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GENETIC DIFFERENTIATION OF *AEDES AEGYPTI* MAINLAND AND ISLAND POPULATIONS FROM SOUTHERN THAILAND

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ABSTRACT. Isozymes of 5 wild-caught collections of *Aedes aegypti* were compared by using starch gel electrophoresis. All collections were collected from Surat-Thanee Province, southern Thailand, an area considered to be a hyperendemic zone for dengue virus. One collection was from Donsak Harbor, whereas the other 4 collections were from 4 districts of Samui Island. The percent polymorphic loci (24.2–33%) in the 4 island collections was lower than in the mainland collection (36.4%). This study revealed a large effective migration rate among all 5 collections. No fixed differences were detected. No significant differentiation was found among the 5 collections from Surat-Thanee Province.

KEY WORDS *Aedes aegypti,* isozyme analysis, genetics, mainland and island populations, Thailand

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF), which are mosquitoborne viral diseases, commonly occur throughout Asia (Gubler 1988). *Aedes aegypti* (L.), an urban mosquito species in Asia, is known to be an important dengue virus vector in Thailand (Bhamarapravati 1990). This species is considered to be a polytypic species worldwide because of morphological, physiological, and geographical variations (Craig and VandeHey 1962, Gouck 1972, McClelland 1974, Trpis and Hausermann 1975, Leahy et al. 1978, Tabachnick et al. 1979, Tabachnick and Powell 1979, Trpis et al. 1995). Thousands of cases of DF and DHF are reported worldwide annually (Henchal and Putnak 1990, Monath 1994, Gubler 1997).

Samui Island is located approximately 740 km from Bangkok and 30 km east of Donsak Harbor in Surat-Thanee Province, southern Thailand (Fig. 1). Samui Island is the 3rd largest island of Thailand, with an area of approximately 280 km^2 and a population of 35,000 inhabitants. Presently, little is known concerning the spread of *Ae. aegypti* between the mainland and Samui Island. Therefore, in this study, we compared genetic profiles of *Ae. aegypti* by using starch gel electrophoresis to determine if any significant differences occurred between mainland and island collections of *Ae. aegypti* in Surat-Thanee Province, southern Thailand.

MATERIALS AND METHODS

Mosquito collections: Five collections of *Ae. aegypti* were made from 2 different geographical zones. One collection was from the mainland from Donsak Harbor and the other 4 collections were from Mae-Nam, Na-Thon, Ma-Ret, and Taling-Ngam districts on Sumui Island (Fig. 1). Mosquitoes were collected as larvae or pupae and reared to adults. An average of 30 adults from each collection was stored at −70°C while awaiting electrophoretic analyses.

Starch gel electrophoresis: Starch gel electrophoresis was used to analyze 24 enzyme systems according to Harris and Hopkinson (1976), Manguin et al. (1995), and Lerdthusnee and Chareonviriyaphap (1999).

Data analysis: Analysis of allele frequencies, heterozygosity, conformity to the Hardy–Weinberg equilibrium, and genetic distance were calculated by using BIOSYS-1 (Swofford and Selander 1989). Differentiation among collections was reported by using F_{ST} (Wright 1978). The effective migration rate (N_em) among the collections was estimated from F_{ST} with equation $N_em \simeq (1 - F_{ST})/4F_{ST}$ (Wright 1978) and N_em values were compared between collections. Nei's (1978) unbiased genetic distance was used for the cluster analysis by unweighted pair group method averaging (UPGMA) to produce a phenogram.

RESULTS

Of 24 enzyme systems screened, 33 putative loci were detected (Table 1). The number of allelic polymorphic loci was 20 in the mainland-Donsak collection, whereas 14, 15, 11, and 16 loci were detected in 4 island collections of Mae-Nam, Nathon, Ma-Rat, and Taling-Ngam, respectively (Table 2). Nine loci (*Est-3, Hk-1, Idh-1, Lap-2, Mdh-1, Mdh-2, Pgd-1, Pgm-1,* and *Pk-2*) showed allelic polymorphism in all collections, whereas the other 13 loci (*Aox-1, Est-1, Est-2, Fum-1, G6p–1, Gpd-1, Gcd-1, Got-2, G3p–1, Mez-1, Mpi-1, Tpi-1,* and *Xdh-1*) were monomorphic in all collections. The frequency of polymorphic loci was also significantly higher ($P < 0.05$) for the mainland-Donsak collection (36.4%) than in 4 island collections (24.2 for Mae-Nam, Na-Thon, and Ma-Ret and 33.3 for

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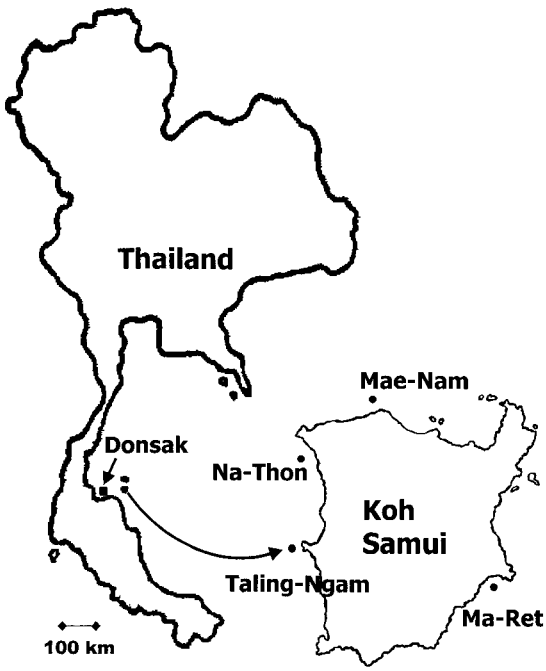

Fig. 1. Map of Samui Island, Surat-Thanee Province, southern Thailand.

Taling-Ngam, respectively; Table 2). Loci *Hk-2, Hk-3, Hk-4,* and *Idh-2* were not detected in this study. Mean expected heterozygosity ranged from 0.107 (0.107 ± 0.035) to 0.155 (0.155 ± 0.038), and the mean number of alleles per locus was between 1.6 and 1.7 for all 33 loci (Table 2).

Departures from Hardy–Weinberg equilibrium

Out of 165 comparisons, 8 significant deviations from the Hardy–Weinberg equilibrium ($P < 0.05$) were observed, and all departures were due to deficiency of heterozygotes (Table 3). However, these represented less than 5% of the expected deviation by chance. Heterozygote deficiency was observed in *Idh-1* and *Pgd-1* in Na-Thon, Ma-Ret, and Taling-Ngam districts. Significant deviation from the Hardy–Weinberg equilibrium in *Est-3* appeared exclusively in collections of Na-thon and Ma-Ret districts.

Estimation of F statistics and N_em

The N_em estimated from F_{ST} (0.055) was 4.29 when all polymorphic loci were considered (Table 4). However, when removing *Mdh-1, Mdh-2,* and *Got-1*, N_em was 9.01. When the mainland-Donsak collection was excluded from the analysis, N_em was 6.32 (Table 4). The N_em values between collections of *Ae. aegypti* were compared (Table 5). The F_{ST} value was largest at the *Mdh-1* locus ($0.25 > F_{ST}$

Table 1. Electrophoretic enzyme systems studied on adult *Aedes aegypti.*

Enzyme system	E.C. number[1]	Symbol	No. loci[2]	Buffer[3]
Aconitase	4.2.1.3	*Acon*	2	TMED
Adenylate kinase	2.7.4.3	*Aks*	2	TCss
Aldehyde oxidase	1.2.3.1	*Aox*	1	LiOH
Arginine kinase	2.7.3.3	*Agk*	2	TCss
Esterase	3.1.1.1	*Est*	3	TMED
Fumarase	4.2.1.2	*Fum*	1	TCss
Glycerol dehydrogenase	1.1.1.72	*Gcd*	1	TMED
Glutamate oxaloacetate transminase	2.6.1.1	*Got*	2	Morph
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*G3pdh*	1	Morph
α-glycerophosphate dehydrogenase	1.1.1.8	*αGpdh*	1	TMED
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6pdh*	1	TMED
β-hydroxyacid dehydrogenase	1.1.1.30	*Had*	1	TMED
Hexokinase	2.7.1.1	*Hks*	1	TMED
Isocitrate dehydrogenase	1.1.1.42	*Idh*	1	Morph
Leucine amino peptidase	3.4.11.1	*Lap*	2	LiOH
Malate dehydrogenase	1.1.1.37	*Mdh*	2	Morph
Malic enzyme	1.1.1.40	*Mez*	1	LiOH
Mannose-6-phosphate dehydrogenase	5.3.1.8	*Mpi*	1	TMED
6-phosphogluconate dehydrogenase	1.1.1.44	*6Pgd*	1	TCss
Phosphoglucomutase	5.4.2.2	*Pgm*	1	Morph
Phosphoglucose isomerase	5.3.1.9	*Pgi*	1	LiOH
Pyruvate kinase	2.7.1.40	*Pks*	2	TCss
Triose phosphate isomerase	5.3.1.1	*Tpi*	1	Morph
Xanthine dehydrogenase	1.2.1.37	*Xdh*	1	LiOH
Total			33	

[1] Enzyme commission number.
[2] Number of scorable bands per phenotype.
[3] Refers to electrophoresis buffer (see Materials and Methods).

Table 2. Measures of genetic variability at 33 loci of 5 populations of *Aedes aegypti.*

Population	Average alleles per locus	No. polymorphic loci	% polymorphic loci[1]	Mean heterozygosity	
				H_{obs}	H_{exp}[2]
Donsak	1.8 (0.2)	20	36.4	0.152 (0.035)	0.155 (0.038)
Mae-Nam	1.6 (0.2)	14	24.2	0.113 (0.034)	0.121 (0.037)
Na-Thon	1.7 (0.2)	15	24.2	0.087 (0.029)	0.109 (0.035)
Ma-Ret	1.7 (0.2)	11	24.2	0.082 (0.028)	0.107 (0.035)
Taling-Ngam	1.7 (0.2)	16	33.3	0.097 (0.030)	0.114 (0.035)

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
[2] Unbiased estimate (see Nei 1978) (standard error is given in parentheses).

> 0.15, average = 0.164), and the *Got-1* locus (0.15 > F_{ST} > 0.05, average = 0.138), whereas the remaining 18 polymorphic loci showed negligible genetic differentiation ($F_{ST} \leq 0.05$; Table 4).

The collection from Samui Island showed a significantly higher frequency (0.53–0.88) of the *Mdh-1-100* allele, whereas a significantly ($P < 0.05$) lower frequency (0.33) was detected in the mainland-Donsak collection. The Na-Thon collection did not contain the *Mdh-1-117* allele, whereas moderate frequencies (0.37–0.67) were found in the other 4 collections. The *Mdh-1-83* allele was not detected in Donsak, Mae-Nam, Ma-Ret, and Taling-Ngam collections but was detected in Na-Thon. The *Got-1-110* allele was only present in the mainland-Donsak collection. Nei's (1978) unbiased genetic distance UPGMA phenogram indicates that all 4 island collections occur in a common genetic cluster separate from the mainland-Donsak collection (Fig. 2).

DISCUSSION

The average expected heterozygosity (H_{exp}) estimated in this study (H_{exp} = 0.121) was slightly lower than those reported in earlier studies. Tabachnick and Powell (1979) analyzed 23 isozyme loci among worldwide collections of *Ae. aegypti* and H_{exp} = 0.152 was found. An H_{exp} = 0.163 was detected by Wallis et al. (1984) among 11 isozyme loci in Puerto Rico. However, H_{exp} among *Ae. aegypti* in this study was higher than those from Asian populations reported by Tabachnick (1991) ($H_{exp} \simeq$ 0.090). However, 4 polymorphic loci (*Hk2, Hk-3, Hk-4,* and *Idh-2)* reported by Tabachnick et al. (1979) were not detected in the present study. In addition, a relatively small sample size could reduce H_{exp}. We also found that H_{exp} was lower in the 4 island collections than in the mainland collection.

The N_em value among all 5 collections was 4.29 reproductive migrants/generation. However, N_em was higher (6.32) among the island collections when the mainland-Donsak collection was excluded from the analysis. The higher N_em among the

Table 3. Loci deviations from Hardy–Weinberg expectation ($P < 0.05$).

Population	Locus	Contingency chi square value	*P*-value
Nathon	*Est-3*	36.271	<0.0001
	Idh-1	18.994	<0.0001
	Pgd-1	9.812	0.02
Ma-Ret	*Est-3*	28.998	<0.0001
	Idh-1	21.623	<0.0001
	Pgd-1	35.497	<0.0001
Taling-Ngam	*Idh-1*	36.578	<0.0001
	Pgd-1	14.333	<0.0001

Table 4. F-statistics analysis of polymorphic loci in 5 populations of *Aedes aegypti.*

Locus	F_{ST}[1]
Acon-1	0.023
Acon-2	0.033
Ak-1	0.054
Ak-2	0.017
Argk-1	0.013
Argk-2	0.014
Est-3	0.031
Got-1	0.138
Had-1	0.085
Hk-1	0.009
Idh-1	0.017
Lap-1	0.017
Lap-2	0.006
Mdh-1	0.164
Mdh-2	0.120
Pgd-1	0.021
Pgi-1	0.036
Pgm-1	0.027
Pk-1	0.054
Pk-2	0.009
Mean	0.055
Nm	4.295
Nm[2]	6.32

[1] F_{ST}, F-statistic. For definitions of loci, see Table 1.
[2] The mainland-Donsak population was not included in the analysis.

Table 5. Pairwise F-statistics at all loci between any of 5 populations of *Aedes aegypti*.

Populations compared	F_{ST}[1]	Effective migration rate (N_em)
Donsak : Mae-Nam	0.036	6.69
Donsak : Na-Thon	0.070	3.32
Donsak : Ma-Ret	0.051	4.65
Donsak : Taling-Ngam	0.033	7.32
Mae-Nam : Na-Thon	0.038	6.32
Mae-Nam : Ma-Ret	0.016	15.37
Mae-Nam : Taling-Ngam	0.017	14.45
Na-Thon : Ma-Ret	0.031	7.81
Na-Thon : Taling-Ngam	0.041	5.84
Ma-Ret : Taling-Ngam	0.009	27.5

[1] F_{ST}, F-statistic. For definitions of loci, see Table 1.

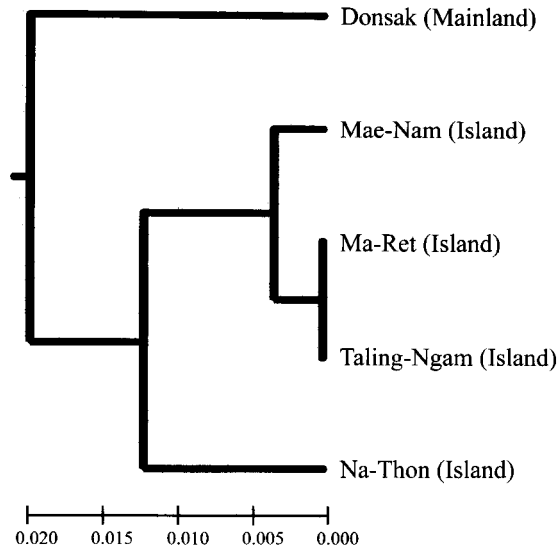

Fig. 2. Unweighted pair group method averaging phenogram from Nei's (1978) unbiased genetic distance matrix among the collections of *Aedes aegypti* (cophenetic correlation = 0.868).

island collections may reflect rapid dispersion among island collections of *Ae. aegypti*. Although flight range of *Ae. aegypti* is less than 1,000 m (Hausermann et al. 1971, PAHO 1994, Edman et al. 1998), the improvement of transportation between the mainland from Donsak Harbor to the Samui Islands could enhance dispersion of *Ae. aegypti*.

The N_em value (6.32 reproductive migrants/generation) among the Koh Samui Island populations of *Ae. aegypti* located within 20 km of one another was lower than the N_em value detected among collections within 200 km of one another in Puerto Rico (9.7–12.2 reproductive migrants/generation) (Apostol et al. 1996). This is probably because density of roads in Koh Samui is much lower than in Puerto Rico. Our estimates of N_em are more similar to those from collections located along a 750-km transect on the northeastern coast of Mexico (5.4–9.0 reproductive migrants/generation) (Gorrochotegui-Escalante et al. 2000).

Nei's (1978) genetic distances among collections ranged from 0.00 to 0.019 and are approximately the same magnitude as Tabachnick and Powell (1979) reported between Asian populations and populations from the West Africa, southeastern U.S., and east Africa. Phenograms produced by using other distance measures, such as modified Rogers (Wright 1978), and Cavalli-Sforza and Edwards chord and arc (Cavalli-Sforza and Edwards 1967) produced nearly identical branching patterns. Noticeably, the Na-Thon collection of the island collections is slightly different from the other 3 collections (Fig. 2). These differences may be due to the fact that the *Ae. aegypti* larval habitats of the Na-Thon population were the town-resident types, whereas the others were rural-resident types. Therefore, this difference in mosquito larval habitats may translate into the intraspecific differences.

The large N_em between mainland and island *Ae. aegypti* populations is probably due to unintentional migration between Koh Samui and Donsak Harbor via commercial exchanges either by boat (ferry from the harbor to either Taling-Ngam or Na-Thon counties), or by bus transportation. Such passive transportation may increase the spread of dengue virus on the island as reported by Failloux et al. (1995). We conclude that a high rate of gene flow occurs among all 5 collections of *Ae. aegypti* between Koh Samui and mainland Thailand.

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