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MOLECULAR CHARACTERIZATION OF MITOCHONDRIAL DNA SEQUENCES OF CALLIPHORID FLIES (CALLIPHORIDAE: DIPTERA)

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ABSTRACT

In the present study mitochondrial DNA sequences i.e. Cytochrome oxidase I (CO I) and Control region (CR) are characterized in order to study the genetic variability and phylogenetic relationships among flies belonging to the family Calliphoridae. Since, these flies are of medical and veterinary importance, therefore, it is imperative to use molecular markers to resolve the evolutionary mechanism responsible for specific genetic variability. The data reveals that there is very little genetic difference among these species. The phylogenetic tree derived by distance and character-state approach shows similar topologies.

KEY WORDS: **Calliphoridae, Control region, Cytochrome oxidase , Phylogeny.**

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INTRODUCTION

The flies belonging to family Calliphoridae are of considerable medical and veterinary importance as the larvae of these flies cause tissue myiasis in animals, especially cattle [1]. Recently mitochondrial genes have been extensively used to unravel genetic relationships among the calliphorids [2-6]. These genes provide informative evolutionary markers because of maternal inheritance, lack of recombination and increased rate of nucleotide substitution[7]. In the present study, two mitochondrial gene sequences *viz*., Cytochrome Oxidase subunit I (CO I) and Control Region (CR) or AT Rich Region (ATRR) were characterized among three species of calliphorids *viz*, *Chrysomya megacephala* (Fabricius), *Hemipyrellia pulchra* (Wiedemann) and *Lucilia cuprina* (Wiedemann), to analyze phylogenetic relationships among the three species.

MATERIALS AND METHODS

Genomic DNA was extracted following the method of Maniatis et al[8] with minor modifications. The DNA was concentrated by ethanol precipitation and resuspended in 100 µl TE buffer (pH-8.0). The primers used for amplification of the two genes, synthesized by Bangalore Genei, are presented in Table 1.

Table 1
The primers used for the two genes in the present study.

Primer no.	Locus	Location	Sequence	Reference
1.	COI	C1-J-2183	CAACATTTATTTTGATTTTTTGG	Simon et al[9]
		TL2-N-3014	TCCAATGCACTAATCTGCCATATTA	Simon et al[9]
2.	CR	T1-N-4	ATTTACCCTATCAAGGTAA	Simon et al[9]
		CMEG-AR	AATCCAGTTAAGAATATCAT	Lessinger et al[10]

The amplification reactions were performed in 25 µl reaction volume containing 2.5 µl 10X buffer, 2 µl dNTP (2.5 mM each), 10 picomole of each primer, 1.5 U Taq DNA polymerase, 30 ng of genomic DNA and rest milli Q water. The amplification profiles were same as followed by Thyssen et al[11]. The amplified products were sequenced by Bangalore Genei and the gene sequences were submitted to GenBank (Table 2). For different statistical and phylogenetic analysis method adopted by Bajpai and Tewari[12] was followed.

Table 2
Species used in the present study and their accession numbers.

S. No.	Species	Sequence	Accession numbers
1	*Chrysomya megacephala*	CO I	FJ842472
2	*Hemipyrellia pulchra*	CO I	FJ842473
3	*Lucilia cuprina*	CO I	FJ842474
4	*Chrysomya megacephala*	CR	FJ946632
5	*Hemipyrellia pulchra*	CR	FJ946634
6	*Lucilia cuprina*	CR	FJ946637

RESULTS AND DISCUSSION

The CO I gene amplified in the present study was 836 bp long with 672 conserved sites and 164 variable sites. The mean nucleotide pairwise distance was 0.119. Average nucleotide composition consists of T=39.6, A=31.4, C=15.4, G=14. The base composition shows high AT content (70 %). In *Drosophila* mitochondrial DNA, it has been suggested that enzymes responsible for transcription and replication functions optimally under high AT content[13]. The AT content of CR was found to be much higher as compared to COI region. This may be due to the AT directional mutation pressure exerted early on the radiation of insects, as suggested by Zhang and Hewitt[14]. Average pairwise nucleotide distance was found to be 0.113. The phylogenetic relationship inferred from Maximum Parsimony method using *D. yakuba* as an outgroup is depicted in figs. 1 and 2. Bootstrap values higher than 70% are indicated in the figures. Greater than 70% bootstrap values generally correspond to a 95% probability that the data consistently support a given clade[15].

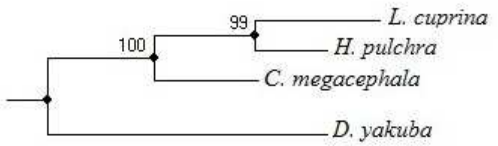

Figure 1
Maximum Parsimony tree obtained for Cytochrome Oxidase I region

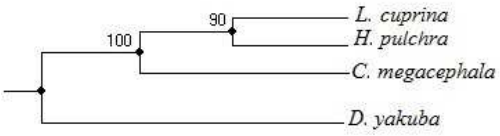

Figure 2
Maximum Parsimony tree obtained for control region

The phylogenetic tree calculated by Maximum Parsimony approach for CO I and CR sequences yield similar results i.e. two clusters were observed, one comprising of *L. cuprina* and *H. pulchra* and the other of *C. megacephala.* In both the procedures gaps were treated as missing data (pairwise deletion). It can be concluded from the present study that the characterization of both COI and CR sequences are effective in elucidating genetic variability and determining phylogenetic relationships among the members of family Calliphoridae.

CONCLUSION

The characterization of a specific mitochondrial DNA region provides an informative marker for genetic variability to understand the phylogenetic relationships among the three species. Phylogenetic trees obtained by using Maximum parsimony of both the regions revealed two lineages, one consisting of, *H. pulchra* and *L. cuprina* and the other of *C. megacephala.* A very close level of genetic identity was observed between *H. pulchra* and *L. cuprina* as compared to *C. megacephala* and

L. cuprina, which reveals a comparative low genetic identity. However, the high genetic identity between *H. pulchra* and *L. cuprina* is supported by reflects that during the evolutionary divergence of these species the accumulation of genetic differences has been more or less at the same rate.

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