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Temporal genetic variation in populations of house fly from Prayagraj, India

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Abstract

Temporal genetic variation analyzed in the three populations of the Musca domestica during two consecutive years. Electrophoretic banding patterns were estimated at the three gene enzyme systems by using polyacrylamide gel electrophoresis (PAGE).Eight loci with fourteen alleles were observed during the present study. Genetic variation of allozymes is influenced by environmental variations. In the present study exceptEST-1 and EST-3 in seasonal cycle 01and ACPH-1seasonal cycle 02 reveal inbreeding which is depicted by the higher Fst values than Fis values. Thus it seems that the house fly populations analyzed showed high level of inbreeding. By comparing Nei's genetic distance (D) and identity (I) values it was found that there is a close similarity between summer and rainy season collections in seasonal cycle 01 and winter and rainy season in seasonal cycle 02.

Keywords: House fly, temporal, allele frequency, electrophoresis, polymorphism.

Introduction

The study of allozyme using electrophoretic technique provide a genetic tool for population genetics, mostly because it provide a direct way to access and calculate the genetic variation in populations through the direct product of gene expression[1,2]. Genetic variation in response to environmental changes has been explored within different insect populations[3-12].

The fly *Musca domestica* Linnaeus, a common house fly,is globally distributed and well known to everyone. The house fly is a dipteran insect belong to family Muscidae and has medical and veterinary importance. Several studies have been done on genetic variation in houseflies in different parts of the world including India[13-19]. This species exhibits great morphological and genetic diversity with the differences in habitat and environmental factors like weather pattern[20-27].

In the present study an attempt has been made to analyze temporal genetic variation of three enzyme patterns viz., Acid phosphatase (ACPH), Esterase (EST) and Malic enzyme (ME) in *Musca domestica* populations. The allelic frequencies were studied during different seasons in two consecutive years (2009 and 2010) to determine the genetic variation.

Materials and Methods

The house flies were collected using sweep nets from George Town locality of Allahabad City, 25°8' North, 81°50' East. The flies were collected during summer (April month), rainy (August month) and winter (December month) season over a 2 year period from 2009to 2010.

Electrophoretic Technique: A random sample of 50 flies was taken from each collection. For sample preparation, individual adult male flies were homogenized in homogenizing tube with 10µl of chilled double distilled water. The homogenate was transferred in centrifuge tube for centrifugation and the supernatant was loaded on the top of polyacrylamide gel. Electrophoresis (PAGE) was performed at 4°C. Three enzyme systems were analyzed in three seasons during two consecutive years (2009, Seasonal Cycle 1 and 2010, Seasonal Cycle 2).

The method of staining to analyze the enzyme activity has been adopted from Ayala et al.[28] and Tsukamoto[29] (Table-1).

Calculation of Data: The Rf (relative mobility) value of each band was calculated by the method of Tsukamoto and Horio[30]. By comparing the relative mobility of the bands the different Electrophoretic genotypes were determined. Conventional method was used for genetic interpretation in which single band is represented as homozygotes and multiple bands are represented as heterozygotes[31]. Chi-square values has been calculated to check whether the enzyme system is in accordance to Hardy-Weinberg equilibrium or not. F_{is} and F_{st} values has also been calculated[32]. Method of Nei[33] was used to calculate Genetic identity (I) and genetic distance (D).

Results and Discussion

In present work enzymes viz., Acid phosphatase (ACPH), Esterase (EST) and Malic enzyme (ME) were examined in the house fly *Musca domestica* from different seasons during 2009 and 2010.

Genetic variation during different seasons of year 2009 (Seasonal Cycle 1): Fourteen putative alleles were observed at eight genetic loci. Malic enzyme represented by only one locus while Acid phosphatase by two loci (ACPH-1 and ACPH-2) and Esterase by five loci (EST-1, EST-2, EST-3, EST-4 and EST-5).

Two loci of ACPH (ACPH-1 and ACPH-2) and three loci of EST viz. EST-1, EST-2, EST-3 are polymorphic in all seasons. .EST 4 and EST 5 were not polymorphic but monomorphic in summer and rainy season and absent in winter season while the malic enzyme activity is monomorphic in winter season and polymorphic in summer and rainy season (Table-2).

Allele frequencies and Chi-square values $(\chi)^2$ are also presented in Table-2. It was found that allele frequencies and electrophoretic phenotype frequencies at one locus in summer season, five loci in rainy season and four loci in winter season were not in accordance of Hardy-Weinberg Equilibrium due to the heterozygote deficiency in the sample population. Sampling error or inbreeding could be the cause of heterozygote deficiency in the population[34]. The data of genetic variation among three collections are presented in Table-3.

A comparison of values described in Table-3 exhibit a very close similarity between populations of summer and rainy season as compared to populations of winter season. The values of Nei's genetic identity (I) and genetic distance (D) also showed similarity between collections of summer and rainy season (Table-4a).

Genetic variation during different seasons of year 2010(Seasonal Cycle 2): Two loci of ACPH (ACPH-1 and ACPH-2) and one loci of EST i.e., EST-3 and Malic enzyme are polymorphic in all season during the year 2010. Enzyme activity at EST-1 was monomorphic in the summer season and polymorphic in rainy and winter season, while EST-2 was monomorphic in winter and polymorphic in rainy and summer season. EST-4 locus was monomorphic in summer and winter season and absent in rainy season. EST-5 locus was monomorphic in summer and rainy season and was absent in winter season. Chi-square and Allele frequencies values are presented in Table-2. In year 2010 Chi square value at two loci in summer season, three loci in rainy and five loci in winter season do not follow Hardy Weinberg Law. This shows that there is deficiency of heterozygotes. Inbreeding in population or sampling error could be the factor for the deficiency of heterozygotes in the sample as suggested by Hartl[34].

The data of genetic variation among three collections are presented in Table-3. Percentage of polymorphic loci and mean effective number of allele shows similarity in summer rainy season collections while Nei's genetic identity (I) and genetic distance (D) values shows similarity between winter and rainy season collections (Table-4b).This shows that there is more closeness in % of polymorphic loci and mean effective number of alleles in flies of summer and rainy season while Nei's genetic identity revealed closeness between flies of winter and rainy season. We can interpret that environmental conditions affected such genetic variations in house fly.

Table-1: Summary of buffers, substrates and dyes used in the present study.

Enzyme	Gel/ electrode buffer	Staining buffer	Substrate/Coenzyme	Dyes	Reference
ACPH	0.1MTris-borate (pH 8.9)	0.1M Acetate (pH 5.0)	Sodium-α-Naphthyl phosphate	Fast Blue BB	28
EST	0.1M Tris-borate (pH 8.9)	0.1MPhosphate (pH6.5)	α-Naphthyl acetate	Fast Blue RR	28
ME	0.1M Tris-HCl (pH 8.5)	0.1M Tris-HCl (pH 7.4)	Malic acid /NADP	NBTPMS	29

Table-2: Chi-square values and Allele frequencies in temporal populations of *Musca domestica*.

Locus	Allele	April 2009 (Summer)	August 2009 (Rainy)	December 2009 (Winter)	April 2010 (Summer)	August 2010 (Rainy)	December 2010 (Winter)
ACPH-1 (n=50)	A	0.56	0.53	0.68	0.51	0.56	0.46
	B	0.44	0.47	0.32	0.49	0.44	0.54
	χ^2	1.17	11.43*	19.99*	1.27	6.15*	9.52*
ACPH-1 (n=50)	A	0.42	0.46	0.45	0.45	0.58	0.46
	B	0.58	0.54	0.55	0.55	0.42	0.54

	χ^2	0.47	6.33*	11.26*	1.27	0.918	18.23*
EST-1 (n=50)	A	0.47	0.60	0.68	1.00	0.39	0.43
	B	0.53	0.40	0.32	-	0.61	0.57
	χ^2	3.25	8.17*	15.60*	-	3.26	6.72*
EST-2 (n=50)	A	0.45	0.55	0.48	0.42	0.53	1.00
	B	0.55	0.45	0.52	0.58	0.47	-
	χ^2	1.27	7.76*	6.40*	8.62*	5.38*	-
EST-3 (n=50)	A	0.42	0.59	-	0.50	0.52	0.55
	B	0.58	0.41	-	0.50	0.48	0.45
	χ^2	0.47	2.30	-	9.68*	11.51*	7.76*
EST-4 (n=50)	A	1.00	1.00	-	1.00	-	1.00
EST-5 (n=50)	A	1.00	1.00	-	1.00	1.00	-
ME (n=50)	A	0.48	0.54	1.00	0.40	0.53	0.42
	B	0.52	0.46	-	0.60	0.47	0.58
	χ^2	3.89*	6.33*	-	1.39	5.04*	8.61*

n= number of individuals (male flies) in each sample; *=Populations deviates from Hardy Weinberg equilibrium.

Table-3: Temporal genetic variability in housefly's populations.

Populations	Sample Size	Total num-ber of loci	Effective no. of alleles(mean)	Polymorphic loci (%)	(H_o)	(H_E)
April 2009 (Summer)	50	08	2.028	67.5%	0.383	0.493
August 2009 (Rainy)	50	08	2.034	67.5%	0.303	0.491
December 2009 (Winter)	50	08	2.155	50.0%	0.215	0.466
Mean	50	08	2.072	61.67%	0.301	0.483
April 2010 (Summer)	50	08	2.031	62.5%	0.320	0.492
August 2010 (Rainy)	50	08	2.034	67.5%	0.303	0.491
December 2010 (Winter)	50	08	2.026	50.0%	0.312	0.493
Mean	50	08	2.030	60%	0.312	0.492

Mean observed heterozygosity (H_o) = No. of heterozygotes / Total no. of individuals, Mean expected heterozygosity (H_E) = 1- Σx_i^2, where x_i is the frequency of ith allele at a locus[33].

Table-4a: Genetic distance (D) and Genetic identity (I) among the three collections of *Musca domestica* (2009).

		(I)		
	Population	Summer	Rainy	Winter
(D)	Summer	-	0.980	0.810
	Rainy	-0.020	-	0-837
	Winter	-0.211	-0.178	-

Table-4b: Genetic distance (D) and Genetic identity (I) among the three collections of *Musca domestica* (2010).

		(I)		
	Population	Summer	Rainy	Winter
(D)	Summer	-	0.875	0.812
	Rainy	0.134	-	0.923
	Winter	0.209	0.080	-

I= Jxy/√Jx Jy, D= -In I, Where, Jx y=$\sum x_i y_i$ over all loci, Jx is the arithmetic mean of ix =$\sum ix_i^2$ over all loci, x_i (or $y_{i)}$ is the frequency of the ith allele in the first (or second) population.

Table-5: F statistics ($F_{is\ and}\ F_{st}$) for all the variable loci.

	2009		2010	
Locus	F_{is}	F_{st}	F_{is}	F_{st}
ACPH-1	0.425	0.17	0.315	0.975
ACPH-2	0.304	.001	0.283	0.015
EST-1	0.420	0.487	0.461	0.325
EST-2	0.462	0.019	0.539	0.278
EST-3	0.145	0.457	0.344	0.002
ME	0.358	-0.494	0.358	0.013

Conclusion

We have analyzed F_{is} and F_{st} values among *Musca domestica* populations in the two consecutive years and found that all the other loci show inbreeding ($F_{is}>F_{st}$) except EST-1 in 2009 and ACPH-1 in 2010 (Table-5). Regarding the negative F_{is} Kimura and Crow[35]have suggested that it indicates random mating. The present work emphasize that the *Musca domestica* populations surveyed are characterized by a high level of inbreeding.

In the present study comparison of genetic identity and genetic distance values among different seasons of the two consecutive years shows that in 2009 the population of summer and rainy season are genetically much closer while in 2010 the populations of rainy and winter season shows closeness. This difference in closeness is due to the environmental changes in the two consecutive years. Our findings has supported by the observations of Mateus and Sene[36] and Prerea et al[12]. Mateus and Sene[36] in their study on *Drosophila antonietae* and Perera et al.[12] on *Helicoverpa Zea* suggest that environmental or climatic factors probably influenced temporal genetic variations of allozymes. Our findings also show that environmental conditions have great impact on the temporal genetic variation of allozymes in house flies.

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