Allozyme Diversity Inforensically Important Indian Species *Chrysomya Megacephala* (Fabricius) (Diptera: Calliphoridae)

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Abstract. The application of electrophoretic technique to study allozyme enzymatic variation has been extensively used to explore hidden genetic variability in natural population and laboratory colonies of many calliphorid flies. Genetic variation at three enzyme loci viz., Alkaline phosphatase (APH), Xanthin dehydrogenase(XDH)and Malate dehydrogenase (MDH) in laboratory colonies of *Chrysomya megacephala*were investigated by using polyacrylamide gel electrophoresis (PAGE). In APH three zones of activity were observed. Which have been designated as APH-1, APH-2, and APH-3 in order of increasing anodal migration. The electrophoretic phenotypes with two codominant alleles were observed at APH-3loci. In MDH and XDH only one zone of activity was observed.

Keywords: Genetic Variation · Allozyme · Electrophoresis · Allozyme Variability

1 Introduction

Enzymatic markers are beneficial gear for estimating genetic variant [1]. The level of genetic variation among populations has received substantial interest, as it's miles indicative of overall species vitality and the capacity for evolutionary responses to environmental changes.

Allozymes have been utilized for making deliberate deductions in bugs for over 20 years [2, 3]. Berlocher (1984b) [4] distinguished three significant errands of such examinations: species segregation, species recognizable proof, and various leveled grouping. The utilization of allozymes to segregate species is currently normal. Models incorporate Polyacrylamide electrophoresis to portray hereditary difference among mysterious types of the Chrysoperla carnea complex in Europe [5] and cellulase acetic acid derivation electrophoresis to give demonstrative characters to distinguishing proof of larval leaf diggers (Liriomyza spp.) [6]. At the lower formal degrees of order (intrageneric), allozymes have demonstrated helpful in numerous bug gatherings (see models in Table 1). At higher ordered positions, allozymes have been less significant in light of the great degree of uniqueness and the probability of homoplasy at the intergeneric reach or more [e.g., in tortricoid moths, Pashley (1983), yet see Brussard et al. (1985).

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The blow flies of the family calliphoridae are distributed worldwide and some of the species belonging to this family are known to be causative agents of animal tissue myasis, causing several losses to sheep and cattle. The technique of isozyme electrophoresis to blow flies genetics was introduction by Bush et al. (1976) [7] in *Cochliomyiahominivorax*. In subsequent years, analysis of genetic variation among calliphorids have been carried out only in the genus *Cochliomyia* and *Calliphora*. Analysis of enzyme variations a valuable and cost effective marker for population genetic studies, despite the advent of several molecular markers in recent years. In the present work allozyme variation of three enzymes Alkaline phosphatase (APH), Xanthin dehydrogenase (XDH)and Malate dehydrogenase (MDH) were studied with a view to unravel enzymatic polymorphism.

2 Materials and Methods

The present work was carried out in *Chrysomya megacephala* (Fabricius) (calliphoridae: Diptera). Specimenswere collected using sweep net from different localities of Allahabad and stock cultures were maintained in the laboratory at 27 ± 1 ºC in insect rearing cages. Studies on enzyme staining patterns were carried out in adult male flies using polyacrylamide gel electrophoresis (PAGE) in tube gel electrophoresis apparatus on 7% polyacrylamide gel and selective enzyme staining protocols. The details of methods are described below.

2.1 Preparation of sample

Flies were taken out of cage and anaesthetized with ether, wings and legs of individual male flies were removed before homogenization in 0.4 ml of chilled double distilled water in a glass and Teflon tissue grinder.

Individual male flies were homogenized in 0.4 ml chilled purified water in a glass and Teflon tissue grinder. The homogenates were centrifuged at 1500 rpm for 5 min. The supernatant was collected for further processing.

2.2 Casting of Gel

For gel casting the lower end of the gel tube (9.0 cm) was moistened with distilled water and fitted in to the rubber grommets of the gel tube stand. The separating rod gel was prepared by mixing the following solutions: 3.5 ml 40% Acrylamide, 2.5 ml 5% N,NMethyleneBisacrylamide(BIS), 5 ml gel buffer, 10 μml N, N, N', N'-Tetramethyl Ethylenediamine(TEMED), 7.5 ml double distilled water, 1.5 ml 0.28% Ammonium persulphate.

The gel tubes were filled carefully up to about 2.0 cm below the top rim with the thoroughly mixed solution and immediately covered with a layer of distilled water to prevent meniscus formation. Water was removed after polymerization and the tubes were carefully screwed in to the rubber grommets of the top buffer chamber. Electrode buffer was poured in to the electrode chamber.

2.3 Electrophoresis

For electrophoresis 20 μl of the sample was loaded on top of the separating gel and covered with 20% sucrose solution mixed with 0.1% Bromophenol blue (in ratio of 9:1). The gel tubes were then filled with electrode buffer up to the brim. Subsequently the upper electrode chamber was also filled with electrode buffer. The power supply unit (Pharmacia, EPS 400/500) was set up and electrophoresis was performed at 4oc at a constant current of 2.5 ma/gel tube. The run was terminated when the dye front reached the lower end of the gel.

2.4 Enzyme Staining

After electrophoresis individual gels were removed and stained in test tubes with specific enzyme staining solution in accordance with the procedures adopted from Ayala et al. (1972a) [8], Tsukamoto (1989) [9] and ManchenKo (1994) [10]. The stained gels were stored in refrigerator in sealed tubes containing 7% acetic acid. For photography the gels were arranged in a Petri dish placed on an illuminator with an opal white screen and photographed with Nikon Coolpixs 400 camera. Conventional method has been used for genetic interpretation of the observations. Single band indicates homozygotes and multiple band/diffuse bands represent heterozygotes [11]. Multiple loci were numbered in order of ascending migration distance from the origin and indicated by hyphenated numeral following the enzyme abbreviation, e.g., APH-1, APH-2. The relative mobility of each band was calculated and expressed as Rf value ($\times 100$), following the method of Tsukamoto and Horio (1985) [12]. Genotypic and allelic frequencies, estimates of genetic variation such as meanheterozygosity (H),percent of polymorphism (P%) and mean number of alleles per locus (AL),as well as deviations from Hardy-Weinbergequilibrium expectations, were calculated basedon the banding patterns.

3 Result and Discussion

One gene- enzyme systems were analysed to reveal genetic variation in *Chrysomya megacephala* in the present work. Only One zone of activity was observed in Xanthin dehydrogenase (XDH)and Malate dehydrogenase (MDH). Three zones of APH activity were observed in zymogram of a single fly homogenate. These zones were designated as APH-1, APH-2, and APH-3 in order of increasing anodal migration. APH-1 is slowest moving zone while the APH-3 is the faster moving zone. Three electrophoretic phenotypes with two codominant alleles were observed at APH-3. Gels incubated in staining solution without the corresponding substrates for all the above mentioned enzymes were used as control. Conventional method of [11] was used for genetic interpretations for staining pattern of all the three gene enzyme systems.

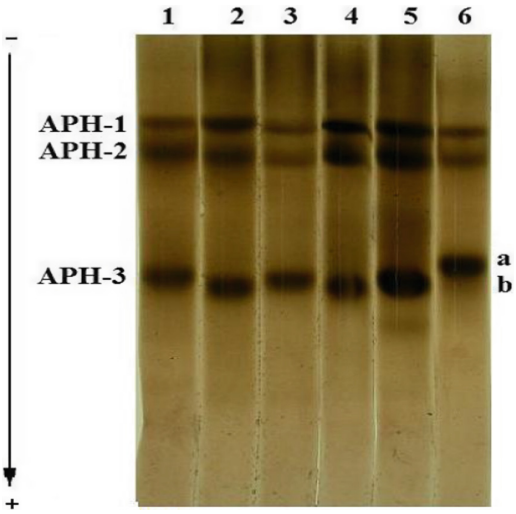

Fig. 1. The banding patterns of APH

Single bands indicate homozygotes and multiple banded phenotypes represent heterozygotes. The observed and expected heterozygosities were calculated by following formulae.

$$\text{Ho} = \text{Observed heterozygosity} = \text{No. of heterozygotes / Total no. of individuals}$$

$$\text{HE} = \text{Expected heterozygosity} = 1 - \sum \text{Xi2}$$

(Nei, 1972) [13].

(Where Xi is the frequency of ithelectromorph). The mean heterozygosity (H) was calculated as the mean of HE over all loci examined. The calculated heterozygosity was compared with the observed heterozygosity (Ho) for deviation from Hardy-Weinberg equilibrium by chi-square test.

The enzyme activity at APH-3 locus is characterized by conspicuous andconsistent bands in all the individuals. At APH-3 locus, three different electrophoretic phenotypes were found, which are assumed to be governed by twoelectromorphs, viz, slow migrating APH-3aand APH-3b (Fig. 1). The heterozygotes were charecterised by broad diffuse bands. The relative mobility of APH-3a electromorphs is 41 and that of APH-3b electromorph is 45. The electromorphs APH-3a and APH-3b are present with a frequency of 0.59 and 0.41, respectively, in the sample analysed during the present study. The observed and expected heterozygosity values were 0.23 and 0.48, respectively. Chi-square test revelled (Table 1) that the distribution of electrophoretic phenotypes were not at Hardy-Weinberg equilibrium.

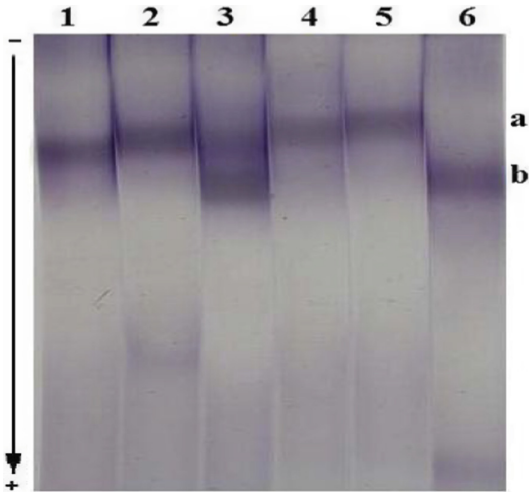

Fig. 2. The banding pattern of MDH enzyme

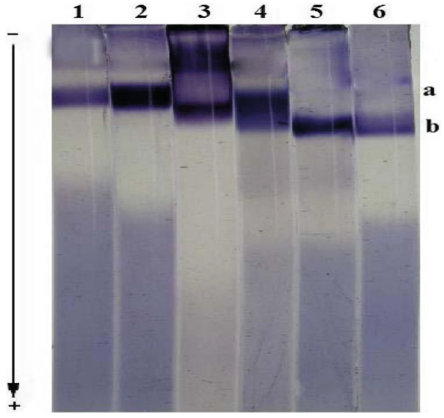

Fig. 3. The banding pattern of XDH enzyme

The enzyme activity at MDH and XDH loci were found to be encoded at a single locus (Fig. 2 and 3). Electrophoretic phenotype distribution, electromorph frequencies, heterozygosities relative mobalities and chisquare values at MDH and XDH locus is given in Table 2 and Table 3.

Genetic variations among calliphorids using allozymes have been estimated only in *Cochliomyiahominivorax* [7, 14], *C.macellaria* [15] and in *Chrysomyamegacephala* (present study).

Table 1. Chi-square values, relative mobilities, electromorph frequencies, heterozygosities, and Electrophoretic phenotype distribution at APH-3 locus in *Chrysomyamegacephala*

	Electrophoretic Phenotype Frequency			Electromorph Frequency		Heterozygosity	
n = 100	aa	ab	bb	a	b	(H_o)	(H_E)
Obs.	48	23	29	0.59	0.41	0.23	0.48
Exp.	34.8	48.4	16.8	41*	45*		

$\chi^2 = 27.19$ ($p > 0.01$)

Table 2. Chi-square values, relative mobilities, electromorph frequencies, heterozygosities, and Electrophoretic phenotype distribution at MDH locus in *Chrysomyamegacephala*

	Electrophoretic Phenotype Frequency			Electromorph Frequency		Heterozygosity	
n = 100	aa	ab	bb	a	b	(H_o)	(H_E)
Obs.	18	55	27	0.44	0.56	0.55	0.49
Exp.	19.36	49.28	31.36	24*	32*		

$\chi^2 = 1.55$ (p between 0.50 and 0.20)

Table 3. Chi-square values, relative mobilities, electromorph frequencies, heterozygosities, and Electrophoretic phenotype distribution at XDH locus in *Chrysomyamegacephala*

	Electrophoretic Phenotype Frequency			Electromorph Frequency		Heterozygosity	
n = 100	aa	ab	bb	a	b	(H_o)	(H_E)
Obs.	36	36	28	0.54	0.46	0.36	0.50
Exp.	29.16	49.68	21.16	17*	25*		

$\chi^2 = 6.06$ (p between 0.05 and 0.01)

The mean observed heterozygosity in *Chrysomyamegacephala* was found to be 0.380 in the present study which is higher than the average value found in invertebrates 0.134 [16] and in other dipterans 0.115 [17] (Tables 4 and 5).

Table 4. Electromorph frequency, heterozygosities and mean heterozygosity at three enzyme loci of *C. megacephala.*

Locus	No. of Individuals (n)	Electromorph frequency			Heterozygosity	
		a	b	c	Observed (H_O)	Expected (H_E)
APH-1 APH-2 APH-3	100 100 100	1 1 0.59	– – 0.41	–	– – 0.23	– – 0.48
MDH	100	0.44	0.56	–	0.55	0.49
XDH	100	0.54	0.46	–	0.36	0.50
Mean het. (H)					0.380	0.490

Table 5. Mean heterozygosity in all the calliphorids with different molecular markers

Species	Molecular markers	Heterozygosity		References
		H_o	H_E	
Cochliomyia hominivorax ” ” ” *populations* *Rafaela* *Valinhos* *Pirassununga* *Caraguatatuba* *Botucatu* *Amparo* *Adamantina*	Allozymes ” Microsatellite RAPD-PCR	0.1404 0.159 0.5647	0.1492 0.165 0.7371 0.123 0.101 0.005 0.119 0.123 0.111 0.140	Taylor and Peterson (1994) [15] Taylor et al. (1996) [14] Torres and Azeredo-Espin (2005) [18] Infante-Malachias et al. (1999) [19]
C. macellaria	Allozyme	0.1540	0.1600	Taylor and Peterson (1994) [15]
Chrysomyaalbiceps	Microsatellite	0.4793	0.6998	Torres and Azeredo-Espin (2008) [20]
C. putoria	”	0.3109	0.5962	Radrigues et al. (2009) [21]
C. megacephala	Allozyme	0.380	0.492	Present Study

4 Conclusion

A perusal of the table indicates that heterozygosity in Ch. Megacephala is similar to other calliphorids. The higher population density leads to greater genetic diversity as compared to small population size which shows low genetic diversity [22, 23]. Several factors such as environmental conditions, genetic drift, population bottle neck, colonization, host availability and reproductive pressures are known to influence genetic variations among populations. It is interesting to note that calliphorids reveal large allelic diversity, a characteristic feature expected from a species with large population size.

However, it is imperative that genetic characterization of geographically diverse populations of different Chrysomya species from India [24] should be The species populations dispensed over a massive style of environmental situations are regarded to be genetically extra heterozygous in comparison to the species with constrained distribution (Narang 1980, Scarpassa and Hamada, 2003, Santos et al. 2005) [25–27] carried out with the help of allozymes and other molecular markers to evaluate the extent of genetic differentiation between population and also to get an insight into the process of bio-geographic patterns of genetic variations.

References

1. H. Harris, Enzyme polymorphisms in man. Proc R Soc Lond B Biol Sci. 164(995), 1966, p. 298-310. DOI: https://doi.org/10.1098/rspb.1966.0032
2. G. Bush and G. Kitto. Application of genetics to insect systematics and analysis of species differences [Pest management]. in: Symposium on Biosystematics in Agriculture. Beltsville, Md.(USA). 8–11 May 1977. 1978
3. D. G. Buth, The application of electrophoretic data in systematic studies. Annual review of Ecology and Systematics. 15, 1984, p. 501-522.
4. S. H. Berlocher, Insect Molecular Systematics. Annual Review of Entomology. 29(1), 1984, p. 403-433. DOI: https://doi.org/10.1146/annurev.en.29.010184.002155
5. D. Thierry, M. Ribodeau, F. Foussard, and M. Jarry, Allozyme polymorphism in a natural population of Chrysoperla carnea sensu lato (Neuroptera: Chrysopidae): A contribution to the status of the constitutive taxons in western Europe. European Journal of Entomology. 94, 1997, p. 311-316.
6. D. W. COLLINS, The separation of *Liriomyza huidobrensis* (Diptera: Agromyzidae) from related indigenous and non-indigenous species encountered in the United Kingdom using cellulose acetate electrophoresis. Annals of applied biology. 128(3), 1996, p. 387-398. DOI: https://doi.org/10.1111/j.1744-7348.1996.tb07100.x
7. G. L. Bush and R. W. Neck, Ecological genetics of the screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae) and its bearing on the quality control of mass-reared insects. Environmental Entomology. 5(5), 1976, p. 821-826. DOI: https://doi.org/10.1093/ee/5.5.821
8. F. J. Ayala and J. R. Powell, Enzyme variability in the *Drosophila willistoni* group. VI. Levels of polymorphism and the physiological function of enzymes. Biochemical genetics. 7(3), 1972, p. 331–345. DOI: https://doi.org/10.1007/BF00484832
9. M. TSUKAMOTO, Enhancement of staining intensity of mosquito larva zymograms after electrophoresis. Journal of UOEH. 11(4), 1989, p. 461-479. DOI: https://doi.org/10.7888/juoeh.11.461

10. G. Manchenko, Handbook of detection of enzymes on electrophoretic gels CRC Press. Inc., Ann Arbor, MI, 1994.
11. M. Harry, I. Galindez, and M. Cariou, Isozyme variability and differentiation between Rhodnius prolixus, R. robustus and R. pictipes, vectors of Chagas disease in Venezuela. Medical and Veterinary Entomology. 6(1), 1992, p. 37–43. DOI: https://doi.org/10.1111/j.1365-2915.1992.tb00032.x
12. M. Tsukamoto and M. Horio, Electrophoretic comparison of the lactate dehydrogenase banding pattern among Japanese mosquito larvae (Diptera: Culicidae). Journal of medical entomology. 22(5), 1985, p. 491-498. DOI: https://doi.org/10.1093/jmedent/22.5.491
13. M. Nei, Genetic distance between populations. The American Naturalist. 106(949), 1972, p. 283-292. DOI: https://doi.org/10.1086/282771
14. D. B. Taylor, R. D. Peterson, and G. E. Moya-Borja, Population genetics and gene variation in screwworms (Diptera: Calliphoridae) from Brazil. Biochemical genetics. 34(1), 1996, p. 67-76. DOI: https://doi.org/10.1007/BF02396241
15. D. B. Taylor and R. D. Peterson, Population genetics and gene variation in primary and secondary screwworm (Diptera: Calliphoridae). Annals of the Entomological Society of America. 87(5), 1994, p. 626-633. DOI: https://doi.org/10.1093/aesa/87.5.626
16. F. Ayala, Genetic polymorphism: from electrophoresis to DNA sequences. Experientia. 39(8), 1983, p. 813-823. DOI: https://doi.org/10.1007/BF01990397
17. D. Graur, Gene diversity in Hymenoptera. Evolution. 39(1), 1985, p. 190-199. DOI: https://doi.org/10.2307/2408528
18. T. T. Torres and A. M. L. DE AZEREDO-ESPIN, Development of new polymorphic microsatellite markers for the New World screw-worm *Cochliomyia hominivorax* (Diptera: Calliphoridae). Molecular Ecology Notes. 5(4), 2005, p. 815–817. DOI: https://doi.org/10.1111/j.1471-8286.2005.01073.x
19. M. E. Infante-Malachias, K. S. C. Yotoko, and A. M. Lima de Azeredo Espin, Random amplified polymorphic DNA of screwworm fly populations (Diptera: Calliphoridae) from Southeastern Brazil and Northern Argentina. Genome. 42(4), 1999, p. 772–779. DOI: https://doi.org/10.1139/g98-164
20. T. T. Torres and A. M. L. DE AZEREDO-ESPIN, Characterization of polymorphic microsatellite markers for the blowfly *Chrysomya albiceps* (Diptera: Calliphoridae). Molecular ecology resources. 8(1), 2008, p. 208–210. DOI: https://doi.org/10.1111/j.1471-8286.2007.01926.x
21. R. A. Radrigues, A. M. L. d. AzeredoEspin, and T. T. Torres, Microsatellite markers for population genetic studiesof the blwflyCnrysomyaputoria (Diptera:Calliphoridae). Mem. Inst. Oswldo Cruz,(104), 2009, p. 1047–1050.
22. E. Krafsur, J. Helm, and W. Black, Genetic diversity at electrophoretic loci in the house fly, Musca domestica L. Biochemical genetics. 30(5), 1992, p. 317-328. DOI: https://doi.org/10.1007/BF02396220
23. E. Krafsur and N. Griffiths, Genetic variation at structural loci in the *Glossina morsitans* species group. Biochemical genetics. 35(1), 1997, p. 1-11. DOI: https://doi.org/10.1023/A:1022252311715
24. S. Agrawal and N. Bajpai, Electrophoretic studies in Indian calliphorids. Int.J. of zoological investigations Vol.8(1), 2022, p. 422–475.
25. S. Narang, Genetic variability in natural populations, evidence in support of the selectionist view. Experientia. 36(1), 1980, p. 50-51. DOI: https://doi.org/10.1007/BF02003964

26. V. M. Scarpassa and N. Hamada, Isozyme variation in four species of the *Simulium perflavum* species group (Diptera: Simuliidae) from the Brazilian Amazon. Genetics and molecular biology. 26, 2003, p. 39-46. DOI: https://doi.org/10.1590/S1415-47572003000100007
27. J. M. M. d. Santos, G. A. D. Rodriguez, J. d. F. Maia, and W. P. Tadei, Variability and genetic differentiation among Anopheles (Ano.) intermedius Chagas, 1908 and Anopheles (Ano.) mattogrossensis Lutz & Neiva, 1911 (Diptera: Culicidae) from the Brazilian Amazon. Memórias do Instituto Oswaldo Cruz. 100, 2005, p. 531–537. DOI: https://doi.org/10.1590/S0074-02762005000600006