



SEASONAL GENETIC VARIATION IN HOUSE FLY POPULATIONS, *Musca domestica* (DIPTERA: MUSCIDAE)

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Abstract

Seasonal genetic variation was assessed in the common house fly *Musca domestica*. Allozymes at six gene enzyme system viz., ACPH, EST, G6PD, ME, AO and XDH were analyzed. Fourteen loci with twenty seven alleles were unraveled. The genetic variations were found to be affected to a great extent by environmental influence. F statistics has been used to calculate genetic variation which revealed that very little genetic variation has occurred among the house fly populations analyzed in the present study. Further, except ACPH-2, G6PD-2 and XDH-1 all the other loci show inbreeding ($F_{is} > F_{st}$). Thus it appears that the house fly populations analyzed are characterized by high level of inbreeding. Nei's genetic identity (I) and distance (D) values reveal a close similarity between summer and rainy season collections.

Key words: *Musca domestica*, allozymes, seasonal variation, genetic variability, genetic identity.

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Abbreviations: **ACPH:** Acid Phosphatase; **AO:** Aldehyde Oxidase; **D:** Genetic Distance; **EST:** Esterase; **Fis:** Interindividual Fixation Index; **Fst:** Subpopulation Index; **G6PD:** Glucose 6 Phosphate Dehydrogenase; **I:** Genetic Identity; **Max:** Maximum; **ME:** Malic Enzyme; **Min:** Minimum; **XDH:** Xanthine Dehydrogenase.

INTRODUCTION

The extent of genetic variability within populations of a species reflects species vitality and its potential for evolutionary response to environmental changes (20). Allozyme variations have unraveled changes in genotype frequencies in relation to spatial, seasonal and temporal variations emphasizing the role of environmental heterogeneity in space and time to maintain genetic polymorphism (2,5,6,8,11,15,16,17, 21,22).

The common house fly *Musca domestica*, a synanthropic fly with world wide distribution, has tremendous capacity to adapt to varying environmental conditions. The spatial, temporal and seasonal genetic variations in house fly populations have been analyzed only in the new world populations from USA, UK and Africa. (3,7,13,14,18,19,24). Seasonal genetic variation in house fly, *M. domestica*, populations from Allahabad, India has been analyzed in the present study.

MATERIALS AND METHOD

Sample collection

The house flies *Musca domestica* L. were collected from George Town locality of Allahabad city, 25° 8'North, 81° 50'East, using sweep nets, brought to the laboratory and kept in insect rearing cages. The flies were collected during summer (June, 2008, Temp. Max. 44°C-Min. 28°C; Relative humidity Max. 35%, Min. 7%), rainy (August, 2008, Temp. Max. 36°C-Min. 26°C; Relative humidity Max. 84%, Min. 48%) and winter seasons (December, 2008, Temp. Max. 28°C-Min. 6°C; Relative humidity Max. 97%, Min. 65%).

Electrophoretic studies

Single male flies were homogenized in 10 µl of chilled double distilled water, homogenates were centrifuged and the supernatant was used for enzyme separation. Electrophoresis was performed on 7% polyacrylamide gel at 4°C. Fifty individuals were assayed for enzyme activity at six gene enzyme systems viz., acid phosphatase (ACPH), esterase (EST), glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), aldehyde oxidase (AO) and xanthine dehydrogenase (XDH). The staining protocols of Ayala *et al.* (1) and Tsukamoto (25) were followed for the analysis of enzyme activity.

Data analysis

The relative mobility of each band was calculated and expressed as R_f values ($\times 100$) following the method of Tsukamoto and Horio (26). Electrophoretic genotypes were determined by comparison of relative mobility of the bands. Genetic interpretations were made by conventional method; single bands were considered to represent homozygotes and multiple banded phenotypes represented heterozygotes (9). At polymorphic loci, groups of similar R_f values were considered to represent a single allele. The mean value for each group was used to designate that particular allele. The genotype information at each locus was used to calculate allele frequencies (4). The genotype information, thus obtained, was used to estimate genetic variability using polymorphic loci, mean observed (H_o) and expected (H_e) heterozygosity (23). Test for conformity to Hardy-Weinberg equilibrium was carried out by Chi-square and Wright's F statistics (27). Genetic identity (I) and genetic distance (D) values were calculated according to the method of Nei (23).

RESULTS AND DISCUSSION

The fourteen genetic loci studied in three collections revealed twenty seven putative alleles at six gene-enzyme systems. The enzyme activity at ME was confined to a single locus while all the other enzymes show activity at multiple loci i.e., ACPH (ACPH-1 and ACPH-2), EST (EST-1, EST-2, EST-3, EST-4 and EST-5), G6PD (G6PD-1 and G6PD-2), AO (AO-1 and AO-2) and XDH (XDH-1 and XDH-2). Enzyme activity at EST-4 and EST-5 was monomorphic in all the samples while all the other loci were

polymorphic. The flies collected during rainy and summer season shared twenty one alleles except six alleles at four genetic loci viz., EST-1, EST-5, AO-1 and XDH-1. The activity at the loci EST-5 and AO-1 was not observed in the population of rainy season, while the activity at the locus EST-1 was totally absent in summer population (Fig.1). XDH-1 is polymorphic in rainy season while it is monomorphic in summer season (Fig.2). Allele frequencies and Chi-square values are presented in table 1. The extent of genetic variation among three collections, number of alleles per locus, percentage of polymorphic loci and the mean heterozygosities are summarized in table 2.

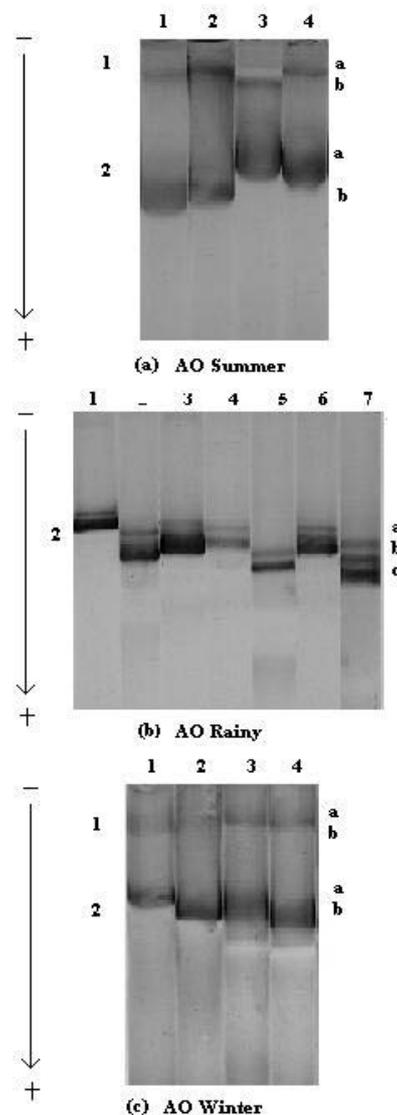


Table 1. Allele frequencies and Chi-square values in seasonal collections of *M. domestica*.

Locus	Allele	Summer	Rainy	Winter
ACPH-1 (n=50)	a	0.55	0.56	0.52
	b	0.45	0.44	0.48
	χ^2	1.14	1.77	1.97
ACPH-2 (n=50)	a	0.68	0.55	0.49
	b	0.32	0.45	0.51
	χ^2	19.99	7.76	2.87
EST-1 (n=50)	a	-	0.46	0.53
	b	-	0.54	0.43
	χ^2	-	9.52*	11.43*
EST-2 (n=50)	a	0.56	0.51	1.00
	b	0.44	0.49	-
	χ^2	6.15*	1.27	-
EST-3 (n=50)	a	0.57	0.46	1.00
	b	0.43	0.54	-
	χ^2	4.69*	6.33*	-
EST-4	a	1.00	1.00	1.00
EST-5	a	1.00	-	1.00
G6PD-1 (n=50)	a	0.45	0.59	0.52
	b	0.55	0.41	0.48
	χ^2	11.26*	2.30	1.97
G6PD-2 (n=50)	a	0.42	0.62	1.00
	b	0.58	0.38	-
	χ^2	0.47	8.23*	-
ME (n=50)	a	0.50	0.66	1.00
	b	0.50	0.34	-
	χ^2	9.68*	7.07*	-
AO-1 (n=50)	a	0.48	-	0.54
	b	0.52	-	0.46
	χ^2	3.89	-	6.33*
AO-2 (n=50)	a	0.42	0.36	0.42
	b	0.24	0.40	0.32
	c	0.34	0.24	0.26
XDH-1 (n=50)	χ^2	28.84*	17.72*	5.71*
	a	1.00	0.53	0.54
	b	-	0.47	0.46
XDH-1 (n=50)	χ^2	-	5.04*	13.36*
	a	0.49	0.40	1.00
	b	0.51	0.60	-
	χ^2	11.51*	1.39	-

n= number of individuals in each sample;

* = Populations not in Hardy Weinberg equilibrium.

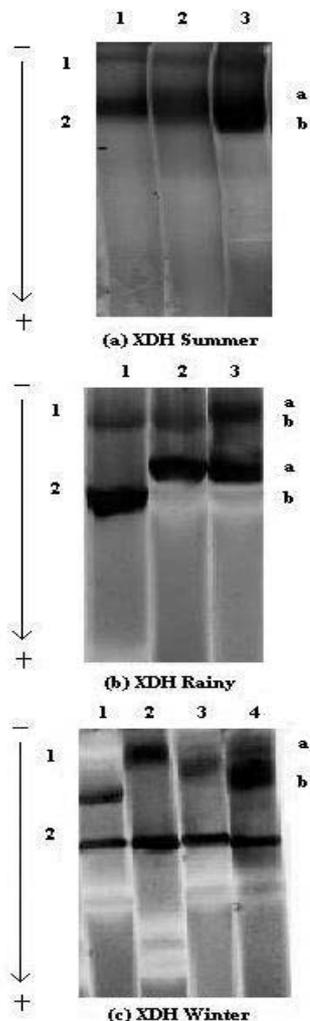
Table 2. Genetic variability in seasonal collections of house flies populations.

Population	Sample Size	Number of loci	Mean effective no. of alleles	Percentage of polymorphic loci	Mean observed hetero-zygosity (H_o)	Mean expected Hetero-zygosity (H_E)
Summer	50	14	2.00	71.43%	0.333	0.504
Rainy	50	14	2.01	78.57%	0.362	0.501
Winter	50	14	1.94	50.00%	0.354	0.520
Mean	50	14	1.98	66.67%	0.350	0.508

 H_o = No. of heterozygote / Total no. of individuals H_E = $1 - \sum x_i^2$ (Nei, 1972), where x_i is the frequency of i th allele at a locus

The mean effective number of alleles per locus ranged from 1.94 to 2.01, with a mean of 1.98. The percentage of polymorphic loci ranged from 50% to 78.57%, with a mean of 66.67%

The mean observed heterozygosity ranged from 0.333 to 0.362, with a mean of 0.350 and mean expected heterozygosity ranged from 0.501 to 0.520, with a mean of 0.508. Allele frequencies and electrophoretic phenotype frequencies at eleven loci were not found to be in accordance with the Hardy-Weinberg equilibrium due to a deficiency of heterozygote in the sampled population. The deficiency could be attributed to sampling error and/or inbreeding in the population (10). The percentage of polymorphic loci was highest in the samples of rainy season and lowest in winter collection.



The present result shows a very close similarity between collections of summer and rainy season, as there is a great deal of similarity in mean effective number of alleles and percentage of polymorphic loci in the two

populations as compared to the winter collections. Thus it may be surmised that the seasonal genetic variations of allozymes is influenced to a great extent by the environmental conditions as has earlier been observed by Mateus and Sene (20) in *Drosophila antonietae*. This contention is further supported by the Nei's genetic identity (I) and genetic distance (D) values which revealed a very high value of genetic identity (0.844) between summer and rainy season collections (Table 3).

Table 3. Genetic identity (I) and distance (D) among the three collections.

Population	(I)		
	Summer	Rainy	Winter
Summer	-	0.844	0.335
Rainy	0.170	-	0.261
Winter	0.715	0.770	-

$$I = J_{xy} / \sqrt{J_x J_y}$$

$$D = -\ln I$$

Where $J_x y$ is the arithmetic mean of $J_x \sum_{i=1}^n x_i y_i$ over all loci, J_x is the arithmetic mean of $J_x \sum_{i=1}^n x_i^2$ over all loci, and x_i (or y_i) is the frequency of the i th allele in the first (or second) population.

Table 3. Genetic identity (I) and distance (D) among the three collections.

Loci	F_{is}	F_{st}
ACPH-1	0.180	0.002
ACPH-2	0.341	0.487
EST-1	0.457	0.005
EST-2	0.254	0.227
EST-3	0.331	0.244
G6PD-1	0.324	0.013
G6PD-2	0.238	0.266
ME	0.410	0.216
AO-1	0.317	0.011
AO-2	0.244	0.011
XDH-1	0.417	0.419
XDH-2	0.327	0.299

Wright's F statistics further supports the fact that very little genetic variation has occurred among house fly populations analyzed in the present study. Except ACPH-2, G6PD-2 and XDH-2 all the other loci reveal inbreeding ($F_{is} > F_{st}$) (Table 4). Kimura and Crow (12) have opined that a negative F_{is} value is indicative of

random mating. Thus, it seems plausible that the house fly populations surveyed in the present study are characterized by a high level of inbreeding.

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REFERENCES

1. Ayala, F.J., Powell, J.R., Tracey, M.L., Mourao, C.A. and Perez-Salas, S., Enzyme variability in the *Drosophila willistoni* group IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics*.1972, **70**: 113-139.
2. Barker, J.S.F., East, P.D., and Weir, B.S., Temporal and microgeographic variation in allozyme frequencies in a natural population of *Drosophila buzzatii*. *Genetics* 1986, **112**: 577-585.
3. Black IV, W.C. and Krafur, E.S., Temporal and spatial trends in allozyme frequencies in house fly populations, *Musca domestica* L. *Theor. Appl. Genet.* 1986, **71**: 673-681.
4. Berlocher, S.H., An Electrophoretic key for distinguishing species of the genus *Rhagoletis* (Diptera: Tephritidae) as larvae, pupae, or adults. *Ann. Entomol. Soc. Am.* 1980, **73**: 131-137.
5. Bublly, O.A., Kalabushkin, B.A. and Imasheva, A.G., Geographic variation of six allozyme loci in *Drosophila melanogaster*: An analysis of data from different continents. *Hereditas* 1999, **130**: 25-32.
6. Cui, F., Qiao, C.L., Shen, B.C., Marquine, M., Weill, M. and Raymond, M., Genetic differentiation of *Culex pipiens* (Diptera : Culicidae) in China. *Bull. Entomol. Res.* 2007, **97**: 291-297.
7. Cummings, M.A. and Krafur, E.S., Spatial diversity in mitochondrial cytochrome oxidase in house fly. *Med. Vet. Entomol* 2005, **19**: 53-59.
8. Felsenstein, J., The theoretical population genetics of variable selection and migration. *Annu. Rev. Genet.* 1976, **10**: 253-269.
9. Harry, M., Galindez, I. and Cariou, M.L., Isozyme variability and differentiation between *Rhodnius prolixus*, *R. robustus* and *R. pictipes*, vectors of chagas disease in Venezuela. *Med. Vet. Entomol.* 1992, **6**: 37-43.
10. Hartl, D.L., *A primer of population genetics*. Sinauer Associates. Inc., Sunderland. Massachusetts. 2000.
11. Hederick, P.W., Ginevan, M.E. and Ewing, E.P., Genetic polymorphism in heterogeneous environments. *Ann. Rev. Ecol. Syst.* 1976, **7**: 1-27.
12. Kimura, M. and Crow, J.F., The measurement of effective population number. *Evolution*.1963, **17**: 279-291.
13. Krafur, E.S., Helm, J.M. and Black IV, W.C., Genetic diversity at electrophoretic loci in the house fly, *Musca domestica* L. *Biochem. Genet.* 1992, **30**: 317-328.
14. Krafur, E.S., Bryant, N.L., Marquez, J.G. and Griffith, N.T., Genetic distances among North American, British and West African house fly populations *Musca domestica* L. *Biochem Genet.* 2000, **38**: 275-284.
15. Kraushaar, U., Goudet, J and Blanckenhorn, W.U., Geographical and altitudinal population genetic structure of two dung fly species with contrasting mobility and temperature preference. *Heredity* 2002, **89**: 99-106.
16. Land, J.V., Van Putten, W.F., Villarroel, H., Kamping, A., and Van Delden, W., Latitudinal variation for two enzyme loci and an inversion polymorphism in *Drosophila melanogaster* from central and South America. *Evolution* 2000, **54**: 201-213.
17. Malacrida, A.R., Guglielmino, C.R., Gasperi, G., Baruffi, L. and Milani, R., Spatial and temporal differentiation in colonizing populations of *Ceratitis capitata*. *Heredity* 1992, **69**: 101-111.
18. Marquez, J.G. and Krafur, E.S., Gene flow among geographically diverse house fly populations (*Musca domestica* L.): a worldwide survey of mitochondrial diversity. *J. Hered.* 2002, **93**: 254-259.
19. Marquez, J.G., Moon, R.D. and Krafur, E.S., Genetic differentiation among populations of house flies (Diptera : Muscidae) breeding at a multiple-barn, egg-laying facility in Central Minnesota. *J. Med. Entomol.* 2001, **38**: 218-222.
20. Mateus, R.P. and Sene, F.M., Temporal and spatial allozyme variation in the South American cactophilic *Drosophila antonietae* (Diptera : Drosophilidae). *Biochem. Genet.* 2003, **41**: 219-233.
21. Milankov, V., Stamenkovic, J., Vujic, A. and Simic, S., Geographic variation of *Chelisia vernalis* (Fallen, 1817) (Diptera: Syrphidae). *Acta Zool. Hung.* 2002, **48**: 255-267.
22. Nayar, J.K., Knight, J.W. and Munstermann, L.E., Temporal and geographic genetic variation in *Culex pipiens quinquefasciatus* (Diptera : Culicidae) from Florida. *J. Med. Entomol.* 2003, **40**: 882-889.
23. Nei, M., Genetic distance between populations. *Am. Nat.* 1972, **106**: 283-292.
24. Stanger, J., Preliminary observations on genetic variation in three colonies of *Musca domestica* (Diptera : Muscidae) isolated from central Alberta. *Quaest. Entomol.* 1984, **20**: 51-59.
25. Tsukamoto, M., Enhancement of staining intensity of mosquito larva zymograms after electrophoresis. *J. UOEH.* 1989, **11**: 461-479.
26. Tsukamoto, M. and Horio, M., Electrophoretic comparison of the lactate dehydrogenase banding pattern among Japanese mosquito larvae (Diptera : Culicidae). *J. Med. Entomol.* 1985, **22**: 491-498.
27. Wright, S., *Evolution and the Genetics of population, vol. 4. Variability within and among natural populations*. Chicago, Illinois, The University of Chicago Press 1978.