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USE OF ALLOZYME MARKERS IN GENETIC VARIATION STUDIES OF HOUSE FLY (*Musca domestica*)

Saumya Srivastava[1a]

[a]Department of Zoology, University of Allahabad, Allahabad, UP, India,
E-mail: ssadventure27@yahoo.co.in

ABSTRACT

Genetic variation within natural populations is a cornerstone of evolutionary biology and vector management strategies. In houseflies (*Musca domestica*), allozyme markers have long served as a reliable tool to assess genetic diversity, population structure, gene flow, and adaptive potential. This review explores the use of allozyme electrophoresis in evaluating genetic variation in *M. domestica* populations globally, with emphasis on Indian studies. It covers enzyme systems employed, statistical measures used (heterozygosity, genetic identity, Hardy-Weinberg equilibrium), and the implications of findings in ecological, medical, and pest control contexts. The persistence of allozyme analysis as a cost-effective and informative technique highlights its continued relevance in contemporary entomogenetics.

KEYWORDS: Allozyme markers, Genetic variations, geographical isolation, House fly, *Musca domestica*

The common house fly, *Musca domestica*, is a synanthropic fly distributed throughout the world, wherever humans and domestic animals are known to occur. The foundation of biochemical population genetics was laid by Lewontin and Hubby (1966), who introduced the technique of gel electrophoresis for the study of genetic variations. Allozyme variations have elucidated the genetic differences in systematics and phylogenetics among several dipterans, especially mosquitoes, Tsetse flies, flesh flies, and fruit flies (Malacrida et al., 1996; Skevington and Dang, 2002; Mateus and Sene, 2007; Hsiao, 1994; Sukontason et al., 2014; Agrawal, 1993). In flies, little work has been carried out on allozyme variations in India, and understanding their genetic variation helps in mapping population connectivity, insecticide resistance evolution, and environmental adaptability (Loxdale and Lushai, 1998; Chakrabarti, 2005; Tripathi et al., 2011). Among various molecular tools, allozyme electrophoresis has been historically valuable due to its simplicity, reproducibility, and ability to reveal codominant genetic differences (Richardson et al., 2012; Kucuktas and Liu, 2007; Anne, 2006).

Allozymes remain a very valuable tool for studies in population genetics due to their cost-effective nature (Murphy et al., 1996). Allozyme variations have unravelled changes in genotype frequencies about spatial, seasonal, and temporal variations, emphasizing the role of environment in space and time to maintain genetic polymorphism, with gene-level regulation influencing responses to genotoxic agents (Barker et al, 1986; Bubliy et al., 1999; Hederick et al., 1976; Kraushaar et al., 2002; Land et al., 2000; Malacrida et al., 1992; Milankov et al., 2002; Nayar et al., 2003; Srivastava et al., 2016). The house fly Musca domestica, a synanthropic fly with worldwide distribution, can rapidly colonize a variety of habitats, implying that they have a tremendous capacity to adapt to environmental conditions. The spatial, temporal, and seasonal genetic variations in house fly populations have been analyzed only in the New World populations from the USA, UK, and Africa (Black et al., 1986; Cummings and Krafsur, 2005; Krafsur et al., 1992, 2000; Marquez and Krafsur, 2002; Marquez et al., 2001; Stanger, 1984). Also, some studies were carried out to determine the genetic variation among three populations of *Musca domestica* from Uttar Pradesh (India) using different allozyme analysis (Srivastava et al., 2012). These studies may also be helpful in genetic characterization of several dipterans by resolving the biochemical and genetic components (Singh and Thakur, 2012; Srivastava et al., 2012; 2013, 2015).

Despite the advent of advanced molecular markers, allozyme analysis remains a robust and cost-effective tool for assessing genetic variation, particularly in non-model organisms like the housefly. Its ability to detect codominant alleles and reveal functional polymorphisms at the protein level offers valuable insights into population structure, gene flow, and evolutionary dynamics. In *M. domestica*, allozyme markers should be extensively employed to understand spatial and temporal patterns of genetic differentiation across varied ecological landscapes. This review synthesizes the current understanding of allozyme-based genetic studies in houseflies, highlighting their relevance in evolutionary genetics, population monitoring, and vector control strategies.

Allozymes as Genetic Markers in Housefly

Allozymes are variant forms of enzymes encoded by different alleles at the same locus. These are separated and identified by their migration patterns on starch or polyacrylamide gels. They allow for direct estimates of allele frequency, polymorphism level,

[1]**Corresponding author**

observed (Ho) and expected (He) heterozygosity, Hardy-Weinberg equilibrium, and genetic distance and identity (Anne 2006; Berg and Hamrick, 1997; Liu and Furnier, 1993). They are particularly informative in revealing functional variation and adaptive potential in wild populations. Numerous enzyme loci have been analyzed in housefly populations, such as Acid Phosphatase (ACPH), Alkaline Phosphatase (APH), Malate Dehydrogenase (MDH), Lactate Dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G6PD), and Malic Enzyme (ME) (Steiner, 1993; Tripathi et al., 2011; Tripathi et al., 2015). These enzymes reflect variation in both glucose-metabolizing and non-glucose-metabolizing pathways. Allozyme-based Nei's genetic identity (I) values often show >0.8 for closely located populations, but drop below 0.6 for populations separated by geographic or anthropogenic barriers (Butlin and Tregenza, 1998; Stacy, 2001). These data help delineate between the isolated and connected populations and selective pressure gradients. Allozyme markers provide a wide range of genetic information in the housefly, assisting the prospects in population studies (Table 1).

Table 1: A list of some commonly Used Allozyme Markers in *M. domestica* and their applications in genetic studies

Enzyme Marker	Locus	Function	Genetic Insight	Reference
Acid Phosphatase	ACPH	Lysosomal activity	Population differentiation	Tripathi et al., 2010
Alkaline Phosphatase	APH	Digestive enzyme	Hardy-Weinberg deviations, allele polymorphism	Srivastava et al., 2012
Malate Dehydrogenase	MDH	Energy metabolism	High heterozygosity, gene flow	Krafsur et al., 1992
Malic Enzyme	ME	NADP-linked metabolism	Adaptive variation	Black & Krafsur, 1985
Lactate Dehydrogenase	LDH	Anaerobic glycolysis	Ecotypic variation	Sharma et al., 2009
Glucose-6-phosphate Dehydrogenase	G6PD	Detoxification pathway	Resistance studies	Tewari & Thakur, 1994

Global and Indian Housefly Populations

Studies from the USA, Europe, and Africa revealed high levels of allozyme polymorphism, significant geographical differentiation, and gene flow across regions. Indian investigations revealed 33–100% polymorphic loci, moderate to high heterozygosity, and deviation from Hardy-Weinberg equilibrium in urban populations, likely due to inbreeding, selection, or founder effects. The genetic variability in houseflies is a requisite for their survival and effective environmental stress response (Sharma et al., 2009). Some research group advocates the use of allozyme heterozygosity as an important measure for the study of population fitness and adaptive potential (Beardmore 1983; Allendorf & Leary 1986; Houle 1989); while some of the studies restraint these genetic data indicating a small portion of the genome, hence not good enough for studies related to adaptive genetic differences (Hedrick and Miller 1992; Reed and Frankham 2001). Allozyme markers have been largely used worldwide as well as in the Indian subcontinent for the assessment of genetic variations among house fly populations (Stanger 1984; Black IV and Krafsur 1985; Krafsur et al. 1992; Tripathi et al. 2010; Tripathi et al. 2011; Tripathi et al. 2012; Tripathi et al. 2015).

In one of the genetic variation studies in *M. domestica*, the genetic identity values among the three populations studied are lower, mainly attributed to the fact that the three populations surveyed were separated by physical barriers that prevent gene flow (Srivastava et al. 2012). Several factors, such as colonization, host and reproductive pressures, cause the genetic variation, subject to the species richness, distribution, and different environmental conditions, leading to genetic heterozygosity compared to restricted species distribution (Narang 1980; Santos et al., 2005). Some studies have suggested that the genetic variability is found to be higher for the non-glucose metabolizing system enzyme group than for the glucose metabolizing system enzyme group in the M. domestica population through allozyme analysis (Tripathi et al. 2015), as elucidated by the neutral theory of Kimura (Kimura 1983).

Significance of Allozymes

Populations showing high variation in detoxification enzymes may exhibit enhanced resistance

profiles. Housefly populations in diverse climates maintain variation necessary for survival under stress (Tripathi et al., 2011). Genetically differentiated fly populations may vary in vectorial capacity and pathogen association, giving useful insights into disease epidemiology, vector control, and ecology. Additionally, allozyme markers are inexpensive, require minimal DNA processing, and are codominant and interpretable, functional protein-level variations (Crozier, 1993; Richardson et al., 2012). Despite its relevant advantages, it carries some limitations, like limited genome coverage, being affected by environmental factors, being less informative than SNPs or microsatellites, or other molecular markers (Al-Samarai and Al-Kazaz, 2015; Helyar et al., Nielsen, 2011; Putman and Carbone, 2014). Although high-throughput genotyping is more comprehensive, allozyme analysis remains a valuable first-tier screening method, especially in resource-limited settings. Combining allozymes with molecular markers can offer multi-dimensional insights into the *M. domestica* population dynamics.

CONCLUSION

Allozyme markers have played a pivotal role in unravelling the genetic diversity and population structure of *M. domestica*. Through electrophoretic analysis of enzyme polymorphisms, researchers have gained insights into the extent of genetic variation, levels of heterozygosity, and evolutionary forces shaping housefly populations across different geographical regions. These studies have revealed both intra- and inter-population diversity, highlighting the influence of ecological conditions, anthropogenic factors, and natural selection. Although newer genomic tools have expanded the scope of population genetics, allozyme analysis continues to be a valuable, accessible, and informative method, especially in resource-limited settings. Future studies integrating allozymes with molecular markers such as microsatellites or SNPs can offer a more comprehensive understanding of adaptive potential and gene flow in this medically important insect species. Allozyme markers continue to be a vital tool for assessing population-level genetic diversity in houseflies. Their application in India and globally has illuminated important evolutionary and ecological trends as depicted by speciation and host-pathogen interactions. As houseflies remain major disease vectors and urban pests, continued genetic monitoring using allozyme and molecular tools is essential for effective management and ecological forecasting.

Conflicts of Interest: The author declare no conflicts of interest.

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