International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **4(10),** 20-23, October **(2015)** *Int. Res. J. Biological Sci.*

Electrophoretic Analysis in two groups of enzymes of *Musca domestica* L. (Diptera: Muscidae)

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Available online at: www.isca.in, www.isca.me
Received 17th August 2015, revised 28th August 2015, accepted 4th October 2015

Abstract

Indian population of Musca domestica was examined by PAGE for allozyme variation. One to five enzyme loci in the glucose metabolizing system (Group I) and six to fourteen enzyme loci in the non-glucose metabolizing system (group II) were assayed. The parameter estimated were the allele frequencies, proportion of polymorphic loci and average heterozygosity for Group I and Group II loci. The present finding is that the genetic variability measured by allozyme variation is much higher for group II than for group I enzymes in M.domestica population. The loci coding for the hydrolytic and other nonspecific enzymes are much more variable than the loci coding for the enzymes of the glycolytic pathway, Kreb's cycle and other specific enzymes.

Keywords: Glucose metabolizing enzyme, non glucose metabolizing enzyme, heterozygosity, *Musca domestica*, genetic variation.

Introduction

Glucose metabolizing enzymes (Group I) show less heterozygosity as compared to the enzymes which are involved in non glucose metabolizing systems (Group II)[1-6]. It was postulated that Group I enzymes are characterized by a singular physiological substrate which is generally generated and utilized intracellularly, while Group II enzymes have multiple physiological substrate which originate from the external environment. Heterozygosity and polymorphism i.e. the proportion of the loci that are genetically variable are the most important measures to compare the amount of genetic variation within different population[7].

Genetic variability in *Musca domestica* populations using electrophoretic technique to detect enzyme variants have revealed that 40% - 60% of the loci examined are polymorphic[8-17]. As most enzymes used have broad substrate specificities, it would be interesting to compare the degree of genetic variability in enzymes known to be active in energy metabolism.

The present study was carried out to compare the amount of polymorphism and the degree of heterozygosity among glucose and non glucose metabolizing enzymes in *Musca domestica* from Allahabad, India.

Material and Methods

Laboratory colonies of *Musca domestica* L. (Diptera: Muscidae) were established in the Cytogenetics research laboratory of Department of Zoology, University of Allahabad, Allahabad, India. In the present study adult male flies of approximately same age were taken and assayed for enzyme activity at 10 gene enzyme systems viz., malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), acid phosphatase (ACPH), esterase (EST), octanol dehydrogenase (ODH), alkaline phosphatase (APH) and aldehyde oxidase (AO). Sample preparation and electrophoretic procedure were according to the method of Tripathi et al. 2010[14]. The staining protocol of Ayala et al.[18] and Tsukamoto[19] were followed for the analysis of enzyme activity.

Band mobilities were measured and expressed as R_f (x 100) as per the method of Tsukamoto and Horio[20]. Electrophoretic genotypes were determined by comparison of the relative mobilities of the bands. The genotype information was used to calculate frequencies of allele for each enzyme[21]. The genotype information, thus obtained was used to estimate genetic variability using polymorphic loci, mean observed (H_o) and expected (H_E) heterozygosity.

Results and Discussion

Ten enzyme systems revealed fourteen (14) loci in *Musca domestica*. Table-1 represents the allelic frequencies and Chi square values. Out of 14 loci, only four are monomorphic and the rest are polymorphic.

The enzymes investigated in the present study were categorized into glucose metabolizing (Group I) and non glucose metabolizing enzymes (Group II) following the Classification of Gillespie and Kojima[1] and Kojima et al [2]. Thus MDH, IDH, G6PD, ME and LDH were categorized as glucose metabolizing enzymes (Group I) and ACPH, EST, ODH, AO and APH were grouped as Non glucose metabolizing enzymes (Group II).

Table-1
Allele frequencies and Chi square values at ten enzyme loci in *Musca domestica*

Locus	(n)	Allele frequencies		Chi square values
		a	B	
MDH	50	0.54	0.46	6.33
IDH	50	0.53	0.47	11.43
LDH	50	0.68	0.32	19.99
G6PD	50	1.00	-	-
ME	50	1.00	-	-
ACPH-1	50	0.58	0.42	17.37
ACPH-2	50	0.46	0.54	9.52
EST-1	50	0.59	0.41	2.30
EST-2	50	0.51	0.49	1.27
EST-3	50	0.46	0.54	6.33
ODH-1	50	1.00	-	-
ODH-2	50	0.60	0.40	8.68
APH	50	0.62	0.38	1.14
AO	50	1.00	-	-

(n)= number of individuals in each sample

In Group I MDH, IDH and G6PD are polymorphic and the average heterozygosity is 0.148 for the five loci surveyed. In Group II among nine loci the activity at the loci APH and AO was confined to single locus, the activity of ACPH and ODH was confined to two loci while the activity of EST was confined to three loci. AO and ODH-1 are monomorphic and ACPH-1, ACPH-2, EST-1, EST-2, EST-3, ODH-2 and APH are polymorphic. Average heterozygosity is 0.253 for the nine loci surveyed (table-2).

A comparison of proportion of the polymorphic loci of two groups revealed that the glucose metabolizing enzymes (Group I) were 60% polymorphic as compared to non glucose metabolizing enzymes (Group-II) which were 77.77% polymorphic. Thus the data shows that the loci in Group I are less polymorphic than the loci in Group II as also opined by Gillespie and Kojima[1]. Similarly the average heterozygosity for glucose metabolizing enzymes is less than the non glucose metabolizing enzymes (table-2). It seems that the enzymes of Group II which include various non-specific hydrolytic enzymes are the most variable, whereas the enzymes of Group I, which are more specific in their mode of action and play a more significant role in cellular physiology, harbor less variation. In other Dipterans[2,3,5,6,22] and other animals (echinoderms and fishes)[23-28] similar pattern of result was observed between the two groups of enzymes.

However, Frydenberg and Simmonson[29] found that glucose metabolizing enzymes in eel pout population tend to be as polymorphic as non glucose metabolizing enzymes. They concluded that the hypothesis of Gillespie and Kojima may not be a general one for animal species. Band[4] findings on *Drosophila melanogaster* populations supported the conclusions of Frydenberg and Simmonson[29]. But in Indian *M. domestica* population greater polymorphism and genetic variability is maintained in the enzymes of group II. Thus the hypothesis proposed by Gillespie and Kojima[1] that glucose metabolizing enzymes tends to be less polymorphic than non glucose metabolizing enzymes, is supported by the data from *Musca domestica*.

Table-2
Average heterozygosities for Group I and Group II enzymes in *Musca domestica*

Locus	**Heterozygosity**	
Group I	H_o (Observed)	H_E (Expected)
MDH	.320	.497
IDH	.260	.498
LDH	.160	.435
G6PD	-	-
ME	-	-
Mean heterozygosity	.148	.286
Group II		
ACPH-1	.200	.487
ACPH-2	.280	.497
EST-1	.380	.484
EST-2	.420	.499
EST-3	.320	.497
ODH-1	-	-
ODH-2	.280	.480
APH	.400	.471
AO	-	-
Mean heterozygosity	.253	.379

Conclusion

It seems that in *M.domestica* the glucose metabolizing enzymes (Group I) are more stringent against electrophoretic variation than that of non-glucose metabolizing enzymes (Group II). These results can be explained by the neutral theory of Kimura[30].

Since the glucose metabolizing enzymes have functional importance therefore functional constraint of these enzymes is much stronger. The enzymes of group II because of a nonspecific and less significant role in the physiology of organism, can tolerate far greater electrophoretic variations.

Acknowledgement

We are grateful to Prof. R.R. Tewari, Cytogenetics Research laboratory, Department of Zoology, University of Allahabad for their useful suggestions, constant encouragement and providing laboratory facilities during the course of present work.

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