RESEARCH NOTE

CHARACTERIZATION OF THREE MICROSATELLITE LOCI FOR *AEDES AEGYPTI* (DIPTERA: CULICIDAE) AND THEIR USE FOR POPULATION GENETIC STUDY

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Dengue fever is a human viral infection of high morbidity and mortality principally distributed in the tropical zone including Southeast Asia, the Pacific Islands, Latin America, Carribean and Africa. It is mainly transmitted by the mosquito species *Aedes aegypti*, which already worlwide population distribution continues to grow with the increasing commercial trade. Several clinical syndromes have been associated with the infection over a wide range of severity (Halstead, 1980). The severity of clinical syndromes has been tentatively associated with specific virus strains, a complex immunological status phenomenon due to the co-circulation of 4 types of dengue virus and, the potential impact of the vector on virus virulence (Gubler *et al,* 1979). However the role of these factors in the occurrence of a specific syndrome in a given area remains poorly understood.

The great variety of *A. aegypti* populations and its potential importance in the virus maintenance and dengue epidemiology (Mattingly, 1957) has induced many studies on genetic populations. Most of these studies are based on *Ae. aegypti* isoenzyme polymorphism (Wallis *et al,* 1983) and more recently RAPD technique allowing the identification of *Ae. aegypti* populations of different geographic areas (Ballinger-Crabtree *et al,* 1992). However, microsatellite loci have been used for genetic differentiation of *Anopheles gambiae* populations and have shown a higher polymorphism than allozyme loci (Lehman *et al,* 1995).

Here we describe the isolation and characterization of three polymorphic microsatellite loci which will be suitable for the study of population structure and genetic relationship among strains of *Ae. aegypti*. Microsatellites sequences were obtained from the GenBank. Trinucleotide microsatellites were identified from the GABA receptor subunit (Rd1) mRNA (accession number: AAU28803; Locus G1G2 and M1M2) and the ecdysteroid receptor mRNA (accession number: AAU02021; Locus E1E2) (Table 1) and, classified as microsatellites sequences according to Jarne and Lagoda (1996). A simplified procedure of DNA extraction with Chelex® was used according to Dumas *et al* (1998). PCR amplifications were performed in 50 ml reactions vials on a Perkin Elmer thermal cycler. Individual mixes contained 20 ml DNA template suspension, 0.2 mM each dNTP, 20 pmol of primer, 1.5 mM $MgCl_2$, 0.5 units *Taq* polymerase (Eurogentec SA) and, 1 X reaction buffer. PCR profiles consisted in initial denaturation at 92°C for 5 minutes and, then 35 cycles: 30 seconds denaturation at 92°C, 30 seconds annealing at 50°C, 1 minute elongation at 72°C. A final 10 minutes elongation step at 72°C was performed. Alleles were resolved on 8% acrylamide gel by ethidium bromide staining.

Six populations of *Ae. aegypti* from Africa (Cameroon, Senegal), Caribbean (Guadeloupe) and Asia (Indonesia and two sites in Thailand distant by 300 km) were studied for allele diversity and observed heterozygosity (Table 2). In our total sample of more than 50 individuals all three loci were polymorphic with 16 alleles identified.

The locus exhibiting the highest allelic diversity is G1G2. The six individuals tested in the Cameroon sample exhibit as much as 12 alleles for the three loci. Interestingly, the two populations of Thailand differ slightly, such difference seeming sufficient to allow population studies.

Further investigations on specimens from different areas should confirm the usefulness of these loci, mainly G1G2 and E1E2 in populations studies. Moreover, the used primer sets have been suc-

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Table 1
Characterization of three repeat array loci and design of *Aedes aegypti* microsatellites primer sets.

Locus	Repeat array	Primer sequences (5´ -> 3´)
G1G2	$(CAG)_3TAG(CAG)_2CAA(ACA)_4AAA(ACA)_2$	CCGAAGAAATTGCGGTGACC
		CCTCTCGGTGTTCGCTAACC
M1M2	$(GAA)_3(GAC)_4(GAA)_3GA(GAA)_3$	CAGGGAAGATCAGACGTACCG
		ATGGTTCCCACTGCTCCGATG
E1E2	$(GTA)_6ACG(GTA)_3ACGGTAACG(GTA)_3$	TGCAGGCCCAGATGCACAGCC
		TCCGCTGCCGTTGGCGTGAAC

Table 2
Allele diversity and heterozygosity in the six *Aedes aegypti* populations and the three selected loci.

	G1G2			M1M2			E1E2			Total
Origin	N	Obs hetero	n	N	Obs hetero	n	N	Obs hetero	n	n
Guadeloupe	10	0	2	11	0	2	11	0	1	5
Senegal	12	8.3	2	11	27.3	3	7	0	1	6
Cameroon	6	50	5	4	0	1	6	33.3	6	12
Indonesia	5	20	2	8	12.5	3	8	0	2	7
Thailand 1	15	0	1	15	0	2	13	8.3	3	6
Thailand 2	11	0	1	10	20	2	9	44.4	4	7
Total	58	15.2[a]	5	59	10.9[a]	3	51	20[a]	8	16

N = No. of individuals.
Obs hetero = Observed heterozygosity.
n = No. of alleles.
[a]Mean observed heterozygosities for populations with more than one allele.

cessfully tested on *Aedes albopictus* individuals collected in Thailand.

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