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Evidence of genetic polymorphism in *Aedes aegypti* population from Delhi, India

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Abstract
Aedes aegypti is a domestic mosquito found mainly inside and around the house causes biting nuisance and also a vector of dengue, chikungunya and zika virus worldwide including India. Overall mosquito-borne diseases have re-emerged as a significant human health problem due to a number of factors, including the lack of progress in vaccine development, the emergence of drug resistance in pathogens and insecticides resistance in mosquitoes. An alternative strategy for vector control could be to exploit observed genetic variability in the vector populations. In the present study RAPD primers were used to understand the genetic polymorphism present in the *Aedes aegypti* populations collected from various locations in Delhi. A total of 50 RAPD primers were screened. Among these, the primer OPAK 12 was selected for further study on the basis of the clear, concrete and scorable fingerprint obtained by it. The statistical analysis was done with the help of POPGENE 1.31 using 17 bands or loci representing each population. The average genetic distances between the populations was 0.5624 ranging from 0.1252-1.1015. The consensus tree generated using the software showed two clusters which are branched as per their geographical distances. In conclusion we propose that our results on RAPD profiles provide evidence that there are enough and significant variations in the genomes of field collected populations from distant locations and the genetic pattern obtained in its various forms appears to be a major differentiating and orienting force for molecular changes in DNA across different populations.

Keywords*:* *Aedes aegypti*, genetic polymorphism, commonwealth games

1. Introduction

Aedes aegypti mosquito, the major vector of dengue and chikungunya in India. It is very difficult to control or eliminate *Ae. aegypti* mosquitoes because they have adaptations to the environment that make them extremely resilient, or with the ability to rapidly bounce back to initial numbers after disturbances resulting from natural phenomena or human interventions. Dengue is the common and the fast spreading mosquito-borne viral disease in the world, chikungunya fever has recently reemerged after an interval of several decades to affect millions of people, mainly in India. No specific antiviral therapy or vaccines are currently available for both dengue and chikungunyas fever, and the treatment remains symptomatic and supportive. Preventive measures at the community and personal levels in the forms vector control measures and avoidance of mosquito bites are the main ways of fighting the epidemics [1]. The ecological disruption brings about a change in the epidemiology of dengue viruses as was observed during Common Wealth Games 2010 held in Delhi, on account of massive construction activities dovetailed with incessant rains. Similarly unprecedented urbanization of the metropolitan cities puts enormous pressure on civic activities leading to shortage of housing, interrupted electricity and water supplies resulting in storage practices resorted by the community thereby making the environment suitable for their growth. For successful vector control programme it is very essential to have the knowledge of the vector and its biology [2]. In addition to the practical understanding required for successful control programs, population genetic studies of introduced disease vectors can provide excellent opportunities to examine evolutionary processes in complex systems. Genetic analyses enable us to locate putative origins of multiple introductions [3, 4] and evaluate differing vectorial capacity across populations [5, 6]. Changes in genetic makeup associated with introductions and expansions [7, 8] as well as new associations with local or introduced hosts and pathogens, create dynamic systems that are amenable to examination and even experimentation [9].

Change can be measured by comparison both to populations in the original range and, in some situations, to the earlier stages of the introduction. Though these systems are invaluable, thorough analyses are rare [10]. The phenomenon of genetic variation has been estimated by several techniques including morphological studies, cytogenetics, protein electrophoresis and direct measurement of DNA variability using various molecular markers such as RAPD, RFLP, ISSR etc. Among these markers the RAPD is a rapid and relatively inexpensive compared with restriction fragment length polymorphism analysis or DNA sequencing [11]. Random amplified length polymorphic DNA (RAPD) [12, 13] is a polymorphism chain reaction (PCR) technique that allows detection of many polymorphisms within the genomic DNA in a short time [14-18]. In the present study, we have used RAPD primers to understand the genetic polymorphism present in the *Aedes aegypti* population collected from different zones from Delhi.

2. Materials and methods

2.1 Test Insect

A total of 6 different locations from Delhi were taken during the present study for the collection of *Ae. aegypti* mosquito. The laboratory reared *Ae. aegypti* has also been taken as a reference population. The collection sites of mosquitoes are listed in table1 and geographic locations of all sampling sites are shown in Fig.1. At every site only one time larvae collections was made from 3-4 closely located water bodies before the commencement of commonwealth games in Delhi, 2010. Larvae collected from each of these study area were pooled together respectively, and a random sample was used for the study in order to minimize relationship between mosquitoes. The adult mosquito emerged from these collected larvae were identified morphologically and representative samples are pinned as the voucher specimen and kept in the laboratory as a reference collection. Mosquitoes were reared until the adult stage under standard conditions (Temp 27º ± 2ºC, relative humidity 80 to 90% and 12-hrs light / dark cycle).The emerging virgin females were used for DNA extraction. The DNA extracted from single adult mosquito was used for further analysis.

Table 1: List of collection sites with Latitude and Longitude

S. No.	Collection sites	(Latitude, Longitude)
1	Vivek Vihar	28° 67’N 77°.31’E
2	Preet Vihar	28° 63’N 77°.29’E
3	Mayur Vihar	28° 61’N 77°.31’E
4	Najafgarh	28° 60’N 76°.99’E
5	Mehrauli	28° 52’N 77°.17’E
6	Saket	28° 52’N 77°.20’E
7	Lab (Gwalior)	26° 20’N 78°.19’E

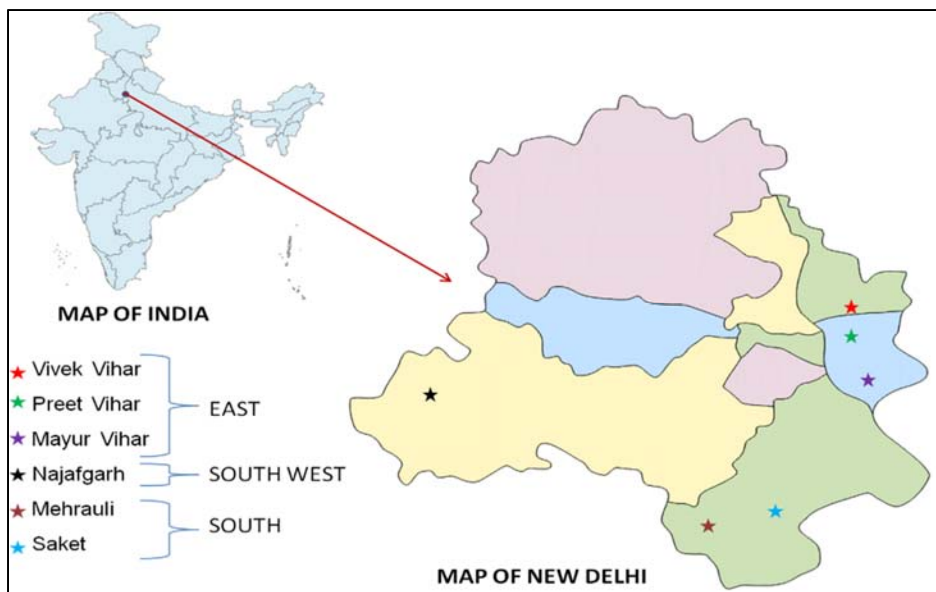

Fig 1: Map of Delhi, India highlighting regions sampled for *Aedes aegypti* population

2.2 Extraction of mosquito DNA

The DNA extraction was done by using modified Coen method [19] and as used in our previous publication [14, 18].

2.3 PCR amplification

A total of 50 Operon grade 10-mer oligonucleotide RAPD primers OPAK11- OPAK20; OPAS11- OPAS20; OPX11-OPX20; OPM11- OPM20 and OPBD11-OPBD 20 (Table 2) were screened with laboratory reared strain of *Ae. aegypti* to get the amplification pattern. The amplification was performed as used in our previous publication [14, 18].

Table 2: Sequences of 50 RAPD-oligonucleotide primers screened for differentiating *Aedes aegypti*

S. No.	Primer	Sequence	S.No.	Primer	Sequence
01	OPAK11	CAGTGTGCTC	26	OPBD16	GAACTCCCAG
02	OPAK12	AGTGTAGCCC	27	OPBD17	GTTCGCTCCC
03	OPAK13	TCCCACGAGT	28	OPBD18	ACGCACACTC
04	OPAK14	CTGTCATGCC	29	OPBD19	GGTTCCTCTC
05	OPAK15	ACCTGCCGTT	30	OPBD20	AGGCGGCACA
06	OPAK16	CTGCGTGCTC	31	OPM11	GTCCACTGTG
07	OPAK17	CAGCGGTCAC	32	OPM12	GGGACGTTGG
08	OPAK18	ACCCGGAAAC	33	OPM13	GGTGGTCAAG
09	OPAK19	TCGCAGCGAG	34	OPM14	AGGGTCGTTC
10	OPAK20	TGATGGCGTC	35	OPM15	GACCTACCAC
11	OPAS11	ACCGTGCCGT	36	OPM16	GTAACCAGCC
12	OPAS12	TGACCAGGCA	37	OPM17	TCAGTCCGGG
13	OPAS13	CACGGACCGA	38	OPM18	CACCATCCGT
14	OPAS14	TCGCAGCGTT	39	OPM19	CCTTCAGGCA
15	OPAS15	CTGCAATGGG	40	OPM20	AGGTCTTGGG
16	OPAS16	AACCCTTCCC	41	OPX11	GGAGCCTCAG
17	OPAS17	AGTTCCGCGA	42	OPX12	TCGCCAGCCA
18	OPAS18	GTTGCGCAGT	43	OPX13	ACGGGAGCAA
19	OPAS19	TGACAGCCCC	44	OPX14	ACAGGTGCTG
20	OPAS20	TCTGCCTGGA	45	OPX15	CAGACAAGCC
21	OPBD11	CAACCGAGTC	46	OPX16	CTCTGTTCGG
22	OPBD12	GGGAACCGTC	47	OPX17	GACACGGACC
23	OPBD13	CCTGGAACGG	48	OPX18	GACTAGGTGG
24	OPBD14	TCCCTGTGAG	49	OPX19	TGGCAAGGCA
25	OPBD15	TGTCGTGGTC	50	OPX20	CCCAGCTAGA

2.4 Estimation the polymorphism and construction of phylogenetic tree

Polymorphism among species and the field samples were analyzed by scoring the polymorphic and monomorphic bands, 'mono' means same and 'morphic' means forms i.e., if same band of DNA is present in all individuals or the sample population under study. Whereas in polymorphic bands 'poly' means many and 'morphic' means forms is defined as discontinuous variation in a single population. The genetic distance and the polymorphism among the population were interpreted by using POPGENE version 1.31 software. A suitable phylogenetic tree was generated with the help of above software.

3. Results

A total of 50 Operon grade RAPD primers were screened with laboratory reared *Aedes aegypti* mosquito. Among these 50 primers only seven primers OPAK 12, OPAS 11, OPAS 12, OPAS 13, OPAS 15, OPM 12 and OPM 13 were able to generate clear, consistent and discrete banding pattern with the laboratory mosquito DNA. All these seven primers revealed the percent polymorphism ranging from 94.7-100%. OPAK 12 primer amplified 32 bands with no monomorphic band and this primer showed 100% polymorphism (Table 1).

From the above seven primers, one primer OPAK 12 was selected for study of polymorphism in the field collected *Ae. aegypti* populations, this primer was selected on the basis of the clear, concrete and scorable fingerprint obtained by it. The DNA isolated from field populations were applied for RAPD-PCR using OPAK 12 primer and their genetic fingerprint was obtained on agarose gel (Fig. 2). For inter population genetic variation studies, POPGENE 1.31 version software was used. RAPD banding information obtained from field population genome fingerprint was coded as a matrix of 1's (band present) and 0's (band absent) and used in computer programs (written in note pad) written specifically for use with data generated by RAPD-PCR. The program RAPPIDIST was used to calculate Nei's genetic distances [20] (Table 3) applying Lynch & Milligan's (1994) [21] correction, between the six field population and the laboratory reared *Aedes aegypti* population.

For the statistical analysis 17 bands or loci representing the each of the seven population using OPAK 12 primer were taken, the average genetic distances between the populations was calculated. The average genetic distance between the populations was 0.5624 ranging from 0.1252-1.1015 (Table 3). Phylogenetic tree was generated with the help of POPGENE 1.31 version (Fig. 3). The cluster analysis technique of unweighed pair- group method of arithmetic averages (UPGMA) and dice coefficient distance matrix method with appropriate bootstrap value was used to develop the trees (Fig. 3). The consensus tree thus developed shows two clusters which are again branched as per their geographical distances.

4. Discussion

Fingerprinting genomes with arbitary primers is a versatile method for detecting genetic polymorphisms useful for population biology [22]. Most RAPD bands are dominant traits and their presence reflects priming sites flanking a segment of

DNA suitable for amplification [13, 23]. RAPD-PCR generates a fingerprint using arbitary selected primers and conditions of reduced stringency so the primer will initiate synthesis on DNA even when the match with the template is imperfect.

The RAPD-PCR method has proved to be valuable in identifying a large number of genetic polymorphisms in several insect species refractory to or little used for classical genetic analysis [24]. Similar work has been used by Sharma *et al*., 2009 [14] for genetic fingerprinting and population variations [25]. They have also proven useful in detection and identification of cryptic species [23, 26-29].

In case of mosquitoes [27] several random primers have been used to distinguish between forest form and domestic form and also for other strains. In an attempt to separate members of the *Anopheles gambiae* complex, some random primers were screened to select profiles identifying each species. Field samples were developed in another species complex (*Anopheles albitarsis* complex), subgenus: Nyssorhipchus in South America [30]. Here, four species including one undescribed species were also identifiable by some primers.

Results presented in our study show that RAPD-PCR profile obtained by using primer OPAK 12 for *Aedes aegypti* population collected from various different locations is capable to demonstrate the polymorphism within this species. The observed genetic polymorphism in the populations may affect the vector competence as the susceptibility of vector to transmit disease has genetic basis. The results of this study using RAPD markers revealed little to high genetic variations in *Ae. aegypti* with reference to their geographic distances between them. A number of similar studies have shown a considerable variation in the mean heterozygosity among the natural vector populations ranging from no demonstrable variations in *Cx. pipiens pipiens* [31] to high level in *Anopheles minimus* [32]. Similar observations for genetic variations based on distance and geographic conditions were reported in *Aedes aegypti* populations at different locations by RAPD markers in Brazil [33] and Argentina [34] and by allozymes in French Polynesia [35]. Besides various extrinsic environmental factors frequent chemical insecticide pressure may also lead the genetic variation among the mosquito populations. In Brazil such relationship were observed among the *Aedes aegypti* populations [33]. The relationship of genetic variation of mosquitoes with its spatial and temporal difference in disease transmission is not fully understood. The possibility of variations in mosquito vectors for transmission of pathogen due to genetic differences among populations can not be ruled out.

5. Conclusion

We propose that our results on RAPD profiles provide evidence that there are enough and significant variations in the genomes of field collected populations from distant locations and the genetic pattern obtained in its various forms appears to be a major differentiating and orienting force of molecular changes in DNA across different populations.

6. Acknowledgement

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7. Conflict Of Interest

All the authors of this research article hereby declare that no conflicts of interest exist among each other's.

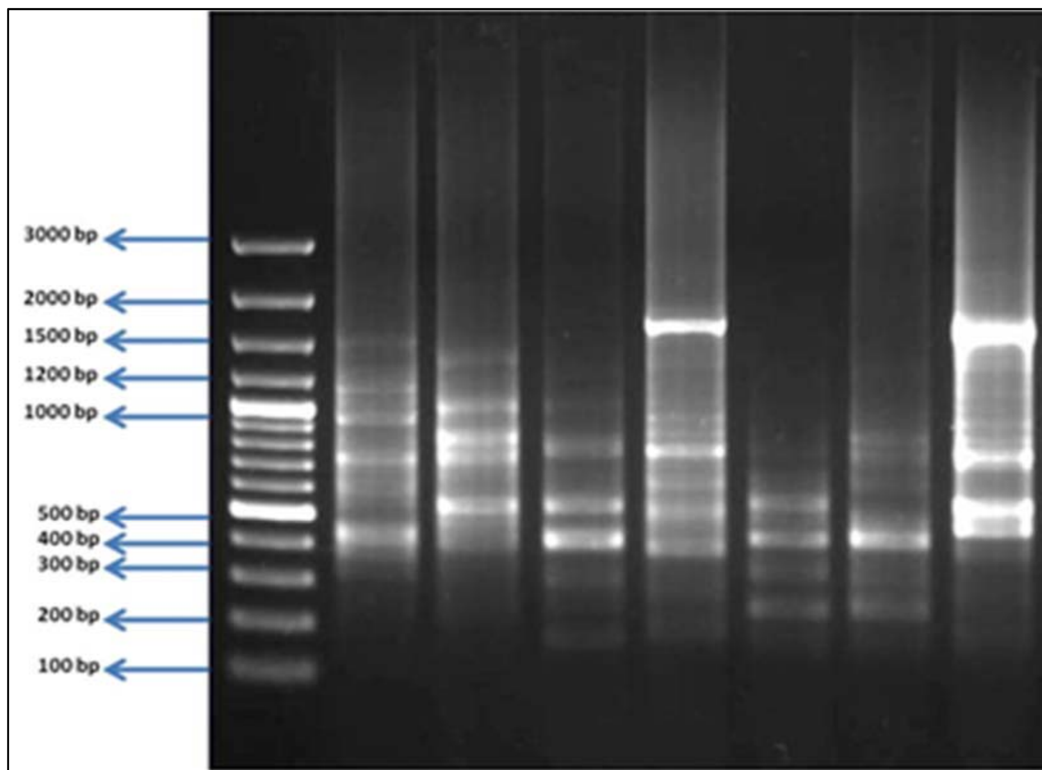

Fig 2: RAPD amplification profile of field population of *Aedes aegypti* using primer OPAK 12.

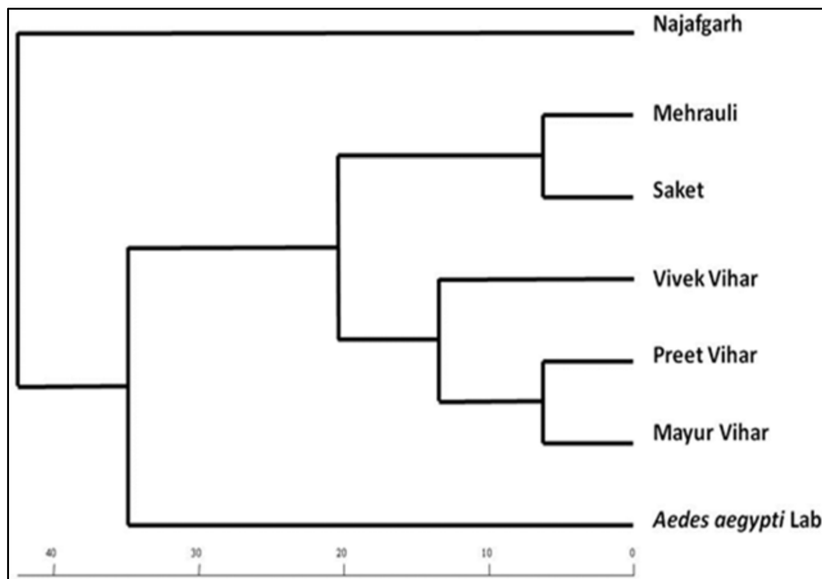

Fig 3: Phylogenetic tree representing Field populations of *Aedes aegypti*

Table 3: Distance matrix and Homology among the field populations

Genetic Identity and Genetic distance

Pop ID	Najafgarh	Mehrauli	Saket	Vivek Vihar	Preet Vihar	Mayur Vihar	Aedes_lab
1	****	0.4706	0.4706	0.3529	0.4706	0.4706	0.3529
2	0.7538	****	0.8824	0.6471	0.6471	0.6471	0.4118
3	0.7538	0.1252	****	0.6471	0.7647	0.6471	0.5294
4	1.0415	0.4353	0.4353	****	0.7647	0.7647	0.6471
5	0.7538	0.4353	0.2683	0.2683	****	0.8824	0.5294
6	0.7538	0.4353	0.4353	0.2683	0.1252	****	0.4118
7	1.0415	0.8873	0.6360	0.4353	0.6360	0.8873	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

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