ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE MOLECULAR BIOLOGY

GENETIC RELATEDNESS AMONG THREE POPULATIONS OF HOUSEFLY *MUSCA DOMESTICA* L. USING RAPD-PCR MARKER

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ABSTRACT

Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique was used to investigate genetic relatedness among populations of housefly *Musca domestica* (L.) from three different regions of the district Allahabad i.e. Arail, Allahabad city and Jhunsi which are separated by rivers Yamuna and Ganga respectively, to analyze whether these rivers act as geographical barriers to gene flow among the three populations. Tools For Population Genetic Analysis (TFPGA) software was used to calculate heterozygosity and Nei's genetic identity. Average heterozygosity ranges from 0.278 to 0.370 showing significant level of genetic variability among these populations. The genetic identity among three populations ranges from 0.467 to 0.694, which reveals that there is a low level of genetic identity reflecting the fact that the rivers appear to act as a barrier to gene flow among these populations.

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

KEY WORDS

RAPD-PCR, *Musca domestica*, housefly, genetic relatedness, heterozygosity.

INTRODUCTION

The house fly, *Musca domestica* Linnaeus, is a synanthropic fly of cosmopolitan distribution. It is the most common species found on hog and poultry farms, horse stables and ranches. These flies are also known to transport disease-causing organisms [1]. Recently, house flies were shown to spread a deadly strain of *Escherichia coli* in Japan [2].

In recent years, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) has been extensively used for population genetic studies in several dipterans [3-10]. In the present study, genetic relatedness among three natural populations of housefly *Musca domestica* from Arail, Allahabad city and Jhunsi regions of district Allahabad has been analyzed on the basis of RAPD-PCR.

MATERIALS AND METHOD

Flies were collected from three different locations of Allahabad district viz., Arail, Allahabad city and Jhunsi (Fig.1).

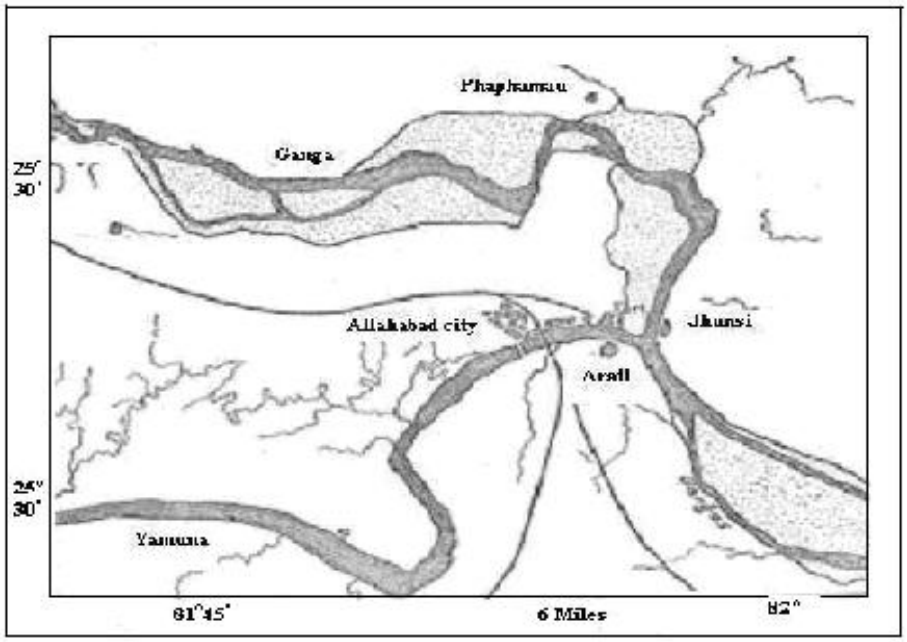

Fig. 1

Fig. 1
Showing the geographical locations of the collecting sites of the three housefly populations under study.

For DNA extraction, amplification, visualization of gels and interpretation of bands, method of Bajpai and Tewari [11] was followed.

A negative control amplification reaction was also performed for each primer. Individuals exhibiting a particular band were assumed to be homozygous or heterozygous for a dominant allele at that locus while the individuals which failed to exhibit a band at that locus were interpreted as homozygous recessive [12]. Average heterozygosity, Nei's [13] genetic identity and distance were calculated by using Tools For Population Genetic Analysis (TFPGA) software [14].

Genetic relationships among the three populations were analyzed by preparing a data matrix by recording the, presence/absence of bands for each primer in 30 individuals from each of the three populations.

RESULTS

The primers used, number of fragments amplified, range of size of amplified fragments, average heterozygosity and number of monomorphic and polymorphic bands among the three populations are presented in Table 1. A total of 98 bands, ranging from 100 bp to 3102 bp were scored among three populations of which twelve bands were found to be exclusive. Only those fragments were considered exclusive which were population specific and observed in more than 70% individuals.

Allahabad city population showed highest percentage of monomorphic bands i.e. 48.14%, while in Arail and Jhunsi populations the percentage of monomorphic bands were 46.66% and 41.81%, respectively. The mean heterozygosity in the three populations ranges from 0.278 to 0.370. The highest mean heterozygosity was observed in case of Jhunsi population.

TABLE 1

The primers used, number of fragments amplified, range of size of the amplified fragments and average heterozygosity among the three populations of *M. domestica*.

S.No.	Sequence (5'-3')	Number of amplified fragments in *Musca domestica* populations			Range of size of amplified fragments in base pair
		Arail N=30	Allahabad city N=30	Jhunsi N=30	
1.	TGATCCCTGG	2(1) [0.250]	2(1) [0.366]	5(3) [0.375]	258-1059
2.	AGGGCGTAAG	3(2) [0.321]	3(2) [0.386]	3(2) [0.448]	273-879
3.	CAGCCCAGAG	5(2) [0.374]	3(2) [0.339]	4(1) [0.383]	192-2478
4.	GTCCCGACGA	6(5) [0.244]	4(3) [0.271]	6(4) [0.413]	214-979
5.	GGTGACGCAG	5(2) [0.358]	2 [0.401]	3(1) [0.374]	202-1425
6.	TGGGGGACTC	3(1) [0.275]	4(1) [0.336]	5(2) [0.435]	196-776
7.	GTAGACCCGT	5(2) [0.347]	5(2) [0.324]	2 [0.396]	144-581
8.	TGCGTGCTTG	3 [0.391]	2 [0.346]	2 [0.439]	100-638

9.	CTCTGGAGAC	5(2) [0.218]	4(1) [0.402]	4(1) [0.433]	283-2334
10.	TCTCCGCTTG	5(4) [0.319]	4(4) [0.427]	4(3) [0.421]	311-1471
11.	TCGTTCCGCA	3(3) [0.000]	4(2) [0.311]	2 [0.407]	489-1622
12.	GGTGCTCCGT	4(1) [0.228]	5(2) [0.348]	5(2) [0.374]	608-2502
13.	ACGGATCCTG	4(1) [0.254]	4(1) [0.274]	3(3) [0.000]	472-2017
14.	CCTGATCACC	4(1) [0.242]	4(1) [0.242]	3(1) [0.342]	487-3102
15.	GGTGATCAGG	3(1) [0.348]	4(4) [0.000]	4(1) [0.314]	195-1375
Mean heterozygosity		0.278	0.318	0.370	

Amplification pattern obtained with primer number 5 and 13 are shown in Figures 2a and 2b which reveal 3 and 2 population specific bands, respectively. Maximum number of population specific bands was observed between Jhunsi and Arail populations i.e. eight bands.

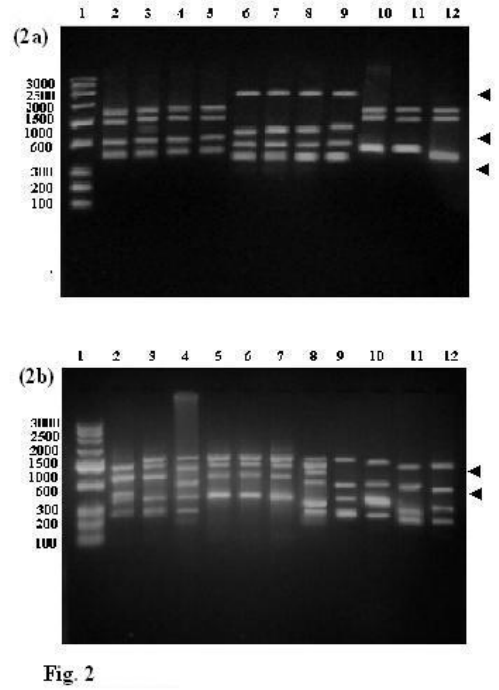

Fig. 2

Fig. 2a
RAPD-PCR pattern derived from primer 5 (5' GGTGACGCAG 3') and 2b primer 13 (5' ACGGATCCTG 3'). Lane 1: Molecular Weight Marker (Low Range DNA ruler), Lane 2-5: *Musca domestica* (Arail), Lane 6-9: *Musca domestica* (Allahabad City), Lane 10-12: *Musca domestica* (Jhunsi)

The mean genetic identity values among the three populations range from 0.467 to 0.694 are represented in Table 2. UPGMA dendogram as revealed by fifteen RAPD primers in the three housefly populations is shown in Fig.3.

TABLE 2
Nei's (1972) genetic identity based on comparison of RAPD patterns among *M. domestica* populations from Arail, Allahabad city and Jhunsi.

	M. domestica (Arail)	*M. domestica* (Allahabad city)	*M. domestica* (Jhunsi)
M. domestica (Arail)	-		
M. domestica (Allahabad city)	0.583	-	
M. domestica (Jhunsi)	0.467	0.694	-

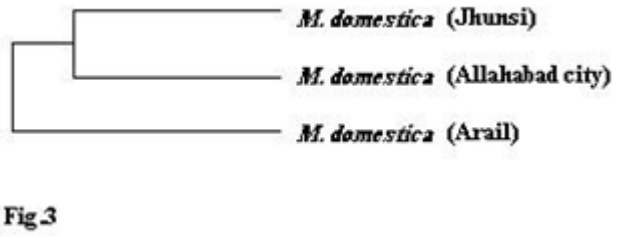

Fig. 3
UPGMA dendogram as revealed by RAPD-PCR data.

DISCUSSION

The RAPD technique consists of amplification by PCR of random segments of genomic DNA using a single short primer of arbitrary sequence. There is no requirement of prior knowledge of the sequence of DNA. Its cost effectiveness provides an advantage in population and evolutionary genetic studies [15].

The mean heterozygosity revealed by RAPD marker (0.322) among the three populations is greater as compared to that of allozyme markers for other muscids [16-18] *viz.*, *Musca domestica* (0.273), *Stomoxys calcitrans* (0.070) and *Haematobia irritans* (0.131). This may be attributed to the fact that RAPD markers show more variability than that of allozyme markers [19]. The average heterozygosity value obtained from RAPD markers in Calliphoridae, Oestridae and Sarcophagidae ranges from 0.08 to 0.168 which show that genetic divergence, in members belonging to these families, is low [20, 21, 11, 22] as compared to the present study (0.322). This may be due to the fact that the aggregate population densities of houseflies are higher in the environment [23]. Presence of variability among populations as well as individuals within a population is essential for their ability to survive and successfully respond

to environmental changes and chemical insecticide pressures [9].

The intra-population genetic identity values were higher than 0.700, this is consistent with the fact that individual of the same population should represent a narrow genetic pool [24]. The genetic identity values among the three housefly populations were low, this could be ascribed to the fact that the three populations surveyed in the present study are separated due to physical barriers i.e. rivers which prevents gene flow among them [25].

REFERENCES

1. Scott, J.G., N. Liu, M. Kristensen and Clark, A.G., A case for sequencing of the genome of *Musca domestica* (Diptera: Muscidae). J. Med. Entomol. 46(2): 175-182, (2009).
2. Sasaki, T., M. Kobayashi, and Agui, N., Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: muscidae) in the dissemination of *Escherichia coli* O157: H7 to food. J. Med. Entomol. 37: 945-949, (2000).
3. Balbino V. de Queiroz , Coutinho-Abreu I. V., Sonoda I. V., Melo M. A., de Andrade P. P., de Castro J. A. F., Rebelo J. M., Carvalho S. M. S.and Ortigao, M. R., Genetic structure of natural populations of the sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae) from the Brazilian northeastern region. Acta Tropica 98: 15-24, (2006).
4. Brito, L.G., Regitano L.C., De A., Huacca, M.E.F., E. Carrilho, M.J. Paes and G.E. Moya-Borja,. Genotype characterization of the *Haematobia irritans* (Diptera: Muscidae) from Brazil, Dominican Republic and Colombia based on randomly amplified polymorphic DNA (RAPD) analysis. Rev. Bras. Parasitol. 17: 179-184, (2008).
5. Castiglioni, L. and Bicudo de Campos H.E.M., Molecular characterization and relatedness of *Haematobia irritans* (horn fly) populations, by RAPD-PCR. Genetica 124: 11-21, (2005).
6. Duncan, G.A., P.H. Alder, K.P. Pruess and Powers, T.O., Molecular differentiation of two sibling species of the black fly *Simulium vittatum* (Diptera: Simulidae) based on random amplified polymorphic DNA. Genome 47: 373-379, (2003).
7. Dvorak, V., A.M. Aytekin, B. Altin, S. Skarupova, J. Votypka and Volf, P., A comparison of the intraspecific variability of *Phlebotomus sergenti* Parrot fly (Diptera: Psychodidae). Journal of Vector Ecology 31 (2): 229-238, (2006).
8. Gonzalez, R., R. Wilkerson., M.F. Suarez, F. Garcia, G. Gallego, H. Cardenas, C.F. Posso and Duque, M.C., A population genetics study of *Anopheles darlingi* (Diptera: Culicidae) from Colombia based on random amplified polymorphic DNA-polymerase chain reaction and amplified fragment length polymorphism markers. Mem. Inst. Oswaldo Cruz., 102: 255-262, (2007).
9. Sharma, A.K., M.J. Mendki, S.N. Tikar, K. Chandel, D. Sukumaran, B.D. Parashar, Vijay Veer, O.P. Agarwal and Shri Prakash, Genetic variability in the geographical populations of *Culex quinquifasciatus* Say (Diptera: Culicidae) from India based on random amplified polymorphic DNA analysis. Acta tropica 112: 71-76, (2009).
10. Zhang, L. and Z.Y. Zhang, Random amplified polymorphic DNA identification of six *Bactrocera* (Diptera: Tephritidae)

species in Yunnan Province of southwest China. Ying Yong Sheng Tai Xue Bio. 18: 1163-1166, (2007).

11. Bajpai, N. and Tewari, R.R., Genetic characterization of sarcophagid flies by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). Natl. Acad. Sci. Lett. 33: 103-106, (2010).
12. Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and Tingey, S.V., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531-6535, (1990).
13. Nei, M., Genetic distance between populations. American Naturalist. 106: 283-292, (1972).
14. Miller, M. N., Tools for population genetic analysis (TFPGA) *(http://bioweb.usu .edu/mpmbio)* (1997).
15. Bardacki, F., Random Amplified Polymorphic DNA (RAPD) Markers. Turk J. Biol. 25: 185-196, (2001).
16. Krafsur, E.S., J.M. Helm and Black IV, W.C., Genetic diversity at electrophoretic loci in the house fly, *Musca domestica* L. Biochem. Genet. 30: 317-328, (1992).
17. Szalanski, A.L., D.B. Taylor and Peterson II, R.D., Population genetics and gene variation of stable fly populations (Diptera: Muscidae) in Nebraska. J. Med. Entomol. 33: 413-420, (1996).
18. McDonald, P.T., L.R. Hilburn, and King, S.E., Genetic similarities among natural populations of the horn fly (Diptera: Muscidae). Ann. Entomol. Soc. Am. 80: 288-292, (1987).
19. Apostol, B., W. C. Black, P. Reiter and Miller, B.R., Population genetics with RAPD-PCR markers: the breeding structure of *Aedes aegypti* in Puerto Rico. Heredity 76: 325-334, (1996).
20. Infante-Malachias, M.E.V., K.S. Yotoko and Azeredo-Espin, A.M.L., Random Amplified Polymorphic DNA of screwworm fly Populations (Diptera: Calliphoridae), from South-east of Brazi l and North of Argentina. Genome 42: 772-779, (1999).
21. Azeredo-Espin, A.M.L. and Lessinger, A.C., Genetic Approaches for Studying Myiasis causing Flies: Molecular Markers and Mitochondrial Genomics. Genetica 126: 111-131, (2006).
22. Bajpai, N., Genetic relationship of sarcophagid flies. D.Phil. Thesis, University of Allahabad, Allahabad, India. (2011).
23. Hartl, D.L., A primer of population genetics. Sinauer Associates Inc., Sunderland, Massachusetts. (2000).
24. Haymer, D. S., H. Mei and McInnis, D. O., Genetic marker analysis of spatial and temporal relationships among existing populations and new infestations of the Mediterranean fruit fly (*Ceratitis capitata*). Heredity 79: 302-309, (1997).
25. Srivastava, S., Genetic variation in *Musca* (Diptera: Muscidae). D.Phil. Thesis, University of Allahabad, Allahabad, India. (2009).