

Mosquito Cytogenetics, Electrophoresis and Cross Mating Studies

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OBJECTIVE : To define and delimit the species or strains of mosquito species in Thailand and Southeast Asia 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 and/or colony populations of the primary vectors with their degree of susceptibility to infection with dengue viruses and/or malaria parasites.

BACKGROUND : These studies are a continuation of projects initiated in 1978 and outlined in a previous report (1). The recognition of sibling or cryptic species in important vector species groups of mosquitoes is steadily increasing with each year, and is essential for developing effective control programs and understanding the epidemiology of vector-borne diseases (2). Cytogenetic and electrophoresis techniques have been shown to be effective in differentiating such cryptic species and in determining the genetic variability (including disease susceptibility) in natural populations of mosquitoes (3). These techniques, when coupled with morphological studies and hybridization experiments, provide the best basis for species and vector strain differentiation.

METHODS : Cytogenetic techniques employed were a modification of the standard chromosome squash technique (4) for salivary polytene chromosome preparations and a modified technique (5) for larval brain metaphase preparations. The electrophoresis techniques employed and the enzyme terminology and abbreviations are those of Steiner and Joslyn (6).

Chromosome maps and electrophoresis starch-gel esterase patterns are being made for selected laboratory colony strains or species. These maps or patterns

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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 *Aedes aegypti* (wild or colony) that exhibit distinct esterase polymorphisms will be tested for susceptibility to infection with dengue viruses.

RESULTS : Two additional colonies of members of the Leucosphyrus Group were established during this period. A colony of the Taiwan strain of *An. balabacensis* was started with the assistance of Dr. J.C. Lien, Taipei, and the U.S. Navy Medical Research Unit No. 2, Taipei, Taiwan. This colony requires forced mating and most females require two blood meals before oviposition. The second colony was acquired from Mr. W.H. Cheong, Institute of Medical Research, Kuala Lumpur, Malaysia. This colony supposedly represents *balabacensis* from an inland locality in Sabah, East Malaysia; however, after a very short time the colony was noted to be self-mating, and a morphological examination of the adult and immature stages showed the colony was the Perlis-Kedah strain (Peninsular Malaysia) of *balabacensis*. This identification was confirmed by personnel at the Medical Entomology Project (MEP), Smithsonian Institution, Washington, D.C.

Efforts to colonize other vector species or potential vectors from Thailand are continuing. Two colonies of *An. maculatus* (Central and Southeast Thai strains) have been started, however, these colonies are still unstable. Efforts are continuing to colonize *nivipes* and/or *philippinensis* from Thailand for a number of studies.

During this report period, the Thai species called *balabacensis* and previously listed (1) as a colony used in these studies, was involved in a taxonomic study which resulted in its being described as a new species, *dirus*, in the Leucosphyrus group from Thailand (7). A more detailed discussion of this new species is provided elsewhere in this annual progress report. This species, *dirus*, is one of the primary species involved in cytogenetic and electrophoresis studies.

Electrophoretic studies on *An. maculatus* were initiated during the year. This species is considered a primary vector of human malaria parasites in Thailand. However, its vector capabilities in the southern peninsular area appear to be different from those in the central and northern areas of Thailand. During the last half of 1978, a large number of adult female *maculatus* were collected in Chumphon Province. Table 1 shows the genetic variation occurring in Chumphon females screened at 9 different electrophoretic enzyme loci. These specimens were screened to help determine the enzyme loci showing allelic variation that can be used in future studies. Actually, no analysis of the Chumphon *maculatus* electrophoretic esterase banding patterns is possible at this time. Chumphon Province probably represents the most northern extension of many Malaysian species in Thailand, thus a number of species, subspecies and other taxa probably overlap in that area. Work is continuing on *maculatus* strains, but, rests, in part, on the establishment of a successful *maculatus* colony from central-northern Thailand.

Electrophoresis studies were also initiated during this period on members of the Leucosphyrus Group. A total of 12 different enzyme loci were screened for genetic variation in colony adults of *An. dirus* and 9 were screened in colony adults of the Perlis strain of *balabacensis* (Table 2). The enzyme loci Hk-1, Me and 6-pgdh were homozygous in these studies, however, larger sample sizes will be used in future studies. Considerable allelic variation was detected in the enzyme loci Aldox, Est-2 and Xdh, and differences were detected between *dirus* and the Perlis strain in frequencies of certain alleles at several enzyme loci. Analyses of these variations and differences are continuing and future comparisons will include the Taiwan strain of *balabacensis*.

A total of 15 enzyme loci were screened for genetic variation in 50 larvae each of 4 colony strains of Thai *Aedes aegypti* (1). Only 6 enzyme loci exhibited allele variation in electrophoretic esterase banding patterns during this study (Table 3). Differences were detected in the *aegypti* strains, particularly at the loci Pgm, Est-1 and Est-2, however, further studies and analysis are necessary for proper interpretation of these differences. Further electrophoretic analyses of the *aegypti* strains are anticipated, coupled with an analysis of each strain's susceptibility to dengue viruses 1-4.

The frequencies of alleles for the enzyme loci, Esterase 1, 2, 3, 4 and Lap 1, 2, 3, 4 in 4 strains of 4th stage larvae of *aegypti* reared at 20° and 35°C are presented in Table 4. The most marked differences were noted in the allele variations at the Est 1, 2, 3 and 4 loci for all 4 strains of *aegypti* larvae reared at 20°C. exhibited striking allele frequency differences at all 4 Est loci, particularly the differences between the Din Daeng F₁ larvae and colonized strains 1, 3 and 4. Differences in allele frequencies at the Lap 1, 2, 3 and 4 loci were observed for all 4 strains of *aegypti*, but were considerably less than those observed for the Est loci. These data indicate that the 4 strains of *aegypti* not only show interstrain differences in allele frequency at a given temperature, but also show intrastrain allele variations when reared at different temperatures.

Cytogenetic studies during this period focused on members of the *Anopheles* Leucosphyrus Group. A limited number of squash preparations were made from adult female ovarian nurse cells; however, this technique did not yield slide preparations of the same quality as salivary gland polytene chromosome preparations. Excellent salivary gland squash preparations have been made for *Anopheles dirus* (Figure 1) and *An. balabacensis* (Perlis strain). Brain metaphase slides have also been prepared from adult specimens of these 2 colonies. Both the salivary and brain metaphase chromosome preparations are currently being analyzed in preparation for publication. A salivary chromosome map has been prepared for *dirus* and is included in a manuscript being prepared for publication in the near future. The specimens of *dirus* used to prepare this map came from a colony which is approximately 15½ years old, and as would be expected, polymorphisms were not observed on the chromosomes. The chromosome map for *dirus* will serve as a "standard" for comparisons with future maps of other taxa in the Leucosphyrus Group. Recently, squash preparations have been started for the Taiwan strain of *balabacensis* and a future comparison of the chromosomes of this strain with those of the Perlis strain and *dirus* is anticipated.

Cross mating experiments between *An. dirus* and *An. balabacensis* (Taiwan strain) are currently in progress. Crossing experiments between *dirus* and *balabacensis* (Perlis strain), and between the Taiwan and Perlis strains will start in the near future.

All aspects of these studies are continuing.

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Table 1. Genetic variation (allele frequencies) at 9 different electrophoretic enzyme loci in feral *Anopheles maculatus* females from Chumphon Province, Thailand (1978)

Enzyme locus	Populations (1)	No. ♀♀	Allele				
			.98	1.00	1.02	1.04	1.06
6-pgdh	07970, 08007 07945, 08003 08004	53	-	.85	.11	.04	-
Hk-1	08003, 08004	5	-	1.00	-	-	-
Hk-3	07945, 08004	11	-	1.00	-	-	-
Xdh	08003, 08007 08003	68	.01	.71	.13	.06	.09
α-gpdh	07970, 08007 07945, 08003	42	-	1.00	-	-	-
Idh	08003, 08004	39	.15	.81	.04	-	-
Aldox	08003, 08004	30	-	-	.97	-	.03
Pgi	08003	29	-	-	-	1.00	
Pqm	08003	29	-	.04	.94	.02 ⁽²⁾	

(1) AFRIMS collection numbers for wild females taken in biting collections on human bait.

(2) Further analysis needed.

Table 2. Presence of genetic(allelic) variation at 12 different electrophoretic enzyme loci in adults from colonies of *Anopheles dirus* and *balabacensis* (Perlis Strain) at AFRIMS, Bangkok.

Enzyme locus	Presence (+) or absence (-) of genetic variation							
	<i>Anopheles dirus</i>				<i>An. balabacensis</i> (Perlis strain)			
	♂	No. Tested	♀	No. Tested	♂	No. Tested	♀	No. Tested
Aldox	+	163	+	202	+	129	+	134
Est-1	-	45	-	103	-----	Not Tested	-----	
Est-2	+	45	+	103	-----	Not Tested	-----	
Est-3	+	30	Not Tested		-----	Not Tested	-----	
Hk-1	-	58	-	48	-	15	-	15
Hk-2	-	58	+	48	-	39	+	38
Hk-3	+	81	-	81	-	15	-	15
Me	-	47	-	30	-	68	-	81
Pgi	-	130	+	219	-	114	+	88
Pqm	+	147	+	194	-	15	-	15
Xdh	+	100	+	144	-	65	+	62
6-pgdh	-	84	-	94	-	58	-	78

Table 3. Genetic variation (allele frequencies) at 6 different electrophoretic enzyme loci in 4th stage larvae of 4 colony strains of *Aedes aegypti* from Thailand (AFRIMS-Bangkok).¹

Enzyme locus	Mosquito Strain	Allele				
		.96	.98	1.00	1.02	1.04
Mdh	<i>aegypti</i> -1	-	.17	.72	.11	-
	-3	-	.20	.80	-	-
	-4	-	.20	.75	.05	-
	-5	-	.10	.80	.10	-
Pgm	<i>aegypti</i> -1	-	-	.50	.40	.10
	-3	-	.09	.50	.32	.09
	-4	-	-	.50	-	.50
	-5	-	-	.60	.30	.10
Idh	<i>aegypti</i> -1	-	-	1.00	-	-
	-3	-	-	.91	.09	-
	-4	-	-	1.00	-	-
	-5	Not Analyzed				
Est-1	<i>aegypti</i> -1	-	-	.90	.10	-
	-3	-	-	.80	.20	-
	-4	-	.25	.70	.05	-
	-5	.20	.30	.20	.30	-
Est-2	<i>aegypti</i> -1	-	1.00	-	(?) Null ²	(?)
	-3	-	-	1.00	(?) Null	(?)
	-4	-	-	-	1.00 Null	(?)
	-5	Not Analyzed				
Pgi	<i>aegypti</i> -1	-	-	1.00	-	-
	-3	-	-	1.00	-	-
	-4	-	-	1.00	-	-
	-5	-	.10	.90	-	-

¹ The following 9 enzyme loci did not exhibit genetic variation during these tests (α -G-pdh, G-6-pdh-F, Ald-M, Ald-F, 6-pgdh, Got-S, Got-F, Me and Lap-F).

² Null (?) - possible overlap in activity between the Est-1 and Est-2 loci.

Table 4. Relative allele frequencies of esterase and lap enzyme loci in 4th stage larvae of 4 strains¹ of *Aedes aegypti* reared at 20°C and 35°C. (AFRIMS, Bangkok)

Locus	<i>Aedes aegypti</i> Strains	No. of Larvae	Allele (20°C)			No. of Larvae	Allele (35°C)		
			.98	1.00	1.02		.98	1.00	1.02
Est-1	<i>aegypti</i> -1	28	.07	.63	.30	23	.16	.61	.23
	-3	16	-	.45	.55	23	.11 ²	.89	-
	-4	26	-	.88	.12	00	ND	ND	ND
	-D.D.	08	.06	.50	.44	08	.05	.25	.69
Est-2	<i>aegypti</i> -1	23	.10	.83	.07	23	-	1.00	-
	-3	16	.07	.76	.17	27	.02	.98	-
	-4	26	.09	.81	.10	00	ND	ND	ND
	-D.D.	08	.13	.68	?	08	-	1.00	-
Est-3	<i>aegypti</i> -1	23	-	.90	.10	23	-	blurry	-
	-3	21	-	.91	.09	24	-	1.00	-
	-4	21	-	1.00	-	00	ND	ND	ND
	-D.D.	03	-	1.00	-	03	-	1.00	-
Est-4	<i>aegypti</i> -1	08	.06	.94	-	23	-	1.00	-
	-3	08	.06	.94	-	24	-	1.00	-
	-4	26	-	1.00	-	00	ND	ND	ND
	-D.D.	08	.13	.87	-	08	-	1.00	-
Lap-1	<i>aegypti</i> -1	28	.05	.95	-	23	-	1.00	-
	-3	16	.04	.96	-	24	-	1.00	-
	-4	18	-	1.00	-	25	-	1.00	-
	-D.D.	08	-	1.00	-	08	.13	.87	-
Lap-2	<i>aegypti</i> -1	28	-	.94	.06	23	.42	.46	.12
	-3	11	-	1.00	-	00	ND	ND	ND
	-4	18	-	.76	.24	00	ND	ND	ND
	-D.D.	10	-	1.00	-	10	-	.93	.07
Lap-3	<i>aegypti</i> -1	28	-	1.00	-	00	ND	ND	ND
	-3	16	-	1.00	-	00	ND	ND	ND
	-4	18	-	1.00	-	00	ND	ND	ND
	-D.D.	08	-	1.00	-	08	-	1.00	-
Lap-4	<i>aegypti</i> -1	23	-	1.00	-	00	ND	ND	ND
	-3	08	-	1.00	-	00	ND	ND	ND
	-4	13	-	.92	.08	00	ND	ND	ND
	-D.D.	03	-	1.00	-	03	-	1.00	-

¹ Generation : Colony 1 (unknown-11 yrs old), Colony 3 (F₁₉),
Colony 4 (F₁₀) and Din Daeng (F₁)

² Not done

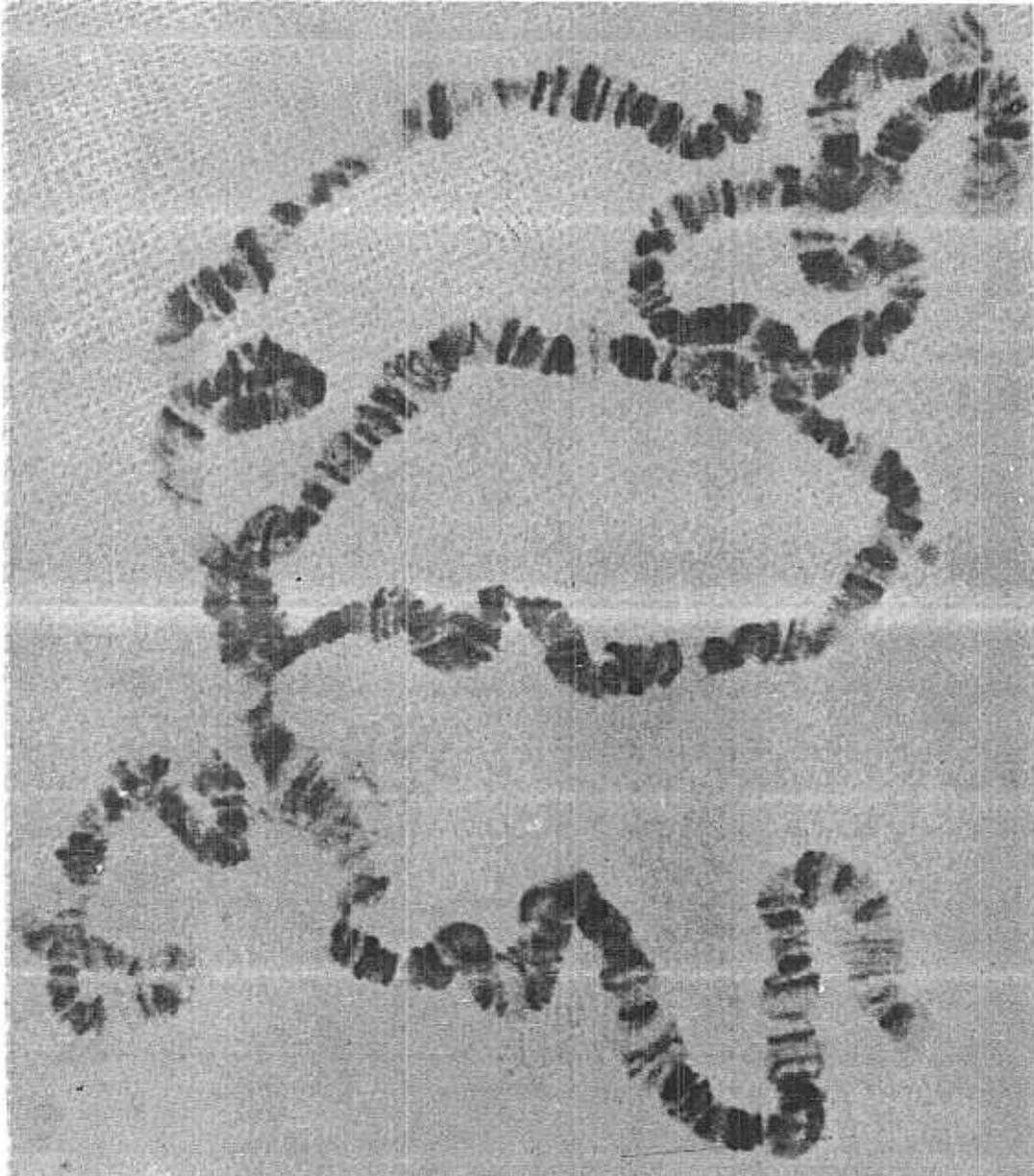


Figure 1. Salivary polytene chromosomes of Anopheles dirus Peyton and Harrison.