

Mosquito Cytogenetic and Electrophoresis Studies

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OBJECTIVE : To define and delimit the species or strains of mosquito species in Thailand that serve as the primary vectors of human pathogens by cytogenetic and enzymatic techniques for :

- (1) a check against current morphological species concepts;
- (2) the accurate determination of the limits of gene pools in natural populations of vector species; and
- (3) the correlation of genetic variation in natural populations of the primary vectors with the degree of susceptibility to dengue viruses and human malaria.

BACKGROUND : Considerable evidence now exists that different biological species of organisms, as defined by Mayr (1), may exist in the absence or near absence of definable morphological differences in all or most life stages. Such cryptic or sibling species may be distinct only on the basis of ecological, behavioral and/or cytogenetic differences. The occurrence of such species in important vector species groups of mosquitoes is well documented (2, 3), and their discovery is essential for developing effective control programs and understanding the epidemiology of vector-borne diseases (4). In addition, the ability of mosquito species or strains of species to transmit certain human pathogens has been shown to be under genetic control (5, 6, 7, 8). Cytogenetic and electrophoresis techniques have been shown to be effective for determining the genetic variability (including disease susceptibility) in natural populations of mosquitoes (9). These techniques, coupled with morphological studies and hybridization experiments, provide the most well founded basis for species and vector strain differentiation.

METHODS : Initially, colony strains (Table 1) of *Aedes aegypti*, *albopictus*, *malayensis*, *Anopheles balabacensis* and *maculatus* were utilized to develop facilities, train personnel and to standardize the cytogenetic and electrophoresis techniques. The cytogenetic techniques employed were : (1) larval salivary

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Table 1. Colony strains of *Aedes* and *Anopheles* mosquitoes used in cytogenetic and electrophoresis studies.

Colony - species	Strain #	Date of Collection	Age of Colony	Location of original collection	Numbers to start strain
<i>Aedes aegypti</i>	1	June-July 1968	122 months	Koh Samui (Island) Surat Thani, Thailand	?
"	3	Aug.-Sept. 1977	12 months	Bangkok, Thailand	many larvae
"	4	November 1977	9 months	Bangkok, Thailand	1 ♀
"	5	June 1978	2 months	Chiang Mai, Thailand	40± larvae
<i>Aedes albopictus</i>	2	May 1978	3 months	Chon Buri, Thailand	many larvae
<i>Aedes malayensis</i>	1	20 April 1968	124 months	Prachuap Kiri Khan, Thailand	many larvae
<i>Anopheles balabacensis</i>	1	February 1964	174 months	Khao Mai Khaeo, Chon Buri, Thailand	many adults and larvae
<i>Anopheles maculatus</i>	1	1966	144± months	Colony - Kuala Lumpur, Malaysia	?

polytene chromosome preparations by a modification of the standard chromosome squash technique (10); (2) larval brain metaphase preparations by a modified technique based on Baimai (11); and (3) ovarian nurse cell polytene chromosome preparations from adult females by the method described by Coluzzi (12). The electrophoresis techniques employed were those of Steiner and Joslyn (13).

Chromosome maps and electrophoresis starch-gel esterase patterns will be initiated for selected laboratory colony strains or species. These maps or patterns will serve as "standards" for later comparisons with other strains and/or species. Once "standard" maps or esterase patterns have been established, wild collected populations will be sampled to survey the variations occurring in natural populations. Wild and/or colony strains of currently recognized morphological species that exhibit sufficient cytogenetic and/or electrophoretic differences will be studied further by hybridization experiments to determine if they are conspecific. Strains of *aegypti* (wild or colony) that exhibit distinct esterase polymorphisms will be tested for susceptibility to infection with dengue viruses.

RESULTS : Squash preparations of the salivary glands of 4th stage larvae produced mixed results. *Anopheles balabacensis* larvae yielded fairly good results with 2-4% of the slides having well-spread chromosome arms. Chromosome preparations of *An. maculatus* were less favorable and those for *Aedes aegypti* were a complete failure. The chromosomes of *balabacensis* have been photographed and work has begun on the development of a standard chromosome map for this species.

Preparations of metaphase chromosomes from larval brains have been quite successful. A study to compare the different karyotypes is in progress.

The development of electrophoresis techniques was expedited by Dr. W.W.M. Steiner, University of Illinois, who spent one month at Mahidol University training personnel and refining his techniques for *Ae. aegypti* and anophelines. Field collected specimens of the Southeast Asian *Anopheles hyrcanus* complex were selected to evaluate the ability of electrophoresis techniques to differentiate very closely related species. According to morphological characteristics, this group is a very closely related assemblage of sibling species, of which at least 8 species occur in Thailand (14). A total of 66 *nigerrimus*, 148 *peditaeniatus* and 140 *sinensis* females were collected from 4 widely separated localities within a 100 km radius of Bangkok, and analyzed for 15, 16 and 16 enzymes respectively. Based on esterase activity, these species exhibited very similar patterns of gene variation, with some polymorphism at different loci. Species patterns were distinct for some loci, which substantiates the current concept that these are very closely related members of a sibling species complex. Further analysis of these data is in progress and additional specimens of these and other species in the complex are being collected for further investigations.

A total of 22 enzymes were tested against 50 larvae each of four colony strains of *Aedes aegypti* and a total of six esterases were detected, although most colonies exhibited only four esterases. The relative frequencies of esterase alleles at the Est-1 and Est-2 loci in 50 larvae each of three strains of *aegypti* and two closely related *Stegomyia* species, *albopictus* and *malayensis*, are tabulated in Table 2. These data demonstrate the ability of electrophoresis

techniques to detect esterase and esterase allele differences between closely related strains (Thai) of *aegypti* and closely related species of *Aedes* (*Stegomyia*) mosquitoes. The data for the other four esterases detected in these colony strains are being analyzed. Additional strains of *aegypti* will be tested in the future in preparation for the selection of strains to test for susceptibility to dengue viruses.

All aspects of this project will be continued, except for the attempted slide preparation of polytene chromosomes from the larval salivary glands of *Ae. aegypti*.

Table 2. Relative frequencies of esterase alleles at the Est-1 and Est-2 Loci in larvae of 3 colonies of *Aedes aegypti* and colonies of *Aedes albopictus* and *Ae. malayensis*.

Locus	Mosquito Colonies - Strains	Allele					
		.92	.94	.96	.98	1.00	1.02
Est-1	<i>aegypti</i> - 1	-	-	-	-	.70	.30
	<i>aegypti</i> - 3	-	-	-	-	.89	.11
	<i>aegypti</i> - 4	-	-	-	.53	.43	.03
	<i>albopictus</i> -2	-	-	.02	.39	.59	-
	<i>malayensis</i> -1	.30	.40	.30	-	-	-
Est-2	<i>aegypti</i> - 1	-	-	-	1.00	-	-
	<i>aegypti</i> - 3	-	-	-	-	1.00	-
	<i>aegypti</i> - 4 ¹	-	-	-	-	Null?	-
	<i>albopictus</i> -2 ²						
	<i>malayensis</i> -1 ²						

¹ This colony has the Est-1, .98 allele overlapping the Est-2 zone of activity, making it difficult to assess the presence of no activity.

² Enzyme activity too low to accurately diagnose the banding patterns.

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