Population structure of *Aedes albopictus* from La Réunion Island (Indian Ocean) with respect to susceptibility to a dengue virus

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Ten F_1 *Aedes albopictus* samples collected from Réunion Island in the Indian Ocean were tested for oral susceptibility to dengue 2 virus and 20 were analysed for genetic polymorphism by starch gel electrophoresis. Data from infection rates defined two distinct geographical areas: east coast vs. west coast. Genetic differentiation was found to be dependent on ecological factors and the biological characteristics of *Ae. albopictus*. These results have implications for the vector ecology and pattern of migration, and have importance in the understanding of dengue transmission.

Keywords: *Aedes albopictus*, dengue, experimental infection, genetic differentiation, La Réunion Island, vector competence.

Introduction

Aedes albopictus (Skuse), the Asian 'tiger mosquito', is considered to be a vector of dengue viruses, the most important arthropod-borne viruses transmitted to humans by mosquitoes. This species seems to have originated in the forests of South-East Asia (Smith, 1956). Its ability to colonize diverse ecological settings may account for its wide distribution; it has spread as far west as Madagascar and the Seychelles and east through the Indomalayan and Oriental regions. However, changes in the urban environment due to human activity may drastically alter the population density of this species. Intensive urbanization created environmental conditions inappropriate for its proliferation. *Ae. albopictus* is commonly found in urban, rural and forested areas, and seems to cluster in and around parks, residential gardens and cemeteries. However, *Ae. albopictus* may be absent from densely crowded cities, which lack vegetation and outdoor breeding sites (Rudnick & Hammon, 1960). *Aedes aegypti*, another vector of dengue viruses, has evolved in close association with man. In contrast, *Ae. albopictus*, a species without ecological specialization, may be involved in the transfer of enzootic forest viruses into inhabited areas. Estimation of differentiation with respect to vector capacity has provided insight into

epidemiological aspects of the disease and can be used to improve disease control (Tran Khanh *et al.*, 1999; Vazeille-Falcoz *et al.*, 1999; Paupy *et al.*, 2000).

Dengue is caused by four serotypes, DEN-1, DEN-2, DEN-3, and DEN-4, which are usually maintained in an urban transmission cycle by *Ae. aegypti*. Since World War II, dengue epidemics have become more frequent and widespread and South-East Asia has experienced large urban dengue haemorrhagic fever (DHF) epidemics. In the last 30 years, the pattern of disease has changed, and the incidence of epidemic DHF has increased in most tropical countries. *Ae. albopictus* has been shown to be responsible for dengue fever in epidemics in South-East Asia (Russel *et al.*, 1969), in southern China (Qiu *et al.*, 1981), in Japan (Sabin, 1952) and in the Seychelles (Metselaar *et al.*, 1980).

In the islands of the south-west Indian Ocean, dengue-like epidemics have been reported since 1872 (Cotholendy, 1873; Pougnet, 1890; MacCarthy & Brent, 1946). Dengue outbreaks were observed in the Seychelles and Réunion between 1976 and 1979 (Metselaar *et al.*, 1980; Coulanges *et al.*, 1979) and in the Comoro Islands in 1984 and 1993 (Boisier *et al.*, 1994). Epidemics due to *Ae. aegypti* have also been detected in East Africa: in Kenya (Johnson *et al.*, 1982), Sudan (Hyams *et al.*, 1986), Somalia (Saleh *et al.*, 1985; Kanesa-Thasan *et al.*, 1994), Djibouti (Rodier *et al.*, 1996) and Mozambique (Gubler *et al.*, 1986). The increase in air travel

since World War II is one of the factors known to be involved in the worldwide resurgence of dengue fever (DF) and the emergence of DHF (Gubler, 1997). The dengue virus is readily disseminated from a dengue-endemic area by competent mosquitoes and/or by dengue-infected travellers. The developing tourism industry in the islands of the south-west Indian Ocean is increasing travel between south-east Asia and Africa, thereby increasing the risk of introducing dengue viruses into dengue-free regions such as Réunion.

The island of Réunion, an overseas administrative region of France, lies in the Indian Ocean about 800 km east of Madagascar. It covers an area of approximately 2500 km^2 and has about 700 000 inhabitants. There are only two distinct seasons: a cool and dry winter from May to October and a hot and rainy summer from November to April. The windward east coast region, which is wetter than the west coast region, is notably less inhabited. Both *Ae. aegypti* and *Ae. albopictus* are present on Réunion. Before the implementation of DDT treatments for malaria control, from 1949 to 1953, *Ae. aegypti* was frequent in the urban coastal area (Hamon, 1953). In contrast, *Ae. albopictus*, which was probably introduced to Réunion by immigrants from Asia in the 17th and 18th centuries, has colonized natural and urban habitats previously occupied by *Ae. aegypti* (Mattingly, 1953). Insecticide treatment, aimed at preventing mosquitoes from breeding in urban areas, has had an effect on *Ae. aegypti* populations, which persist today as residual wild populations in the west coast region (Salvan & Mouchet, 1994). The decline in *Ae. aegypti* populations was associated with *Ae. albopictus* infestation of unoccupied breeding sites. *Ae. albopictus* is now present at high densities colonizing both urban and rural biotopes. An epidemic of dengue fever started in the Seychelles in December 1976, in which dengue 2 virus and *Ae. albopictus* were implicated (Metselaar *et al.*, 1980). In 1977, the epidemic reached Réunion and was attributed to *Ae. albopictus* (Kles *et al.*, 1994); 30–35% of the island's inhabitants, most of whom were living in the east coast region, showed DF symptoms (Mora, 1979). No dengue epidemic has since been recorded on Réunion. The dramatic increase in the incidence of dengue in tropical countries especially over the last several decades may be attributed to the increase in human travel and to uncontrolled population growth and urbanization. The problem of importation of mosquitoes and dengue viruses from viremic travellers (potential source of infection for local *Ae. albopictus*) into dengue-free zones is a major concern that should receive serious attention.

Ae. albopictus and *Ae. aegypti* are both present on Réunion. As both are effective vectors for dengue viruses, it is important to estimate the competence of mosquito populations and their dispersal rate (and indirectly, virus dissemination) by analysing the genetic differentiation of mosquito populations. Such studies are necessary for risk assessment before the implementation of any control programme.

Materials and methods

Mosquito samples

Twenty *Ae. albopictus* samples were collected from Réunion in October 1999 (Table 1, Fig. 1). Larvae or pupae were maintained until the adult stage in laboratory conditions (temperature $25 \pm 1°C$, relative humidity $80 \pm 10\%$ and photoperiod 12 h). The adults (F_0 generation) obtained were allowed to blood feed on mice to enable them to produce eggs. Larvae were reared until the imago (F_1 generation). The F_0 imagoes were stored at $-80°C$ until genetic analysis. We investigated the oral susceptibility to dengue 2 virus of F_1 females. The Paea strain of *Ae. aegypti* was established from adults caught in the Paea district of Tahiti (French Polynesia), and has been maintained in our laboratory since 1994. This strain was used as a control for mosquito oral susceptibility to dengue viruses and as a mobility control for isoenzyme electrophoresis. The *Ae. albopictus* strain, Mada 15, established from a sample collected in March 1999 from Madagascar, was also used as a mobility control for isoenzyme electrophoresis.

Experimental infection of mosquitoes

For each F_1 mosquito sample, two infection assays were performed at two-day intervals, when sufficient mosquitoes were available. The 5- to 7-day-old and non-blood-fed females were starved for 24 h before infection, in small plastic containers. They were allowed to feed for 20 min on an artificial meal maintained at 37°C in glass feeders covered with chicken skin (Vazeille-Falcoz *et al.*, 1999). The infectious meal consisted of 2/3 washed rabbit erythrocytes, 1/3 virus suspension and a phagostimulant, ATP (5×10^{-3} M). The viral suspension used for feeding had a final titre of $10^{8.2}$MID$_{50}$ (50% mosquito infectious dose for *Ae. aegypti*) per mL of a dengue-2 strain isolated in 1974 from a human serum sample from Bangkok (Thailand). Viral stocks were prepared as described by Vazeille-Falcoz *et al.* (1999). Fully engorged females were incubated for 14 days and allowed to feed on a 10% sugar solution. Dengue 2 virus was detected by an indirect immunofluorescence assay (IFA) using head squashes from surviving females. The Paea strain was used as an infectivity control (Vazeille-Falcoz *et al.*, 1999). The viral titre resulting in a mean of 90% infection of the Paea strain was determined before

Table 1 Sites from which *Aedes albopictus* was sampled on Réunion in October 1999

No.	Sample	Location	Collection date	Breeding site
East coast				
1	AND	Saint André	10/08/99	Flower vase
2	BEN	Saint Benoît	10/07/99	Flower vase
3	BRU1	Grand Brûlé	10/09/99	Tyre
4	BRU2	Grand Brûlé	10/09/99	Tyre
5	GLI	Ravine Glissant	10/07/99	Tyre
6	LAM	La Marine	10/07/99	Abandoned boat
7	MAR	Sainte Marie	10/08/99	Empty oil drum
8	PAN	Bras-Panon	10/07/99	Flower vase
9	PRO	La Providence	10/11/99	Palm leaf
10	ROS	Sainte Rose	10/07/99	Flower vase
11	SUZ	Sainte Suzanne	10/08/99	Flower vase
West coast				
12	ERM	L'Ermitage	10/11/99	Tyre
13	LEU	Saint Leu	10/11/99	Rock hole
14	LOU	Saint Louis	10/07/99	Tyre
15	PAU	Saint Paul	10/08/99	Tree hole
16	PIE1	Saint Pierre	10/07/99	Tyre
17	PIE2	Saint Pierre	10/07/99	Empty oil drum
18	POR	Le Port	10/08/99	Flower vase
19	POS	La Possession	10/06/99	Rock hole
20	TAM	Le Tampon	10/09/99	Flower vase

the infection of F_1 females. This titre was determined by infecting mosquitoes with serial dilutions of the viral stock. We determined the feeding suspension virus titre that resulted in the infection of 90% of the females and this titre was used to infect field-collected mosquitoes.

Electrophoresis

Each individual mosquito was ground in 25 μL of distilled water. The sample was centrifuged (20 000 g for 4 min at 4°C) and the supernatant, which contained the soluble proteins, was subjected to starch gel electrophoresis in Tris-malate-EDTA (pH 7.4) buffer. Nine enzyme systems were studied: glutamate oxaloacetate transaminases (Got1 and Got2), glycerol-3-phosphate dehydrogenase (G-3-pdh), hexokinases (Hk1 and Hk2), malate dehydrogenase (Mdh), malate dehydrogenase (NADP) (Mdhp-1), phosphoglucoisomerase (Pgi) and phosphoglucomutase (Pgm). *Aedes aegypti* Paea and *Ae. albopictus* Mada 15 were used as controls of electrophoretic mobility. For field-collected samples, alleles were numbered according to their mobility relative to that of the most common allele obtained at each locus for the control strains.

Genetic analysis

Genetic differentiation was analysed using GENEPOP software (version 3.1), as described by Raymond &

Rousset (1995). F_{IS} and F_{ST} were calculated according to the formula of Weir & Cockerham (1984). Heterozygote deficits were tested using a exact test procedure (Rousset & Raymond, 1995). Genotypic associations between pairs of loci were tested for each sample. Genotypic differentiation was tested for each population by calculating the *P*-value of the F_{ST} estimate. The overall significance of multiple tests was estimated by Fisher's combined probability test (Fisher, 1970). The significance level of each test was adjusted for the number of tests run, using the sequential method of Bonferroni (Holm, 1979). Isolation by distance (Slatkin, 1993) was investigated in computing rank correlations between F_{ST} estimates calculated between pairs of samples and geographical distances. Partial Mantel one-sided tests in which *P*-values were determined by permutation procedures were used to estimate the significance of correlations (Smouse *et al.*, 1986; Leduc *et al.*, 1992).

Results

Mosquito infection rates

For the 10 mosquito F_1 samples tested, infection rates were between 18% ((18)POR a) and 59% ((12)ERM b), Table 2. The rates obtained were lower in each case than those obtained for the Paea control (68.5% to 95.5%). For samples with two replicates ((12)ERM (13)LEU (16)PIE1 and (18)POR), infection rates were compared,
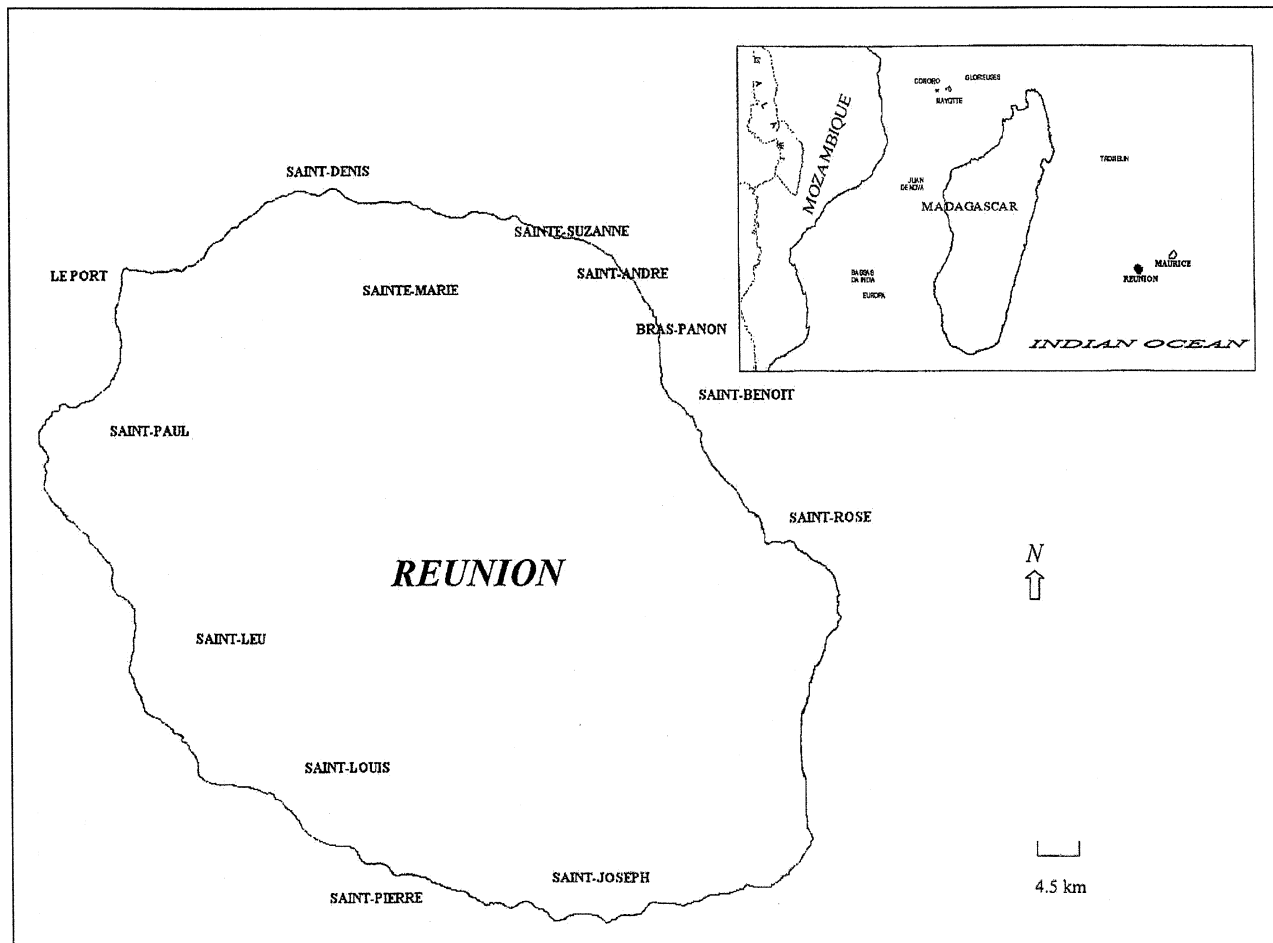
Fig. 1 Geographical locations on Réunion (Indian Ocean) from which *Aedes albopictus* was collected in October 1999.

using Fisher's exact test; *P*-values were not significant ($P > 0.05$), indicating that the replicates were homogeneous. We therefore pooled the two replicates of each sample for further analysis. The overall *P*-value obtained for analysis of all the samples was highly significant ($P < 10^{-4}$). We investigated the reasons for the observed differences in infection rate, by assigning samples to three different geographical groups: (1) the first group comprised four sites ((6)LAM (7)MAR (8)PAN and (11)SUZ) and corresponded to samples from the east coast; (2) the second group consisted of six samples ((12)ERM (13)LEU (15)PAU (16)PIE1 (18)POR and (19)POS) from the west coast; and (3) the third group representing a subgroup of the west coast corresponded to samples from the north-west coast ((12)ERM (13)LEU (15)PAU (18)POR and (19)POS). The null hypothesis of homogeneous infection rates was rejected ($P < 10^{-4}$) for the samples from the west coast and from the north-west coast, whereas the samples collected from the east coast showed no population differentiation ($P = 0.46$).

Population structure

Two of the nine loci investigated (*G-3-pdh* and *Mdhp1*) were monomorphic or displayed limited polymorphism in most samples and were therefore not used in further analyses. *Got1*, *Got2*, *Hk1*, *Hk2*, *Mdh*, *Pgi* and *Pgm* showed codominant segregation in all samples.

Genotypic associations between pairs of loci within each sample were ruled out in the total analysis for all samples, taking multiple testing into account (Bonferroni sequential test $P > 0.05$). We made 250 comparisons, and non-random associations between pairs of loci were rejected in all samples, taking multiple testing into account, except for the *Hk1–Hk2* combination for the (12)ERM and (2)BEN samples. We investigated whether non-random allelic associations between pairs of polymorphic loci were due to selection or genetic drift by breaking down the total linkage disequilibrium (D_{IT}) into four indices, as described by Ohta (1982): D_{IS} and D'_{IS} correspond to the disequilibrium created within populations, and D_{ST} and D'_{ST} correspond to the

Table 2 Infection rates with dengue 2 virus of 10 *Aedes albopictus* F$_1$ samples from Réunion

| No. | Sample | Replicate | Percentage of infected females | | Probability of homogeneity‡ |
			Assay	Control†	
East coast					
6	LAM	a	51.5 (17/33)	85.7 (30/35)	*0.002*
7	MAR	a	37.2 (19/51)	79.6 (39/49)	*< 10⁻⁴*
8	PAN	a	50.0 (15/30)	95.5 (43/45)	*< 10⁻⁴*
11	SUZ	a	52.4 (11/21)	72.2 (13/18)	0.322
West coast					
12	ERM	a	56.4 (22/39)	84.0 (42/50)	*0.008*
		b	59.1 (39/66)	91.6 (76/83)	*< 10⁻⁴*
13	LEU	a	41.2 (14/34)	88.0 (22/25)	*0.001*
		b	30.1 (19/63)	79.6 (39/49)	*< 10⁻⁴*
15	PAU	a	31.9 (26/84)	68.6 (59/86)	*< 10⁻⁴*
16	PIE1	a	36.7 (22/60)	84.0 (42/50)	*< 10⁻⁴*
		b	40.0 (22/55)	91.5 (76/83)	*< 10⁻⁴*
18	POR	a	18.0 (7/39)	88.0 (22/25)	*< 10⁻⁴*
		b	22.2 (14/63)	79.6 (39/49)	*< 10⁻⁴*
19	POS	a	38.7 (24/62)	68.6 (59/86)	*0.001*

†*Ae. aegypti* Paea strain.
‡Significant values are in italics.

disequilibrium between populations. For all pairs of loci, D_{IS} was lower than D_{ST} and D'_{IS} was larger than D'_{ST} (Table 3), indicating that the non-random genotypic associations found among *Ae. albopictus* populations were due to genetic drift rather than selection.

Hardy–Weinberg equilibrium was tested for each locus in each sample using the probability test. We tested each locus for each sample (120 probability tests); 29 tests were significant ($P < 0.05$), 20 of which were related to the *Hk1* locus (Table 4). Hardy–Weinberg equilibrium (H0: random mating) was tested, the

alternative hypothesis (H1) being heterozygote excess. The significant P (< 0.05) and F_{IS} values (close to $+1$) obtained at the *Hk1* locus for all samples indicated heterozygote excess at this locus, which was probably subject to selection. Global tests considering all populations for each locus or considering all loci for each population were also performed: significant deviation from Hardy–Weinberg equilibrium ($P < 10^{-4}$) was observed in 18 of 20 samples. If *Hk1* was excluded from the analysis, only three samples ((7)MAR (18)POR and (3)BRU1) gave significant P-values. After

Table 3 Variance components of linkage equilibrium. Ohta's statistic values estimated for all combinations of loci

| Comparison | Within sample | | Between samples | | Total D_{IT} |
	D_{IS}	D'_{IS}	D_{ST}	D'_{ST}	
PGM–PGI	0.00390	0.05706	0.01672	0.00017	0.05724
PGM–GOT1	0.00161	0.05175	0.01444	0.00001	0.05175
PGM–GOT2	0.00326	0.05906	0.01802	0.00033	0.05939
PGM–MDH	0.00722	0.05831	0.01369	0.00044	0.05876
PGM–HK1	0.00071	0.02819	0.00925	0.00003	0.02822
PGI–GOT1	0.00074	0.03626	0.01088	0.00001	0.03627
PGI–GOT2	0.00156	0.05165	0.01641	0.00006	0.05171
PGI–MDH	0.00302	0.04800	0.01376	0.00030	0.04830
PGI–HK1	0.00074	0.02025	0.00857	0.00009	0.02034
GOT1–GOT2	0.00047	0.04515	0.01229	0.00000	0.04516
GOT1–MDH	0.00110	0.04300	0.01127	0.00006	0.04306
GOT1–HK1	0.00013	0.01373	0.00559	0.00000	0.01373
GOT2–MDH	0.00269	0.05582	0.01540	0.00036	0.05618
GOT2–HK1	0.00046	0.02639	0.00922	0.00003	0.02642
MDH–HK1	0.00100	0.02280	0.00770	0.00010	0.02290

Table 4 Allelic frequencies and deviations from Hardy–Weinberg expectations observed at six loci encoding isoenzymes in 20 *Aedes albopictus* samples collected from Réunion in October 1999

Locus	Got1					Got2						Hk1					
	1	2	N	F_{IS}	P	1	2	6	N	F_{IS}	P	2	4	6	N	F_{IS}	P
1. AND	0.104	0.896	96	−0.106	—	0.000	0.979	0.021	96	−0.011	—	0.000	0.500	0.500	96	−0	$<10^{-4}$
2. BEN	0.000	1.000	32	—	—	0.000	0.875	0.125	32	−0.011	—	0.125	0.500	0.375	32	−0.67	0.0002
3. BRU1	0.000	1.000	46	—	—	0.000	0.957	0.043	46	−0.023	—	0.000	0.500	0.500	46	−1	$<10^{-4}$
4. BRU2	0.095	0.905	42	−0.081	—	0.000	0.905	0.095	42	−0.081	—	0.000	0.500	0.500	42	−1	$<10^{-4}$
5. GLI	0.000	1.000	26	—	—	0.000	0.923	0.077	26	−0.043	—	0.115	0.500	0.385	26	−0.67	0.002
6. LAM	0.021	0.979	96	−0.011	—	0.000	0.958	0.042	96	−0.033	—	0.000	0.500	0.500	96	−1	$<10^{-4}$
7. MAR	0.063	0.938	96	−0.056	—	0.000	0.948	0.052	96	−0.044	—	0.000	0.500	0.500	96	−1	$<10^{-4}$
8. PAN	0.091	0.909	44	−0.077	—	0.000	0.886	0.114	44	−0.105	—	0.000	0.500	0.500	44	−1	$<10^{-4}$
9. PRO	0.111	0.889	90	+0.111	—	0.000	0.878	0.122	90	−0.128	—	0.000	0.500	0.500	90	−1	$<10^{-4}$
10. ROS	0.038	0.962	26	—	—	0.000	0.846	0.154	26	−0.143	—	0.000	0.500	0.500	26	−1	0.0008
11. SUZ	0.073	0.927	96	−0.068	—	0.000	0.906	0.094	96	−0.093	—	0.000	0.500	0.500	96	−1	$<10^{-4}$
12. ERM	0.048	0.952	42	−0.026	—	0.024	0.952	0.024	42	−0.013	—	0.167	0.357	0.476	42	−0.60	0.0002
13. LEU	0.022	0.978	90	−0.011	—	0.000	0.844	0.156	90	−0.173	—	0.000	0.500	0.500	92	−1	$<10^{-4}$
14. LOU	0.115	0.885	96	−0.119	—	0.000	0.906	0.094	96	−0.093	—	0.000	0.500	0.500	96	−1	$<10^{-4}$
15. PAU	0.021	0.979	96	−0.011	—	0.000	0.880	0.120	92	+0.082	—	0.010	0.490	0.500	96	−0.96	$<10^{-4}$
16. PIE1	0.031	0.969	96	−0.022	—	0.000	0.969	0.031	96	−0.022	—	0.000	0.510	0.490	96	−0.96	$<10^{-4}$
17. PIE2	0.000	1.000	46	—	—	0.000	1.000	0.000	46	—	—	0.000	0.500	0.500	46	−1	$<10^{-4}$
18. POR	0.073	0.927	96	−0.068	—	0.000	0.969	0.031	96	−0.022	—	0.000	0.500	0.500	96	−1	$<10^{-4}$
19. POS	0.000	1.000	96	—	—	0.000	0.896	0.104	96	−0.106	—	0.000	0.500	0.500	96	−1	$<10^{-4}$
20. TAM	0.048	0.952	62	−0.034	—	0.000	0.661	0.339	62	+0.080	—	0.032	0.500	0.468	62	−0.88	$<10^{-4}$
All				−0.045	—					−0.057	—					−0.960	$<10^{-4}$

					N	F_{IS}	P						N	F_{IS}	P				N	F_{IS}	P	
1.	AND	0.021	0.792	0.188	96	+0.224	—	0.010	0.094	0.760	0.135	0.000	96	+0.113	—	0.031	0.917	0.052	96	−0.058	—	$<10^{-4}$
2.	BEN	0.063	0.750	0.188	32	−0.091	—	0.000	0.031	0.688	0.281	0.000	32	+0.193	—	0.125	0.844	0.031	32	−0.119	—	—
3.	BRU1	0.043	0.783	0.174	46	−0.202	—	0.048	0.095	0.524	0.333	0.000	42	−0.481	0.01	−0.000	0.957	0.043	46	+1	0.022	$<10^{-4}$
4.	BRU2	0.000	0.619	0.381	42	−0.415	—	0.048	0.000	0.762	0.190	0.000	42	+0.024	—	0.024	0.833	0.143	42	−0.148	—	0.003
5.	GLI	0.000	0.750	0.250	24	+0.294	—	0.038	0.038	0.846	0.077	0.000	26	+0.200	—	0.115	0.885	0.000	26	−0.091	—	0.044
6.	LAM	0.021	0.809	0.170	94	+0.198	—	0.000	0.073	0.729	0.198	0.000	96	+0.126	—	0.021	0.865	0.115	96	−0.123	—	$<10^{-4}$
7.	MAR	0.023	0.614	0.364	88	−0.007	—	0.021	0.094	0.740	0.146	0.000	96	−0.173	—	0.031	0.896	0.073	96	+0.355	0.02	$<10^{-4}$
8.	PAN	0.024	0.762	0.214	42	−0.003	—	0.000	0.114	0.727	0.159	0.000	44	−0.027	—	0.068	0.909	0.023	44	−0.057	—	0.006
9.	PRO	0.056	0.644	0.300	90	−0.028	—	0.044	0.000	0.789	0.167	0.000	90	+0.053	—	0.022	0.900	0.078	90	+0.163	—	$<10^{-4}$
10.	ROS	0.038	0.731	0.231	26	−0.104	—	0.000	0.154	0.615	0.231	0.000	26	−0.091	—	0.192	0.769	0.038	26	−0.210	—	—
11.	SUZ	0.031	0.656	0.313	96	−0.318	—	0.010	0.042	0.688	0.260	0.000	96	+0.100	—	0.115	0.781	0.104	96	−0.156	0.010	$<10^{-4}$
12.	ERM	0.100	0.625	0.275	40	−0.120	—	0.000	0.167	0.690	0.143	0.000	42	+0.281	—	0.214	0.762	0.024	42	−0.125	—	0.029
13.	LEU	0.023	0.640	0.337	86	−0.110	—	0.011	0.130	0.565	0.283	0.011	92	+0.265	0.007	0.033	0.924	0.043	92	−0.050	—	$<10^{-4}$
14.	LOU	0.033	0.707	0.261	92	−0.198	—	0.042	0.031	0.677	0.250	0.000	96	+0.092	—	0.021	0.938	0.042	96	−0.041	—	$<10^{-4}$
15.	PAU	0.033	0.711	0.256	90	−0.184	—	0.011	0.074	0.681	0.234	0.000	94	+0.161	0.003	0.032	0.862	0.106	94	+0.401	0.011	$<10^{-4}$
16.	PIE1	0.011	0.728	0.261	92	−0.126	—	0.042	0.063	0.813	0.083	0.000	96	+0.056	—	0.000	0.969	0.031	96	−0.022	—	$<10^{-4}$
17.	PIE2	0.000	0.696	0.304	46	−0.419	—	0.000	0.174	0.674	0.152	0.000	46	+0.314	—	0.022	0.848	0.130	46	−0.132	—	$<10^{-4}$
18.	POR	0.021	0.781	0.198	96	−0.120	—	0.073	0.104	0.667	0.156	0.000	96	−0.001	—	0.031	0.927	0.042	96	+0.254	—	$<10^{-4}$
19.	POS	0.056	0.711	0.233	90	−0.159	—	0.010	0.042	0.781	0.167	0.000	96	−0.315	0.006	0.010	0.948	0.042	96	+0.382	—	$<10^{-4}$
20.	TAM	0.145	0.532	0.323	62	−0.294	—	0.065	0.016	0.629	0.290	0.000	62	−0.110	0.003	0.097	0.903	0.000	62	−0.091	—	$<10^{-4}$
	All					−0.137	—							+0.057	$<10^{-4}$					+0.072	—	$<10^{-4}$

N, sample size.

F_{IS} is the inbreeding coefficient and measures the reduction of heterozygosity of a subpopulation due to nonrandom mating.

P is the probability for rejecting Hardy–Weinberg equilibrium when H1 = heterozygote deficit.

−, non-significant *P*-value (*P* > 0.05).

application of the Bonferroni procedure, these results were not significant. For this reason, *Hk1* was excluded from the analysis.

Significant differentiation, evaluated by estimating F_{ST}, was observed in analysis of the 20 samples ($F_{ST} = +0.019$, $P < 0.001$). We investigated the pattern of differentiation by considering various groups of samples defined on the basis of geographical and environmental factors (Table 5). The west coast of Réunion is drier and more heavily populated than the very humid east coast. The two samples from Saint-Denis, the largest town on the island ((9) PRO and (7)MAR) were not significantly differentiated ($F_{ST} = +0.001$, $P > 0.05$). If samples from the west and east coasts of Réunion (excluding (9)PRO and (7)MAR) were analysed separately, high F_{ST} values ($+0.025$ for the west coast and $+0.013$ for the east coast) with significant *P*-values ($P < 0.05$) were obtained. However, the estimated F_{ST} value for the west coast was twice that for the east coast. The relationship between gene flow and geographical distances can be examined by the rank correlation between $F_{ST}/(1 - F_{ST})$ estimates and log distance computed for all pairs of samples. This correlation was negative when considering samples from the west coast (b $= -0.005$) and positive when analysing samples from the east coast (b $= +0.0005$). However, in both cases, the correlation was not significant ($P > 0.05$) indicating that intensity of gene flow was independent of distance between samples. Samples collected from the north-west coast ($F_{ST} = +0.015$, $P < 0.001$) and the north-east coast ($F_{ST} = +0.01$, $P < 0.05$) were less differentiated than those from the south-west coast ($F_{ST} = +0.049$,

$P < 0.001$) and the south-east coast ($F_{ST} = +0.023$, $P < 0.001$).

We analysed samples from the north-west coast as a function of the ecological characteristics of the breeding sites sampled (rural vs. urban). Genetic differentiation was high ($F_{ST} = +0.025$) and significant ($P < 0.05$) for samples collected in urban areas, whereas samples from rural areas were not differentiated ($F_{ST} = +0.0009$, $P > 0.05$).

Discussion

In this study, we investigated the population structure of *Ae. albopictus* on Réunion using phenotypic (susceptibility to dengue 2 virus) and genotypic (isoenzyme polymorphism) markers. We found that extrinsic factors (climatic, geographical and human density) and intrinsic factors related to mosquito bionomics (host preference) affected the genetic structure and vector competence of *Ae. albopictus* populations.

The oral susceptibility of *Ae. albopictus* to dengue virus depends on the serotype and geographical origin of the virus (Rosen *et al.*, 1985). The vector competence of *Ae. albopictus* populations for dengue viruses varies widely (Boromisa *et al.*, 1987). In our study, the 10 populations experimentally infected with dengue 2 virus differed in infection rates. These data suggest that there is a higher level of variability in the oral susceptibility to dengue 2 virus of mosquito populations on Réunion and therefore, that there is intensive local differentiation of mosquitoes. Populations from the east coast seem to be more susceptible to dengue than those from the west coast. In addition, heterogeneity was observed in the

Table 5 Population differentiation of *Aedes albopictus* on Réunion

| Comparison | N | F_{ST} | | | | | |
		Got1	*Got2*	*Mdh*	*Pgi*	*Pgm*	Total
All samples	20	+0.019**	+0.049**	+0.011*	+0.022**	+0.013**	+0.019**
Main city (Saint Denis)	2	+0.004	+0.021	−0.005	−0.013	+0.006*	+0.001
West coast	9	+0.025*	+0.081**	+0.009*	+0.029**	+0.017**	+0.025**
North-west coast	5	+0.018*	+0.022*	+0.006	+0.034**	+0.013*	+0.015**
Urban habitat	2	−0.011	−0.011	+0.029	+0.112*	−0.006	+0.025*
Rural habitat	3	+0.001	−0.004	+0.001	+0.008	+0.022	+0.009
South-west coast	4	+0.033*	+0.184**	+0.016*	+0.032**	+0.032**	+0.049**
East coast	9	+0.019*	+0.011	+0.011	+0.019*	+0.011*	+0.013**
South-east coast	5	+0.029	+0.008	+0.017	+0.029*	+0.028*	+0.023**
North-east coast	4	+0.005	+0.021*	+0.012	+0.019	+0.002	+0.010*

F_{ST}, fixation index.
N, sample size.
*$P < 0.05$; **$P < 0.001$.

infection rates of samples from the west coast whereas those from the east coast displayed similar infection rates. During the dengue 2 outbreak in 1977–78, the more heavily populated west coast presented fewer DF cases than the east coast (Mora, 1979). Most international trade passed via the main harbour, Le Port, and the international airport at Saint-Denis. The most likely sites for the introduction of dengue viruses from dengue endemic/epidemic zones, as occurred in 1977, are therefore the harbour and the airport, where mosquitoes have been found to be less susceptible to dengue infection.

Réunion is a very hilly volcanic island. This results in climatic differences between the west and east coasts. The east coast, which is not protected by the coral barrier reef, has a narrow coastal area, limiting urban development. This coast is subjected to the trade winds, which bring large amounts of rainfall, creating numerous natural breeding sites suitable for *Ae. albopictus*. Larvae colonize cut bamboo, leaf axils, rock pools and tree holes. Genetic exchanges frequently occur between close populations and the level of population differentiation tends to be low throughout the year. This pattern of extensive dispersal of the mosquito (i.e. low differentiation) is consistent with a high level of dispersal of dengue-infected *Ae. albopictus* and thus, with a large number of human dengue cases. In the east coast region, the number of available breeding sites does not seem to be restricted but human density may affect the level of dispersal of mosquitoes, which must find hosts for blood feeding. However, if human hosts are not available, as in the uninhabited south-east region (with the volcano, Le Piton de la Fournaise), *Ae. albopictus* feeds instead on pigs, dogs and chickens (Sullivan *et al.*, 1971). This leads to a higher level of differentiation (twice that in the north-east region) expressed as the preference of *Ae. albopictus* mosquitoes for mammalian hosts other than humans. This species seems to be an opportunistic blood feeder capable of feeding on a wide variety of hosts.

The lack of precipitation on the west coast during the dry season causes the drying up of natural breeding sites. Recurrent extinction events tend to reduce intra-natural site genetic variability and increase inter-natural site variability. The presence of the coral reef in the west coast has led to intensive urbanization of this area and the development of tourism infrastructures. In urban areas, *Ae. albopictus* is able to colonize the human-built habitat, altering its natural ecology. This species is present in parks, residential gardens and cemeteries. Factors other than the ecological characteristics of the area seem to influence the distribution of *Ae. albopictus*, indicating that humans may be involved in the spread of this species. On the west coast, the availability of human hosts in which the mosquito can complete its gono-

trophic cycle reduces species dispersal. In addition, due to good sanitation conditions, which limit the number of artificial breeding sites, the main cities are unsuitable for the proliferation of *Ae. albopictus* populations. Mosquitoes tend to identify only oviposition sites similar to those on which they were born, and to lay their eggs in the same or neighbouring breeding sites. So, differentiation among populations is intense, even if populations are separated by only a few kilometres. Black *et al.* (1988a) also showed that *Ae. albopictus* samples collected from neighbouring areas within a city were 3–4 times more differentiated than populations from other areas, suggesting extensive local differentiation. Genetic drift was found to be associated with the establishment of a small number of founding individuals, in some cases subjected to insecticidal control, which is probably more common in populated areas.

The most typical larval habitats of *Ae. albopictus* appear to be man-made containers and tree-holes. In the urban environment, discarded plastic containers and tyres collect rainwater, making ideal larval habitats. On the north-west coast, the mosquito populations sampled from artificial breeding sites were more highly differentiated than those collected from natural breeding sites. In rural areas, *Ae. albopictus* breeds in a large variety of containers so the distribution of oviposition sites is considerably more continuous than that in cleaned urban areas (Black *et al.*, 1988b). This results in a higher level of differentiation in populations from urban areas.

Ae. albopictus often shows a discontinuous distribution, with localized breeding units, probably due largely to the low dispersal rate of this species (a mean distance of 104 m) (Bonnet & Worchester, 1946; Mori, 1979). Local selection and low levels of migration facilitate the emergence of genetically different populations. However, extrinsic factors, such as changes in climatic and geographical characteristics, and human density appear to modulate these patterns. To identify more clearly the factors that affect the genetic structure of *Ae. albopictus* populations, studies should be carried out on populations from isolated areas, such as islands in the Indian Ocean. The influence of humans on *Ae. albopictus* genetic structure should be studied in a geographical situation in which the physical barriers to gene flow are readily distinguishable and thus the role of human displacements in mosquito genetic exchanges could be determined.

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