecular markers have been applied to resolve the discrepancies regarding identification which include allozyme, RAPD-PCR, PCR-RFLP, sequencing of mitochondrial and nuclear regions etc. These methods can be applied to identify an unknown species also. In this review, an attempt has been made to summarize different molecular work performed on Indian Calliphorids.

Keywords: Calliphoridae, Allozyme, Electrophoretic analysis, Sequencing, Cytochrome Oxidase subunit I (COI)

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Introduction

The insect order Diptera includes many families of immense medical, veterinary and economic importance. The blow flies of the family Calliphoridae are distributed worldwide and some of the species belonging to this family are known to be causative agents of animal tissue myiasis (Hall and Wall, 1995). Their larvae are used for biological control by parasitizing invertebrates especially locusts and grasshoppers. These flies also serve as scavengers by cleaning the environment. Blow flies have great significance in forensic studies also, as the age of oldest maggot give estimation of PMI (post mortem interval) (Crosskey and Lane, 1993). However, there exist diagnostic adult morphological characters for identification of all described species of Calliphorids but for identification of larvae or for incomplete specimen morphological characters are not useful (Catts and Goff, 1992). Therefore, different electrophoretic techniques based studies involving protein and DNA markers (eg. Allozyme, RAPD-PCR, PCR-RFLP and sequencing of mitochondrial and nuclear region) have been applied to resolve the discrepancies about accurate identification. The application of electrophoretic technique to study variation has been extensively used to explore hidden genetic variability in natural population and laboratory

colonies of many dipterans (Bajpai and Tewari, 2010a, b, 2012; Bajpai *et al*., 2011; Bajpai, 2016a, b, 2019). Electrophoretic techniques involve both protein based (allozyme) and DNA based (RAPD-PCR, PCR-RFLP, sequencing of mitochondrial and nuclear regions) markers. Both these markers are important markers that enhanced many fields of study in Biological Sciences. As compared to DNA markers, protein markers are cheap and much easier to develop. Protein markers produce single or multi locus banding pattern. On the other hand, DNA markers are also useful while we have small amount of insect material. However, DNA based molecular methods are considerable expensive than protein based markers and also complex instruments and protocols are required for DNA based markers. Studies of genetic variation by these markers have contributed a great deal in elucidating intra- and inter-specific discrimination, forensic studies, genetic polymorphism, gene flow among populations and phylogenetic relationships. In this review, an attempt has been made to briefly describe protein based and DNA based studies on Indian flies belonging to family Calliphoridae.

Allozyme studies in Calliphorids:

Among Calliphorids the first estimate of genetic variation by allozyme was done by Kiran *et al.* (2012) in which five gene enzyme system i.e. LDH, APH, ACPH, ME and XDH were analysed using Poly Acrylamide Gel Electrphoresis (PAGE) among laboratory colonies of *Chrysomia megacephala*. The result revealed a very low level of genetic variability with APH-2, APH-3 and ACPH-2 allozyme loci to be less heterozygous which could be due to the phenomenon of inbreeding (Singh *et al*., 2012). Agrawal *et al.* (2013) assessed single gene enzyme system i.e. EST (Esterase) pattern among *Chrysomya megacephala* in which five zones of activity were observed namely EST-1, EST-2, EST-3, EST-4 and EST-5. Except EST-5, all the loci were monomorphic while EST-5 was polymorphic (Agrawal *et al*., 2013). Agrawal (2015) further elaborated allozyme studies in *Chrysomya megacephala* populations by assessing EST and ACPH enzyme activity. Both these enzyme systems revealed low level of heterozygosity and low level of genetic variability (Agrawal, 2015). Further Agrawal *et al.* (2016) and Agrawal and Gaur (2017) elaborated allozyme studies by analyzing ADH, ACPH, G6PD and AO gene enzyme system in which low level of genetic variation was found. Table 1 represents electromorphs frequency and heterozygosity values for different gene enzyme system.

DNA based studies in Calliphoridae:

Mitochondrial COI and CR (Control Region) of three different Calliphoridae flies viz. *Chrysomia megacephala, Hemipyrellia pulchra* and *Lucilia cuprina* were characterized by Bajpai *et al*. (2013). The analyzed COI region was 836 base pair long with high AT content. Mean nucleotide distance was 0.119. However, with CR region average pair wise nucleotide distance was 0.113. The characterization provides information about phylogenetic relationships i.e., *Hemipyrellia pulchra* and *Lucilia cuprina* are close as compared to *Chrysomia megacephala* (Bajpai *et al*., 2013).

Sharma and Singh (2014) collected dried specimens of *Chrysomia rufifacies* and *Chrysomia megacephala* from different regions of North West India for characterization of mitochondrial COI region in which they aligned 480 sites. The value of sequence divergence among taxa ranges from 0 to 12.2 % while with the same species divergence value was found to be from 0 to 1.1 % for *Chrysomia megacephala* and 0 to 1.5 % for *Chrysomia rufifacies*. This study conclude that small region of mitochondrial gene is quite enough to show intra-specific and geographical variation (Sharma and Singh, 2014).

Bharti and Singh (2017) analyzed 22 specimens belonging to nine species and three genera viz. *Calliphora*, *Chrysomia* and *Lucilia* for mitochondrial COI region. Less than 0.3 % intra specific divergence value was observed in these flies while inter-specific divergence ranged from 0.1% to 18.14%. This study also confirms the reliability of COI gene in identification purposes,

Table 1: Electromorphs frequency and heterozygosity values for different gene enzyme system in Calliphorids

Enzyme	Electromorph frequency			Heterozygosity		References
	Alleles					
	A	b	c	Ho	He	
LDH-1	0.44	0.56		0.56	0.49	Singh *et al.* (2012)
ACPH-2	0.48	0.52		0.24	0.48	
APH-2	0.53	0.47		0.26	0.50	
APH-3	0.8	0.2		0.20	0.32	
EST-5	0.60	0.40		0.14	0.50	Agrawal *et al.* (2013)
ACPH-1	0.52	0.48		0.31	0.49	Agrawal *et al.* (2015)
EST-5	0.60	0.40		0.14	0.50	
ACPH-1	0.52	0.49		0.31	0.49	Agrawal *et al.* (2016)
AO-1	0.36	0.37	0.27	0.55	0.67	
G6PD-1	0.55	0.45		0.28	0.49	
ADH	Monomorphic					
ACPH-1	0.52	0.49		0.31	0.49	Agrawal *et al.* (2017)
G6PD-1	0.55	0.45		0.28	0.49	
ADH	Monomorphic					

Table 2: Regions analyzed, size, T:C:A:G ratio, intra- and inter-specific genetic divergence value for Calliphorids

Region/locus analyzed	Size in base pair (bp)	T:C:A:G ratio	Intra-specific genetic divergence (%)	Inter-specific genetic divergence (%)	References
COI	836	39.6:15.4:31.4:14	-	0.119	Bajpai *et al.* (2013)
CR	-	-	-	0.113	Bajpai *et al.* (2013)
COI	-	-	0-1.1	0-12.2	Sharma and Singh (2014)
COI	1423	-	<0.3	0.11-18.14	Bharti and Singh (2017)
COI	658	38.3:15.5:30.55:15.6	5.23 (pair wise) 6.54(K_2P)	-	Abd-Algalil and Zambare (2017)

where these flies have forensic importance (Bharti and Singh, 2017).

Abd-Algalil and Zambare (2017) tested 658 bp long COI region in four species of *Chrysomia* viz. *C. megacephala, C. rufifacies, C. saffranea* and *Chrysomia* new species for molecular identification. The COI region was found to be AT rich. The sequence divergence value for pair wise distance within genes was found to be 5.23 % while with k2p model it was 6.54 % (Abd-Algalil and Zambare, 2017). Table 2 represents regions analysed, Size, T:C:A:G ratio, intra- and inter-specific genetic divergence value for Calliphorids.

Conclusion

On the basis of literature surveyed it is clear that only few allozyme and DNA bases studies are available on these medically, veterinary and forensically important calliphorids. However, allozyme studies are performed in flies of limited regions. It is imperative that upcoming research work should be performed by involving some more species of different regions to explore

genetic diversity, while, DNA based studies in Calliphorids are totally based only on mitochondrial COI region avoiding many other valuable genes which could also provide useful information regarding identification. The molecular characterization once again confirms the utility of COI gene as an identification purposes. Allozyme (protein) and DNA based markers constitute different genetic source of organism and both markers can be used for genetic characterization purpose. However, it is imperative that additional molecular markers have been utilized to genetically characterize these flies because combination of such informative markers may give better information.

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