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GENETIC DIFFERENTIATION OF *AEDES AEGYPTI,* THE VECTOR OF DENGUE VIRUS IN FRENCH POLYNESIA

ANNA-BELLA FAILLOUX,[1,2] HELENE DARIUS[1] AND NICOLE PASTEUR[3]

ABSTRACT. In recent years the incidence of dengue fever epidemics has increased and transmission has tended to be established over a geographically expanding area, including French Polynesia. An increase in air transportation contributes to the diffusion of the dengue virus from Southeast Asia, a region considered to be a hyperendemic dengue zone, to the Pacific region. Presently, little is known about the role of the vector (*Aedes aegypti*) in the diffusion of the dengue fever virus. A study on the genetic structure of vector populations was conducted using allozyme polymorphism. This study showed a low level of genetic exchange between mosquito populations on different islands. It is concluded that the occurrence of dengue hemorrhagic fever in French Polynesia during the last few years was likely due to the dispersal of the dengue virus *via* viremic people rather than *via* infected vectors.

INTRODUCTION

During the last 2 centuries, dengue outbreaks occurred at intervals of a few decades in Asia and the Americas (Monath 1994). The rate of spread of the dengue virus was relatively slow until World War II. Rapid postwar population growth and urbanization in developing countries has created situations that are highly favorable to the range expansion of the dengue vector, *Aedes aegypti* (Linn.). The recent increase in air transportation contributes to the movement of viremic people from Southeast Asia, which is considered to be a hyperendemic dengue zone.

In French Polynesia, the interepidemic periods have shortened since 1963, coincidental to the establishment of an international airport in Tahiti. Four dengue virus serotypes occurred in epidemics during the last 50 years: dengue 1 in 1944 and 1975–76, dengue 2 in 1971, dengue 3 in 1964–69, and dengue 4 in 1979. From 1979 to 1988, serotype 4 was endemic (Chungue et al. 1989). An unusually severe manifestation of dengue hemorrhagic fever (DHF) due to serotype 3 developed between September 1989 and February 1990. Out of the 36,330 recorded cases, 213 presented severe clinical manifestations (about 70% concerning children under 15 years of age) and 7 patients died (Chungue et al. 1990).

Comparisons of dengue virus DNA sequences indicated that each serotype can be separated into multiple subclasses with distinct evolutionary lineages (Lanciotti et al. 1994). The present geographic distribution of dengue virus serotypes can only be explained by wide circulation of the viruses *via* infected humans (Gubler and Trent 1994) or vectors.

This study was undertaken to investigate the role of the vector in the changing pattern of the disease in French Polynesia. We have analyzed the genetic structure of *Ae. aegypti* in an attempt to analyze the pattern of gene flow between Polynesian islands.

MATERIALS AND METHODS

Mosquitoes: Aedes aegypti females were collected on 4 islands of the Society Archipelago (Tahiti, Huahine, Raiatea, and Bora-Bora), 2 islands of the Tuamotu Archipelago (Rangiroa and Aratika), and one island of the Austral Archipelago (Tubuai), using human baits. They were stored in liquid nitrogen until used.

Electrophoresis: Enzyme polymorphism was studied by starch gel electrophoresis as described by Pasteur et al. (1988), using TME 7.4 (Tris-Maleate-EDTA) buffer systems. Electrophoretic variation was recorded for 3 esterase (EC 3.1.1.1.) loci (namely *Est1, Est2,* and *Est3,* in order of decreasing mobility of their allozymes), 2 glutamate-oxaloacetate transaminase (EC 2.6.1.1.) loci (*Got1* with allozymes presenting a positive migration, and *Got2* with allozymes presenting a negative migration), and a phosphoglucomutase (EC 2.7.5.1.) locus (*Pgm*). From the staining properties of their allozymes in the presence of both alpha- and beta-naphthyl acetates, our *Est1* locus most probably corresponds to the *Est6* locus studied by previous authors (Saul et al. 1976).

Statistical analyses: Hardy-Weinberg proportions, population differentiation, and genotypic linkage disequilibrium were tested using GENEPOP (version 2) software (Raymond and Rousset 1995a). The significance level for each test was adjusted to take into account the other tests

[1] Institut Territorial de Recherches Médicales Louis Malardé, B.P. 30, Papeete, Tahiti, Polynésie Française.

[2] Present address: Institut Pasteur, Unité d'Ecologie des Systèmes Vectoriels, 25 rue du Dr. Roux, 75724 Paris 15 France.

[3] Institut des Sciences de l'Evolution (CNRS, URA 327), Laboratoire de Génétique et Environnement, Université de Montpellier II (CC 65), 34095 Montpellier, France.

Table 1. Allelic frequencies at the *Est1, Est2, Est3,* and *Pgm* loci observed in *Aedes aegypti* samples from different French Polynesian islands. *Got1* and *Got2* loci were monomorphic for the same allele in all samples except in the Aratika sample where a rare allele (frequency = 0.03) was observed at the *Got1* locus and in one Tahiti sample (1).

	Society Archipelago						
	Tahiti			Huahine			
Locus	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Raiatea	Bora Bora
Est1							
n	30	28	29	15	28	46	5
A	0.07	0.11	0.10	0.43	0.63	0.25	0.4
B	0.03	0.00	0.00	0.00	0.05	0.00	0.00
C	0.28	0.09	0.22	0.00	0.00	0.00	0.00
D	0.38	0.34	0.38	0.37	0.27	0.39	0.4
E	0.23	0.47	0.29	0.20	0.05	0.36	0.20
(Fis)	+0.03	+0.41	+0.19	−0.01	+0.41	+0.21	+0.47
(*P*)[1]	0.44	0.008	0.42	0.49	0.003	0.02	0.16
Est2							
n	29	27	31	27	28	25	5
A	0.90	0.46	0.89	0.63	0.61	0.22	0.4
B	0.00	0.00	0.00	0.24	0.25	0.00	0.00
C	0.09	0.16	0.03	0.09	0.14	0.48	0.6
D	0.00	0.00	0.00	0.00	0.00	0.30	0.00
E	0.00	0.37	0.06	0.04	0.00	0.00	0.00
F	0.02	0.00	0.00	0.00	0.00	0.00	0.00
G	0.00	0.00	0.02	0.00	0.00	0.00	0.00
(Fis)	−0.37	+0.89	−0.08	+0.26	−0.09	+0.07	+0.27
(*P*)	1.00	0.51	0.73	0.006	0.71	0.22	0.62
Est3							
n	30	18	32	27	30	43	5
A	0.77	0.36	0.73	0.41	0.65	0.62	0.50
B	0.00	0.00	0.00	0.57	0.25	0.15	0.30
C	0.23	0.00	0.11	0.00	0.00	0.00	0.00
D	0.00	0.00	0.00	0.02	0.10	0.23	0.20
E	0.00	0.11	0.05	0.00	0.00	0.00	0.00
F	0.00	0.50	0.00	0.00	0.00	0.00	0.00
G	0.00	0.06	0.11	0.00	0.00	0.00	0.00
(Fis)	−0.29	+0.58	+0.15	+0.14	+0.16	+0.41	+0.73
(*P*)	1.00	0.093	0.094	0.28	0.27	$\underline{<10^{-3}}$	0.05
Pgm							
n	30	33	32	28	30	46	5
A	0.92	0.94	0.94	0.87	0.82	0.53	1.0
B	0.00	0.00	0.03	0.04	0.07	0.10	0.00
C	0.00	0.00	0.00	0.00	0.00	0.04	0.00
D	0.08	0.06	0.03	0.09	0.12	0.33	0.00
(Fis)	−0.07	−0.05	−0.03	−0.09	+0.27	+0.17	—
(*P*)	1.00	1.00	1.00	1.00	0.04	0.012	—
All							
(Fis)	−0.13	+0.31	+0.20	+0.11	+0.18	+0.23	+0.51
(*P*)	0.40	0.001	0.60	0.02	0.006	$\underline{<10^{-4}}$	0.006

[1] Probability testing the significance of heterozygote deficit (when Fis > 0). The underlined characters indicate values that remained significant when taking into account multiple tests at the locus.

Table 1. Extended.

Tuamotu Archipelago		Austral Archipelago	
Rangiroa	Aratika	Tubuai	All
4	21	27	
0.50	0.12	0.46	
0.00	0.00	0.00	
0.00	0.00	0.00	
0.25	0.83	0.46	
0.25	0.05	0.07	
+1	+0.52	+0.55	+0.26
0.03	0.03	$<10^{-3}$	0.00
4	17	21	
1.00	0.41	0.52	
0.00	0.15	0.00	
0.00	0.44	0.48	
0.00	0.00	0.00	
0.00	0.00	0.00	
0.00	0.00	0.00	
0.00	0.00	0.00	
—	−0.02	+0.07	−0.01
—	0.63	0.22	0.35
4	23	27	
0.50	0.61	0.87	
0.12	0.15	0.00	
0.00	0.02	0.00	
0.37	0.22	0.13	
0.00	0.00	0.00	
0.00	0.00	0.00	
0.00	0.00	0.00	
+0.67	+0.17	−0.13	+0.20
$<10^{-4}$	0.12	1.00	0.001
4	30	30	
0.75	0.87	0.63	
0.00	0.00	0.00	
0.00	0.02	0.00	
0.25	0.12	0.37	
−0.20	+0.02	+0.30	+0.13
1.00	0.41	0.18	0.02
+0.63	+0.19	+0.28	+0.16
0.004	0.001	0.002	$<10^{-5}$

using the sequential Bonferroni method as described by Holm (1979). The overall significance of multiple tests for each locus was estimated by Fisher's combined probability test as described by Raymond and Rousset (1995a). The Fis and Fst values were estimated according to Weir and Cockerham (1984). The number of effective migrants per generation (Nm) was estimated from the F statistics of each locus according to the equation Nm = (1/Fst − 1)/4 (Wright 1969). This formula assumes the neutrality of the polymorphic genes and an island model of migration (Hartl and Clark 1989).

RESULTS

Among the 6 loci examined, *Est1, Est2, Est3,* and *Pgm* were polymorphic for 4–7 alleles (Table 1), *Got1* was polymorphic for a rare allele in a single population (Aratika), and *Got2* was monomorphic in all samples except in one Tahiti sample. *Got1* and *Got2* will not be considered further. Significant ($P < 0.05$) deviations from Hardy-Weinberg expectations were observed in one or more populations at all loci and they always corresponded to heterozygote deficits. When taking into account multiple tests (Bonferroni sequential test, Holm 1979), only 3 deviations remained significant at the 5% level (at the *Est1* locus on Tubuai, and at the *Est3* locus on Raiatea and Rangiroa). Over all samples, *Est1, Est3,* and *Pgm* loci displayed a heterozygote deficit (Fis = +0.26, +0.20, and +0.13, respectively) that was significant ($P < 0.05$).

Possible nonrandom allelic associations between pairs of polymorphic loci were tested using Fisher's exact test on contingency tables (Raymond and Rousset 1995b), considering each sample independently. The random association of alleles was rejected ($P < 0.05$) when taking into account multiple tests for each pair of loci (Bonferroni sequential test, Holm 1979) between alleles of the *Est2* and *Pgm* loci in the sample from Raiatea. In this case, the linkage disequilibrium corresponded to a preferential association of the Pgm^{D} and $Est2^{A}$ alleles. To determine whether nonrandom associations were due to selection or genetic drift, the total linkage disequilibrium (Dit) was divided into 4 components following Ohta (1982): Dis and D'is correspond to the part created within populations, and Dst and D'st correspond to the part created between populations. For all pairs of loci Dis was lower than Dst and D'is was larger than D'st, indicating (Table 2) that the nonrandom gametic associations observed were due to genetic drift rather than to selection (Ohta 1982).

Genetic differentiation (Table 3), tested over all loci, was significant ($P < 0.01$) when con-

Table 2. Ohta's D statistics values (1982) computed for all pairs of loci.

Loci comparison	Within population		Between populations		Total population
	Dis	D'is	Dst	D'st	Dit
Pgm–Est1	0.00991	0.32058	0.07503	0.00314	0.32373
Pgm–Est2	0.02735	0.34252	0.10330	0.01489	0.35741
Pgm–Est3	0.00668	0.43820	0.11000	0.00275	0.44095
Pgm–Got1	0.00028	0.16010	0.03600	0.00002	0.16012
Pgm–Got2	0.00002	0.15673	0.03521	0.00003	0.15676
Est1–Est2	0.02356	0.35451	0.08187	0.01507	0.36958
Est1–Est3	0.03338	0.30544	0.08016	0.00874	0.31417
Est1–Got1	0.00000	0.35334	0.09046	0.00000	0.35334
Est1–Got2	0.00037	0.35436	0.08733	0.00012	0.35448
Est2–Est3	0.02567	0.47900	0.11977	0.01142	0.49042
Est2–Got1	0.00000	0.41273	0.12611	0.00000	0.41273
Est2–Got2	0.00181	0.41609	0.12303	0.00019	0.41628
Est3–Got1	0.00000	0.42142	0.12425	0.00000	0.42142
Est3–Got2	0.00066	0.41224	0.11887	0.00026	0.41250
Got1–Got2	0.00000	0.00215	0.00049	0.00000	0.00215

sidering all samples, the 3 Tahiti samples or the 2 Huahine samples, or when considering groups of samples to test differentiation between islands, or groups of islands. In order to analyze this differentiation, genetic exchanges within the islands of Tahiti and Huahine or between islands or groups of islands were estimated by computing the number of effective migrants (Nm) from the Slatkin's private allele method (Barton and Slatkin 1986), and from the Fst estimates computed according to Weir and Cockerham (1984). Within islands, Nm values were similar on Huahine and Tahiti using Slatkin's method and higher on the small island of Huahine (75 km^2) than on the large island of Tahiti (1,042 km^2) with the Fst method (Nm = 6 and 1.9, respectively). Between islands, when considering all islands or islands from the Society or Tuamotu archipelagoes, Nm values were between 1.1 and 1.6 with the Fst method, and between 0.3 and 0.5 with the Slatkin's method. Isolation by distance, suggested by the negative slope (−0.053) obtained in computing the regression slope between Nm and geographic distances, both expressed in logarithms (Slatkin 1993), was not confirmed statistically when computing rank correlation between Fst estimates and distances ($\rho = -0.12$; Mantel test: $P > 0.66$; see Pasteur et al. 1995).

DISCUSSION

Our study of the genetic structure of *Ae. aegypti* populations using electrophoretic polymor-

Table 3. Population structure of *Aedes aegypti* populations in French Polynesia.

Comparison	n[1]	Fst				
		Est1	*Est2*	*Est3*	*Pgm*	Total[2]
Between samples						
All samples	10	0.118	0.206	0.155	0.116	0.150
Tahiti	3	0.015	0.216	0.192	−0.006	0.114
Huahine	2	0.028	−0.016	0.127	−0.010	0.040
Between islands						
All islands	7	0.126	0.184	0.100	0.135	0.135
Society Archipelago	4	0.103	0.209	0.109	0.169	0.141
Tuamotu Archipelago	2	0.372	0.316	−0.063	−0.008	0.187

[1] Number of samples considered.
[2] Total refers to the multiloci estimates.
[3] Number of effective migrants per generation estimated from Fst and Slatkin private alleles.

phism disclosed a significant differentiation among samples collected on the same island (Tahiti or Huahine) or on different islands of French Polynesia. Genetic exchanges, estimated by the number of effective migrants (Nm) using 2 methods, appeared higher within than between islands. They were uniformly low between islands and independent of geographic distances. These results are very similar to those obtained by Tabachnick and Wallis (1985) in the Caribbean, although they were based on different loci except for *Pgm*. It is worth noting that Fst estimates over all samples are similar in the 2 studies at the *Pgm* locus (0.096 in the Caribbean and 0.116 in the present study) as well as over all loci (0.154 in the Caribbean and 0.150 in French Polynesia). As observed in French Polynesia, genetic differentiation based on Fst estimates tended to be higher on large islands than on small ones, an observation that Tabachnick and Wallis (1985) attributed to a greater heterogeneity of habitats. These authors also observed that Fst estimates were higher on islands with an efficient *Ae. aegypti* control program than on those with poor control. For Tabachnik and Wallis, the absence of organized genetic structure of *Ae. aegypti* on the Caribbean islands was the direct result of the recent species history, which has been dominated by human efforts to control it and has resulted in many islands having periods of reduced population size, followed by population increase when control efforts were relaxed.

In French Polynesia, *Ae. aegypti* control is done by the Public Health Service, which routinely controls mosquitoes in the vicinity of the International Airport and in urban areas on the island of Tahiti. This control is intensive during dengue fever outbreaks. On other islands, control by the Public Health Service is based on specific demands by the districts, and is mostly limited to periods of dengue fever outbreaks and to the islands frequented by tourists in the Society Archipelago (i.e., Moorea, and to a lesser extent Raiatea and Bora-Bora). It is possible that, as proposed for the Caribbean, the higher Fst estimates observed in Tahiti as compared to Huahine are due to the different regimes of control on the 2 islands. However, we think that outside Tahiti, control is not sufficient to explain the high genetic differentiation observed between islands, and that it is related to a low degree of gene flow, itself associated with a low level of migration. The similarity of Nm values observed between islands of the Society Archipelago and between islands of the Tuamotu Archipelago further suggests that unintentional transport of *Ae. aegypti* is equivalent in the 2 groups of islands despite the large existing differences of air and maritime travel. Commercial exchanges by both airplanes and boats are extensive among islands of the Society Archipelago studied here, but are very scarce between the 2 islands of the Tuamotu Archipelago considered. Aratika has no airport, and is visited by a single scheduled boat every month. Although Nm estimates concern only migrants that reproduce, these results suggest that the circulation of the dengue virus between French Polynesian islands is not explained by the migration of infected *Ae. aegypti,* and that most probably the disease is spread by infected people who travel from island to island. It thus appears that the changes in dengue fever epidemiology that have recently occurred in French Polynesia are related to increased movements of dengue virus carried by viremic people and are associated with the growth of both local and international air travel following the development of airports (between 1964 and 1979 the number of domestic passen-

Table 3. Extended.

Probability of homogeneity					Nm[3] from	
Est1	*Est2*	*Est3*	*Pgm*	Total[2]	Fst	Private alleles
$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-5}$	1.4	0.5
0.06	$<10^{-4}$	$<10^{-4}$	0.33	$<10^{-5}$	1.9	3.9
0.06	0.55	$<10^{-3}$	0.74	0.002	6.0	2.4
$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-5}$	1.6	0.4
$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-5}$	1.5	0.3
0.002	0.012	0.79	0.39	0.01	1.1	0.5

gers grew from 45,000 to almost 500,000 per year and has remained around 400,000 since then [Atlas de Polynésie française 1993]). Within each island, an increase in *Ae. aegypti* densities, related to an enhanced use of nonbiodegradable containers, may also contribute to the local explosion of dengue fever.

In view of the importance of dengue epidemics in French Polynesia, planning of dengue prevention might be helped by investigations aimed at determining whether the strong genetic differentiation observed among *Ae. aegypti* populations from different islands is associated with different abilities to harbor and transmit the dengue virus.

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