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A study on identification of genetic polymorphism of *Aedes aegypti* using Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR).

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Abstract

Amplification of random regions of genomic DNA using 10-base primers in the randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) was used to identify the genetic polymorphisms of *Ae. aegypti* mosquito populations based on genetic variation. Genomic DNA was extracted from individual mosquitoes from 10 populations of *Ae. aegypti* and amplified in PCR reaction using single primers of arbitrary nucleotide sequence. RAPD fragments were produced using 8 different primers. The genetic relatedness of the populations were estimated using Nei and Li pair-wise genetic distances similarity index analysis.

Key word : Genetic Polymorphism of *A. ageyptic*

1. Introduction

Mosquito-borne diseases are responsible for significant human mortality throughout the world. *Aedes aegypti* is the principal vector of dengue viruses, responsible for a viral infection and yellow fever virus that has become a major public health problems concerned in Asia (Failloux *et. al.*, 1995). *Ae. aegypti* is a complex species. Morphological, physiological and behavioral variation is extensive and complexed.

The probable divisions at sub specific level on the ability of the mosquito to transmit specific sero-types of dengue and unveiling the genetic structure of the vector population are considered as important areas worthy of investigation. Novel molecular biological techniques such as RFLP, RADP, AFLP and SSRP provide

ample opportunities for rapid identification of gentic markers and accurate assessment of genetic diversity among plant and animal populations.

Recent advances in genetic research have included the development of an adaptation of the polymerase chain reaction Known as the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) that involves the amplification of random segments of genomic DNA using a single primer of arbitrary nucleotide sequence. The technique is fast, relatively easy to perform, requires minuscule tissue sample, requires no prior sequence information and reveals large numbers of polymorphism. This techniques has been used to detect polymorphism in amplified DNA fragments, termed Random Amplified Polymorphic DNA(RAPD), in a variety of a organisms (Mary *et. al.*, 1992). This paper describes a preliminary study aimed at investigating the genetic diversity by detection of RAPDs in ten populations of *Ae, aegypti* in Sri Lanka.

2. Materials and Methods

Ten populations of adult *Ae. aegypti* which were collected from Borella (B), Chilaw (C), Gangodawila (G), Maharagama (M), Kurunegala (K), Modara (M), Nugegoda (N), Ragama (R), University of Sri Jayawardenapura premise (U) and Wadduwa (W), were used to detect the polymorphism. Genomic DNA was extracted from adult mosquitoes. Individual mosquitoes were crushed and re-suspended in 300µl of lysis buffer (100mM Tris-HCl, pH 8.0, 50mM EDTA, 50mM NaCl, 1% sodium dodecyl sulfate) and 5µl of a 20mg/ml solution of proteinase K. Suspensions were incubated overnight at 50°C and gently extracted twice with buffered phonol, pH 8.0 (Sigma). DNA was precipitated by the addition of 0.2 volumes of 10M ammonium acetate (Sigma) and 2.0 volumes of ethanol (BDH) at room temperature. Solutions were gently mixed and centrifuged for 5min. to pellet the DNA. After removal of the supernatant, the pellets were air-dired for 5min, resuspended in 20µl of sterile distilled water, and stored at 4°C.

Eight primers (OPA02, OPA04, OPA14, OPB10, OPB15, OPC05, OPD15 and OPE07) pre-slected after preliminary screening of 20 Operon primers were used for PAPD-PCRs with all the ten DNA samples. Amplification reactions were performaed according to the protocol reported by Williams and others. Each 25µl reaction contained 10mM Tris-HCl, pH 8.3, 50mM KCl, 2mM $MgCl_2$, 0.001% gelatin, 0.1mM each of dATP, dCTP, dGTP, and dTTP (Pharmacia), 15ng of primer (Operon Technologies), 0.5 units of Taq DNA polymerse (Promega). Reactions were overlaid with 50µl of mineral oil and amplified in the PTC-100 thermal cycler programmed for one cycle at 94°C for 4min., 45 cycle at 94°C for 1min., 36°C for1min., 72°C for 2min., and one cycle at 72°C for 5min.

3. Results

Extracting genomic DNA from an individual mosquito by the method described above gave reproducible results and produced sufficient DNA to amplification reactions. Amplified DNA was analyzed by electroporesis on 1.2% agarose gels. Gels were stained with ethidium bromide and observed under ultraviolet tranilluminator. Bands were scored for presence (1) absence (0) of bands (Table -1).

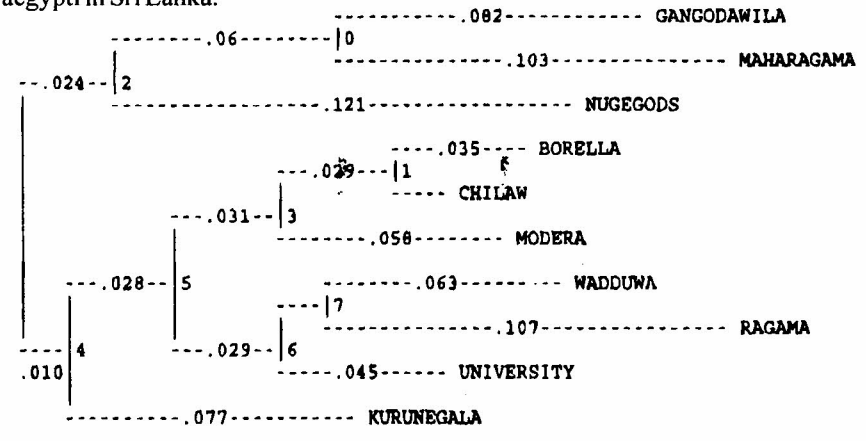
Table :1 RAPD-PCR fragments amplified by OPERON primers.

Name of primer	Fragment number	B	C	D	H	K	M	N	R	U	W
OPA 02	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	0	0	0	1	0	0	0	1
	3	1	1	0	0	0	1	0	0	0	1
	4	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1
OPA 04	1	1	1	0	0	1	1	0	0	1	1
	2	1	1	0	0	1	1	1	1	1	1
	3	1	1	0	0	1	1	1	1	1	1
	4	1	1	0	0	1	1	1	1	1	1
	5	0	1	1	0	1	1	0	1	1	1
	6	1	1	1	1	0	1	1	0	0	0
	7	0	0	0	0	1	0	0	0	0	0
OPA 14	1	1	0	0	0	0	1	0	1	0	0
	2	1	0	0	0	0	0	0	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	0	1	1	1	0	1	1	1
	5	1	1	0	1	1	1	0	1	1	1
	6	0	0	0	0	0	0	1	0	0	0
	7	1	1	1	1	1	1	1	0	1	0
OPA 10	1	1	1	0	0	0	1	1	1	0	0
	2	1	1	0	0	0	0	0	0	0	0
	3	1	1	1	0	1	1	1	1	1	1
	4	1	1	0	0	0	0	1	0	1	0
	5	1	1	1	0	0	1	0	0	1	0
	6	1	1	0	1	0	1	0	0	1	0
	7	0	0	0	0	1	0	0	1	1	1
	8	1	1	1	1	1	1	1	1	1	1
OPA 15	1	1	1	1	0	0	1	1	0	1	1
	2	0	0	0	0	0	0	0	1	0	0
	3	1	1	1	1	1	0	1	0	0	0
	4	1	1	1	1	1	1	0	0	1	1
	5	0	0	0	1	0	1	0	0	0	0
	6	1	1	1	1	1	1	0	1	1	1
	7	1	1	1	1	1	1	1	1	1	1
	8	0	0	0	1	0	0	0	0	0	1
OPA 05	1	1	1	0	0	1	1	1	1	1	1
	2	1	1	0	0	0	0	0	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1
OPA 15	1	1	0	1	0	0	0	0	0	0	0
	2	1	1	1	1	0	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	1	1	0	0	0	1	0	0	1	1
OPA 07	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1
	4	0	0	0	0	0	1	0	0	0	1
	5	0	0	1	1	1	1	0	0	0	0
	6	1	1	1	1	1	1	1	1	1	1
	7	1	1	1	1	1	1	1	0	0	0

The PCR products were electrophoressed in 1.2% Agarose at 40V constant voltage for 4 hour, stained with ethidium bromide and scored by scanning the gels using Photo-print Gel Documentation system and Photo-Capt software.

The fragment profiles for each individual in the study was compared with that of every other individual in a pair-wise fashion and the similarly index between each pair were calculated according to the Nei and Li s' formula. A dendrogram depicting the relationship of the accessions were computed using the software "RAPDistance". (Fig. 1)

Fig. 1 - Dendrogram illustrating the genetic relatedness of 10 populations of Aedes aegypti in Sri Lanka.



The distance matrix revealed a genetic distance between mosquito populations in Sri Lanka ranging from 0.05-0.37.The dendrogram resulted from above matrix reveals the genetic relatedness of mosquioes as an assemblages of two major groups.

4. Discussion

The goal of this study was to develop protocols for the application of RAPD-PCR technology to the identification of genetic polymorphism of mosquito populations. The procedures described for extracting and amplifying mosquito genemic DNA were consistently reproducible. The extracted DNA should be highly purified to answer the RAPD-PCR. DNA samples were not frozen at anytime in the procedure to prevent degradation due to repeated freezing and thawing.

The RAPD-PCRs performed with DNA preparation of *Ae. aegypti* gave an average of 6.4 bands per primer, which is well within expected number of amplification in RAPD studies (Williams *et. al.*, 1990). The size of bands ranged from 0.5-2.5kb. The number of polymorphic bands was very high that out of the 51 fragments

amplified 48 exhibited polymorphisms. Detection of number as large as 48 polymorphisms with the use of just eight primers and ten DNA samples clearly indicated the potential of this technique to develop and screen an excessively large number of DNA markers to tag mosquitoes at sub specific levels with different affinities to harbour specific dengue serotypes.

Reproducibility is an important requirement in RAPD-PCR and therefore considerable care should be taken to avoid possible artifacts in amplification and scoring of such in assays. Temporal repeating of experiment and scoring only reproducible bands in one way of using RAPDs effectively in genome analysis. One of the major problems with the RAPDs technology is the scoring of the resulting gels. The main problem is in deciding on the presence of weakly amplifying fragments that do not occur in all tracks on the gel.

When scoring RAPD gels, it is more important to exclude suspicious data from the data base than to try and increase the total amount of data.

The populations of *Ae. aegypti* show the genetic polymorphism and RAPD-PCR method can be used successfully to identify the polymorphism between mosquito populations. The highest distance is the 0.37 shows between Ragama and Gangodawila populations and Ragama and Maharagama populations. Between Wadduwa and Gangodawila and Wadduwa and Maharagama (0.35) and Wadduwa and Nugegoda (0.33) also show the high genetic distance. Gangodawila, Maharagama and Nugegoda population show the clear cluster of difference than other populations.

The overall results of this investigation very clearly indicated the enormous potentials of the RAPD-PCR technique for ready detection of DNA polymorphisms in *Aedes aegypti* for charactization of mosquitoes strains having specific affinities to harbour particular serotypes of dengue vires and for identification of individual mosquito and populations at sub specific level, which will be very valuable of epidemiological investigations of the vector.

5. Acknowlegdements

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