Variation in the vector competence of *Aedes polynesiensis* for *Wuchereria bancrofti*

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SUMMARY

The vector competences of 6 geographic strains of *Aedes polynesiensis* for *Wuchereria bancrofti* were studied using two types of experimental infections. Experimental infection of laboratory-bred mosquitoes fed on the carriers' forearms with different levels of microfilaraemia showed that microfilariae (mf) uptake was directly proportional to the carrier's mf density and, as mf densities decreased, concentration capacity of *Ae. polynesiensis* increased. It was also shown that infection has an important effect on mosquito mortality, and that the mortality rate differed among mosquito strains. In infections using artificial feeders, the mf uptake was closely regulated, thus showing differences in the vectorial efficiency of *Ae. polynesiensis* related to the geographic origin of the mosquito strain. The mosquitoes from the Society archipelago were more efficient intermediate hosts than geographically distant strains when infected with *W. bancrofti* from an island within the archipelago (Tahiti). Mosquito strains from the Society archipelago developed the highest proportion of infective-stage larvae and exhibited the lowest mortality rate when infected with sympatric Tahitian *W. bancrofti*.

Key words: vector competence, geographic variations, filariasis, *Wuchereria bancrofti*, *Aedes polynesiensis*, experimental infections, French Polynesia.

INTRODUCTION

Wuchereria bancrofti, Cobbold, 1877 the causative agent of bancroftian filariasis, has been spread around the world during mass migrations of its human hosts. Thus the parasite had to overcome the difficulties of establishing new infections in new geographical regions where novel species of mosquito were available as potential vectors. In the islands of the South Pacific, where *W. bancrofti* was probably spread from west to east during the historical Polynesian migrations (Laurence, 1989), its success in establishing itself has been due to its ability to develop in several species of the *Aedes* (*Stegomyia*) *scutellaris* subgroup. Marks (1954) and Belkin (1962) have suggested that in the Pacific, dispersal of this mosquito subgroup also accompanied human migrations from the larger land masses in the west to the islands in the east, and the fragmentation of land masses may have played an important part in its diversification (more than 30 species). In French Polynesia, *Aedes polynesiensis* Marks, 1951 was reported as the main vector of *W. bancrofti* (Kessel, 1957). In contrast to other species of the *Ae. scutellaris* subgroup which seek their bloodmeal during the night, *Ae. polynesiensis* is a day-biting mosquito (Buxton & Hopkins, 1927), and it is worth noting that its geographical distribution coincides with that of the subperiodic form of *W. bancrofti*.

A high prevalence of filariasis, including its extreme manifestation elephantiasis, has long been noted in the Society islands (Kessel, 1957; Laurence, 1989). In 1949, 30% of the population from Tahiti harboured microfilariae (mf) (Galliard, Mille & Robinson, 1949). Between 1950 and 1970, mass chemotherapy programmes based on the distribution of diethylcarbamazine (DEC) were implemented with success, reducing the prevalence to less than 1%. But the interruption of systematic administration of DEC in 1982 has resulted in a dramatic increase of mf prevalence in humans (21·4%, Cartel *et al.* 1992) and in mosquitoes (8·96%, Lardeux *et al.*, unpublished observations).

Control of *Ae. polynesiensis* by insecticides is not feasible because this mosquito is essentially a forest-dweller which breeds in a very wide range of small and cryptic breeding sites (crab-holes, tree-holes, coconut shells, etc); therefore, alternative techniques are required to limit or eliminate the disease. Macdonald (1962) suggested that the most promising technique would be the replacement of a vector population by a microfilaria-refractory population. New developments in the manipulation of mosquito

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Table 1. Strains of *Aedes polynesiensis* tested for experimental infections with *Wuchereria bancrofti*

Strain*	Origin locality	Archi-pelago	Collection date (month, year)	Distance from Tahiti (km)
(1) Tahiti	Papara	Society	1991	0
(2) Raiatea	Opoa	Society	01, 92	220
(3) Tikehau	Tikehau	Tuamotu	07, 92	320
(4) Nuku-Hiva	Taiohae	Marquesas	11, 92	1100
(5) Rangiroa	Avatoru	Tuamotu	10, 92	350
(6) Mangareva	Rikitea	Gambiers	06, 92	1600
(7) Rurutu	Unaa	Australs	10, 92	570

* Named in relation to the island of collection.

genomes (Crampton *et al.* 1993) may render this possible in the future, considering that susceptibility to filaria parasites has a simple mendelian inheritance in several mosquito species. This is the case of susceptibility to *Brugia malayi*, *B. pahangi* and *W. bancrofti* in *Aedes aegypti* (f^m gene, Macdonald, 1962; Macdonald & Ramachandran, 1965), *Dirofilaria immitis* in *Ae. aegypti* (*ft* gene, MacGreevy, MacClelland & Lavoipierre, 1974) but contrasts with the expression of the *Plasmodium* susceptibility in *Anopheles gambiae* which was proved to be under the control of two distinct but linked genes (Vernick, Collins & Gwadz, 1989).

The purpose of the present study was to investigate whether *Ae. polynesiensis* from different Polynesian islands present differences in vectorial efficiency toward *W. bancrofti*, both to achieve a better understanding of the disease epidemiology and to further research on the genetic mechanisms underlying variations in vectorial efficiency.

MATERIALS AND METHODS

Mosquito strains

The experiments were carried out with 7 laboratory strains of *Ae. polynesiensis* (Table 1, Fig. 1.) founded from 10–50 females caught on human bait (Bonnet & Chapman, 1958). Adults were maintained in cages (30 × 30 × 30 cm) at 25 ± 1 °C with 80 ± 10 % relative humidity, and fed with a 10% (w/v) sucrose solution. Once a week, females were allowed to feed on a restrained mouse. For infecting experiments, batches of eggs were hatched in 200 ml of water with a low concentration of dissolved oxygen induced with a vacuum air pump to provide larvae of the same age. Larvae were reared in pans containing 1·5 l of tap water and provided with 0·5 mg bovine liver powder (ICN Biomedicals Inc, USA). Suitable densities (200–400) of larvae were kept to obtain a good larval development without replacing the medium, and females of homogeneous size (Failloux *et al.* 1991). Five to ten-day-old females were used for the experimental infections. Their mortality was always below 20% during the 15 days following feeding on uninfected blood.

Human microfilariae carriers

Three human carriers (A, B, and C) from Tahiti were recruited and gave informed consent for feeding experiments. The density of circulating mf in the human carriers was determined before and after each mosquito feeding period from a 1 ml venous blood sample, concentrated using the nucleopore filtration technique (Desowitz, 1971; Desowitz & Southgate, 1973). Human carrier (A) was 50 years old and had 800 mf/ml. Human carrier (B) was 40 years old and had an average of 1700 mf/ml venous blood. Human carrier (C) was 25 years old and had approximately 8000 mf/ml. The three human carriers had received no microfilarial treatment for at least 1 year before the experiments; each volunteered for feeding mosquitoes on their forearms. Carrier (C) also agreed to have a blood sample taken periodically for artificial feeding of mosquitoes.

Blood feeding on carriers' forearms

Four strains of *Ae. polynesiensis* were used in each series of experiments: Tahiti, Raiatea, Tikehau and Nuku-Hiva. Five to ten-day-old females were deprived of sugar for at least 12 h before being allowed to blood-feed for 15 min on a human carrier forearm introduced inside the cage. With each carrier and each experiment, two strains of 300–400 mosquitoes per cage were blood-fed simultaneously (one on each arm of the human carrier), and the four strains were processed within 2 h (09.00–11.00 h), thus insuring homogeneity of microfilariae conditions within each experiment. The females were collected after complete repletion, and held in cages with a 10% sugar solution. Every day, dead females were removed and dissected to count developing filarial larvae and to detect possible worm degeneration that is known to start slowly within about 2 days following blood-feeding (Macdonald, 1963). On day 15, the surviving mosquitoes were killed and dissected for direct examination of infective larvae. Two replicates using the 4 mosquito strains were performed for each carrier.

Artificial blood-feeding

Six strains of mosquitoes were used: Tahiti, Raiatea, Nuku-Hiva, Rangiroa, Mangareva and Rurutu. The 5–10-day-old non-blood fed females of each strain were placed in 0·5 l plastic-screened containers in groups of 50. They were allowed to feed for 1 h on the infected blood heated to 37 °C using glass feeders covered with Parafilm membrane stretched on the

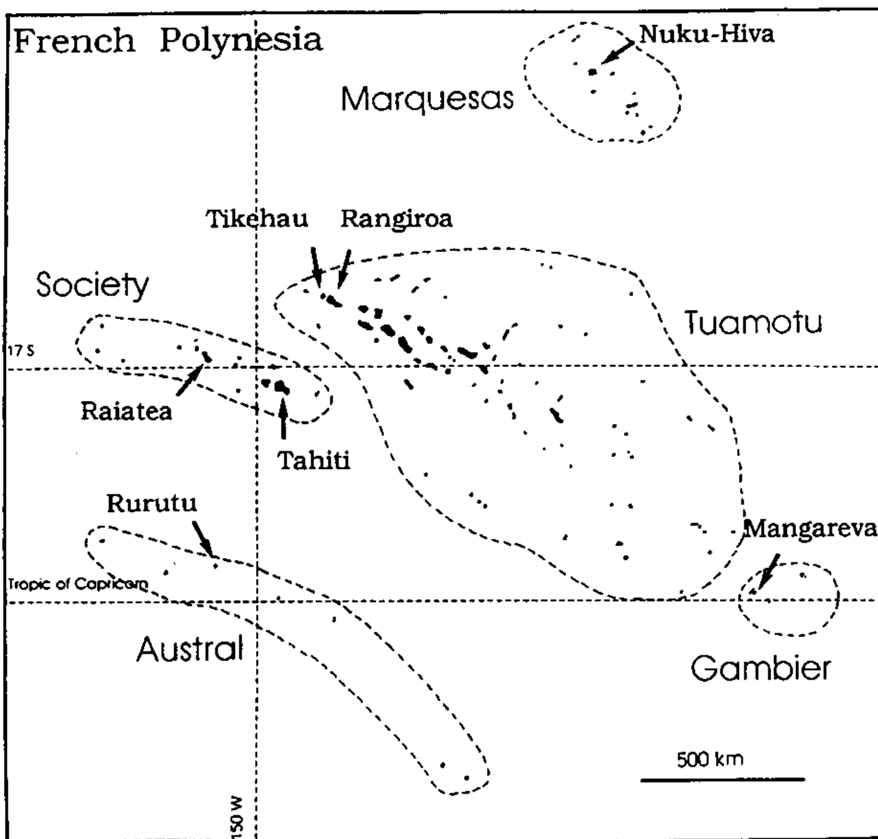

Fig. 1. Geographical origin of *Aedes polynesiensis* samples collected.

opening base (Rutledge, Ward & Gould, 1964; Failloux *et al.* 1991). An infectious blood meal was made using an appropriate amount of venous blood diluted with a 10% sucrose solution to give a density of 15000 mf/3·5 ml in each feeder. Venous blood was collected in Vacutainers containing EDTA (1 mg/ml) as an anticoagulant (Becton Dickinson, England). Females which were fully engorged were kept in plastic cups (20/cup) and provided a 10% sugar solution until day 15. At least 2 replicates were carried out for each strain.

Blood meal volumes

These were estimated for females fed on carriers' forearms. The method was based on the complete conversion of haemoglobin from lysed erythrocytes to a stable complex, haemoglobincyanide (Briegel, Lea & Klowden, 1979). The absorbance of the complex was read at 256 nm. Ten to twenty females fed until repletion were killed with chloroform. Their abdomens were thoroughly ground in 0·5 ml of Drabkin's solution with a glass pestle to insure the complete elution of haemoglobin; the homogenates were then incubated at room temperature (23–25 °C) for at least 20 min with appropriate reagents as described by Briegel *et al.* (1979). To assess the relationship between absorbance and blood meal volume, a calibration curve was performed using various amounts of uninfected human blood.

Vector competence

The developmental cycle of *W. bancrofti* in the mosquito lasts 12–15 days (Beaver & Jung, 1985) depending on the temperature. To assess the vector competence, the mosquitoes were searched for parasites: (1) at the time of mosquito infection within 1 h after the blood meal, and (2) at the end of the parasite cycle, i.e. 15 days after infection on the surviving females to identify infective larvae. In freshly blood-fed females, mf were searched and counted by examining the midgut contents after lysis of the blood meal in distilled water (Brengues & Bain, 1972). Ingested microfilariae reach the haemocele in high proportions 24 h after blood feeding depending on the mf uptake. This phenomenon described as the limitation is specific to the *Ae. polynesiensis–W. bancrofti* combination (Bain, 1976; Prod'hon *et al.* 1980). The thoraces of females which had died during the 10 days following blood-feeding were dissected in saline solution, and muscles were teased apart to count larvae and determine their developmental stage. For females dying 10 days or more after the blood meal, and all surviving females, head, thorax and abdomen were dissected separately in RPMI 1640 solution (Gibco Ltd, Scotland) and examined for infective larvae under a dissecting microscope. A control was conducted with mosquitoes from Tahiti strain engorged on an uninfected person.

Table 2. Vector efficiency of 4 *Aedes polynesiensis* strains following the ingestion of *Wuchereria bancrofti* microfilariae (mf) on infected carriers' forearms

	Replicate	Carrier A	Carrier B	Carrier C
Microfilaraemia mf/ml*	1	768/810 (789)	1560/1080 (1320)	9940/6902 (8421)
	2	527/680 (603)	2220/2040 (2130)	9928/8534 (9231)
% of infected females				
Tahiti	1	78 (32)†	100 (14)	100 (40)
	2	93 (40)	100 (28)	100 (42)
Raiatea	1	87 (30)	70 (20)	97 (39)
	2	85 (39)	88 (48)	95 (42)
Tikehau	1	91 (33)	89 (27)	100 (40)
	2	88 (40)	90 (30)	98 (42)
Nuku-Hiva	1	77 (30)	97 (34)	90 (41)
	2	82 (44)	97 (30)	100 (42)
% of infective females				
Tahiti	1	92 (47)	79 (14)	93 (45)
	2	88 (48)	92 (25)	95 (40)
Raiatea	1	89 (27)	85 (27)	93 (42)
	2	91 (21)	100 (43)	100 (28)
Tikehau	1	83 (57)	93 (30)	100 (41)
	2	90 (56)	100 (8)	100 (28)
Nuku-Hiva	1	73 (58)	77 (13)	98 (41)
	2	90 (51)	87 (39)	100 (28)
mf/infected female‡				
Tahiti	1	6·8 ± 7·0 (25)†	10·3 ± 10·3 (14)	22·5 ± 28·0 (40)
	2	6·6 ± 6·8 (37)	15·3 ± 16·9 (28)	35·0 ± 4·9 (42)
Raiatea	1	4·5 ± 4·2 (26)	9·7 ± 8·9 (14)	15·3 ± 14·7 (38)
	2	5·2 ± 5·5 (33)	15·2 ± 14·5 (42)	32·3 ± 34·3 (40)
Tikehau	1	4·2 ± 3·8 (30)	24·7 ± 18·8 (24)	23·3 ± 21·3 (40)
	2	7·9 ± 7·2 (35)	14·4 ± 14·2 (27)	28·7 ± 27·5 (41)
Nuku-Hiva	1	5·2 ± 5·7 (23)	17·7 ± 14·5 (33)	12·4 ± 12·7 (37)
	2	6·5 ± 6·2 (36)	17·3 ± 17·2 (29)	38·4 ± 38·0 (42)
Infective larvae/infective female‡				
Tahiti	1	5·6 ± 4·5 (43)†	6·9 ± 3·8 (11)	16·1 ± 13·3 (42)
	2	5·9 ± 4·6 (42)	12·0 ± 8·6 (23)	19·8 ± 13·6 (38)
Raiatea	1	4·2 ± 2·4 (24)	6·4 ± 5·1 (23)	10·6 ± 7·2 (39)
	2	4·7 ± 2·7 (19)	13·1 ± 9·4 (43)	7·5 ± 5·1 (28)
Tikehau	1	3·9 ± 2·7 (47)	11·2 ± 7·4 (28)	10·9 ± 8·1 (41)
	2	6·7 ± 5·3 (50)	6·2 ± 3·9 (8)	11·8 ± 6·0 (28)
Nuku-Hiva	1	4·3 ± 3·4 (42)	3·5 ± 2·3 (9)	10·4 ± 6·5 (40)
	2	6·2 ± 4·8 (46)	9·4 ± 7·5 (34)	13·2 ± 5·1 (28)
Blood meal volume (μl)‡				
Tahiti	1	3·8 ± 0·8 (19)†	3·7 ± 0·6 (7)	3·9 ± 0·9 (9)
Raiatea	1	—	3·1 ± 0·8 (9)	3·0 ± 0·7 (7)
Tikehau	1	3·6 ± 1·2 (26)	4·0 ± 1·1 (11)	3·0 ± 0·7 (7)
Nuku-Hiva	1	3·8 ± 1·6 (10)	3·0 ± 0·6 (14)	2·5 ± 0·6 (7)
Expected mf/infected female§				
Tahiti	1	3·0	4·9	32·8
Raiatea	1	—	4·1	25·1
Tikehau	1	2·9	5·2	25·4
Nuku-Hiva	1	3·0	3·9	21·3

* Number of circulating mf at the beginning and the end, respectively, of the feeding period; the mean of these numbers is in parenthesis.
† Number of mosquitoes tested.
‡ Mean and standard deviation.
§ Calculated from the mean number of mf/ml in the carrier blood and the blood meal size.

Statistical analyses

Variations in the proportions of infected females (day 0) and of infective females (day 15) were compared using the R × C Fisher's exact test for each carrier and for each replicate. The subprogram STRUC was used to compute an unbiased estimate of the exact *P*-value (Raymond & Rousset, 1995). An

Table 3. Probabilities testing the homogeneity in the proportions of infected (day 0) and infective (day 15) females of *Aedes polynesiensis* from strains Tahiti, Raiatea, Tikehau and Nuku-Hiva fed on carriers' forearms

(Significant ($P < 0{\cdot}05$) values are in bold type.)

	Carrier			
Replicate	A	B	C	All
Infected females				
1	0·37	**0·008**	**0·047**	**0·007**
2	0·52	0·16	0·62	0·43
All	0·51	**0·010**	0·13	**0·022**
Infective females				
1	0·75	0·11	0·28	0·28
2	0·86	0·83	0·31	0·81
All	0·93	0·32	0·30	0·57

overall test was constructed by combination of test-probabilities using Fisher's method. The mean numbers of parasites (i.e. of mf at day 0 or of infective larvae at day 15) per infected or infective females were compared using ANOVA analysis. The daily cumulative mortalities of mosquitoes were analysed according to COX's model from Statistica software (Statsoft, Tulsa, USA). This model assumes that the underlying hazard rate is a function of the independent variables. The homogeneity of blood meal volumes among females of each strains was tested using the Kruskal–Wallis test provided by Statgraphics. Variations in mf densities in carrier venous blood (microfilaraemia) were tested using Student's *t*-test from Statgraphics (Plus Ware Products, USA). The expected number of mf ingested per female was calculated from the mf density of each carrier and the mean blood meal volume of each mosquito strain.

RESULTS

Wuchereria bancrofti cycle in *Ae. polynesiensis* lasts 12–15 days (Beaver & Jung, 1985), and vectorial efficiency depends on the parasitic yield (i.e. the ability of mosquito females to ingest microfilariae (mf) during the infected blood meal at day 0, and to ensure their maturation until the infective stage when they can be transferred to a new human host at day 15), as well as on the rate of survival of the infected insects until maturation of the parasite (i.e. at least until day 15).

Parasitic yield after feeding on carriers' forearms

At day 0, the proportions of infected females varied between 70 and 100% when considering all experiments (i.e. 24 experiments corresponding to 4 strains tested twice on 3 carriers – see Table 2). Homogeneity of these results was rejected when considering all 24 experiments ($P = 0{\cdot}022$, Table 3) and in a few experiments between strains ($P = 0{\cdot}008$ and 0·047 in replicate 1 with carriers B and C). After 15 days, the proportions of infective females varied between 72 and 100%; homogeneity between the tests was not rejected either when considering all experiments ($P = 0{\cdot}57$), or when considering each replicate ($P > 0{\cdot}20$, Table 3).

The mean number of parasites observed in mosquitoes also displayed large variations. There were between $4{\cdot}2 \pm 3{\cdot}8$ and $38{\cdot}4 \pm 38{\cdot}0$ mf/infected female at day 0, and between $3{\cdot}5 \pm 2{\cdot}3$ and $19{\cdot}8 \pm 13{\cdot}6$ infective *W. bancrofti* larvae/infective female at day 15. Carriers and replicates explained the larger part of the variances of these results (carrier contribution: $F = 39{\cdot}4$ and 35·9, respectively, 2 D.F., $P < 10^{-4}$; replicate contribution: $F = 4{\cdot}6$ and 3·4, respectively, 4 D.F., $P = 0{\cdot}01$), whereas strains had no contribution ($F = 1{\cdot}1$, 3 D.F., $P = 0{\cdot}44$).

To understand better how carriers influenced these results, parasitic yield was examined in relation to the carrier microfilaraemia (number of mf/ml of venous blood) at the time of feeding. Spearman rank correlations (r) were computed with data from the first replicate of each carrier ($N = 12$ tests). A significant ($P < 0{\cdot}05$) positive correlation was observed between the carrier microfilaraemia and all the parasitic yield parameters: percentages of infected females ($r = 0{\cdot}62$), percentages of infective females ($r = 0{\cdot}67$), mean number of mf/infected female ($r = 0{\cdot}73$), and mean number of infective parasites/infective female ($r = 0{\cdot}74$). In these experiments, the mean volume of blood ingested by females varied between $2{\cdot}5 \pm 0{\cdot}6$ and $4{\cdot}0 \pm 1{\cdot}1$ ml. The strong contribution of strains in the overall variance ($F = 3{\cdot}92$, 3 D.F., $P = 0{\cdot}01$), and the absence of correlation between blood meal volumes and carrier microfilaraemia (Spearman rank correlation $r = -0{\cdot}443$, $P > 0{\cdot}05$) prompted the comparison of observed number of ingested mf with what could be expected from blood volumes and carrier microfilaraemia. Mosquito females which had fed on carriers with a low microfilaraemia (i.e. 800 mf/ml for carrier A and 1700 mf/ml for carrier B) ingested 2–5 times more mf than expected, whereas those feeding on the carrier with high microfilaraemia (carrier C, 8000 mf/ml) ingested 1·1–1·7 times less mf than expected.

Parasitic yield after artificial blood-feeding

The strong influence of carrier microfilaraemia on parasitic yield parameters may have obliterated variations due to strain differences in the previous experiment. To avoid this source of variation, mosquito females were fed on artificial feeders which

Table 4. Vector efficiency of 6 strains of *Aedes polynesiensis* following the ingestion of *Wuchereria bancrofti*-infected blood using artificial feeding

Strain replicate	Infected females at day 0 (%)	Mf/infected female at day 0	Infective females at day 15 (%)	Infective larvae/infective female at day 15
Tahiti				
1	95 (20)*	8·1 (19)	78 (9)	6·6 (7)
2	94 (17)	7·2 (16)	90 (19)	5·2 (17)
1 and 2		7·3 ± 5·0		5·6 ± 4·0
Raiatea				
1	100 (22)	9·6 (22)	100 (9)	9·2 (9)
2	100 (19)	8·9 (19)	71 (21)	6·3 (15)
1 and 2		9·3 ± 4·4		7·6 ± 3·9
Rangiroa				
1	100 (20)	10·0 (20)	58 (12)	3·6 (7)
2	100 (22)	8·2 (22)	87 (12)	4·7 (20)
1 and 2		9·0 ± 5·8		4·4 ± 2·1
Rurutu				
1	100 (18)	10·9 (18)	43 (21)	3·3 (9)
2	100 (12)	10·7 (12)	82 (11)	4·2 (9)
1 and 2		10·8 ± 5·6		3·6 ± 2·0
Nuku-Hiva				
1	100 (19)	8·4 (19)	65 (20)	4·5 (13)
2	100 (22)	8·4 (22)	92 (22)	4·3 (20)
1 and 2		8·4 ± 4·4		4·4 ± 2·9
Mangareva				
1	100 (17)	9·2 ± 7·1 (17)	46 (46)	3·6 ± 3·7 (21)

* Number of mosquitoes examined.

allowed monitoring of the quantity of mf in the infected blood. After ensuring that mf were homogeneously distributed in each feeder, females of 6 strains were fed on blood containing about 4300 mf/ml. The experiment with each strain was replicated once (Table 4). At day 0, all blood-fed females except 1 in the 2 replicates with the Tahiti strain were infected, and homogeneity of the mean number of ingested mf/infected female (range: 8·1–10·9) was not rejected by the Fisher's exact test ($P > 0{\cdot}05$). In addition, a variance analysis showed that strains did not significantly contribute to the variance of mf number/infected female ($F = 1{\cdot}6$, 5 D.F., $P = 0{\cdot}16$).

In contrast, at day 15, large differences in the percentage of infective females (range: 43–100%) and in the mean number of infective larvae/female (range: 3·3–9·2) were observed. In the two cases, strains contributed significantly to the variance of these two parameters (Fisher's test: $P < 0{\cdot}01$; ANOVA: $F = 34{\cdot}67$, 10 D.F., $P = 10^{-4}$ for infective females, and $F = 4{\cdot}72$, 5 D.F., $P < 10^{-4}$ for number of infective larvae), but not the replicates ($P > 0{\cdot}05$). Mosquitoes from the Society archipelago (Tahiti and Raiatea) allowed the development of a larger number of infective parasite stages, respectively 5·1 ± 4·0 and 7·6 ± 3·9, than did those from other archipelagoes.

Mortality rates of infected mosquitoes

These were recorded every day during the 15 days following the infection on mosquitoes which had fed on carrier forearms. Two parameters, mortality in relation to the day of death and mortality in relation to the number of parasites/dead mosquito, were submitted to a variance analysis to determine how carrier, replicates and mosquito strains contributed to the overall variations. Mosquito strains had a significant contribution in the variance mortality in relation to the day of death ($F = 9{\cdot}19$, 3 D.F., $P = 0{\cdot}03$), but not carriers ($F = 1{\cdot}48$, 2 D.F., $P = 0{\cdot}28$) or replicates ($F = 1{\cdot}90$, 4 D.F., $P = 0{\cdot}11$, respectively). In contrast, carriers contributed significantly to the variance of mortality in relation to the number of parasites ($F = 16{\cdot}18$, 2 D.F., $P = 0{\cdot}001$), but not strains ($F = 1{\cdot}77$, 3 D.F., $P = 0{\cdot}29$) or replicates ($F = 1{\cdot}43$, 4 D.F., $P = 0{\cdot}22$). In addition, for the same carrier, mortality rates were less important in mosquitoes from the Society archipelago (Tahiti and Raiatea), and this effect decreased with increasing microfilaraemia of carriers (Fig. 2).

In experiments with artificial feeding, mortalities recorded only at day 15 ranged from 20% (Rangiroa strain) to 60% (Raiatea strain, data not shown).

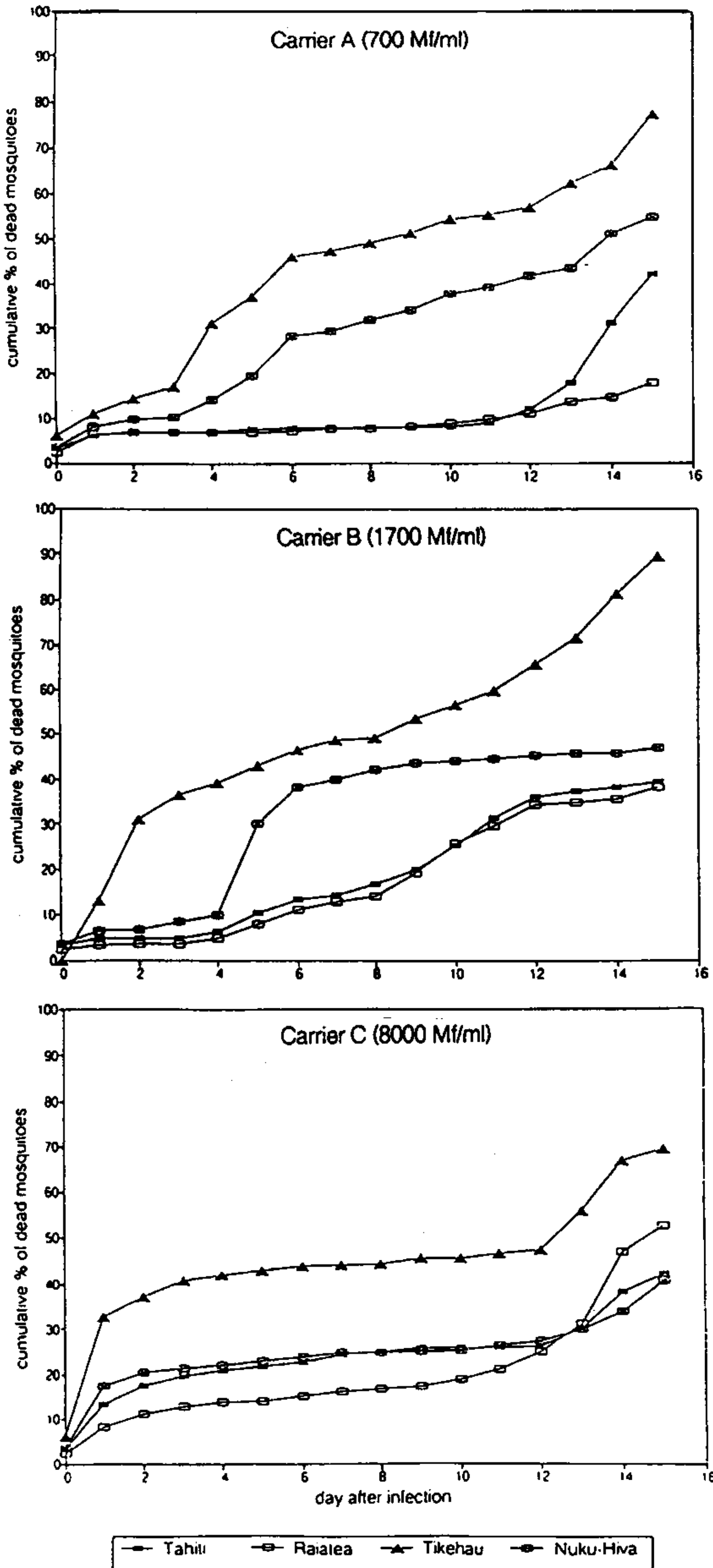

Fig. 2. Cumulative mortality rates on successive days after feeding on carriers' forearms with different mf densities during the development of *Wuchereria bancrofti* in 4 strains of *Aedes polynesiensis*.

DISCUSSION

Our study has brought clear evidence that *Ae. polynesiensis* strains from the Society islands (Tahiti and Raiatea) have a better vectorial efficiency for *W. bancrofti* microfilariae from Tahiti than do strains from other archipelagoes, namely Tuamotu, Marquesas, Gambiers and Australs. This higher efficiency was characterized by a higher parasitic yield, at day 15, as well as by a higher rate of survival of infected females.

Differences in parasitic yield among mosquito strains could be demonstrated only after standardizing the number of microfilariae in the blood which females engorged (e.g. by artificial feeding). Feeding on human carriers disclosed that all parameters influencing parasitic yield (proportions of infected and infective females, microfilariae uptake, microfilariae reaching the infective stage) are significantly ($P > 0{\cdot}05$) and positively correlated to the concentration of microfilariae present in the blood of the donor, despite the existence of a regulation in the rate of microfilariae uptake. This regulation appeared to be density dependent as previously reported by various authors (Rosen, 1955; Pichon, 1974; Samarawickrema *et al.* 1985; Lowrie *et al.* 1989), since mosquitoes feeding on blood with a low concentration of microfilariae (800–1800 mf/ml) tend to concentrate them by a factor of 2–5, whereas those feeding on blood with a high microfilariae concentration (8000 mf/ml) tend to ingest fewer parasites than expected. To our knowledge, no explanation for this density-dependent phenomenon has been proposed, and its seems unlikely that it can only be due to an uneven distribution of microfilariae in skin capillaries as put forward to explain why female concentrate microfilariae (Gordon & Lumsden, 1939; Hairston & Jachowski, 1968; Beaver, 1970).

The key factor of vector competence is the production of infective parasites in sufficient number to insure transmission (Ramachandran & Zaini, 1968), knowing that only 20% of *W. bancrofti* larvae are leaving the mosquitoes at the end of their maturation, and only 6·6% are successful in entering the final host (Zielke, 1976). As shown by our results, *W. bancrofti* from Tahiti were able to reach the infective stage in all *Ae. polynesiensis* strains, independently, of their geographical origin but, all other factors being equal, more numerous infective larvae were obtained with strains from Tahiti and Raiatea (means: 5·2–9·2) than with strains from Rangiroa, Rurutu, Nuku-Hiva or Mangareva (means: 3·3–4·7).

In the mosquito, the first line of defence against filarial infection is the presence of cibarial and pharyngeal armatures that may damage mf as they enter the midgut (Coluzzi & Trabucchi, 1968; MacGreevy *et al.* 1978; Bryan & Southgate, 1988). In the midgut, the rapid formation of crystals may block the migration of mf into the haemocele, resulting in their death and eventual elimination in faeces. The peritrophic membrane, whose formation begins within minutes after blood-feeding, also has been proposed as a barrier to migration of the parasites through the midgut epithelial cells (Bertram & Bird, 1961). Mosquitoes may also develop extracellular or/and intracellular melanization of the parasite during its development, as encountered in some species which are refractory to filarial infection (Desowitz & Chellapah, 1962; Hairston & De Meillon, 1968). The present study was not aimed at investigating the mechanisms of parasite elimination, but it can nevertheless be noted that melanized worms were never observed in the dissections done at day 15, or in those of females which had died before day 15.

The probability that an infected vector would transmit infective parasites is closely related to the physiological state of the mosquitoes. Two factors have been pointed out as being particularly important: the infection rate and the survival rate (Defoliart, Grimstad & Watts, 1987; Paulson & Hawley, 1991). Both factors are, at least partially, dependent on the environmental conditions experienced during larval development. It is well known that, in mosquitoes, adult body size is correlated to the quality of the larval diet (as for example in *Ae. aegypti*, Hare & Nasci (1986)), and that many life-history traits are related to adult body size, including the volume of the blood meal, blood-feeding behaviour, duration of gonotrophic cycles, and longevity (Reisen, 1975; Ichimori, 1989; Kitthawee, Ehman & Sattabonghot, 1990). In our experiments, all mosquitoes were reared under standardized laboratory conditions, and differences during larval life were minimal, so that observed differences are essentially due to events occurring during adult life and/or to intrinsic characteristics of the mosquitoes. As expected, survival was dependent on the parasitic load in all strains, with higher mortalities associated with higher parasitic loads, as often observed (Ramachandran, 1966; Kartman, 1954; Brengues, 1975). However, at comparable parasitic loads, mortality was always lower in mosquitoes from the Society archipelago (Tahiti and Raiatea) than in those from other islands (Rangiroa and Nuku Hiva).

The differences in vector efficiency toward *W. bancrofti* observed between *Ae. polynesiensis* strains raise several questions. (1) Do they reflect differences existing in natural populations? (2) Are they restricted to the parasites from Tahiti or general for all *W. bancrofti* independently of their geographical origin? (3) How can the difference between mosquitoes from the Society islands and other islands be explained?

The mosquito strains used in the present study were maintained in the laboratory for about 20

generations before our experiments, and part of their genetic variability may have been lost at the time of colonization (sampling effect) or in later generations (genetic drift). However, we presume that the differences observed in our study exist in natural populations, even if they concern only part of these populations.

At present it is not possible to answer the question concerning the geographical origin of the parasites. All three *W. bancrofti* carriers had spent the larger part of their life on the island of Tahiti, thus it is likely that the large majority, if not all, of their filariae derived from the Tahitian *W. bancrofti* population. Co-adaptation of hosts and parasites has been reported in many species (May & Anderson, 1983), and it is possible that *Ae. polynesiensis* from the Marquesas (for example) are as efficient vectors toward *W. bancrofti* from Marquesas, as are Tahitian mosquitoes toward Tahitian parasites. Future studies will have to be undertaken to examine this very important problem for the understanding of filariasis epidemiology.

Recently, studies on the genetic structure of *Ae. polynesiensis* in French Polynesia based on electrophoretic polymorphism of various enzymes (Failloux, 1994) have revealed that gene flow is more intense among islands of the Society archipelago than between islands of different archipelagoes. This implies that mosquito populations from Tahiti and Raiatea share more genetic similarities than do populations from Tahiti and Rangiroa or Rurutu. The present results on vectorial efficiency confirm these genetic studies, and suggest that vectorial efficiency variations have a genetic support. In the past, it has been shown that certain traits of medical importance in several sand flies and mosquito species are associated with enzyme electrophoretic variations (Tabachnick *et al.* 1985; Wu, 1989). It is not likely that electrophoretic variations are directly responsible for these characters, but rather that their genes are closely linked to those of certain enzymes. Looking for such associations may help in the identification of various parameters of vector efficiency (Kassem *et al.* 1993).

In conclusion, much work remains to be done to understand the underlying genetic basis of vectorial efficiency of *Ae. polynesiensis* from different islands. The present study has shown that to progress, it is absolutely necessary to monitor the number of microfilariae present in the blood meal of the insect, and that this is possible by artificial feeding procedures. Our results help to understand filarial transmission and may be of direct concern for filariasis control in the field. First, they point out that the residual carriers, who have a low microfilaria after microfilaricidal treatments, may still constitute an important pool for the infection of mosquitoes as previously mentioned by Lowrie *et al.* (1989), both because mosquitoes that feed on them will concentrate microfilariae, and because they have a high probability of surviving the infection. Second, the lower competence and higher mortalities of vectors when infected by allopatric worms may restrain the introduction of new filarial strains from endemic zones in areas which have been already eradicated by long-term mass prophylaxis.

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