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### **Amplifications of orthologous DNA fragments in three *Drosophila* species endemic to India.**

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The footprint of evolution is very often reflected at the molecular organisation in the closely related taxa (Takano, 1998). Such footprints can easily be traced by the identification of conserved DNA sequences across different taxa (otherwise known as orthologous DNA fragments). These conserved DNA sequences can then be utilized to reconstruct phylogenetic positions of these taxa (Goodstadt and Ponting, 2006) and to infer detailed evolutionary history of a group of closely related taxa. Thus, much attention has been paid in recent years to use of single-locus homologous DNA markers for these kinds of study. However, it has been shown that evolutionary history reconstruction through the use of multilocus DNA fragments provides robust inference to this aspect (Kopp and Barmina, 2005).

To this respect, the model organism *Drosophila* has been used to test this hypothesis in great detail. Since wide varieties of *Drosophila* species are sympatric to many tropical habitats of the globe, it is interesting to understand evolutionary history of these species and thus to correlate with ecological adaptations. Evolutionary history inferred from multilocus DNA fragments, that too using putatively neutral markers are especially important, as demographic events related to species differentiation could be inferred barring the effect of natural selection. *Drosophila* species, due to manifold advantages for use in both classical and molecular genetic work, are organisms of choice to test the differential evolutionary hypotheses and also the role of different evolutionary forces in speciation.

India harbours a wide range of *Drosophila* species. Species that are found in domestic or semi-domestic areas are often common. Among these, three different species of *Drosophila* are of fairly common in occurrence in the wild; *Drosophila ananassae*, *D. malerkotliana* and *D. melanogaster*. While the first species is fairly common in almost all seasons, *D. malerkotliana* is widely available for collection during rainy season of the year and *D. melanogaster* during winter. However, the latter species is becoming rare in the wild in India. Ecologically, *D. melanogaster* and *D. ananassae* almost utilize a similar food resource and both are found to be strictly domestic, while *D. malerkotliana* is semi-domestic and mostly seen in orchards, gardens, and places just close to human habitation, but rarely inside houses. Thus, while the almost-common ecological habitat of all the three species in India provides an opportunity to understand similarities at the DNA level, it is of high interest, on the other hand, to look for genetic dissimilarities as well, by which each have adapted to different ecological, seasonal conditions in India.

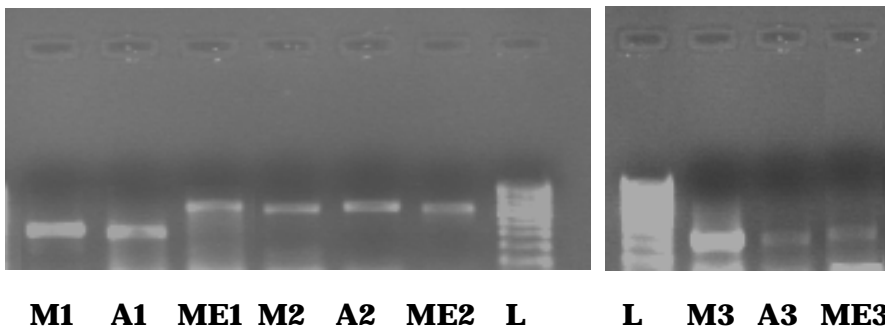


Figure 1. Agarose-gel electrophoretic pictures showing three PCR-amplified nuclear DNA fragments in the genomic DNA of three species of *Drosophila* endemic to India. L = 100 base pair DNA ladder; M1 to M3 = *D. malerkotliana* DNA fragment no. 1 to 3; A1 to A3 = *D. ananassae* DNA fragment no. 1 to 3; ME1 to ME3 = *D. melanogaster* DNA fragment no. 1 to 3.

The present study, a part of the long-term genomic understanding of speciation history of common *Drosophila* species in India focuses on identifying common nuclear DNA fragments and utilizes these markers to understand evolutionary history of these species in India.

To initiate such a kind of study, we have utilized the whole genome sequence information (Adams *et al.*, 2000) of *D. melanogaster* to design primers in the coding regions of different genes (exon parts) to amplify the

flanking non-coding introns. As stated above, such types of DNA fragments serve as putatively neutral nuclear DNA markers (Das *et al.*, 2004). We have designed as many as 20 primer pairs based on the *D. melanogaster* whole genome sequence information of the X-chromosome and could successfully amplify only three fragments in all the three species so far (Figure 1). Considering a large reported divergence time among these three species (Kopp and Barmina, 2005), the data on the low amplification rate of DNA fragments across three different species is not surprising, even though the primers are designed in the coding regions of the genome. These fragments will further be sequenced followed by sequence alignments and phylogenetic tree construction which is in process in this laboratory.

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