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Genetic Variation in Aedes Aegypti Mosquito Populations Along the West Cost of India and Their Susceptibility to Insecticides and Dengue Virus

KEYWORDS: Aedes aegypti, Genetic variation, Dengue virus, Vector competence, Insecticide, Enzymes, Resistance.

Mourya DT
Microbial Containment Complex, National Institute of Virology, Sus Road, Pashan, Pune 411 021, India.

Kumar R
Microbial Containment Complex, National Institute of Virology, Sus Road, Pashan, Pune 411 021, India.

Barde PV
Microbial Containment Complex, National Institute of Virology, Sus Road, Pashan, Pune 411 021, India.

Gokhale MD
Microbial Containment Complex, National Institute of Virology, Sus Road, Pashan, Pune 411 021, India.

Yadav PD
Microbial Containment Complex, National Institute of Virology, Sus Road, Pashan, Pune 411 021, India.

ABSTRACT *Genetic characteristics of twelve Aedes aegypti mosquito populations collected from differnt geographical areas along the westcoast of India was studied using bioassays, biochemical assays and dengue-2 virus susceptibility. Polymorphism in the populations was also studied using random amplification of polymorphic DNA (RAPD) polymerase chain reaction (PCR), amplification of mitochondrial DNA fragments from cytochrome-b gene and isoenzyme profile. Resistance to DDT and Malathion was detected in majority of strains while none developed deltamethrin resistance. Dengue-2 virus infection rates among the populations ranged between 13.8% and 47.2%. Isoenzyme profile and RAPD-PCR studies demonstrated a higher genetic heterozygosity among the populations at 19 presumptive loci. Phylogenetic analysis based on cytochrome-b gene identified three well-supported clades. The study neither showed any association of genetic composition with insecticide resistance or dengue-2 virus susceptibility nor any association could be demonstrated for insecticide susceptibility staus and dengue virus susceptibility.*

INTRODUCTION

Genetic delineation of vector populations according to geographic regions facilitates assessment of the potential threat of invading populations (Tabachnick et al 1982). Population genetic approaches provide information on genetic connectedness among populations distributed over a large area in a geographic region (Tabachnick & Powell 1979; Aposto et al 1996; Gorrochoteigui-Escalante et al 2000;Ravel et al Ravel et al 2002; Yan et al 1999). The amount of genetic exchange between groups provides estimates of gene flow within mosquito populations and indicates the type of population movement. Knowledge of patterns of gene flow among populations associated with estimations of their abilities to harbor and transmit dengue viruses is necessary for the development of effective control strategies. Recent population genetic studies on Aedes aegypti suggested its possible application to understand the impact of mosquito genetic differentiation on the epidemiological pattern of dengue (Failloux et al 2002). Surveys conducted along the western coast of India recently have shown wide dispersal and distribution of Ae aegypti. Attempts were therefore made to understand the probable association of genetic composition of differnt mosquito populations along the westcost to insecticide and dengue-2 virus susceptibility.

MATERIALS AND METHODS

Study area: The geographical areas selected for the study were ports, sub-coastal towns/cities and towns/villages on the highways leading to ports etc extending from Madavi in Gujarat to Cochin (Fig 1). Ae aegypti larvae were collected from natural breeding sites (Table 1) and transported to the National Institute of Virology, Pune where they were reared to adults. Either the F0 generation adults or F1, F2 generation adults were used for the study.

Figure I: Map of west coast showing sampling sites.

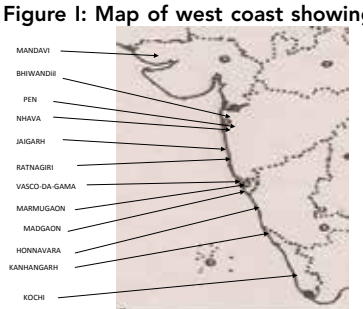

Table I: Geographic location of Aedes aegypti collection

Locality	Geo-coordinates N° E°	Nature of Town/village	Locality (Type of Settlement)	Collection site
Pune	———	City	Laboratory	Laboratory
Ratnagiri (R)	17°-01.7'-73°-20.75'	Intermediate Port	MIDC depot (Non Residential)	Tires
Jaigadh (R)	17-29.19, 73-21.83	Major Port	(Residential)	Plastic Barrel
Pen (R)	18-47.25, 73-08.54	Highway Junction	MIDC (Non Residential)	Tires
Nhava (R)	18-57.69, 72-59.16	Minor Port	(Residential)	Metal container
Bhiwandi (U)	19-28.28, 73-06.06	Industrial Town	Sidhi Nagar (Residential)	Plastic Barrel
Marmugao (R)	15-26.72, 73-54.05	Major Port	Bapanaga (Residential)	Plastic Barrel
Vasco da gama (U)	———	Railway Termitus	Fish Dungal (Residential)	Earthenware
Madgaon (U)	15-28.09, 73-59.87	Railway Junction	El-Mesco (Residential)	Plastic tub
Kanhangad (R)	12-07.03, 75-09.79	Highway township	Non residential	Tyre dump
Honnavara (R)	14-20.06, 74-30.56	Highway township	Residential	Domestic setting
Kochi (U)	10-08.67, 76-27.66	Thripunithura village	Non-residential	Plastic sheet
Madavi (U)	22-52.5, 69-24.85	Small port	Marsh, residential	Discarded tyres and tin cans

Insecticide bioassays: Bioassays were carried out with DDT

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(4%), Malathion (0.8%), Deltramethrin (0.05%) and Propoxure (0.1%). Insecticide impregnated papers were prepared locally as described by Busvine & Nash (1953) and the tests were performed as per WHO (1981) protocol. In brief, batches of adult female mosquitoes (n=2025) were exposed to insecticide impregnated papers, maintained on 10% glucose solution at 28±2oC with 8090% humidity and percent mortality count was taken at 24hr post exposure.

Biochemical assays:
Enzyme assays were performed on female mosquitoes. The mosquitoes were homogenized individually in distilled water with the help of plastic pestle (Kontes) in microfuge tubes using a battery operated hand held homogenizer, centrifuged at 10,000 RPM for 10 min, collected supernatanats and protein content was estimated as described by Lowry et al (1951) from 40µl of individual homogenates. A standard protein curve was prepared using bovine serum albumin (BSA) fraction V. The methods followed for esterase (Est A & B), Acetylcholinesterase and Glutathione stransferase were as below:

Esterase (EST): Assays were performed following the method described by Hemingway et al11 with 2 x 20µl aliquots of individual supernatants using 1 and 2-naphthyl acetate as substrates. After incubation for 10 min at 37oC, the reaction was stopped by the addition of fast blue RR in 5% sodium dodecyl sulphate solution and the end-point absorbency was measured at 490 nm in an UVmax microplate reader. Results were converted to absolute units by analysis against standard curves for 1 and 2-naphthol and adjustment for protein concentration.

Acetylcholinesterase (AChE): Normal and propoxur-inhibited AChE activity was determined as described by ffrench-Constant and Bonning (1989) in 2x30µl aliquots of individual supernatants. In these assays, final concentration of Propoxur was 0.2mM as higher concentrations totally inhibited AChE activity in Ae aegypti. (Hemingway et al 1986; Mourya et al 1993; 1994).

Glutathione s-transferase (GST): GST activity was determined as described by Habig et al (1974). In brief, 80µl of a mixture of freshly prepared reduced glutathione (0.1M) in phosphate buffer (pH 6.5) and 0.48µl of 3,4,-chloro-dinitrobenzene (CDNB) in methanol (15 nMol) was added to 25µl of individaual supernatant. Rate reactions were measured for 5 min at 37oC. Enzyme activity was calculated with the extinction co-efficient for CDNB I>E= 9.5/mM/cm and the protein concentration of the sample. Enzyme activity was expressed as activity/min/mg protein.

Vector susceptibility:
Dengue-2 virus (DENV-2) (strain#9012384), isolated from a dengue hemorrhagic fever patient from Jammu & Kashmir at the 8th mouse passage level was used in the study. A stock of blood virus mixture was prepared by mixing virus suspension and defibrinated chicken (white leghorn fowl) blood at 1:1 ratio, distributed in different feeding cups and allowed the different mosquito populations to feed on infected blood through an artificial membrane (American National Can, Greenwich, USA) as described by Harada et al (1996). Dengue virus in brain of infected mosquitoes was detected on 14th day post infection using indirect immunofluorescence antibody technique.

Polymorphism in Ae aegypti mosquito populations:
Enzyme based polymorphism in mosquito populations: Isoenzyme loci were assayed from soluble proteins extracted from each mosquito sample and enzyme polymorphism was estimated on polyacrylamide gel electrophoresis as described by Munstermann (1979). Six enzymes were studied, viz., glucose-6-phosphate dehydrogenase (G6PD), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), Octanol dehydrogenase (ODH) and EST on different Ae aegypti populations (Table II). Laboratory strain of Ae aegypti was used as control. For field-collected samples, alleles were numbered according to their mobility relative to that of the most common allele obtained at each locus for the control strains. Genetic analysis was performed with BIOSIS-2 (version 1.7) software (University of Illinois). Linkage disequilibrium between pairs of loci was tested for each population using Fisher's test on contingency tables. Genetic differentiation between populations was determined using Fisher's exact test (1970) for each locus.

Table II: Cellular isoenzymes with their attendant buffer and stains.

Cellular isozymes	Gel buffer Molarity	Composition of staining solution
LDH	TC 0.045	50mM Lithium lactate, 0.754 mM NAD, 0.568 mM NBT in 0.1M Tris-HCl (pH 8.5), PMS 10mg.
MDH	TC 0.045	10mM Malic acid, 0.754 mM NAD, 0.568 mM NBT in 0.1M Tris-HCl (pH 8.5). PMS 10mg
G6PD	TC 0.045	1.85 mM Glucose-6-phosphate, 0.673 mM NADP, 1.2 mM $MgCl_2$, 0.568 mM NBT in 0.1M Tris-HCl (pH 8.0) PMS 10mg
IDH	TC 0.045	3 mM DL Isocitric acid, 1.2 mM $MgCl_2$, 0.673 mM NADP, 0.568 mM NBT in Tris-HCl 0.1M, (pH 8.5) PMS 10mg
Est A	TBE 0.1	0.7 mM α-Naphthyl acetate, Fast Blue B, 2.6 mM in Tris-HCl (pH 8.5) (α-naphthyl acetate is first dissolved in acetone and then added to staining solution).
Est B	TBE 0.1	0.7 mM β-Naphthyl acetate, Fast Blue B, 2.6 mM in Tris-HCl (pH 8.5) (β-naphthyl acetate is first dissolved in acetone and then added to staining solution).

Nucleic acid based polymorphism:
Random amplification of polymorphic DNA (RAPD) based population genetics was used to study polymorphism among different populations of Ae aegypti. DNA from individual mosquitoes was isolated as described by Black and DuTeau (1997) and amplified as per Ocampo and Wesson (2004) method. Three primers were used viz., A02: 5'-TGC CGA GCT G-3', B03: 5'-CAT CCC CCT G-3' and B13: 5'-TTC CCC CGC T-3'. Appropriate negative controls were included in all runs which constituted all components as the test samples and 5µl of DEPC water in place of the DNA template. RAPD product was evaluated on 1.2% agarose gel in Tris-acetate EDTA buffer (0.04 M Tris-acetate, 1mM EDTA), stained with ethidium bromide and visualized on ultraviolet transilluminator at 302nm. The molecular weights of the scored bands were confirmed using molecular weight standards and comparability between experiments was assured by analyzing products from different PCRs on the same gels. Gels of the RAPD products were scored for the presence or absence of chosen bands (loci) and all data were imported into the FORTRAN programs BIOSYS-2 to estimate the allelic frequencies.

Mitochondrial cytochrome-b gene fragment: Polymorphism analysis of variation in a 500bp region of cytocrome-b gene from mitochondrial DNA (mtDNA) was performed following the protocol described by Lyman et al (1999). The two primers used included CYT BF: 5'GGA CAA ATA TCA TTT TGA GGA GCA ACA G3' and CYT BR: 5'ATT ACT CCT CCT AGC TTA TTA GGA ATT G3'. Amplified DNA was evaluated on 1.2% agarose gel in Tris-acetate EDTA buffer (0.04 M Tris-acetate, 1mM EDTA), stained with ethidium bromide and visualized on ultraviolet transilluminator at 302nm. Molecular weights of the scored bands were confirmed using molecular weight standards. Bands of interest were recovered using StrataPrep DNA Gel Extraction Kit, according to the manufacturer's protocol. Direct sequencing of the amplified product

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was performed using an ABI 3100 automated DNA sequencer using Big Dye terminator kit (Applied Biosystems, Inc., Foster City, CA). The sequences were aligned on line using ClustalW at ebi site for generating trees. MEGA software and Juke Cantor and Kimura 2 parameter algorithm and neighbor joining tree method were used for tree building.

RESULTS

Insecticide Susceptibility: Adult bioassays showed all the Ae aegypti populations except Mandavi were resistant to DDT. Similarly, all the populations except Mandavi and Ratnagiri were resistant to Malathion. Propoxur resistance was noticed only in six populations. However, all the populations were susceptible to Deltamethrin (Table III). Larval bioassays also showed similar results (data not shown). When biochemical assays were performed, 8.3% to 41.7% of populations from Nhava, Bhiwandi, Marmugao, Pen, Ratnagiri, Vasco-da-gamma and Madgaon showed elevated GST enzyme levels; 8.3% to 92% of populations from Jaigadh, Nhava, Bhiwandi, Vasco-da-gamma, Kanhangad, Honnavara, Madgaon and Kochi showed elevated EST enzyme levels (Table IV). High esterase activity was found in Jaigadh, Nhava, Bhiwandi, Marmugao, Vasco-da-gamma, Kanhangad, Honnavara, Madgaon and Kochi as compared to laboratory colony and the other three populations (Table IV).

Table III: Adult bioassay studies on Ae aegypti strains from western coast of India.

Locality	Insecticides (% mortality at 24 hour post exposure)			
	DDT (4%)	Malathion (0.8%)	Deltamethrin (0.05)	Propoxur (0.1)
Lab Colony	100	100	100	100
Ratnagiri	52	100	100	100
Jaigadh	90.66	90.66	100	94.66
Pen	74.66	80	100	84
Nhava	74.66	84	100	85.33
Bhiwandi	85.33	80	100	100
Marmugao	70.66	85.33	100	100
Vasco-da-gama	74.66	80	100	100
Madgaon	85.33	70.66	100	100
Kanhangad	74.66	60	100	85.33
Honnavara	96	65.33	100	85
Kochi	60	70.66	100	76
Mandavi	100	100	100	100

Table IV: Characteristic of Aedes aegypti Populations for Biochemical assays

Town/ Village	GST			Est-A			Est-B		
	Avg. activity*	SD	% Resistant**	Avg. activity***	SD	% Resistant	Avg. activity***	SD	% Resistant
Lab Colony	300	120	0.0	3.6	0.9	0.0	2.9	0.9	0.0
Ratnagiri	325.4	142.6	41.7	2.8	1.1	0.0	2.9	1.0	0.0
Jaigadh	267.3	55.4	0.0	4.0	2.5	8.3	4.3	1.4	33.3
Pen	310.3	57.7	8.3	3.0	0.7	0.0	2.9	0.6	0.0
Nhava	379.7	65.5	41.7	3.6	0.9	8.3	3.9	1.3	25.0
Bhiwandi	335.9	65.0	41.7	3.6	0.9	8.3	3.8	0.7	8.3
Marmugao	321.2	126.5	16.7	3.4	0.9	0.0	3.3	1.3	8.3
Vasco-da-gama	318.7	69.0	8.3	3.6	1.5	16.7	3.6	1.7	25.0
Madgaon	355.6	60.5	25.0	3.9	0.6	8.3	7.5	4.6	58.3
Kanhangad	ND	ND	ND	5.5	2.3	83.0	6.1	1.9	75.0
Honnavara	ND	ND	ND	4.7	2.0	50.0	5.2	1.0	58.0
Kochi	ND	ND	ND	5.7	2.3	92.0	5.5	2.1	75.0
Mandavi	245	97	0.0	2.2	0.8	0.0	2.2	0.7	0.0

* = Enzyme activity/min/mg protein
** = Based on Biochemical analysis
***= nMol activity/min/mg protein
ND = Not Done

Susceptibility to DEN-2 virus: All the populaitons were susceptible to DEN-2 and infection rates ranged between 13.8% and 47.2% (Table V). Jaigadh, Ratnagiri, Kanhangad and Kochi populations showed high infection rates. No correlation of virus susceptibility to geographical areas was observed. Though certain populations were found highly susceptible to DENV-2 but a correlation with insecticide susceptibility/resistance could not be established.

Table V: Susceptibility of different populations of Ae aegypti to DENV-2

Locality	Number positive/number tested	% Positive	RR	Z Value
Lab Colony	5/36	13.8		
Ratnagiri	14/36	38.8	2.80	1.80
Jaigadh	21/48	43.75	3.15	**2.31***
Pen	6/24	25.0	1.80	0.76
Nhava	14/48	29.10	2.10	1.24
Bhiwandi	9/36	25.0	1.91	0.76
Marmugao	11/36	30.5	2.20	1.24
Vasco-da-gama	15/36	41.6	3.00	**2.00***
Madgao	9/36	29.1	1.80	0.76
Kanhangad	17/36	47.2	3.40	**2.36***
Honnavara	7/36	19.4	1.40	0.45
Kochi	13/36	36.1	2.60	1.66
Mandavi	5/36	13.8	1.00	0.00

RR=Relative Risk
*=Significant given at $p<0.05$
Titre of virus: 3.1log/0.02ml MID_{50} in infant mice.

Polymorphism in mosquito populations:

Genetic Differentiation: The survey was conducted during a span of 2-3 years and hence no attempts were made to calculate gene flow between the populations. Figure I shows the Ae aegypti populations used for genetic variability analysis. Isoenzyme based polymorphism showed loci were polymorphic in most samples (major allele frequency, <0.95). Genotypic association between pairs of loci was analyzed for each sample. Hardy-Weinberg equilibrium was tested for each locus in each population using the probability test (Table VI). The level of genetic differentiation is displayed in Table VII. The population differentiation is found in all loci that contributed to the divergence. A different pattern was obtained displaying a high level of genetic differentiation except between Daman and Ratnagiri and between Madgao and Vasco-da-gamma (Figure II). Considering each locus separately, a high level of differentiation was found at GPD and EST loci. Highest percent loci were found polymorphic in Honnavar followed by Pen, Jaigadh, Nhava, Madgaon and Mandavi (Table VI).

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Table VI: Genetic variability in different Aedes aegypti populations to six enzymes.

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Direct-count	Hdy/Wbg expected**
1. Marmugao	14.0	1.9	42.9	0.204	0.173
	(0.0)	(0.3)		(.080)	(.066)
2. Lab Colony	14.0	2.4	71.4	0.276	0.238
	(0.0)	(0.2)		(.067)	(.053)
3. Bhiwandi	14.0	2.1	71.4	0.286	0.243
	(0.0)	(0.3)		(.073)	(.060)
7. Pen	13.0	2.3	85.7	0.374	0.312
	(0.0)	(0.2)		(.070)	(.053)
8. Jaigadh	14.0	2.4	85.7	0.367	0.307
	(0.0)	(0.3)		(.069)	(.056)
9. Nhava	14.0	2.3	85.7	0.265	0.231
	(0.0)	(0.3)		(.073)	(.059)
10. Ratnagiri	14.0	2.0	57.1	0.184	0.162
	(0.0)	(0.3)		(.068)	(.056)
11. Madgao	14.0	2.3	85.7	0.296	0.255
	(0.0)	(0.3)		(.079)	(.062)
12. Mandavi	14.0	2.3	85.7	0.286	0.244
	(0.0)	(0.4)		(.081)	(.064)
13. Vasco	14	2.3	57.1	0.235	0.202
	(0.0)	(0.3)		(.081)	(.065)
14. Honnaver	15.0	2.6	100.0	0.295	0.257
	(0.0)	(0.3)		(.066)	(.051)
15. Kochi	15.0	2.3	57.1	0.286	0.239
	(0.0)	(0.4)		(.095)	(.078)
16. Kanhangad	15.0	2.4	71.4	0.324	0.270
	(0.0)	(0.4)		(.094)	(.076)

* Locus considered polymorphic when frequency of the most common allele did not exceed 0.95
** Unbiased estimate (Nei,1978)

Table VII: Allele frequencies and genetic variability measures in Ae aegypti populations.

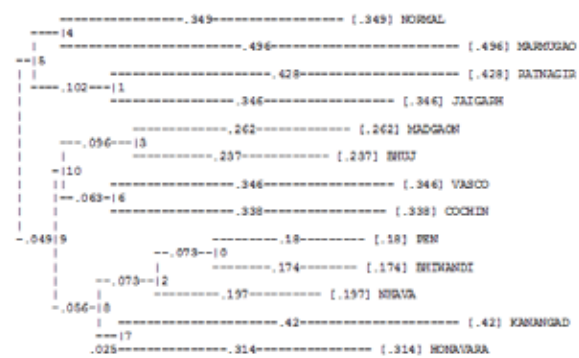
Locus and sample size							
Allele	GPD-1 14	GPD-2 14	IDH-1 14	LDH-1 14	MDH-1 14	Est-1 14	Est-2 14
Population: Marmugao							
A	1.000	786	786	964	964	786	1.000
B	000	214	214	036	036	143	000
C	000	000	000	000	000	071	000
H	000	337	337	069	069	337	000
Population: Lab Colony							
A	786	786	893	964	893	750	964
B	179	179	107	036	107	214	036
C	036	036	000	000	000	036	000
H	349	349	191	069	191	393	069
Population: Bhiwandi							
A	893	786	821	964	750	786	1.000
B	107	214	143	036	143	214	000
C	000	000	036	000	107	000	000
H	191	337	304	069	408	337	000
Population: Pen							
A	885	692	808	846	769	731	962
B	115	192	192	154	231	192	038
C	000	115	000	000	000	077	000
H	204	470	311	290	355	423	074
Population: Jaigadh							
A	750	821	857	786	750	750	1.000
B	214	179	143	179	179	214	000
C	036	000	000	036	071	036	000
H	390	293	245	349	401	390	000
Population: Nhava							
A	929	929	821	893	750	750	1.000
B	071	071	179	071	179	179	000
C	000	000	000	036	071	071	000
H	133	133	293	196	401	401	000
Population: Ratnagiri							
A	893	1.000	893	857	964	750	1.000
B	107	000	071	143	036	214	000
C	000	000	036	000	000	036	000
H	191	000	196	245	069	390	000
Population: Madgao							
A	821	929	679	893	857	786	1.000
B	107	071	179	107	143	107	000
C	071	000	143	000	000	107	000
H	309	133	487	191	245	380	000
Population: Mandavi							
A	821	929	857	786	929	679	1.000
B	179	071	143	179	071	179	000
C	000	000	000	036	000	107	000
D	000	000	000	000	000	036	000
H	293	133	245	349	133	495	000
Population: Vasco							
A	750	929	821	964	964	750	1.000
B	214	071	143	036	036	179	000
C	036	000	036	000	000	071	000
H	390	133	304	069	069	401	000
Population: Honnaver							
A	867	933	867	900	700	767	933
B	133	067	067	100	167	200	067
C	000	000	067	000	100	033	000
D	000	000	000	000	033	000	000
H	231	124	245	180	471	371	124
Population: Kochi							
A	733	800	1.000	767	733	967	1.000
B	167	167	000	167	200	033	000
C	100	033	000	067	067	000	000
H	424	331	000	380	418	066	000
Population: Kanhangad							
A	867	767	1.000	1.000	833	833	767
B	167	133	000	000	100	133	233
C	133	100	000	000	067	033	000
D	033	000	000	000	000	000	000
H	309	384	000	000	291	297	359

RAPD-PCR polymorphisms at 19 presumptive loci were used to examine the genetic structure of the mosquito populations. The average genetic heterozygosity was higher in RAPD than the level detected in isoenzyme analysis as large number of polymorphism was revealed by the former. The laboratory strain had unique heterozygosity suggesting that few mosquitoes established this population (Figure-III).

Figure II: Genetic distance of various Aedes aegypti populations, dendrogram based on Nei's unbiased genetic distance.

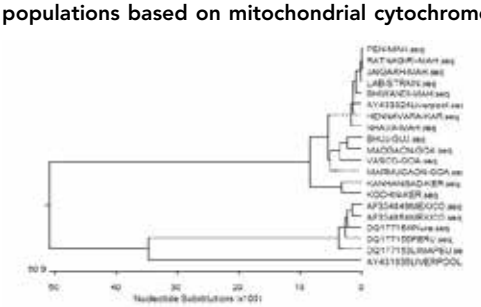
Total length of tree = 1.062. Branch lengths and linkages for unrooted Wagner network, Wagner tree produced by rooting at midpoint of longest path (After optimization).

Figure III: Genetic distance of various Ae aegypti populations using RAPD- PCR.



Phylogenetic study based on the 500bp region of cytochrome-b gene from the mtDNA identified three well-supported clades. This indicated that populations are isolated by distance (Figure IV).

Figure IV: Genetic distance of various Aedes aegypti populations based on mitochondrial cytochrome b gene.



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DISCUSSION

The present study highlights development of insecticide resistance in mosquitoes. Preent study demonstrates the development of resistance to DDT, Malathion and Propoxur by Ae aegypti. Deltamethrin is still found potent as all the populaitons were found susceptible. However, it is interesting to note that Ae aegypti populations from Mandavi is susceptible to all the groups of insecticides used in the study. Similary, Ratnagiri population also found susceptible to all the groups of insecticides except DDT. Among the other populations, Jaigarh population is still susceptibile as resistance was demonstrated by only a small percentage (<10%). Results of the present study is an eye opener as Ae aegypti mosquitoes are developing resistance to the commonly used insecticides and their continued application will lead to development of a totally resistant populaiton. This could create a major concern especially when Ae aegypti borne viruses, viz. dengue and chikungunya are emerging as a national problem. There is an urgent need to find alternative methods for control of Ae aegypti applying integrated methods with community participation.

The populations, which showed resistance to organophosphorus compounds and DDT were due to elevated enzyme levels of esterases and GST respectively. Our earlier studies on different populations of Ae aegypti from Maharashtra and Uttar Pradesh have shown that DDT resistance was GST based (Mourya et al 1993; 1994).

There is a wide variation in the susceptibility of mosquito populations to DENV-2, which was not found associated with any geographical areas. Ratanagiri, Jaigarh and Kanjahad populations were found to be highly susceptible giving 47, 44 and 38% positivity while populaitons such as Madavi etc were found highly refractile (Table V). The differnce in susceptibility among the populations is difficult to understand as dengue transmission is quite complex and various factors influence Ae aegypti and DENV interactions. This could probably influence in differences in infection rates between mosquito populations and virus serotypes. It is becoming evident that intraspecific variation in vector competence is related to several barrier systems that prevent infection of various tissues in mosquitoes. These barrier systems are known to be under genetic control and may be present to various extents within a given mosquito species, thereby affecting the epidemiological pattern of arboviral diseases. However, none of the three methods used to understand genetic structure of populations in the present study showed correlation with either to DENV susceptibility or insecticide susceptibility. These methods only documented the genetic structures of the populations based on different parameters.

Similarly, none of the three methods used for determining polymorphism also showed correlation with geographic origin (coastal, sub-coastal, town and village) of populations. The similarities and dissimilarities observed among various populations with the three methods were also different. Earlier studies on Ae aegypti mosquito populations from French Gayana and Southeast Asia showed highest level of genetic similarity (Failloux et al 2002; Paupy et al 2003; Fouque et al 2001; Smith 1956). However, population genetic studies carried out on Ae aegypti formosus identified three clusters and differences in population dynamics (Fouque et al 2001). Vector competence surveys also showed a drastic difference between these two forms as Ae (aegypti) agypti is more susceptible to DENV-2 than Ae aegypti formosus (Fouque et al 2001). Humans are not only DENV reservoirs and disseminators but also shape the ecology of Ae aegypti, modulate its demography through vector control activities, and increase mosquito efficiency in transmitting dengue viruses.

In the present study, no association of insecticide susceptibility status and DENV-2 susceptibility is found associated with geographical origin of the populations. Analysis by RAPD is based on the following assumptions: i) the genomic regions amplified by RAPD-PCR segregate as dominant alleles; ii) each sub-population is in Hardy-Weinberg equilibrium; iii) alleles in homozygous recessive individuals are identical; and iv) dominant amplified alleles are similar. RAPD method shows polymorphism from genome region irrespective to the coding and non-coding regions hence for understanding polymorphism in the populations it is the method of choice. In the present study, the technique has revealed a large number of polymorphism in the populations. However, more studies are needed either alone or in conjuction with other techniques to demonstrate polymorphism in Ae aegypti populations.

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