AEDES AEGYPTI IN SOUTH VIETNAM: ECOLOGY, GENETIC STRUCTURE, VECTORIAL COMPETENCE AND RESISTANCE TO INSECTICIDES

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Abstract. In Vietnam, dengue hemorrhagic fever has been detected since the 1950s. In Southeast Asia, urban centers expanded rapidly in an uncontrolled and unplanned way. The *Aedes aegypti* populations and dengue viruses thrived in these new ecological and demographic settings. The result of these changes was a greatly extended geographic distribution, increased densities of *Ae. aegypti* and the maintenance of the four dengue serotypes leading to a dramatic increase in dengue transmission. To assess the role of the vector in the changing pattern of the disease in Southeast Asia, we studied the ecology of *Ae. aegypti*, genetic differentiation, variability in competence as a vector for dengue 2 virus, and resistance to insecticides.

INTRODUCTION

Dengue viruses are transmitted by mosquitos, mainly *Aedes aegypti* and *Aedes albopictus*. The four dengue serotypes co-circulate in Southeast Asia, where outbreaks occur mainly in the rainy season when mosquito densities peak. Hyperendemicity is known to be a result of ecological changes related to a rapid, uncontrolled and unplanned expansion of urban centers in Southeast Asia.

Before the 1950s, only dengue fever (DF) was reported in Asia. Dengue hemorrhagic fever (DHF) was first described in Vietnam in 1958, in Hanoi (Mihov *et al*, 1959). In 1960, 60 cases were recorded in South Vietnam along the Mekong River. During the same period, cases were described near the Cambodian border along the Mekong River. A second dengue outbreak occurred in South Vietnam in 1963, also along the Mekong River (Halstead *et al*, 1965). River transportation was incriminated in dengue diffusion, particularly into districts located along the Mekong River (Phong, 1967). Moreover, in 1960, an outbreak occurred in the region around the Red River delta. The disease was probably brought into Vietnam from Thailand, where dengue was highly endemic (Mirovsky *et al*, 1965). Since the 1960s, DHF incidence has steadily increased, from 41.02 per 100,000 inhabitants in 1981 to 462.24 in 1987 (Do Quang Ha, personal communication).

Two types of dengue transmission can be described: urban and rural. In towns, *Ae. aegypti* is the main vector and colonizes water storage containers and small temporary breeding sites leading to mosquito proliferation during the rainy season. In the outskirts and rural areas, *Ae. aegypti* is associated with *Ae. albopictus*, which is generally more abundant. Introduced in Vietnam in 1915 (Stanton, 1920), *Ae. aegypti* contributes to maintain hyperendemicity in most cities in tropical Asia. *Ae. aegypti*, with its limited spread, is known to be genetically structured and to react differently to infection by a pathogen (Tabachnick, 1991). In Vietnam, dengue incidence varies from one region to another (*eg* 1996-1997: 62.2-70% of DHF cases were recorded in South Vietnam, 2-8.5% in the

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north (Do Quang Ha, personal communication)). Furthermore, dengue serotypes varied from one outbreak to another. Serotype 3 appeared only in 1995, coinciding with an increase in DHF cases and deaths. Since 1997, serotype 4 was isolated in South Vietnam after six years of silence. The number of DHF cases is also subject to seasonal variations, with a maximum of cases in the rainy season (16,000 to 18,000 cases per month) and a minimum during the dry season (less than 2,000 cases per month).

In order to assess the role of *Ae. aegypti* in the dynamic of dengue transmission in Ho Chi Minh City, we studied: (1) the species ecology, (2) genetic differentiation, (3) variability in competence towards dengue 2, and (4) resistance to insecticides.

MATERIALS AND METHODS

Mosquito samplings

Mosquito sampling was carried out by catching adults outside houses for 15 minutes and by collecting larvae in domestic containers. In Ho Chi Minh City and in some provinces, 30 houses were sampled every 15 days.

Genetic structure

The genetic structure of the populations can be analyzed by studying the distribution of genetic variations. The F_{ST} parameter was used to estimate genetic differentiation. F_{ST} varies from 0 (no differentiation) to 1 (maximal differentiation) (Wright, 1931). Twenty mosquito samples were collected from February to May 1998: 9 from the center of Ho Chi Minh City and 11 from the outskirts. F0 adults were subjected to isoenzyme analysis (seven enzyme systems revealed) by starch gel electrophoresis (Pasteur *et al*, 1987). Only four loci were sufficiently polymorphic to be considered (Tien *et al*, 1999).

Vectorial competence

F1 generations of *Ae. aegypti* were subjected to oral infection with dengue 2 virus. Fourteen days after the infecting meal, surviving females were tested for the presence of dengue virus by indirect fluorescent assay on head squashes (Vazeille-Falcoz *et al*, 1999). Infection rates were compared according to mosquito geographical location.

Insecticides resistance

The resistance rate is evaluated using toxicological tests, which consist of applying increasing doses of permethrin to a mosquito population (L4 larvae) as described by WHO (1975). *Ae. aegypti* ROCK, collected before the use of organophosphorus insecticides, was used as a sensitive reference. Comparison of the mortality curves obtained allows definition of the resistance ratio.

RESULTS

Ecology of *Ae. degypti* in south Vietnam

Distribution: Between 1975 and 1998, 35.7% of caught adults and 93.3% of larvae collected in Ho Chi Minh City (Table 1), were *Ae. aegypti*. Two other species, *Ae. albopictus* and *Culex quinquefasciatus*, were also found. *Cx. quinquefasciatus* larvae were rarely present in big water jars because of the low organic content.

Seasonal variations: *Ae. aegypti* densities fluctuate according to season. During the dry season, the density is low (a mean number of 1-2 females caught per house). At the beginning of the rainy season (when the breeding sites are being filled up with rainwater), the density increases gradually to reach a maximum in June and July (3-4 females per house) (Table 2).

Ecological preferences - Breeding sites: In towns, 90% of *Ae. aegypti* breeding sites are jars and cisterns used for water storage (Table 3). However, in the center of Ho Chi Minh City, the water supply tends to limit the use of jars. Therefore, the most frequent breeding sites are temporary ones composed of discarded plastic containers, bowls used as ant traps and flower vases. In the surrounding provinces, or on the outskirts, the lack of a

Table 1
Percentage of adult mosquitos caught inside houses and larvae collected in Ho Chi Minh City.

Year	*Ae. aegypti*		*Ae. albopictus*		*Cx. quinquefasciatus*	
	Adults	Larvae	Adults	Larvae	Adults	Larvae
1975-1978	48.7	96.0	0.6	1.1	50.0	2.4
1979	37.2	99.5	0.3	0.08	62.4	0.4
1980	53.5	99.2	0.3	0.7	46.2	0
1982	30.9	100.0	0.6	-	68.5	-
1983	36.3	99.5	0.1	0.3	63.2	0.2
1984	22.5	98.9	0.05	0.7	75.3	0.4
1985	36.6	84.4	0.4	10.0	62.9	5.6
1986	40.6	94.0	0.67	4.9	58.7	0.7
1987	28.6	99.0	0.01	0.02	71.3	1.0
1988	18.8	98.8	0.01	1.1	81	0.2
1989	30.3	97.8	0	0.7	67.2	1.3
1990	24.6	96.2	0.1	3.8	75.2	0
1991	16.0	83.3	0.2	6.7	82.8	1.0
1992	21.8	-	0.02	-	77.9	-
1993	26.0	60.1	0	1.1	73.6	38.6
1994	68.9	-	0	-	29.5	-
1995	49.7	-	0	-	47.7	-
1996	49.8	-	0	-	46.6	-
1997	34.9	-	0	-	65.0	-
1998	37.8	-	0	-	62.0	-
Mean	35.7	93.3	0.2	2.4	63.3	4.0

Table 2
Seasonal variations of *Ae. aegypti* densities in Binh Duong and Long An Provinces in 1998 (mean number of females caught per house).

Month	Province (district)		
	Binh Duong (Thuan An)	Long An (Can Duoc)	Long An (Moc Hoa)
January	0.2	0.4	0.4
February	0.4	1.0	1.1
March	1.3	3.0	0.2
April	1.7	2.3	0
May	1.6	1.1	0.7
June	1.8	2.3	0.8
July	1.7	3.7	3.0
August	1.4	1.8	1.2
September	0.8	1.5	1.0
October	0.6	1.3	2.3
November	-	0.8	0.4
December	-	0.7	0

Table 3
Aedes aegypti breeding sites: percentage of different types of water storage containers in South Vietnam.

	Province (Village)				
	Ho-Chi-Minh City Banlieue-(Hung Long)	Long An (Nhi Thanh)	Kien Giang (Nam Yen)	Kien Giang (Van Khanh)	An Giang (Binh Long)
Year	1996	1993	1998	1998	1994
Big jar	77	77	74.5	84	28
Small jar	12	12	0.8	0.7	66
Cistern	9	4	14.7	10.1	4
Other	2	7	10.0	5.2	2

Table 4
Population differentiation of *Ae. aegypti* in Ho Chi Minh City revealed by analysis of isoenzyme polymorphism.

Comparison	No. of samples analyzed	F_{ST} (total)	Probability of homogeneity
Samples	20	+0.099	$<10^{-6}$
City center	9	+0.071	$<10^{-6}$
Outskirts	11	+0.125	$<10^{-6}$
Cu Chi	2	+0.033	0.0001
Hoc Mon	2	+0.104	$<10^{-6}$
Binh Chanh	5	+0.167	$<10^{-6}$
Nha Be	2	+0.051	0.059

F_{ST}: Fixation index.

piped water supply forces people to store drinking water in uncovered jars.

Genetic structure

The results highlight two areas (Table 4): (1) the center of Ho Chi Minh City with highly differentiated populations (F_{ST}=+0.071, P< 10^{-6}) and (2) the outskirts with globally highly-differentiated mosquitos; however, two districts present less differentiated populations (Cu Chi: F_{ST}=+0.033; Nha Be: F_{ST}=+0.051). Despite geographic distances separating them, populations from the outskirts are less differentiated (*ie*, have a higher dispersion rate) than mosquitos from the center of Ho Chi Minh City.

Vectorial competence

Populations from the center had differentiated infection rates (p=0.001), whereas samples from the outskirts were not differentiated (p=0.35). These results (Table 5) emphasize the impact of vector ecology and, indirectly, ecological disturbances due to human activities on vectorial competence (Tien *et al*, 1999).

Resistance to insecticides

When comparing permethrin doses that cause 50% mortality in assays compared with the reference, we observed that mosquitos in the center of Ho Chi Minh City had higher resistance ratios than mosquitos in the outskirts and in Long An Province (Table 6). As *Ae. aegypti* is an endophagic and endophilic mosquito, the common use of agricultural insecticides in provinces has a low impact on its populations. However, in Nha Be Province,

Table 5
Infection rates for dengue 2 virus of *Ae. aegypti* collected in Ho Chi Minh City. F1 females were subjected to oral infection.

Sample deviation	% infected females (N)		P (standard deviation)
	Assay	Control	
City center			
PHU	99.1 (114)	100 (51)	1 (0)
	96.5 (145)	100 (50)	0.335 (0.003)
KHIE	92.2 (179)	96.1 (52)	0.533 (0.005)
ARR	94.5 (55)	90.8 (65)	0.504 (0.004)
KHA	92.6 (68)	94.7 (38)	1 (0)
QUA	88.8 (143)	93.7 (80)	0.333 (0.005)
THU	98.7 (149)	87.5 (32)	0.010[a] (0.001)
BIH	98.5 (66)	93.7 (80)	0.225 (0.003)
THA	92.3 (39)	93.7 (80)	0.718 (0.003)
Total			0.001[a]
Outskirts			
NHA	96.8 (62)	100 (51)	0.503 (0.003)
	96.5 (86)	100 (50)	0.294 (0.003)
NBE	93.5 (92)	94.7 (38)	1 (0)
BIN	100 (18)	90.8 (65)	0.332 (0.003)
CHI	98.8 (163)	90.8 (65)	0.009 (0.001)
BCH	100 (36)	87.5 (32)	0.05 (0.001)
LOI	100 (20)	93.7 (80)	0.579 (0.002)
HAI	96 (50)	87.5 (32)	0.206 (0.003)
Total			0.035

P: Probability of homogeneity (Fisher exact test).
[a]significant p-values (<0.05).
Control corresponds to *Ae. aegypti* Paea strain (Tahiti, French Polynesia).

described as a malaria area, people use permethrin-impregnated nets; indoor mosquitos tend to be resistant to insecticides.

DISCUSSION

The emergence of DHF in the 1950s upset the dengue epidemiological profile. Various factors, viral, vectorial and human, may contribute to maintaining an outbreak. It is now clear that the change in the pattern of dengue transmission are partly related to replacement of the endemic vector. Before the 1950s, *Ae. albopictus* was the main vector of dengue viruses in Southeast Asia. It colonized natural as well as artificial breeding sites, and was responsible of local outbreaks in rural areas. It was not a good vector in urban areas. In 1913, *Ae. aegypti* was first described in Hué (Bernard and Bauche, 1913) and later, in Saigon (Stanton, 1920; Pirot, 1926). Ecological and demographical changes occurring after the Vietnam War (1959-1973) favored the proliferation of domestic mosquitos. Anthropophilic, endophagic and perfectly adapted to human housing, *Ae. aegypti* has progressively replaced *Ae. albopictus* in urban areas.

Our studies contribute to highlighting the role of human activities in *Ae. aegypti* genetic structuring and, therefore, in dengue epidemiological changes. In densely populated towns, *Ae. aegypti* colonizes numerous temporary breeding sites in the vicinity of high human densities favoring the persistence of geneti-

Table 6
Sensitivity of *Aedes aegypti* to permethrin in South Vietnam (December 1999).

Insecticide	Permethrin	
	LC50	RR50
Ho Chi Minh City		
City center		
HCM 1	0.0050	1.4
HCM 2	0.0122	3.4
HCM 3	0.0135	3.8
Outskirts		
Bin 1	0.0048	1.4
Nha Be 1	0.0186	5.3
Nha Be 2	0.0099	2.9
Outside Ho Chi Minh City		
Long An 1	0.0075	2.1
Long An 2	0.0100	2.9
Long An 3	0.0037	1.0

LC50: concentration required to kill 50% of tested larvae.
RR50: resistance ratio: LC50 (assay) / LC50 (control).

cally isolated populations. In the outskirts, mosquitos colonize larger breeding sites filled with water throughout the year. They are larvae-productive throughout the year, even during the dry season, and are less subjected to insecticidal treatments.

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