Review Paper

Review: Isozyme Variation in Indian Muscid flies

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Available online at : www.isca.in, www.isca.meReceived 19th June 2025, revised 10th July 2025, accepted 8th August 2025

Abstract

Isozymes or isoenzymes are multiple molecular forms of an enzyme that perform the same catalytic function but differ in their biochemical, structural and electrophoretic properties. These variants of isoenzymes are of great importance to population genetics studies and evolutionary studies. Isozyme variation has been studied in a large and diverse group of flies of the family Muscidae. Isozyme studies have been used to explore polymorphism, seasonal, temporal and spatial genetic variations. This review emphasizes the relevance of isozyme analysis in understanding genetic, spatial and environmental diversity in Muscid flies offering a road map for future research.

Keywords: Muscidae, Isozyme, *Musca domestica*, house fly, polymorphism, seasonal variation, spatial variation.

Introduction

The insect order Diptera includes many families such as Glossinidae, Simulidae, Calliphoridae, Tephritidae, Sarcophagidae, Muscidae etc., and all are of immense medical, veterinary and ecological importance due to their role in carrion decomposition and their potentials as vectors of pathogens. The family Muscidae is a large and diverse family, comprising over 5,000 described species under 170 genera and is distributed in different bio geographical regions of the world¹. Among these, there are 263 species under 35 genera are known from India². The flies of this family are of great importance in medical, veterinary and agricultural sciences. Most of the adult flies are very important because of their relationship with men and dwellings. They are carriers of the pathogens causing diseases in humans and animals³⁻⁶. Kettle⁷ reported various pathogens for diseases such as typhoid, tuberculosis, leprosy, dysentery, anthrax etc. which are carried by these flies.

The house fly, *Musca domestica*, a synanthropic fly with worldwide distribution are able to rapidly colonize a variety of habitats implying that they have tremendous capacity to adapt to different environmental conditions⁸. It is the most common species found on hog and poultry farms, horse stables and ranches. This fly is also known to transport disease-causing organisms⁹.

Recently, house flies were shown to spread a deadly strain of *Escherichia coli*^{10,11}. *Musca autumnalis*, commonly known as face fly or autumn fly, not widely distributed as common housefly, is a common pest of cattle and horses. These flies while feeding on animal's face and other body parts can transmit eye inhabiting nematodes and pathogenic bacteria that

cause pink eye¹²⁻¹⁴.

The pepper fruit fly, *Atheriogona orientalis*, a member of the muscidae, is highly polyphagus. Larvae feed and develop on decaying plant materials faeces and carrion¹⁵⁻¹⁷. There are many records where *A. orientalis* is considered a secondary host and sometimes reported as a primary pest of certain crops of the family Solanaceae^{18,19}. The Muscid flies belonging to genus *Lispe* usually inhabit moist habitats, and are predators feeding on small aquatic insects, and the adults may be helpful in reducing mosquito populations²⁰⁻²². The Stable fly, *Stomoxys calcitrans* is a widespread, economically important pest of man and animals²³⁻²⁵. Biting activity of these flies reduces weight gain and feed efficiency in cattle^{26,27}. The horn fly, *Haematobia irritans* is an obligate haematophagus, cosmopolitan insect pest. The economic importance of these flies is based on its role as an obligate blood sucking ectoparasite that causes annual losses in cattle production and decreased milk production²⁸⁻³⁰.

In view of the above characteristics of various Muscid flies it is imperative that a thorough study with regard to species discrimination, genetic polymorphism, gene flow among populations and phylogenetic relationships involving members of this family should be carried out in order to formulate strategies for their control. To study genetic polymorphism, Lewontin and Hubby introduced the technique of gel electrophoresis³¹. There has been an exponential increase in investigations both in amount and refinements in methods in a wide variety of organisms. Basically, the technique is based on the migration of proteins under an electrical field in a support medium. The application of electrophoretic techniques to study the genetic variation within and between species made it possible to obtain genetic information even in those organisms

which were not amenable for breeding experiments and have a prolonged life cycle.

This aroused tremendous enthusiasm in biochemical genetics and, to date, data on species discrimination, polymorphisms in natural populations and phylogenetic relationships with the help of electrophoretic technique have become available in a wide variety of organisms³²⁻⁴⁰. In addition, electrophoretic study of isozymes has also been extensively used by geneticists to estimate differential gene expression during development, effect of gene dosage on enzyme structure and function and the evolution of genes and organisms⁴¹⁻⁴³.

Despite the development of a plethora of molecular markers in recent past, isozymes still remain very valuable and costeffective markers for population genetic studies. Analysis of isozyme variation has contributed a great deal to elucidate the genetic differences in systematics and evolution among species in several dipterans especially Glossina-Glossinidae⁴ Drosophila-Drosophilidae 46,47. Simulium-Simulidae⁴⁵, Sarcophaga – Sarcophagidae 48-52, Chrysomya – Calliphoridae 53,54, Bactrocera-Tephritidae 55-57 and house flies Musca domestica-Muscidae. In 1968 McDonald et al., introduced the technique of isozyme electrophoresis to housefly genetics⁵⁸. Several workers, subsequently, analysed genetic variability in *Musca domestica* populations^{8,40,59-72}. The present review describes the isozyme variation studies in Indian Muscid flies and deals with the importance of these flies used for assessing polymorphism, species differentiation, environmental variation and spatial genetic variation etc., considering Musca domestica as a model organism.

Analysis of Polymorphism

Tripathi et al., described genetic variation in the house fly *Musca domestica* from Prayagraj, India. Isozyme variation was estimated at eleven gene-enzyme systems and 63.6% loci were found to be polymorphic⁸. In this study genetic variability analyzed was found to be greater than all the other Muscid flies. While in another study very little genetic differences were observed among four populations of *M.domestica*⁶⁷.

Analysis of Temporal and Seasonal variation

In several cases, enzyme polymorphisms exhibit changes in frequencies which are often correlated with environmental conditions, such that the genetic polymorphism increases with the increase in environmental heterogeneity. For example., seasonal genetic variation in house fly *Musca domestica* at sixgene enzyme system viz., acid phosphatase (ACPH), esterase (EST), glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), aldehyde oxidase (AO) and xanthine dehydrogenase (XDH) showed fourteen loci with twenty-seven alleles. The study supports that genetic variations were affected to a great extent by environmental influence. F statistics revealed that very little genetic variation has occurred among the house fly populations. Further, except three loci all the other loci show inbreeding (Fis>Fst). Thus, it appears that the house

fly populations analyzed are characterized by high level of inbreeding. Nei's genetic identity (I) and distance (D) values reveal a close similarity between summer and rainy season collections⁷³.

Further, temporal genetic variation analyzed at three gene enzyme systems of the *Musca domestica* during two consecutive years, showed eight loci with fourteen alleles. This study also proved that genetic variation of isozymes is influenced by environmental variations. Except two loci in seasonal cycle 01 and one locus in seasonal cycle 02 reveal inbreeding which is depicted by the higher Fis values than Fst values. In this study also house fly populations showed high level of inbreeding. Comparison of Nei's genetic distance (D) and identity (I) values rendered that there is a close similarity between summer and rainy season collections in seasonal cycle 01 and winter and rainy season in seasonal cycle 02⁴⁰.

Analysis of Spatial / Geographical variation

It is interesting to note that survey from different geographic location in Musca domestica exhibit variation in allele frequency and level of heterozygosity. During Spatial genetic variation assessment in the house fly Musca domestica, isozymes at three gene enzyme system unravelled ten loci which revealed seventeen alleles in four populations of house fly from Prayagraj, India. F statistics revealed that except ACPH-1 and EST-3 all the other loci show inbreeding (Fis>Fst). Persistent heterozygosity was observed in ACPH-1(Fis= -0.140) which indicate random mating for this locus⁷⁴. In addition to this when natural populations of house fly Musca domestica from three different places of Uttar Pradesh (India), were analyzed for eight genetic loci, scores nineteen putative alleles, and the amount of mean observed heterozygosity (Ho) was reported greater for Varanasi population than the Mirzapur and Phaphamau. Nei's genetic identity (I) and distance (D) values reveal that the house fly populations from Mirzapur and Varanasi are clustered closely (I = 0.736) as compared to other populations⁶⁹.

Group I and Group II Enzyme Variation

Analysis of isozyme variation in Indian population of *Musca domestica* showed that there is a difference in amount of polymorphism and the degree of heterozygosity among glucose metabolizing enzymes (Group I) and non-glucose metabolizing enzymes (Group II). The study of Tripathi et al., revealed that the genetic variability measured by isozyme variation is much higher for Group II than for Group I enzymes in *M.domestica* population⁶⁸. This type of isozyme polymorphism in specific functional and non-functional enzymes was also found in other dipterans⁷⁵⁻⁷⁷.

Conclusion

Since Muscidae are cosmopolitan and synanthropic to human being, they are exposed to almost same environmental conditions. Several studies on basic genetics, physiological genetics, genetics of resistance and physiology in Muscidae surmised that the fly *Musca* exhibit extensive morphological and genetic variability. Although, very little work has been done on isozyme variation in *Musca domestica* in India as compared to temperate or other part of the world^{64-66,71,72,78}. The review strongly urges the use of other Muscidae as an alternate model system apart from *Musca domestica* in isozyme studies. The integration of isozyme analysis with molecular markers contributes to deeper understanding of population genetic studies and also unravels further insights into this diverse and ecologically important family.

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