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**RESEARCH ARTICLE**

# Genetic variability among populations of Crysomya megacephala (Diptera: calliphoridae) in India.

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## *Abstract*

Genetic variation at four gene enzyme systems was analyzed in Crysomya megacephala. The three enzymes namely acid phophatase (ACPH), aldehyde oxidase(AO),glucose-6-phosphate dehydrogenase (G6PD) and alcohol dehydrogenase (ADH) were found to express activity only in a single zone indicating that they are encoded at single locus .Alcohol dehydrogenase was monomorphic while acid phoshatase and glucose-6-phosphate dehydrogenase were polymorphic.



## Introduction

Blow flies of the family calliphoridae are distributed worldwide and some of the spices belonging to this family are known to be causative agents of animal tissue myiasis and transmission of enteric pathogens and parasites causing severe loss to live stoke(El-Azazy, 1989). Enzyme electrophoresis has contributed a great deal to elucidate genetic differences in calliphorids (Bush and Neck 1976 , Whitten 1980, Richardson et al 1982 ,Krafsur and Whitten 1993, Taylor and Peterson 1994, Taylor et al 1996, Wallman and Adams,1997,2001).

However in the genus *Chrysomya* genetic variation has been analysed only by using microsatellite markers in Chrysomya albiceps (Torris and Azeredo-Espin, 2008) and *C.Putoria* (Rodrigues et al 2009) from Brazil. In the present study an attempt has been made to analyze genetic variation in *Chrysomya megacephala* from Allahabad (India).

## Material And Methods:-

Specimens of Chrysomya megacephala (Fabricious) were collected using sweep net from Allahabad. Single male flies were homogenized in 40µl of chilled double distilled water, homogenate was centrifuged and the supernatant was used for enzyme separation.Electrophoresis was perphormed on 7% polyacrylamide gel in a tube gel electrophoresis apparatus at 4ºC. The enzyme systems buffers and staining mixtures for all the gene enzyme systems analyzed are represented in Table 1.

**Table-1:-** Summary of electrophoresis and staining protocols followed in the present study

| Enzyme | Gel/electrode buffer | Staining buffer | Substrate/ Coenzyme* | Dyes | Reference |
|---|---|---|---|---|---|
| ACPH (E.C. 3.1.3.2) | 0.1M Tris-borate (pH 8.9) | 0.1M Acetate (pH 5.0) | Sodium-α- Naphthyl phosphate | Fast Blue BB | Ayala et al (1972) |
| ADH (E.C. 1.1.1.1) | 0.05M Tris- HCl (pH 8.5) | 0.05M Tris-HCl (pH 8.5) | Ethanol/ NAD | NBT PMS | Manchenko (1994) |
| G6PD (E.C.1.1.1.49) | 0.1M Tris- HCl (pH 8.5) | 0.1M Tris- HCl (pH 8.5) | Na-glucose 6-phosphate/NADP | NBT PMS | Tsukamoto (1989) |
| AO (E.C. 1.2.3.1) | 0.1M Tris- HCl (pH 8.5) | 0.1M Tris- HCl (pH 7.4) | Benzaldehyde | NBT PMS | Tsukamoto (1989) |

The relative mobility of each band was calculated and expressed as Rf value (X100)as per following the method of Tsukamoto and Horio (1985).

$$Rf = \frac{Migration\ distance\ of\ a\ band}{Migration\ distance\ of\ the\ (buffer)\ front} x\ 100$$

Electrophoretic genotypes were determined by comparison of relative mobilities of the bands. Genetic interpretation was carried out following the method of Harry et al (1992) thus single band indicates homozygotes and multiple bands/diffuse bands represent heterozygotes.On the basis of electromorph frequencies, the genetic variability was estimated using the polymorphic loci (P), mean observed (Ho) and expected (He) heterozygosity (Nei,1972) and test for conformance to Hardy-Weinberg equilibrium by Chi-squere test.

## Result & discussion:-

Four gene enzyme systems were analyzed Genetic variation in the Chrysomya megacephala . All the four enzymes namely acid phophatase (ACPH),aldehyde oxidase(AO), glucose-6-phosphate dehydrogenase (G6PD) and alcohol dehydrogenase (ADH) were found to express activity only in a single zone indicating that they are encoded at single locus .Alcohol dehydrogenase was monomorphic while acid phoshatase, aldehyde oxidase and glucose-6-phosphate dehydrogenase were polymorphic.

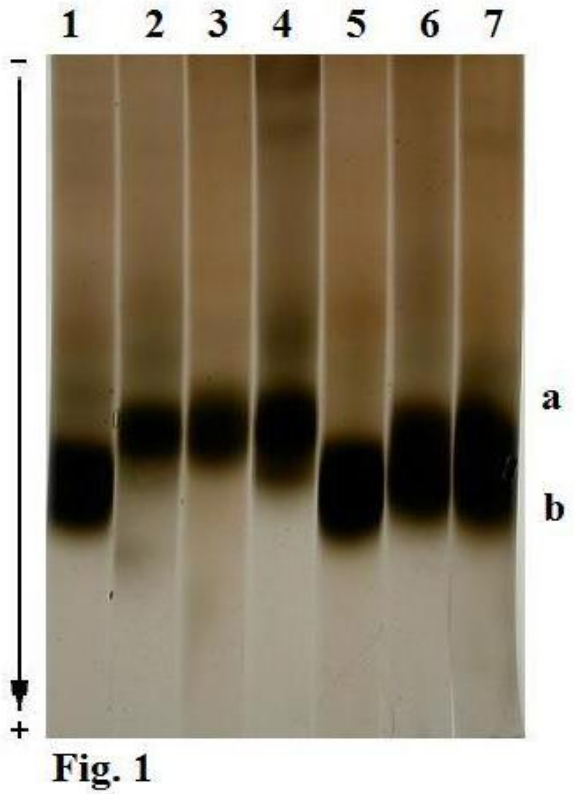


Fig. 1

**Fig. 1:**- The banding pattern of ACPH enzyme

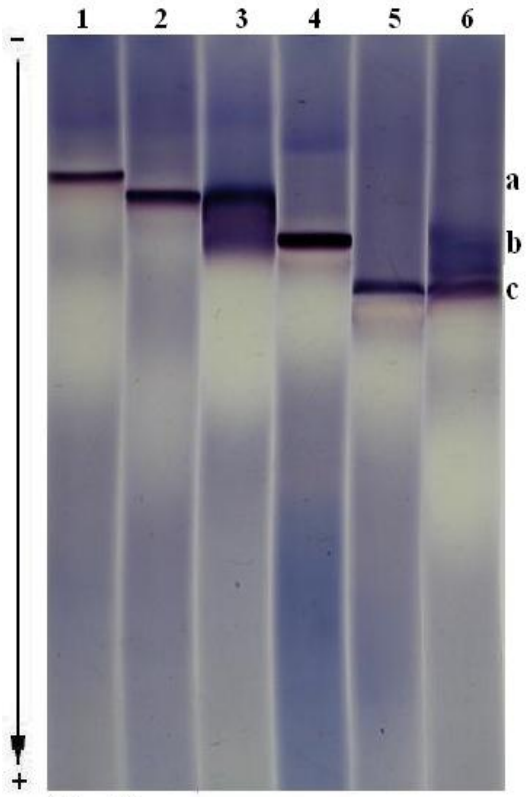


Fig. 10

**Fig. 2:**- The banding pattern of AO enzyme

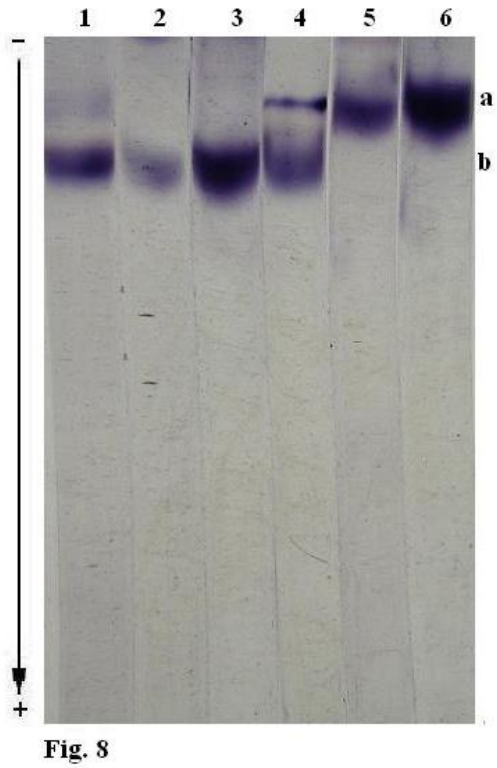


**Fig. 3:-** The banding pattern of G6PD enzyme

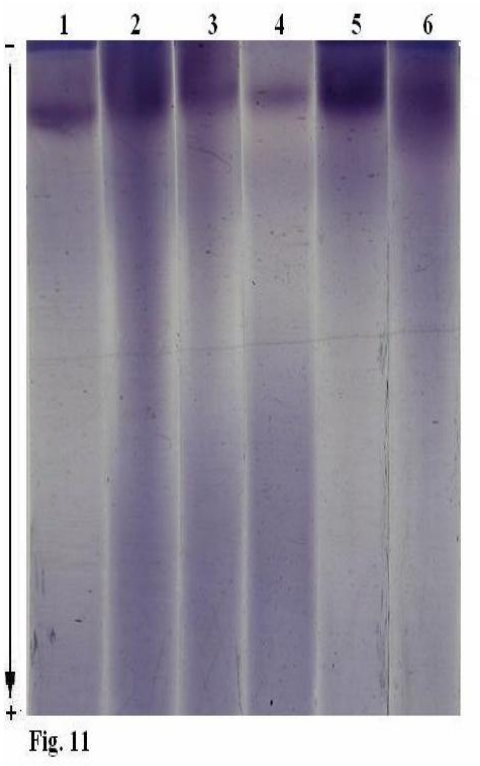


**Fig. 4:-** The banding pattern of ADH enzyme

**Table -2:**-The electromorph frequencies and heterozygosities at all four loci are presented in

| Locus | No. of Individuals (n) | Electromorph frequency | | | Heterozygosity | |
|---|---|---|---|---|---|---|
| | | a | B | c | Observed ($H_o$) | Expected ($H_E$) |
| ACPH | 100 | 0.52 | 0.49 | - | 0.31 | 0.49 |
| AO | 100 | 0.36 | 0.37 | 0.27 | 0.55 | 0.67 |
| G6PD | 100 | 0.55 | 0.45 | - | 0.28 | 0.49 |
| Mean het.(H) | | | | | 0.38 | 0.55 |

The distribution of electrophoretic phenotypes did not conform to Hardy –Weinberg equilibrium for acid phophatase (ACPH),aldehyde oxidase(AO), glucose-6-phosphate dehydrogenase (G6PD).This may be attributed to a deficiency of heterozygotes to sampling error and/ or inbreeding in the population (Harti,2000).

The mean observed heterozygosity(Ho) 0.38 in the present study is found to be higher than the average value found in invertebrates 0.134 (Ayala 1983) and in other dipterans 0.115 (Graur 1985).

Genetic variations among calliphorids using allozymes have been estimated only in *Cochliomyia hominivorax* (Taylor and Peterson, 1994; Taylor et al., 1995), *C. macellaria* (Taylor and Peterson,1994) and the present study.These flies were characterized by large heterozygosities as compared to other dipterans (Selander ,1976; Santos et al ,2005; Tripathi et al 2010) Large population size is responsible for greater genetic diversity as compared to small population size diversity (Krafsur et al., 1992, 2005, Krafsur and Griffiths 1997). Several factors eg. environmental conditions, genetic drift, population bottle neck, colonization, host availability and reproductive pressures are known to influence genetic variations within and among populations. In general the species populations distributed over a large variety of environmental conditions are known to be genetically more heterozygous as compared to the species with restricted distribution (Narang, 1980, Scarpassa and Hamada, 2003, Santos et al., 2005). It is interesting to note that all the calliphorids reveal large allelic diversities and microsatellite heterozygosities,( Torres and Azeredo- Espin,2005; Torres and Azeredo- Espin, 2008), a characteristic feature expected for a species with large population size. However, it is imperative that genetic characterization of geographically diverse populations of different *Chrysomya* species from India should be carried out with the help of allozyme and other molecular markers to evaluate the extent of genetic differentiation between population.

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