Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Population genetic structure of *Culex quinquefasciatus* in India by ISSR marker

Mendki MJ[1], Sharma AK[1*], Vijay Veer[1], Agrawal OP[2], Shri Prakash[1], Parashar BD[1]

[1]*Entomology Division, Defence R & D Establishment, Jhansi Road, Gwalior, Madhya Pradesh, India*
[2]*School of Studies in Zoology, Jiwaji University, Gwalior, Madhya Pradesh, India*

ARTICLE INFO

Article history:
Received 12 October 2010
Received in revised form 27 December 2010
Accepted 15 January 2011
Available online 20 May 2011

Keywords:
Genetic structure
Culex quinquefasciatus
ISSR marker
India

ABSTRACT

Objective: To characterize the genetic structure of various populations of *Culex quinquefasciatus (Cx. quinquefasciatus)* from India representing different geoclimatic locations. **Methods:** Inter simple sequence repeat (ISSR) markers were used. A set of 20 primers were screened with the laboratory populations of mosquito species. Finally the IS 40 primer was chosen based on the scorable banding pattern showing 100 percent polymorphism among the various populations. The statistical analysis was done using POPGENE 1.31 software. The consensus tree was generated based on UPGMA modified from NEIGHBOR procedure of PHYLIP Version 3.5. **Results:** The cluster analysis shows the main cluster which is divided into two sub cluster representing all the populations separated as per their phylogeographic and geoclimatic condition. **Conclusions:** The findings will be helpful in understanding the population variation under different ecological conditions and development of effective vector management strategies.

1. Introduction

Culex quinquefasciatus (Cx. quinquefasciatus) are commonly prevalent near the human inhabitation, causing severe biting nuisance. They are vectors of various diseases such as Lymphatic Filariasis (LF), West Nile virus (WNV) encephalitis, Japanese encephalitis (JE) and many more. Among these diseases LF is the second most common vector-borne disease found in 80 tropical and subtropical countries. WHO estimated that about 120 million people are affected with LF, with one billion at risk. One-third of the people infected with LF live in India[1]. WNV, a flavivirus, currently spreading throughout the North America with 1%-4% mortality among confirmed cases[2].

The prevalence of vector-borne disease depends upon a number of factors involving the insect vector, the human and the pathogen. Genetic variability and environmental factors are amongst the important factors that may influence the refractoriness, propagation of pathogen and transmission of disease[3]. Although the exact mechanism of genetic basis of susceptibility to pathogen in vectors is not understood but the environmental variants of various genes for such divergence are very well documented. Investigations on the occurrence of genetic variations among vector populations could address several persistent questions with respect to vector competence and also provide rich insight into vector species complexes.

Unprecedented advancement in the modern molecular biology, particularly those of DNA marker technology, have created a wealth of know how that finds useful applications of these markers in molecular ecology or population genetics studies in mosquitoes. Amongst the various markers, Inter simple sequence repeats (ISSR) are the one that permits the detection of DNA variations in microsatellite. These are best suited for population genetic structure as they are relatively easy and inexpensive to use and evolve rapidly enough to be variable. Microsatellite markers have been successfully applied for genetic diversity studies in *Aedes aegypti (Ae. aegypti)*[4-6], *Anopheles funestus (An. funestus)*[7], *Culex pipiens (Cx. pipiens)*[8] and in *Bombyx mori (B. mori)*[9,10], *Mayetiola* sp [11], Blackflies[12], honey bee[13] and necrophagous flies[14].

The present study was undertaken with the objective to determine the genetic structure and phylogenetic

*Corresponding author: Sharma AK, Entomology Division, Defence R & D Establishment, Jhansi Road, Gwalior-474002, Madhya Pradesh, India.
Tel: +917512233493
Fax: +917512341148
E-mail: agarima2001@yahoo.com; ajaymolbio@gmail.com

relationship among the various geographical populations of *Cx. quinquefasciatus* from India using ISSR primers. The population genetic studies of geographically dispersed *Cx. quinquefasciatus* populations across the India are critical both for delineating the genetically distinct groups or populations and for the eventual design of effective vector control strategies.

2. Materials and methods

2.1. Mosquito collection

The locations and sample sizes of *Cx. quinquefasciatus* larvae collected from natural oviposition sites in each of the thirteen locations representing different geographical zones of India. These included Sri Ganganagar, Bikaner, Jodhpur, Barmer (Rajasthan state); Bathinda, Amritsar (Punjab state); Pathankot, Nagrota (Jammu and Kashmir); Bhuj, Gandhinagar (Gujrat state); Hathigarh, Masimpur (Assam, North-eastern part of India) and Leh (Cold dry climatic part of Jammu and Kashmir) as shown in Figure 1. The mosquito collected from each site has been identified morphologically and representative samples are pinned as a voucher specimen and kept in the laboratory as reference collection. Mosquitoes were reared until adult stage under standard conditions (27±2 ℃, relative humidity 80% to 90% and 12 h light/dark cycle). Pupae were isolated and emerging virgin females were used for DNA extraction.

Figure 1. Collection sites of *Cx. quinquefasciatus* mosquitoes in India, 1- Gandhinagar, 2- Bhuj, 3-Jodhpur, 4- Barmer, 5- Bikaner, 6- Sriganganagar, 7- Bathinda, 8- Amritsar, 9- Pathankot, 10- Nagrota, 11-Leh, 12- Hathigarh, 13- Masimpur.

2.2. DNA extraction

The DNA extracted from single adult mosquito was used for further analysis. The DNA extraction was performed by using modified Coen method[15]. Each single mosquito is homogenized in 100 μL lysis buffer (Tris HCl 0.1 mol/L pH 9.1 containing NaCl 0.1 mol/L, EDTA 0.05 mol/L, Sucrose 0.2 mol/L Sodium dodecyl sulfate 0.05%) using motorized homogenizer (Sigma Aldrich, USA) and kept in water bath at 65 ℃ for 30 min. Subsequently, 30 μL of 5 mol/L potassium acetate was added and immediately kept on ice for 1 h. Samples were then centrifuged at 13 000 rpm for 15 min at 10 ℃ and DNA was precipitated by adding double volume of chilled ethanol to the collected supernatant and then centrifuged again at 13 000 rpm, for 15 min. The collected DNA was then washed twice with 70% ethanol and finally dissolved in 50 μL of TE buffer (Tris HCl 10 mmol/L pH 8.0, 1 mmol/L EDTA). Qualitative analysis of DNA was done by running the DNA on 1% agarose gel for 1 h using 0.5×TAE Buffer (Tris acetate 40 mmol/L pH 7.6, EDTA 1 mmol/L).

2.3. PCR amplification with ISSR primers

A total of 20 ISSR primers (Table 1), procured from University British Colombia (UBC), Canada, were screened with laboratory reared strain of *Cx. quinquefasciatus* to get the amplification pattern. The amplification was performed in a total of 10 μL of reaction mixture consisting of Tris HCl 10 mmol/L pH 9.0, 50 mmol/L $MgCl_2$, 2 mmol/L dNTP, 0.2 mmol/L 10 pmoles of primer, 0.5 U of Taq DNA polymerase (MBI Fermentas) and 10 ng of genomic DNA. Reactions were performed in a (iCycler, Bio-Rad PCR System) thermal cycler. The PCR condition consisted of initial Denaturation step for 4 min at 94 ℃ followed by 35 cycles of 1 min at 94 ℃, 1 min at 50-54 ℃, and 2 min at 72 ℃. A final extension step was performed at 72 ℃ for 10 min. The PCR products were analyzed by electrophoresis in 1% (w/v) agarose gel run at 100 V for 1 h followed by 80 V for about 3 h using 0.5×TAE Buffer (Tris acetate 40 mmol/L pH 7.6, EDTA 1 mmol/L).

2.4. Estimation of polymorphism and construction of phylogenetic tree

The binary scoring of the profiles was done on the basis of presence or absence of a band at a particular locus. From the binary data, the genetic diversity among the individual populations was analyzed. The genetic distance and the polymorphism among the population were interpreted by using POPGENE version 1.31 software. Necessary assumptions like genotype frequencies at ISSR loci are in Hardy-Weinberg proportion and genetic variability of ISSR-PCR technique was taken into account. The program POPGENE was used to calculate Nei's genetic distances[16] applying Lynch & Milligan's[17] correction, between the 13 field collected populations of *Cx. quinquefasciatus*. The population genetic indexes like Shannon index and genetic diversity index were also recorded. A cluster analysis technique of unweighted pair-group method of arithmetic averages (UPGMA) was used to develop the phylogenetic tree.

3. Results

A total of twenty ISSR primers (summarized in Table1) were initially screened using laboratory reared strains of *Cx. quinquefasciatus*, *Ae. aegypti*, *Ae. albopictus* and *An. stephensi in* order to select a set of ISSR primers which produce scorable fingerprinting for the further study with field mosquito populations. Out of these 20 primers 15 primers did not show any amplification or poor amplification or well beyond the capacity of amplification by Taq DNA polymerase used. Among these primers only IS40 was chosen for further study as it produced clear reproducible banding pattern with *Cx. quinquefasciatus* mosquito species. This primer gave scorable PCR products and yielded a total of 29 polymorphic bands ranging from 200 bp to 3.1 kb showing a polymorphism of 100%.

For statistical analysis 29 bands or loci were taken representing all field population under study using IS 40 primers (Figure 2). The average genetic distance observed between the populations was 0.26 which was ranging from 0.071 5 to 0.659 2 (Table 2). The genetic diversity among the populations indicated the Shannon index of 0.36 and genetic diversity index (*n*) of 0.21 which significantly demonstrate the rich genetic diversity among the *Cx. quinquefasciatus* populations. The phylogenetic tree was generated with the help of POPGENE 1.31 version (Figure 3) based on Nei's[16]

Table 1

The sequences of 20 ISSR-oligonucleotide primers screened to see genetic variability in *Cx. quinquefasciatus* populations collected from different parts of India.

S. No.	Primer	UBC code	Sequence	Tm(℃)
1	IS 23	UBC 830	(TG)8G	57.19
2	IS 24	UBC 848	(CA)8RG	58.76
3	IS 25	UBC 851	(GT)8YC	58.76
4	IS 26	UBC 855	(AC)8YT	56.48
5	IS 27	UBC 856	(AC)8YA	56.48
6	IS 28	UBC 857	(AC)8YG	58.76
7	IS 29	UBC 861	(ACC)6	64.46
8	IS 30	UBC 864	(ATG)6	50.79
9	IS 31	UBC 866	(CTC)6	64.46
10	IS 32	UBC 867	(GGC)6	78.12
11	IS 33	UBC 868	(GAA)6	50.79
12	IS 34	UBC 869	(GTT)6	50.79
13	IS 35	UBC 871	(TAT)6	37.12
14	IS 36	UBC 874	(CCCT)4	64.40
15	IS 37	UBC 890	VHV(GT)7	56.39
16	IS 38	UBC 891	HVH(TG)7	55.58
17	IS 39	UBC 881	GGGT(GGGGT)2G	64.37
18	IS 40	UBC 895	AGAGTTGGTAGCTCTTGATC	58.35
19	IS 41	UBC 899	CATGGTGTTGGTCATTGTTCC	60.61
20	IS 42	N. A.	GGATGGATGGAT	43.73

Table 2

Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between *C. quinquefasciatus* populations collected from different parts of India.

POP	SNGR	BHT	BKN	JD	PKT	NGR	ASR	BDR	LEH	BHUJ	GN	HGR	MSM
1	****	0.827 6	0.862 1	0.896 6	0.689 7	0.827 6	0.827 6	0.827 6	0.793 1	0.758 6	0.931 0	0.793 1	0.827 6
2	0.189 2	****	0.758 6	0.862 1	0.655 2	0.758 6	0.724 1	0.793 1	0.758 6	0.724 1	0.896 6	0.827 6	0.862 1
3	0.148 4	0.276 3	****	0.827 6	0.620 7	0.793 1	0.758 6	0.758 6	0.862 1	0.689 7	0.862 1	0.793 1	0.758 6
4	0.109 2	0.148 4	0.189 2	****	0.655 2	0.896 6	0.862 1	0.862 1	0.758 6	0.724 1	0.896 6	0.827 6	0.862 1
5	0.371 6	0.422 9	0.476 9	0.422 9	****	0.551 7	0.517 2	0.586 2	0.620 7	0.586 2	0.758 6	0.620 7	0.655 2
6	0.148 4	0.276 3	0.231 8	0.109 2	0.594 7	****	0.896 6	0.827 6	0.724 1	0.620 7	0.793 1	0.793 1	0.758 6
7	0.189 2	0.322 8	0.276 3	0.148 4	0.659 2	0.109 2	****	0.724 1	0.689 7	0.655 2	0.758 6	0.827 6	0.724 1
8	0.189 2	0.231 8	0.276 3	0.148 4	0.534 1	0.189 2	0.322 8	****	0.689 7	0.655 2	0.827 6	0.758 6	0.793 1
9	0.231 8	0.276 3	0.148 4	0.276 3	0.476 9	0.322 8	0.371 6	0.371 6	****	0.689 7	0.862 1	0.793 1	0.827 6
10	0.276 3	0.322 8	0.371 6	0.322 8	0.534 1	0.476 9	0.422 9	0.422 9	0.371 6	****	0.827 6	0.758 6	0.724 1
11	0.071 5	0.109 2	0.148 4	0.109 2	0.276 3	0.231 8	0.276 3	0.189 2	0.148 4	0.189 2	****	0.862 1	0.896 6
12	0.231 8	0.189 2	0.231 8	0.189 2	0.476 9	0.231 8	0.189 2	0.276 3	0.231 8	0.276 3	0.148 4	****	0.827 6
13	0.189 2	0.148 4	0.276 3	0.148 4	0.422 9	0.276 3	0.322 8	0.231 8	0.189 2	0.322 8	0.109 2	0.189 2	****

1-SNGR-Sri Ganganagar, 2-BHT-Bathinda, 3-BKN-Bikaner, 4-JD-Jodhpur, 5-PKT-Pathankot, 6-NGR-Nagrota, 7-ASR-Amritsar, 8-BDR-Barmer, 9-LEH-Leh, 10-BHUJ-Bhuj, 11-GN-Gandhinagar, 12-HGR-Hathigarh, 13-MSM-Masimpur populations respectively.

genetic distance and unweighed pair group method of arithmetic averages (UPGMA) modified from Neighbour procedure of Phylip version 3.5. The highest distance of 0.66 was observed between the Amritsar and Pathankot followed by 0.594 between Pathankot and Nagrota; 0.534 between Pathankot and Barmer whereas, lowest distance of 0.071 was observed between Gandhinagar and Sriganganagar population. The highest genetic identity was found between Sriganganagar-Gandhinagar and Sriganganagar-Jodhpur; Jodhpur-Nagrota; Jodhpur-Gandhinagar; Bathinda-Gandhinagar and Masimpur-Gandhinagar ranging from 0.89-0.93. On the other hand the lowest identity was observed between Pathankot-Amritsar (0.517 2) followed by 0.55-0.58 between Pathankot-Nagrota, Pathankot-Barmer, Pathankot-Bhuj (Table 2).

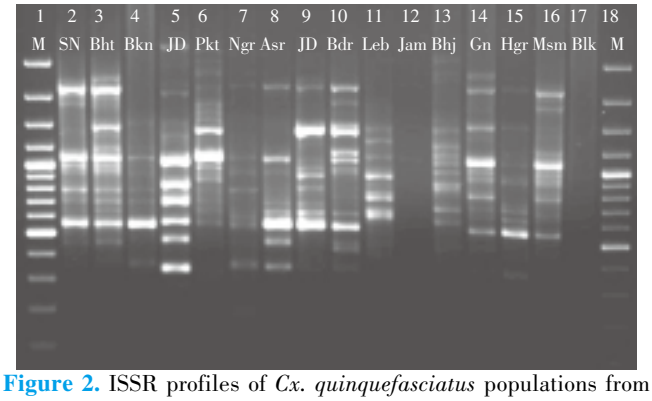

Figure 2. ISSR profiles of *Cx. quinquefasciatus* populations from different locations with Primer IS 40.
M-molecular weight marker 100 bp plus (100 bp to 3 kb), SN-Sriganganagar, Bht-Bathinda, Bkn-Bikaner, JD-Jodhpur, Pkt-Pathankot, Ngr-Nagrota, Asr-Amritsar, Bdr-Barmer, Leh-LEH, Bhj-Bhuj, Gn-Gandhinagar, Hgr-Hathigarh, Msm-Masimpur, Blk-Blank.

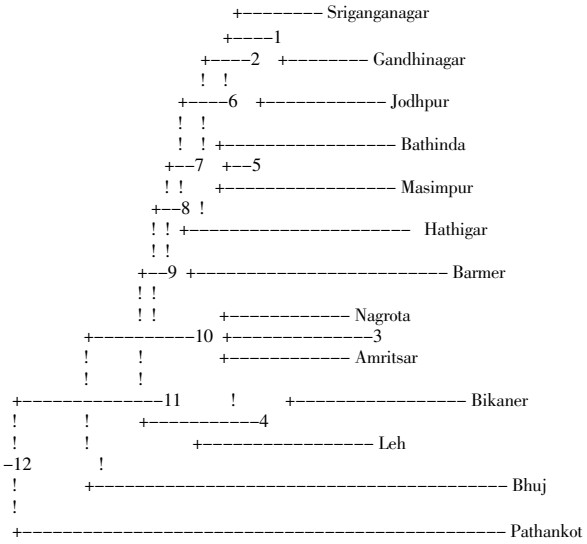

Figure 3. Phylogenetic tree generated by using POPGENE 1.31 version for different *Cx. quinquefasciatus* populations resulting from UPGMA cluster analysis.

The cluster analysis shows the one main cluster which is sub divided into two sub clusters. The one sub cluster represents the population from Sriganganagar, Nagrota, Gandhinagar, Jodhpur and Bathinda which are geographically closely placed and shows almost similar climatic conditions of semi- arid environment. In the same cluster the HGR & MSM are placed together showing genetic identity of 0.83 and genetic distance of 0.19, belongs to hot and humid climatic condition in the North Eastern region of India. The another sub cluster represents the population from cold climatic condition *i.e.* Nagrota, Amritsar and Leh (cold desert) which are placed along with Pathankot as a separate branch, although belong to the cold climatic region.

4. Discussion

This work is an early report of ISSR based polymorphism for studying the genetic diversity among the *Cx. quinquefasciatus* of different geographical locations in India. To our knowledge this is a first report of ISSR based genetic diversity particularly in this mosquito species. In this regard very little information is available on the use of ISSR markers in mosquito species[18].

Our results indicated a varying degree of genetic diversity in populations of *Cx. quinquefasciatus* in relation to the geographical locations. The phylogenetic estimation revealed a phylogeographic and geoclimatic relationship of *Cx. quinquefasciatus* with its distribution. Among the different populations studied the genetic relatedness was demonstrated by the genetic distance as the least genetic distance with high homology among the populations. In this study the high homology was found between Sriganganagar and Gandhinagar with least genetic distance of 0.071 5. On the contrary low homology with high genetic distance was found between Amritsar and Pathankot followed by Nagrota and Pathankot. Similar findings were also reported in *Ae. aegypti* populations at different geographic locations by using RAPD markers in Argentina[19] and Brazil[20,21] and enzyme based genetic divergence and gene flow in *Cx. pipiens* and *Cx. quinquefasciatus* in Argentina[22].

The phylogenetic analysis of *Cx. quinquefasciatus* based on ISSR markers revealed the genetic variations based on the geographical locations in India. All the locations under study were broadly divided into two clusters where Pathankot population showing isolation from the all others. The populations of Gandhinagar-Jodhpur, Hathigarh-Masimpur and Amritsar-Nagrota resulted high degree of homology which corroborate with RAPD markers studies representing these populations[23]. The Gandhinagar-Jodhpur populations belong to semi-arid zones and Hathigarh-Masimpur belongs to hot-humid climatic conditions in the North East region from India.

The microsatellite based population genetic studies were reported in different mosquito species viz. *Ae. aegypti*[4-6], *An. funestus*[7], *Cx. pipiens*[8]. However, the ISSR based genetic diversity studies among different geographical populations are limited. The ISSR markers were widely used in silkworm Bombyx mori for the genome mapping, germplasm screening and strain improvement

programmes[9,10].

Other than mosquito species the ISSR were widely used in determination of genetic variations between and within species in some of the geographic distribution in black flies[12], Mayetiola species[24], necrophagous flies in China[14] and honey bee in Lithuania[13].

The genetic variations are believed due to the various intrinsic and extrinsic factors which includes geoclimatic conditions, host, pathogen interactions *etc*. The effect of host could be one of the factors which influence the genetic variability as demonstrated in a polyphagous agriculture pest *Helicoverpa armigera* collected from tomato, blackgram, redgram, ladiesfinger, chilly and cotton[25]. Mutational changes such as base substitution, insertions or deletions can create new motifs also the non-random patterns of base substitution would cause the increase in the length and abundance of particular repeats[26]. The sequences which evolve a repetitive pattern can recombine more often and its elimination is avoided. The occasional mutations alter the local characteristics of a sequence. The emerging local pattern is determined by unequal crossing over involving large number of repeats acting as a long range of ordering force, on the other hand slippage (Slipped Strand Mispriming) likely to be more restricted to the shorter distances[27].

In disease transmission vector- pathogen interaction plays an important role followed by the vector-host interactions. The different populations of *Culex* mosquitoes show varying degree of competence of pathogen acquisition and disease transmission. The studies carried out in various laboratory and different geographical strains of various *Culex* species showed susceptibility to Japanese encephalitis virus infection, West Nile virus in various parts of the world *i.e*. Japan[28], Southern Asia and Far East[29], USA[30-32]. Apart from the different mosquito strains and their vector competence, seasonal variation may also affect susceptibility to infection as reported in *Cx. pipiens* from California[33]. The studies on the genetic diversity of the *Cx. quinquefasciatus* field population and its vector competence is unexplained. However, the possibility of the genetic variations in mosquito vectors and its relationship to disease transmission cannot be ruled out. The location taken in our study *i.e*. Gandhinagar, Jamnagar, and Bhuj of coastal region and Hathigarh and Masimpur (Hot and humid North East region) were reported to be affected by lymphatic filariasis[34,35].

The use of quantitative trait loci (QTL) as a genetic determinant of vector pathogen interaction have been demonstrated in tans-ovarian transmission of La Crosse virus in *Ochloretetus trisariatus*, mid gut infection and escape of dengue virus in *Ae. aegypti*, Plasmodium resistance in *An. gambiae*[36-41]. The application of such genetic tools may help us as a foundation for developing biomarkers of vector competence to pathogen and novel targets for genetic manipulation and also for priorities for vector control. Further studies on the phenotypic and genotypic temporal variations will help to get insight into understanding the genetic factors responsible for vector competence/susceptibility and disease transmission (both filarial and arboviral ones) of the mosquito vectors.

The present study is a step forward towards harnessing highly informative microsatellite loci that provide a ubiquitous marker system for characterization of the geographical variations in the *Cx. quinquefasciatus* populations. This investigation could be helpful to understand the population variations with respect to geographical location and the role of natural selection, environmental stress, insecticide pressure, vector-host and vector-pathogen interactions.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to Dr. R. Vijayaraghavan, Director, DRDE, Gwalior, Madhya Pradesh, India for interest and providing all necessary facility to conduct this research work. Sincere thanks also due to the scientists and supportive staff of Entomology Division for their kind cooperation for carrying out the above work

References

[1] WHO. Research on rapid geographical assessment of bancroftian filariasis. In: *TDR/TDF/COMDT/98.2*. Geneva: World Health Organization; 1998.

[2] CDC (Centre for Disease Control and Prevention). *West Nile virus: statistics, surveillance, and control*. [online]. Available from: http://www.cdc.gov/ncidod/westnile/surv&control.htm#casedefinition. [Accessed on October 22, 2009]

[3] Hill SM, Crampton JM. DNA based methods for the identification of insect vectors. *Ann Trop Med Parasitol* 1994; **88**: 227-250.

[4] Ravel S, Herve JP, Diarrassouba S, Kone A, Cuny G. Microsatellite markers for population genetic studies in *Aedes aegypti* (Diptera: Culicidae) from Côte d'Ivoire: evidence for a microgeographic genetic diffrentiation of mosquitoes from Bouaké. *Acta Trop* 2002; **82**: 39-49.

[5] Ravel S, Monteny N, Olmos V, Escalante D, Verdugo J, Cuny G. A preliminary study of the population genetics of *Aedes aegypti* (Diptera: Culicidae) from Mexico using microsattelite markers and AFLP markers. *Acta Trop* 2001; **78**: 241-250.

[6] Endersby NM, Hoffman AA, White VL, Lowenstein S, Ritchie S, Johnson, PH, et al. Genetic structure of *Aedes aegypyi* in Australia and Vietnam revealed by microsatellite and exon primed intron crossing markers suggests feasibility of local control options. *J Med Entomol* 2009; **46**: 1074-1083.

[7] Temu EA, Hunt RH, Coetzee M. Microsatellite DNA polymorphism and heterozygosity in the malaria vector mosquito *Anopheles funestus* (Diptera: Culicidae) in East and southern Africa. *Acta Trop* 2004; **90**: 39-49.

[8] Huang S, Theodore G, Andreadis G. Genetic insights into population structure of *Culex pipiens* (Diptera: Culicidae) in the Northeastern United States by using microsatellite analysis. *Am J*

Trop Med Hyg 2008; **79**: 518–527.

[9] Nagaraja NJ, Ranganath HA. Molecular phylogeny of the nasuta subgroup of Drosophila based on 12S rRNA, 16S rRNA and COI mitochondrial genes, RAPD and ISSR polymorphisms. *Genes Genet Syst* 2004; **79**: 293–299.

[10]Nagaraju NJ, Reddy D, Nagaraja GM, Sethuraman BN. Comparison of multilocus RFLP's and PCR-based marker systems for genetic analysis of the silkworm, Bombyx mori. *Heredity* 2001; **86**: 588–597.

[11]Khemakhem MM, Marrakchi M, Makni H. Genetic diversity of *Mayetiola destructor* and *Mayetiola hordei* (Diptera: Cecidomyiidae) by intersimple sequence repeats (ISSRs). *Afr J Biotech* 2005; **4**: 601–606.

[12]Dusinsky R, Kudela M, Stloulova V, Jedlicka L. Use of inter-simple sequence repeat (ISSR) markers for discrimination between and within species of blackflies (Diptera: Simuliidae). *Biologí a* 2006; **61**: 299– 304.

[13]Paplauskiene V, Ceksteryte V, Pasakinskiene I, Tamasauskiene D, Racys J. The use of ISSR method for the assessment of bee genetic diversity. *Biologija* 2006; **3**: 16–20.

[14]Hu JL, Zheng XL, Wang Q, Chen XG, Huang YP. Identification of necrophagous fly species from 12 different cities and regions in China using inter-simple sequence repeat molecular markers. *J Southern Med Univ* 2008; **28**: 524–528.

[15]Coen ES, Stracha T, Dover G. Dynamics of concerted evolution of ribosomal DNA and histone gene families in the melanogaster species subgroup of Drosophila. *J Mol Biol* 1982; **158**: 7–35.

[16]Nei M. Genetic distance between populations. *The Am Nat* 1972; **106**: 283–292.

[17]Lynch M, Milligan BG. Analysis of population genetic structure with RAPD markers. *Mol Ecol* 1994; **3**: 91–99.

[18]Abbot P. Individual and population variation in invertebrates revealed by inter- simple sequence repeats (ISSRs). *J Insect Sci* 2001; **1**: 1–3.

[19]de Souza GB, Blanco A, Gardenal CN. Genetic Relationships among *Aedes aegypti* Diptera: Culicidae) Populations from Argentina using Random Amplified Polymorphic DNA polymerase chain reaction markers. *J Med Entomol* 2001; **38**: 371–375.

[20]Ayres CFJ, Santos Melo MAV, Cava Sole AM, Furtado AF. Genetic Differentiation of *Aedes aegypti* (Diptera: Culicidae), the major dengue vector in Brazil. *J Med Entomol* 2003; **40**: 430–435.

[21]Paduan KS, Araujo-Junior JP, Ribolla Paulo EM. Genetic variability in geographical populations of *Aedes aegypti* (Diptera, Culicidae) in Brazil elueidated by molecular markers. *Genet Mol Biol* 2006; **29**: 391–395.

[22]Humeres SG, Almiron WR, Sabattini MS, Gardenal CN. Estimation of genetic divergence and gene flow between *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Argentina. *Mem Inst Oswaldo Cruz* 1998; **93**: 57–62.

[23]Sharma AK, Mendki MJ, Tikar SN, Chandel K, Sukumaran D, Parashar BD, et al. Genetic Variability in geographical populations of *Culex quinquefasciatus* Say (Diptera: Culicidae) from India based on random amplified polymorphic DNA analysis. *Acta Trop* 2009; **112**: 71–76.

[24]Khemakhem MM, Marrakchi M, Makni H. Genetic diversity of *Mayetiola destructor* and *Mayetiola hordei* (Diptera: Cecidomyiidae) by intersimple sequence repeats (ISSRs). *Afr J Biotech* 2005; **4**: 601–606.

[25]Subramanian S, Mohankumar S. Genetic variability of the bollworm, *Helicoverpa armigera*, occurring on different host plants. *J Insect Sci* 2006; **6**: 1–8.

[26]Levinson G, Gutman GA. Slipped strand mispairing: a major mechanism for DNA sequence evolution. *Mol Biol Evol* 1987; **4**: 203–221.

[27]Stephen W, Cho S. Possible role of natural selection in the formation of tandem- repetitive noncoding DNA. *Genetics* 1994; **136**: 333–341.

[28]Takahashi M. Variation in susceptibility among colony strains of *Culex tritaeniorhynchus* to Japanese encephalitis virus infection. *Jpn J Med Sci Biol* 1980; **33**: 321–329.

[29]Ahmed T, Hayes CG, Baqar S. Comparision of vector competence for West Nile virus of colonized populations of Culex tritaeniorhynchus from southern Asia and Far East. *Southeast Asian J Trop Med Publ Health* 1979; **10**: 498–504.

[30]Vaidyanathan R, Scott TW. Geographic variation in vector competence for West Nile virus in the *Culex pipiens* (Diptera: Culicidae) complex in California. *Vector Borne Zoo Dis* 2007; **7**: 193–198.

[31]Hayes CG, Baker RH, Baqar S, Ahmed T. Genetic variation for West Nile virus susceptibility in *Culex Tritaeniorhynchus*. *Am J Trop Med Hyg* 1984; **33**: 715–724.

[32]Goddard LB, Amy ER, Reisen WK, Scott TW. Vector competence of California mosquitoes for West Nile virus. *Emerging Inf Dis* 2002; **8**: 1385–91.

[33]Vaidyanathan R, Scott TW. Seasonal variation in susceptibility to West Nile virus infection in *Culex pipiens pipiens* (L.) (Diptera: Culicidae) from San Joaquin Country, California. *J Vector Ecol* 2006; **31**: 423–425.

[34]Kumar P, Prajapati PB, Saxena D, Kavishwar AB, Kurian G. An evaluation of coverage and compliance of mass drug administration 2006 for elimination of Lymphatic filariasis in endemic areas of Gujrat. *Indian J Commun Med* 2008; **33**: 38–42.

[35]Sabesan S, Vanamail P, Raju KHK, Jambulingam P. Lymphatic filariasis in India: epidemiology and control measures. *J Postgrad Med* 2010; **50**(3): 232–238.

[36]Bosio CF, Fulton RE, Salasek ML, Beaty BJ, Black WC. Quantitative trait loci that control vector competence for dengue-2 virus in the mosquito *Aedes aegypti*. *Genetics* 2000; **156**: 687–698.

[37]Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(1): 43–48.

[38]Kitvatanachai S, Apiwathnasorn C, Leemingsawat S, Wongwit W, Overgaard HJ. Lead levels of *Culex* mosquito larvae inhabiting lead utilizing factory. *Asian Pac J Trop Biomed* 2011; **1**(1): 64–68.

[39]Nikkon F, Habib MR, Saud ZA, Karim MR. *Tagetes erecta* Linn. and its mosquitocidal potency against *Culex quinquefasciatus*. *Asian Pac J Trop Biomed* 2011; **1**(3): 186–188.

[40]Zheng L, Wang S, Romans P, Zhao H, Luna C, Benedict MQ. Quantitative trait loci in *Anopheles gambiae* controlling the encapsulation response against *Plasmodium cynomolgi* Ceylon. *BMC Genet* 2003; **4**: 16.

[41]Gomez-Machorro C, Bennett KE, de Lourdes Munoz M, Black WC. Quantitative trait loci affecting dengue midgut infection barriers in an advanced intercross line of *Aedes aegypti*. *Insect Mol Biol* 2004; **13**: 637–648.