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Genome fingerprinting of two populations of *Aedes (Stegomyia) albopictus* Skuse (Asian Tiger) using random primers (Diptera: Culicidae)

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Abstract: The present paper deals with RAPD-PCR based genomic characterization of two allopatric populations of *Aedes (Stegomyia) albopictus* Skuse which is a major vector of dengue fever and a source of many other pathogenic infections in man. For the present purpose one population was collected from village Dhunater, Distt. Hamirpur in Himachal Pradesh (pop.A) while the other was procured from Panjab University campus (pop.B). The genomic DNA from the legs of individual specimens was amplified by using three random primers *viz*: Primer I- 5'-TTTGCCCGGA-3', Primer II- 5'-GTCCCGACGA-3' and Primer III- 5'-CAGGCCCTTC-3'. The amplification of the DNA of pop.A with primer I, produced a total of 7 bands ranging from 230-880 bp while 3 bands ranging from 450-820 bp were produced from pop. B. Similarly, with primer II a total of 8 bands were produced from pop. A, which ranged from 220-800 bp while 9 were produced from the DNA of the individuals of pop. B, which ranged from 200-900 bp. With primer III, a total of 15 bands were produced from pop. A, with a base pair composition varying from 210-1031 bp while 5 were produced from pop. B with a range of 210-370 bp. From the hierarichial cluster sharing analysis of bands, primers I and II were found to ideal for the differentiation of the individuals at the population level studies of this species of considerable epidemiological significance.

Keywords: *Ae. Albopictus*, Allopatric populations, Genome fingerprinting, RAPD-PCR.

INTRODUCTION

A gene has been identified as a dynamic component of genetic material whereby the study of the gene, nucleic acids and chromosomes has become an integral part of the subject of molecular biology. As a consequence of these developments, the understanding of the genetic structure and the plausible explanations for the various phenomena of genetics operating in the natural populations cannot be understood without probing the variations at the level of nucleotides. The studies carried out so far on comparative cytogenetics of species has shown that there is no single population which represent the entire gene pool of a species, therefore the parameters of genomic studies have been under going changes with more sophisticated experimental techniques. In the last few years, the techniques of molecular biology have become the more acceptable means of species differentiation for supplementing the data of morphological, biochemical, cytogenetical and physiological researches of taxonomic value (Carlson and Service, 1980; Rao and Rai, 1988; Mathiopoulos *et al.*, 1995; Munstermann, 1995; Chaudhry, 1999; Chaudhry *et al.*, 2006). In mosquitoes there is a unique phenomenon of species complexes in which a single species may exist in the form of two or more sibling species or intra-specific variants with doubtful or overlapping taxonomic characters (Rudnick and Chan, 1965; Craven *et al*., 1988; Sambrook *et al.*, 1989; Welsh and McClelland, 1990; Kambhampati *et al.*, 1992; Kumar and Rai, 1993; Knudson *et al*., 1996; Sucharit and Komalamisra, 1997; Shouche and Patole, 2000). In this context, the Polymerase Chain Reaction (PCR) based techniques of DNA diagnostics have become quite useful in the detection of genetic variations at the nucleotide level of individual member of a species for which the polymorphism in DNA acts as a source of RAPD markers (Welsh and McClelland, 1990; Mitchell, 1995; Black and Du Teau, 1996; Mutebi *et al.*, 1997). In reference this, the present paper deals with RAPD-PCR based genomic characterization of two allopatric populations of *Aedes (Stegomyia) albopictus* Skuse which is a major vector of dengue and a source of many other pathogenic infections in man (Rai, 1991). With a dubious distinction as 'Asian Tiger' this particular species has remained a native of South- east Asia. However, in a brief span of about 30 years it invaded several parts of the western world including Brazil and

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USA. This remarkable adaptability of this vector to newer geographic regions has created serious epidemiological implications (Conn *et al.*, 1993; Narang *et al.*, 1993; Favia *et al.*, 1994; Manguin *et al.*, 2002). In addition to the analysis of RAPD based composition and the identification of ideal primer markers, the nearest-neighbour joining (NJ) analysis was also employed to develop a "hierarchical cluster analysis".

MATERIALS AND METHODS

Out of the two populations used in the present research work, one (pop.A) was collected from village Dhunater, Distt. Hamirpur Himachal Pradesh (Latitude: 76º 18' - 76º 44' E; Longitude: 31º 25' to 31º 52' N) while the other (pop.B) was procured from Panjab University campus, Chandigarh (Latitude: 30°43'N; Longitude: 76°47'E). The genomic DNA was extracted from the legs of individual specimen by following a standard phenol-chloroform extraction method with minor modifications (Ausubel *et al.*, 1999; Chaudhry, 1999; Kaura *et al.*, 2009). The purified and quantified samples of DNA were amplified by using three random primers *viz*: Primer I- 5'-TTTGCCCGGA-3', Primer II- 5'-GTCCCGACGA-3' and Primer III- 5'-CAGGCCCTTC-3'. In addition to the master mix a negative control containing all the contents of this reaction mixture, except the genomic DNA, was also run for ruling out the experimental errors. The amplified DNA products were subjected to 2% horizontal agarose gel electrophoresis for the separation of variable size DNA fragments which appeared in various lanes and the details of base pair length of each band and their electrophoretic mobility were determined by making use of the standard curve plotted for gene ruler (lane-M). For this the electrophoretic mobility of each fragment was calculated by using the following formula (Sambrook *et al.*, 1989)

Relative mobility = Distance traveled by the DNA / Distance traveled by the dye BPB

In addition to the genomic analysis presented here, the nearest-neighbour joining (NJ) analysis was also employed to develop a "hierarchical cluster analysis". This was based on the proximity, intensity, thickness and relative mobility of all the bands. Depending upon the presence or absence of DNA bands available from the individuals of two different populations of *Ae. albopictus,* the data was scored as 1 for the presence of the band and 0 for its absence. From these values the band sharing coefficient between individuals was calculated by employing the formula $D = 2N_{ab}/(N_a+N_b)$ which is based on the principle that increase in the value of band sharing coefficient increases the genetic relatedness among the members of different populations of the same species. Here, D denotes band sharing coefficient, N_{ab} - number of bands shared by individuals a and b, Na - number of bands obtained from a and N_b -number of bands obtained from b.

RESULTS AND DISCUSSION

The amplification of the DNA of pop. A with primer I, produced a total of 7 bands ranging from 230-880 bp while 3 bands of 450-820 bp were produced from pop. B (Figs.1,4). Similarly, with primer II a total of 8 bands were produced from pop. A, which ranged from 220-800 bp while 9 were produced from the DNA of the individuals of pop. B, where they varied from 200-900 bp (Figs. 2,5). With primer III, a total of 15 such bands were generated from pop. A, with a base pair composition varying from 210-1031 while 5 bands wereproduced from pop. B with a range of 210-370 bp (Figs. 3, 6).

Based on the data of presence/absence of bands and those unique to each population, it was found that primers I and II were ideal for the genomic differentiation of the individuals of the two populations as they produced maximum number of population specific bands (Tables 1, 2).

In addition to the identification of present, absent and the unique bands, the dendrograms of genetic relatedness between the two allopatric populations of *Ae. albopictus* were also constructed for which the homologies and differences in the DNA profiles were taken into consideration. This data was subjected to Nearest Neighbour Joining (NJ) analysis of single linkage method meant for closely placed bands. In the present studies, this was useful in selecting a specific

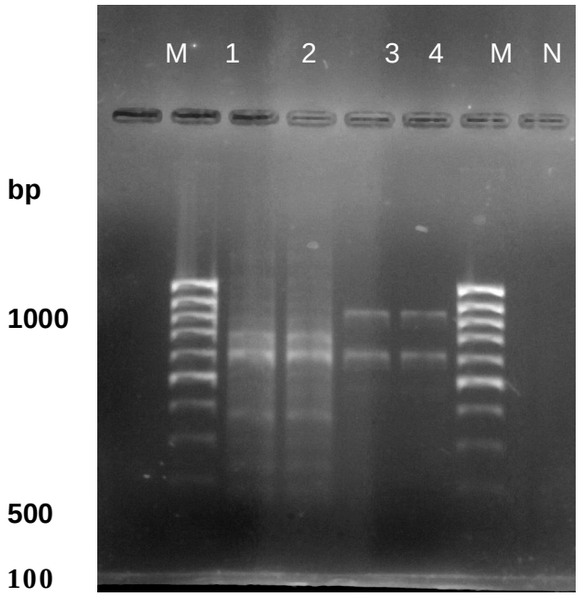

Fig. 1. *DNA bands generated with primer I. Lane M-gene ruler, N-Negative control, 1- DNA of pop. A, 2- DNA of pop. A, 3-DNA of pop. B, 4-DNA of pop. B of Ae. albopictuts.*

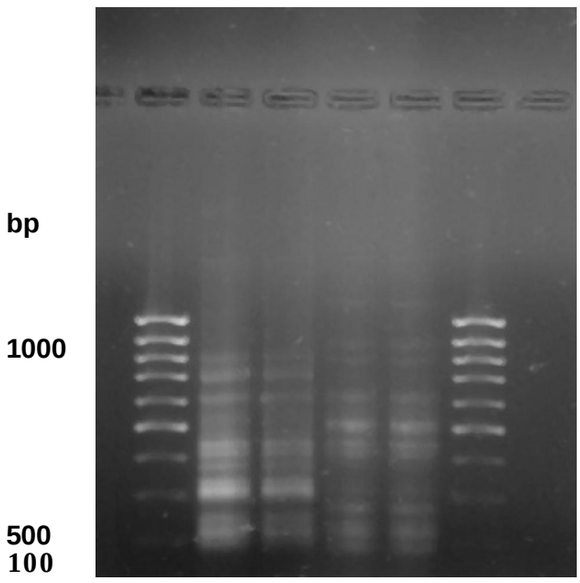

Fig. 2. *DNA bands generated with primer II. Lane M-gene ruler, N-Negative control, 1- DNA of pop. A, 2- DNA of pop. A, 3-DNA of pop. B, 4-DNA of pop. B of Ae. albopictuts.*

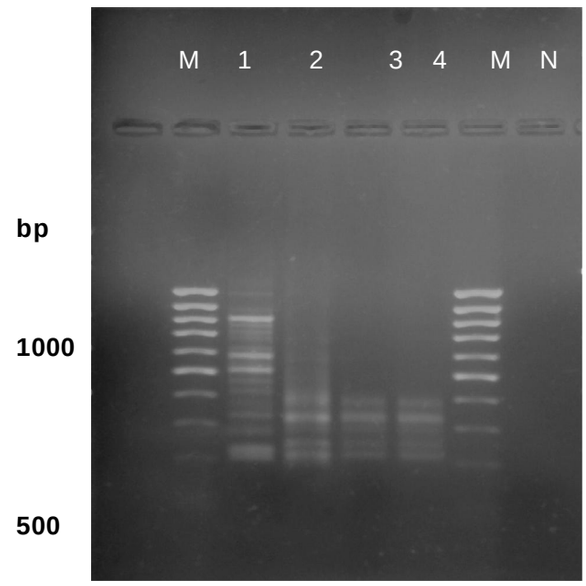

Fig. 3. *DNA bands generated with primer III. Lane M-gene ruler, N-Negative control, 1- DNA of pop. A, 2- DNA of pop. A, 3-DNA of pop. B, 4-DNA of pop. B of Ae. albopictuts.*

group of amplified fragments which could be used to differentiate the two populations of the present species in particular and other populations of related species within the genus *Aedes* of the Oriental region. As per the present cluster sharing analysis, primers I and II were once again found to be quite reliable markers for the differentiation of the individuals at the population level studies (Table 1). The band sharing coefficient value of 1 indicates maximum similarity among the individuals of the same population of *Ae. albopictus*. In relevance to the results of these two primers as molecular markers, it is pertinent to add that, due to their adaptation to different sets of environmental conditions and their allopatric status, the lack of gene flow has permitted the accumulation of genetic novelities which could be detected by the application of the present parameters of study. It is also logical to state that differences in their DNA profiles are attributed to the spacial segregation which tends to influence the genotype qualities of populations. The changes or the variations at the genome level have produced differences in the RAPD profiles which also revealed the genome as a source of constantly changing sequences among the populations and vector

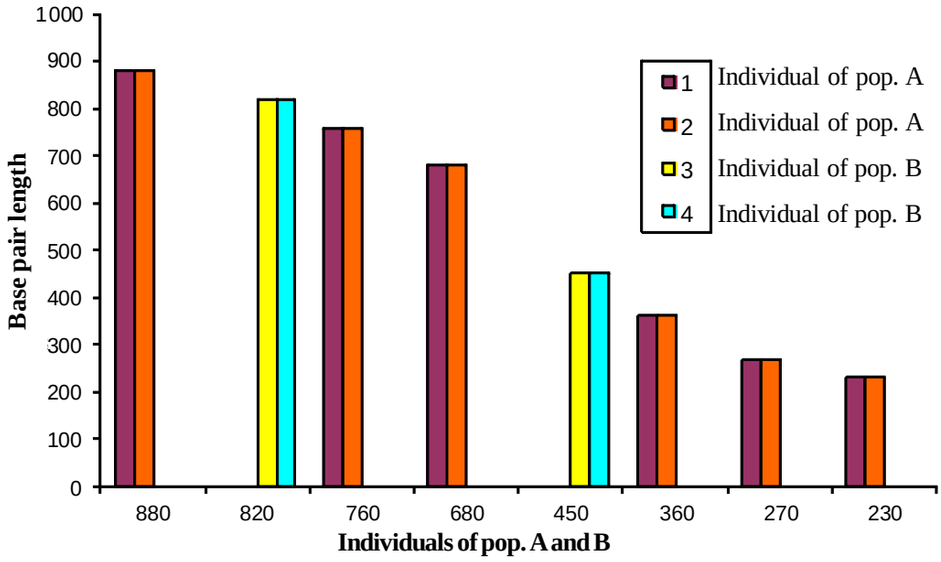

Fig. 4. *Base pair length variation in individuals number 1 and 2 of pop.A and 3 and 4 of pop.B with primer I.*

Table 1. Data of all the DNA bands produced with primers I, II and III from populations A and B of *Ae. albopictus.*

Primer number	No. of bands	Populations			
		pop.A	pop.A	pop.B	pop.B
Primer-I					
	800	+	+	-	-
	820	-	-	+	+
	760	+	+	-	-
	680	+	+	-	-
	600	+	+	+	+
	450	-	-	+	+
	360	+	+	-	-
	270	+	+	-	-
	230	+	+	-	-
Primer-II					
	900	-	-	+	+
	820	+	+	-	-
	800	+	+	-	-
	700	-	-	+	+
	640	+	+	-	-
	600	-	-	+	+
	520	+	+	+	+
	450	-	-	+	+
	380	-	-	+	+
	340	+	+	-	-
	310	+	+	-	-
	300	+	+	-	-
	280	-	-	+	+
	240	+	+	-	-
	230	-	-	+	+
	220	+	+	-	-
	200	-	-	+	+
Primer-III					
	1031	+	-	-	-
	840	+	-	-	-
	800	+	-	-	-
	750	+	-	-	-
	700	+	-	-	-
	650	+	-	-	-
	570	+	-	-	-
	500	+	-	-	-
	460	+	-	-	-
	370	+	+	+	+
	310	+	+	+	+
	280	+	+	+	+
	240	+	+	+	+
	210	+	+	+	+

Presence (+), absence (-)

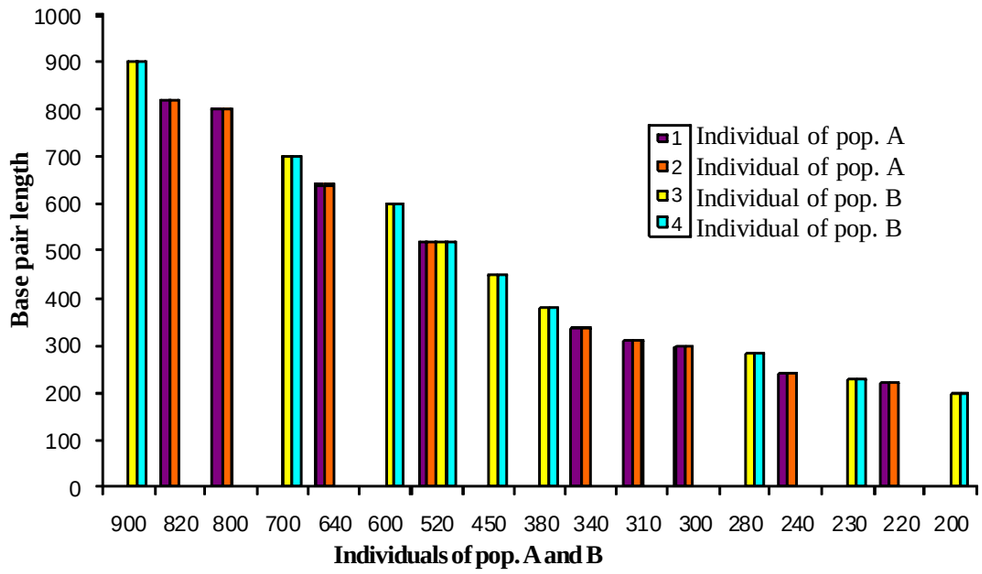

Fig. 5. *Base pair length variation in individuals number 1 and 2 of pop.A and 3 and 4 of pop.B with primer II.*

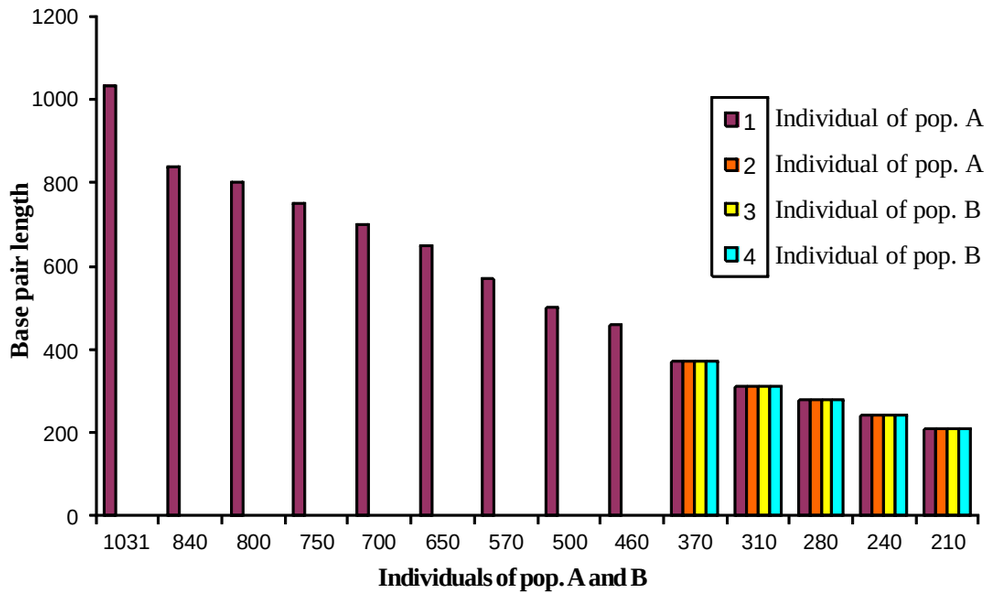

Fig. 6. *Base pair length variation in individuals number 1 and 2 of pop.A and 3 and 4 of pop.B with primer III.*

migrations (Kengne *et al.*, 2001; Kaura *et al.*, 2009). The classic works on the distribution, bionomics, epidemiology and genetics of this taxon provide enough impetus to analyse the genome organization of as many populations of the species as possible to understand the patterns of sequence variations (Narang *et al.*, 1993; Mitchell, 1995; Ayres *et al.*, 2002). The potential of RAPD markers in the discrimination of species, subspecies and populations of genus Aedes can be gauged from some of the classic works of Ballinger *et al.*, 1992, Mutebi *et al.*, 1997, de Sousa *et al.*, 2001 and dos Santos *et al.*, 2003. For example, Mutebi *et al.*, 1997 used six RAPD markers which resulted in the identification of as many as six polymorphic markers. Since *Ae albopictus* has remarkable potential of invading different geographic regions with matching genetic changes, the present studies are considered valuable in the documentation of population level studies of this species of considerable epidemiological significance.

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Table 2. Band sharing coefficient between individual specimens of populations A and B of *Ae. albopictus.*

Primer No.	Individuals	Band sharing coefficient
I	Ind.1 and 2 (pop. A)	1
	Ind.1 (pop.A) and Ind.3 (pop.B)	0.2
	Ind.1 (pop.A) and Ind.4 (pop.B)	0.2
	Ind.2 (pop.A) and Ind.3 (pop.B)	0.2
	Ind.2 (pop.A) and Ind.4 (pop.B)	0.2
	Ind.3 and 4 (pop. A)	1
II		
	Ind.1 and 2 (pop. A)	1
	Ind.1 (pop.A) and Ind.3 (pop.B)	0.1
	Ind.1 (pop.A) and Ind.4 (pop.B)	0.1
	Ind.2 (pop.A) and Ind.3 (pop.B)	0.1
	Ind.2 (pop.A) and Ind.4 (pop.B)	0.1
	Ind.3 and 4 (pop. A)	1
III		
	Ind.1 and 2 (pop. A)	0.5
	Ind.1 (pop.A) and Ind.3 (pop.B)	0.5
	Ind.1 (pop.A) and Ind.4 (pop.B)	0.5
	Ind.2 (pop.A) and Ind.3 (pop.B)	1
	Ind.2 (pop.A) and Ind.4 (pop.B)	1
	Ind.3 and 4 (pop. A)	1

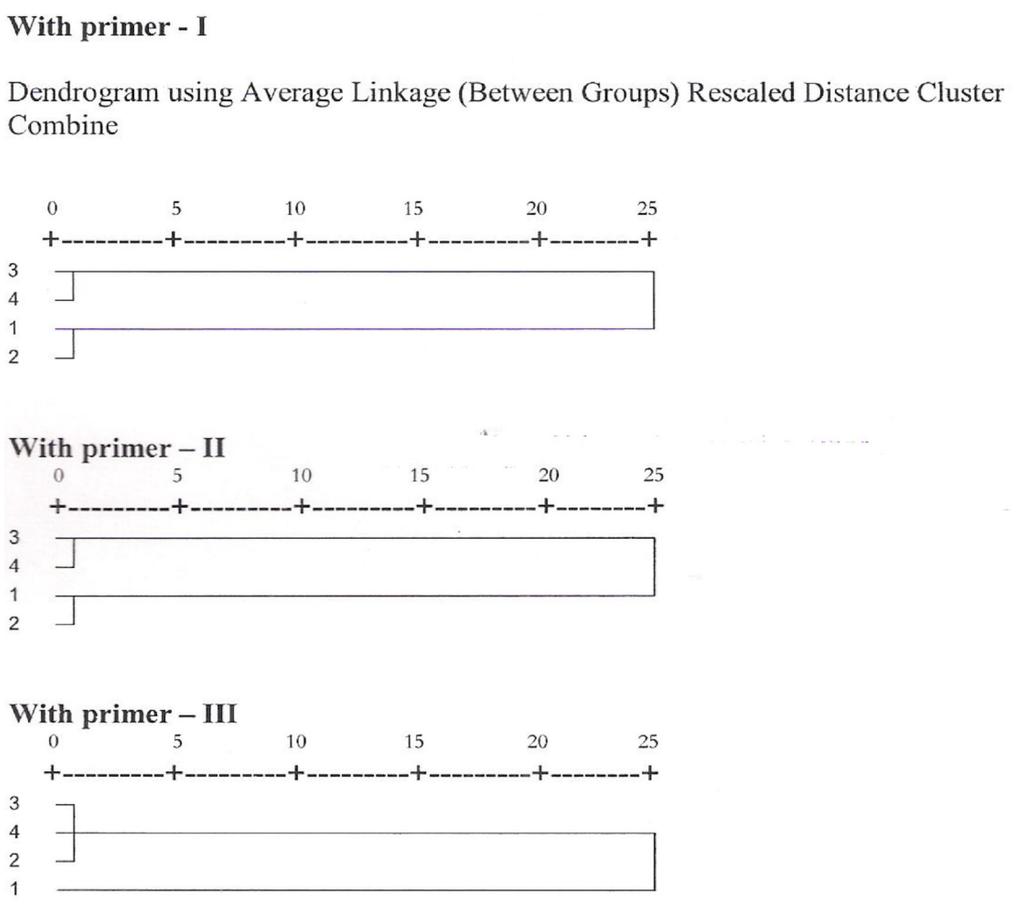

Fig. 7. *Dendograms of genetic relatedness of populations A and B of Ae. albopictus.*

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