ISSN 0975-6299 Vol 3/Issue 2/April – June 2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE MOLECULAR BIOLOGY

A SURVEY OF ISOZYME POLYMORPHISM IN LABORATORY POPULATION OF *S.DUX* (DIPTERA, SARCOPHAGIDAE)

KIRAN SINGH*[1] AND SASYA THAKUR[2]

1,2 Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology & Sciences – Deemed University, Allahabad–211007, India

KIRAN SINGH

Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology & Sciences – Deemed University, Allahabad–211007, India

ABSTRACT

A survey of isozyme polymorphism among glucose and non-glucose metabolizing enzymes was carried out in the laboratory population of *Sarcophaga dux*. Polymorphic levels were found to be within the range recorded for other dipterans. Polymorphism among glucose-metabolizing enzymes were found to be on an average less variable (H_o=0.186; H_e=0.163) than the non-glucose metabolizing enzymes (H_o=0.275; H_e=0.485). Out of five loci, three loci (LDH-1, ACPH-1 and APH-1) were found to be polymorphic and these displayed significant deficiency of heterozygotes. These results supported the earlier findings that the substrate-specific enzymes with functional constraints have lower heterozygosity than the non-specific enzymes. They also added to the evidence obtained from other animals like *Drosophila*, echinoderms and some fish species.

KEYWORDS

isozyme polymorphism, glucose metabolizing enzymes, non-glucose metabolizing enzymes, functional constraint, neutral mutation, *Sarcophaga dux*

INTRODUCTION

While it is well established that the allozyme polymorphisms are ubiquitous, their adaptive significance still remains a matter of controversy. Among the various factors which have been implicated in maintaining the genic variation in any population, a very attractive hypothesis was postulated by Gillespie and Kojima[1] and later examined in detail by Kojima et al[2] and Band[3]. They proposed that enzymes involved in glucose-metabolizing systems (Group I) will be, on an average less variable than enzymes which are not involved in the above mentioned systems (Group II). Their argument was that, for Group I enzymes, substrates *in vivo* are restricted in most cases to a single species of molecule which is the product of a previous enzyme reaction and does not come directly from the environment. On the other hand, the Group II enzymes act on a class of molecules, many of which come from the external environment. Thus the substrate environment of the Group II enzymes will be quantitatively more variable than that of the Group I enzymes.

The purpose of this paper is to report a survey of five loci in the laboratory population of *Sarcophaga dux*, to compare heterozygosity among glucose and non-glucose metabolizing enzymes with estimates obtained for other dipteran populations[2,4-8] and other animal populations[9-13].

MATERIALS AND METHODS

In the present study adult male flies were taken from laboratory colonies of *Sarcophaga dux* (Thompson). Flies of approximately same age were homogenized individually in 0.2 ml of chilled double distilled water in a glass and Teflon tissue grinder. The homogenate was centrifuged at 3000 rpm for 15 minutes and only the supernatant was used for the assay of enzyme activity. Electrophoresis was carried out on 7% polyacrylamide gel at 4°C. The run was terminated when the front marked with Bromophenol blue reached the lower end of the gel. Five randomly chosen allozymic systems, the staining solutions and gel and electrode buffers used are as follows:

1*. Acid Phosphatase (ACPH, E.C. 3.1.3.2)*
Gel buffer: 0.1 M Tris-borate (pH 8.9)
Electrode buffer: 0.1 M Tris-borate (pH 8.9)
Staining solution: 50 ml 0.1 M Acetate buffer (pH 5.0), 250 mg PVP, 50 mg Fast blue BB,
50 mg sodium α naphthyl phosphate

2*. Malic Enzyme (ME, E.C. 1.1.1.40)*
Gel buffer: 0.1 M Tris-HCl (pH 8.5)
Electrode buffer: 0.1 M Tris-HCl (pH 8.5)
Staining solution: 50 ml 0.1 M Tris-HCl buffer (pH 7.4), 25 mg malic acid, 10 mg NBT,
13 mg NADP, 12 mg $MnCl_2$, PMS< 1mg

3*. Lactate Dehydrogenase (LDH, E.C. 1.1.1.27)*
Gel buffer: 0.1 M Tris-HCl (pH 8.5)
Electrode buffer: 0.1 M Tris-HCl (pH 8.5)
Staining solution: 50 ml 0.1 M Tris-HCl buffer (pH 7.4), 1 ml D, L Sodium lactate,
15 mg NBT, PMS < 1 mg, 25 mg NAD

This article can be downloaded from www.ijpbs.net

4. *Xanthine Dehydrogenase (XDH, E.C. 1.2.1.37)*
Gel buffer: 0.1 M Tris-HCl (pH 8.5)
Electrode buffer: 0.1 M Tris-HCl (pH 8.5)
Staining solution: 50 ml 0.1 M Tris-HCl (pH 8.0)

Solution A: 15 mg hypoxanthine, 10 ml 0.1 M Tris, EDTA < 1 mg, 15 mg NAD

Solution B is added to Solution A and gels incubated at 38ºC

Band mobilities were measured and expressed as R_f (X100) as per the method of Tsukamoto and Horio[14].Electrophoretic genotypes were determined by comparison of the relative mobilities of the bands. The genotype information was used to calculate the frequencies of electromorphs for each enzyme[14].

RESULTS AND DISCUSSION

The five enzymes examined yielded five loci in *S.dux.* The schemes of zymograms are shown in the Figure 1. It can be seen that all the five enzymes were found to be encoded by a single locus. Of these ME and XDH were monomorphic as no electrophoretic variation was observed

Solution B: 10 ml d. water, 15 mg NBT, PMS < 1mg
.**5. *Alkaline Phosphatase (APH, E.C. 3.1.3.1)***
Gel Buffer: 0.1 M Tris–borate (pH 8.9)

Electrode buffer: 0.1 M Tris–borate (pH 8.9)
Staining solution: 50 ml 0.05 M Tris-HCl buffer (pH 8.6), 250 mg Polyvinylpyrrolidone (PVP), 50 mg Fast Blue BB, 50 mg Sodium-α-Naphthyl phosphate, 30 mg $MgCl_2$, 30 mg $MnCl_2$,
2 gm NaCl

While LDH, APH and ACPH revealed polymorphism for two electromorphs and three electrophoretic phenotypes. The enzymes of the LDH-1, APH-1 and ACPH-1 suggest their monomeric structure. The XDH and ME loci do not provide information about the structure of the enzyme as they are monomorphic.
The information about the genotypic and allelic frequencies is presented in Table 1. All the polymorphic loci presented two alleles and three genotypes. The estimation of genetic variation in this population for three analysed polymorphic loci was: mean observed heterozygosity (H_o = 0.222), mean expected heterozygosity (H_e = 0.292), proportion of polymorphic loci (P = 60%) and mean number of alleles per locus (A_L) = 1.6.

Table 1.
Average heterozygosity for groups of enzyme loci (Group I and Group II) in S.dux (H_o, observed heterozygosity; H_e, expected heterozygosity)

Enzyme	Heterozygosities	
	H_o	H_e
A. Glucose metabolizing enzymes (Group I enzymes)		
LDH-1	0.56	0.49
XDH-1	0	0
ME-1	0	0
Mean Value	**0.186**	**0.163**
B. Non-Glucose metabolizing enzymes (Group II enzymes)		
ACPH-1	0.26	0.49
APH-1	0.29	0.48
Mean Value	**0.275**	**0.485**

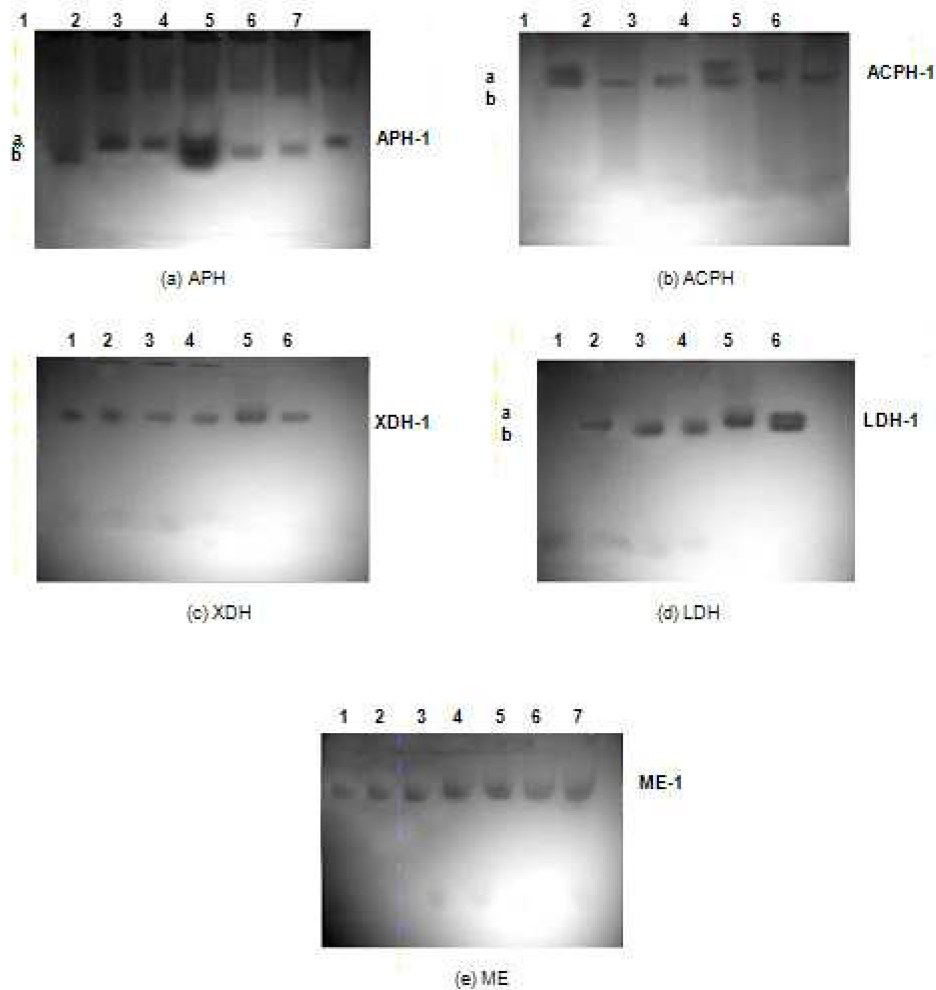

Fig.1 (a-e): Different banding patterns found for each enzyme in 50 analyzed *Sarcophaga dux*. Different alleles and genotypes for each polymorphic locus are shown. (The zones of activity are indicated on the right and electromorphs are indicated on the left).

The polymorphism statistics for each of the two groups is presented in Table 1, where it is clearly shown that the loci in Group I are less polymorphic than the loci in Group II. Thus our data seems to be in accordance with the hypothesis of Kojima et al[2]. Data from various groups (Table 2) were also found to be compatible with the proposal of Kojima et al[2].

Table 2.
Average heterozygosity for groups of enzyme loci (Group I and Group II) as proposed by Kojima et al (1970)*

Enzyme Group	Group I		Group II		Group III	
Loci group	**All Loci**	**Polymorphic loci**	**All loci**	**Polymorphic loci**	**All loci**	**Polymorphic loci**
Drosophila	0.118 (16)	0.118 (16)	0.224 (10)	0.224 (10)	0.071	-
Other insects	0.199 (15)	0.214 (14)	0.256 (9)	0.256 (9)	0.139	-
Non insect invertebrates	0.126 (16)	0.134 (15)	0.142 (8)	0.189 (6)	0.122	-
Fish	0.057 (15)	0.077 (11)	0.121 (8)	0.161 (6)	0.016	-
Amphibians	0.099 (8)	0.132 (6)	0.118 (4)	0.118 (4)	0.100	-
Reptiles	0.038 (11)	0.051 (8)	0.067 (5)	0.084 (4)	0.028	-
Birds	0.109 (10)	0.181 (6)	0.088 (3)	0.265 (1)	-	-
Mammals (excluding man)	0.052 (14)	0.066 (11)	0.038 (9)	0.043 (8)	0.06	-
Man	0.050 (45)	0.237 (11)	0.130 (20)	0.273 (10)	0.162 (12)	0.216 (9)
Total #	**0.087**	**0.136**	**0.140**	**0.186**	**0.092**	-

(Except for Group III, the means is weighted by the number of loci.
Group III contains non-enzymatic proteins. Number in parenthesis represents the number of loci).
**** Taken from Singh[15]***

Gillespie and Kojima[4] were first to point out that both average heterozygosity and the proportion of polymorphic loci were less for glucose-metabolizing enzymes than for non-glucose metabolizing enzymes. These studies were later on extended on other dipterans[2,4,5-8,16-19] as well as on other animals[6,9-13] and the similar pattern of results was observed between the two groups of enzymes.

At variance with the above results, Frydenberg and Simonsen[9] found that glucose metabolizing enzymes in eel pout populations along the Danish coast tended to be as polymorphic as non-glucose metabolizing enzymes. They concluded that the hypothesis of Gillespie and Kojima[1] may not be a general one for all animal species. Findings of Band[3] for the South Amherst *Drosophila melanogaster* population supported the conclusions of Frydenberg and Simonsen[9].Further with respect to the relationship between enzyme function and heterozygosity, Yamazaki[20] and Gojobori[21] showed that the substrate-specific enzymes with functional constraint have lower heterozygosity than the non-specific enzymes. These results can be explained by the neutral theory of Kimura[22].According to this theory, the glucose metabolizing enzymes have functional importance, and therefore functional constraint of these enzymes is much stronger than that of

non-glucose metabolizing enzymes. The more strictly functional constraint decrease neutral regions of the molecules, and thus the probability of neutral mutation would be smaller for the glucose-metabolizing enzymes than for non-glucose metabolizing enzymes.

The use of laboratory strains often leads to limitations in the conclusions drawn from the data. Clearly genetic variability could be reduced in laboratory strains even if loss of heterozygosity is not a required phenomenon[23].The establishment of a breeding stock from founder individuals corresponds to a bottleneck. However, Frankel and Soule[24] emphasized that “a bottleneck will not, by itself, erode much of the genetic variance” and that the effects of these bottlenecks on allelic diversity are moderate[25].

CONCLUSION

Thus it seems that continued simultaneous studies on biochemical polymorphisms in the population may give us important clues to the adaptive roles of enzyme polymorphisms. Biochemical polymorphism in the field populations of *S.dux* and other species of Sarcophagidae with more gene-enzyme systems may provide further evidence on the tendency of their populations to maintain higher levels of heterozygosity among glucose-metabolizing enzymes.

REFERENCES

1. Gillespie JH and Kojima KI, The degree of polymorphism in enzymes involved in energy production compared to that in non-specific enzymes in two *Drosophila ananassae* populations.Proc.Natl.Acad.Sci.U.S. 61, 582-585, (1968).
2. Kojima KI, Gillespie JH and Tobari YM , A profile of *Drosophila* species’ enzymes assayed by electrophoresis.I.Number of alleles, heterozygosities and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochem.Genet. 4, 627-637, (1970).
3. Band HT, A survey of isozyme polymorphism in a *Drosophila melanogaster* natural population. Genetics 80, 761-771, (1975).
4. Ayala FJ and Powell JR, Enzyme variability in the *Drosophila willistoni* group VI. Levels of polymorphism and the physiological function of enzymes. Biochem.Genet. 7, 331-345, (1972).
5. Prakash S, Patterns of gene variation in central and marginal populations of *Drosophila robusta*. Genetics 75, 347-369, (1973).
6. Gillespie JH and Langley GH, A general model to account for enzyme variation in natural populations. Genetics 76, 837-848, (1974).
7. Thakur S, Allozyme variation in fleshflies (Diptera: Sarcophagidae, D.Phil. Dissertation, University of Allahabad, Allahabad, (1992).
8. Tripathi M, Genetic variation in house fly, *Musca domestica* (Muscidae: Diptera). D.Phil. dissertation, University of Allahabad, Allahabad, (2006).
9. Frydenberg O and Simonsen, Genetics of *Zoarces* populations V. Amount of protein polymorphism and degree of genic heterozygosity. Hereditas 75, 221 – 232, (1973).
10. Matsuoka N and Nakamura Y, Enzyme variation within the population of the sea-urchin, *Glyptocidaris crenularis*, from Japanese waters. Comp.Biochem.Physiol.B. 96(2), 335-338, (1990).

11. Matsuoka N and Hatanaka T, Molecular evidence for the existence of four sibling species within the sea urchin, *Echinometra mathaei* in Japanese waters and their evolutionary relationships. Zool.Sci. 8, 121-133, (1991).
12. Asanuma T and Matsuoka N, Phylogenetic relationships among seven fish species of the order Clupeiformes inferred from allozyme variation. Bull. Fac. Agric. & Life Sci., Hirosaki University. 4, 1-15, (2002).
13. Matsuoka N, Molecular Phylogeny and Allozyme Variation of the Five Common Fish Species of the Suborder Percoidei. Bull.Fac.Agric. & Life Sci. Hirosaki Univ. No. 5, 17-22, (2003).
14. Tsukamoto M and Horio M, Electrophoretic comparison of the lactate dehydrogenase banding pattern among Japanese mosquito larvae (Diptera: Culicidae). J.Med.Entomol. 22, 491-498, (1985).
15. Singh RS Substrate specific enzyme variation in natural populations of *Drosophila pseudoobscura*. Genetics 82, 507-526, (1976).
16. Richmond RC, Enzyme variability in the *Drosophila willistoni* group III. Amounts of variability in the superspecies *D.paulistorum*. Genetics 70, 87 – 112, (1972).
17. Singh K, Genetic variation in carrion-breeding blowfly, *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and flesh fly, *Sarcophaga dux* (Thompson, 1869) (Diptera: Sarcophagidae). Ph.D. Dissertation, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad, (2011).
18. Singh K, Thakur S and Ramteke PW, Electrophoretic analysis of genetic variability in carrion-breeding blowfly, *Chrysomya megacephala* Fabricius. Int.J.Pharma.Biosci. 3(1), B538-B546, (2012).
19. Singh K and Thakur S, A Survey of Isozyme Polymorphism in Laboratory Population of *C.megacephala* (Diptera, Calliphoridae). In: 99th Indian Science Congress, pp. 157.
20. T.Yamazaki. Enzyme polymorphism and functional difference: mean, variance and distribution of heterozygosity. In: M. Kimura (eds.), *Molecular Evolution and Polymorphism*, Mishima: National Institute of Genetics, Mishima, 1977, pp., 127-147.
21. T. Gojobori. Means and variances of heterozygosity and protein function. In: M.Kimura (eds.), Molecular *Evolution, Protein Polymorphism and the Neutral Theory*, Japan Scientific Socities Press, Springer-Verlag, Berlin ,1982 pp. 137-148.
22. Kimura M, The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge (1983).
23. Mason LJ, Pashley DP and Johnson SJ, The laboratory as an altered habitat: phenotypic and genetic consequences of colonization. Florida Entomol. 70, 49-58, (1987).
24. Frankel OH and Soule ME, Conservation and evolution Cambridge University Press, Cambridge. (1981).
25. Vernon P, Cariou ML and Deunff J, Genetic variability in the wingless subantarctic genus *Anatalanta* (Diptera, Sphaeroceridae): a preliminary approach. Polar Biol. 18(6), 384-390, (1997).