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The role of passive migration in the dispersal of resistance genes in *Culex pipiens quinquefasciatus* within French Polynesia

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Summary

We have investigated the distribution of resistance genes in relation to genetic structure and gene flow between various islands of French Polynesia in *Culex pipiens quinquefasciatus*. We show that (1) resistance to organophosphorous insecticides, mediated by A2–B2 esterases, is present in all islands, (2) A2–B2 frequency decreases as distance from Tahiti increases, and (3) genetic differentiation (measured as estimates of the F_{st} parameter from allozyme polymorphism) between islands is significantly correlated to geographic distances which is also correlated to air or sea commercial traffic. These data are discussed in relation to A2–B2 invasion of French Polynesian islands.

1. Introduction

Amplification of esterase B gene is a major resistance mechanism to organophosphorous insecticides (OP) in mosquitoes of the *Culex pipiens* complex. In each of the presently known amplifications (B1, B2, B4 and B5), the structural gene is coamplified with its flanking sequences, and the whole amplified region, or haplotype, presents a RFLP (restriction fragment length polymorphism) pattern which is specific. Molecular variability of esterase B haplotypes in susceptible mosquitoes (with a single haplotype copy) is very large and, until now, none of the amplified haplotypes have been observed in the non-amplified state. Raymond *et al.* (1991) concluded that the occurrence of each amplified form more than once was highly improbable, and that the present geographic distribution of each amplified esterase B haplotype was the result of gene flow. In the case of amplified esterases B1 and B2, which are now found on at least three continents (Raymond *et al.* 1991; Qiao & Raymond, 1995), it has to be assumed that these resistance genes were unintentionally transported by man across oceans. Here we attempt to determine the role of man in *Culex pipiens* genetic exchanges. To distinguish gene flow by natural flight activity from gene flow associated with human activities, it was necessary to identify a geographic situation where the two factors can be clearly characterized. French Polynesia seemed ideal in that there are numerous islands which (1) are separated by large stretches of sea water in discrete units, and (2) present a wide diversity in intensity of commercial exchanges between islands. After a first survey of two islands (Tahiti and Moorea) in 1990 to determine whether resistance genes existed in French Polynesia, a second survey covering the Society (five islands), the Tuamutu (two islands) archipelagoes and Tubuai island was undertaken. Investigations of populations collected during this second survey included a study of resistance to various insecticides (two organophosphates, one pyrethroid and one carbamate), an analysis of resistance gene frequencies, and a study of population structure and differentiation based on the polymorphism of supposedly neutral genes. Data were analysed in order to answer the following questions. Is the pattern of resistance to insecticides acting on different targets similar over the geographic region investigated? Have resistance genes invaded all islands? How can the geographic distribution of resistance genes be explained in relation to gene flow? Is it possible to assess the importance of commercial traffic in the amount of gene flow between islands?

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2. Materials and methods

Culex pipiens quinquefasciatus larvae or pupae were collected in 28 breeding sites from Tahiti and Moorea islands in 1990 and 1992, and from Huahine, Raiatea, Bora Bora, Rangiroa, Manihi and Tubuai islands in 1992 (Fig. 1). Some of the larvae were immediately used for bioassays. The rest were reared to the imaginal stage and stored in liquid nitrogen for biochemical genetic investigations.

Bioassays were performed on 4th-instar larvae with temephos (American Cyanamid, Princetown NJ), chlorpyrifos (Dow Chemical, Midland Mich.), propoxur (Bayer AG, Leverkusen, Germany), and permethrin (Riedel de Haen, Germany) as described by Raymond *et al.* (1986). S-LAB susceptible strain (Georghiou *et al.* 1966) was used as reference. Mortality data were analysed with the PROBIT software (version 3·3) of Raymond *et al.* (1993*a*).

Electrophoretic studies were conducted on adult homogenates as described by Pasteur *et al.* (1988), using starch gels. The homogenate of each mosquito was split in two parts, one to investigate the polymorphism of aspartate-amino-transferases (AAT), esterases, hexokinase (HK), malate-dehydrogenases (MDH), and phosphoglucomutase (PGM) with TME pH 7·4 buffer systems, the other to analyse the polymorphism of glucose-phosphate-isomerase (GPI), glycerophosphate-dehydrogenase (GPD), isocitrate-dehydrogenases (IDH), and mannose-phosphate-isomerase (MPI) with TC pH 8·0 buffer systems. Strains used for electrophoretic mobility references were SELAX (Wirth *et al.* 1990), TEM-R (Raymond *et al.* 1993*b*), BARRIOL (Chevillon *et al.* 1995), ES-SAYADA (Ben Cheikh & Pasteur, 1993), and EVORA (M.R., unpublished).

Conformity of genotype frequencies to Hardy–Weinberg proportions, populations differentiation and genotypic linkage disequilibrium were tested using GENEPOP *v.* 1·2 software (Raymond & Rousset, 1995). Multiple sample heterozygote deficits were tested using an exact test procedure described by Rousset & Raymond (1995). F_{is} and F_{st} estimates were computed according to Weir & Cockerham (1984). The number of effective migrants (Nm) was estimated using the F statistics of each locus according to the equation $Nm = (1/F_{st} - 1)/4$ (Wright, 1969). This formula assumes the neutrality of the polymorphic genes and an island model of migration (see e.g. Hartl & Clark, 1989). The significance level for each test was adjusted to take into account the other tests 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 (Fisher, 1970).

Partial Mantel one-sided tests in which P values were determined by permutation procedures (Smouse *et al.* 1986; Leduc *et al.* 1992) were used to evaluate the significance of correlations between genetic differentiation (i.e. estimates of F_{st}) and three explanatory variables: geographic distance and air or sea traffic.

Two series of indices measuring the intensity of commercial exchanges between pairs of Polynesian islands were computed: one evaluated air traffic and was based on the 1992 Air Tahiti and Air Moorea flight schedules; the other evaluated maritime traffic and used the 1992 records published by the Papeete Port Autonome. During a round trip, an aircraft or a boat was considered to 'connect' two islands if it did not stop elsewhere more than once. The number of 'connexions' between two islands for ships or aircraft was used as a traffic index. In French Polynesia, commercial traffic increased between 1960 and 1979 with the building of airports in many islands and the starting of a ferry-boat service between Tahiti and Moorea in the mid 70s. It remained fairly constant during the 80s (Atlas de la Polynésie française, 1993), so that the traffic indices estimated for 1992 are representative of the last decade.

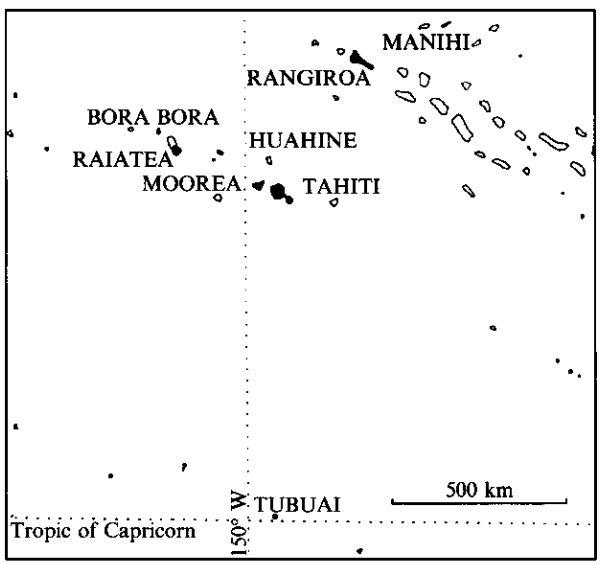

Fig. 1. Geographic localization of the French Polynesian islands studied.

2. Results

(i) *Insecticide susceptibility of Polynesian populations*

Bioassays were conducted on samples collected in islands of the Society and Tuamutu archipelagoes. They showed a resistance to temephos, chlorpyrifos and permethrin (Table 1) in several islands, but not to propoxur (data not shown).

In the 1990 survey of Tahiti and Moorea, resistance to temephos (defined by the resistance ratio or RR, i.e. LD_{95} value relative to that of the susceptible S-LAB reference strain) varied between 3- and 9-fold in samples from the two islands. In 1992, this resistance varied between 9- and 23-fold in Tahiti, and between 4- and 12-fold in Moorea, suggesting that temephos resistance has increased between the two surveys. This increase was confirmed in two cases by comparing

Table 1. *Resistance ratios to three insecticides and frequency of the overproduced esterases A2–B2 in* Culex p. quinquefasciatus *sampled in 1990 and 1992 in islands of French Polynesia*

Samples				Resistance ratio[a]			Esterase A2–B2	
No	Site	Island	Date	Temephos	Chlorpyrifos	Permethrin	A2–B2 frequency	Sample size
1.	Mahina	Tahiti	1990	2·7	—	—	0·62[b]	74
2.	Mahapé	Tahiti	1990	3·6	—	—	0·80	69
3.	Carrière	Tahiti	1990	8·9#*	—	—	0·82	60
4.	FAAA	Tahiti	1990	4·1	—	—	0·85	33
5.	Tautira-1	Tahiti	1990	6·8#*	—	—	0·67[b]	51
6.	EPHE	Moorea	1990	4·2#*	—	—	0·75	57
7.	Economie	Moorea	1990	8·7#*	—	—	1·00	31
8.	Pk 29	Moorea	1990	2·7	—	—	0·27	62
9.	Papenoo	Tahiti	1992	8·6#*	6·2#*	3·7	0·77	30
10.	Jasmin	Tahiti	1992	12#*	9·9#*	2·8	0·83	65
11.	Papara	Tahiti	1992	23#*	14#*	—	0·75	45
12.	Taravao	Tahiti	1992	13#*	4·3#*	3·8	0·75	28
13.	Tautira-2	Tahiti	1992	13#*	7·4#*	3·8	0·65	43
14.	Hauru	Moorea	1992	4·2#*	5·4#*	12#*	0·41	61
15.	Manuir	Moorea	1992	—	—	—	0·50	59
16.	Lycée	Moorea	1992	13	8·2#*	14#*	0·60	30
17.	Dortoir	Huahine	1992	> 3·6	—	—	0·07	30
18.	Tabu	Huahine	1992	4·2	—	1·3	0·58	31
19.	Tortue	Huahine	1992	—	—	—	0·15	27
20.	Faafau	Raiatea	1992	1·1	1·8	1·3	0·12	42
21.	Alexandre	Raiatea	1992	7·1	> 2·9	1·9	0·23	30
22.	Opoa	Raiatea	1992	—	—	—	0·06	31
23.	Apoomau	Raiatea	1992	—	—	—	0·07	41
24.	Vallée	Bora Bora	1992	2·1#*	1·8	1·1	0·04	29
25.	Avatoru	Rangiroa	1992	—	—	—	0·13	30
26.	Décharge	Rangiroa	1992	3·1#*	1·2	—	0	30
27.	Manahune	Manihi	1992	1·4	3·5	2·8#*	0·03	30
28.	Mahu	Tubuai	1992	—	—	—	0·07	28

[a] Resistance ratios (RR) were calculated at LD_{95} relative to the S-Lab susceptible reference strain. S-Lab LD_{95} was 0·0028 mg/l for temephos, 0·0017 mg/l for chlorpyrifos and 0·027 mg/l for permethrin. # Indicates that the 95% confidence limits of RR could be calculated and * that these confidence limits did not include the value 1 which indicates a statistically significant resistance.

[b] In Mahina and EPHE samples, 2 and 1 mosquitoes, respectively, had also an overproduced esterase B1.

samples from Tahiti collected in 1990 and 1992 in breeding sites separated by less than 500 m: samples no. 3 (1990) and no. 11 (1992) differed by 1·5-fold (95% confidence limits CL = 1·1–2·8), and samples no. 5 (1990) and no. 13 (1992) by 1·9-fold (CL = 1·3–3·2).

In 1992, resistance to temephos was around 4-fold in Huahine, 7-fold in one sample of Raiatea (the other Raiatea sample was susceptible), 2–3-fold in Bora Bora and Rangiroa, and 1·4-fold in Manihi. The 95% confidence limits of resistance ratios (RR) in Bora Bora and Rangiroa samples did not include the value 1, indicating that they were slightly but significantly more resistant than the susceptible reference S-LAB strain.

Resistance to chlorpyrifos was observed in all 1992 samples collected in Tahiti (RR = 4–14-fold), and in some samples from the other islands. As for temephos, resistance to chlorpyrifos appeared to be higher in Tahiti and Moorea than in the other islands although this could not be tested statistically. Resistance to permethrin was highest in Moorea (RR = 12–13-fold); it also was present in Tahiti (3–4-fold) as observed previously (Failloux *et al.* 1994), and in Manihi (3-fold).

(ii) *Resistance genes*

No attempt to investigate variation at the *Ace* locus (coding the target acetylcholinesterase of organophosphorous insecticides) was made because the available test (Raymond *et al.* 1985; Raymond & Marquine, 1994) is based on activity differences in the presence of propoxur to which no resistance was observed.

Electrophoretic studies revealed the presence of amplified esterases B1 and A2–B2 (Table 1). Esterase B1 was observed in only three of the almost 1200 mosquitoes analysed. It was found only in the 1990 survey in both Tahiti and Moorea islands, and its activity on starch gel was much lower than that of the reference TEM-R mosquitoes.

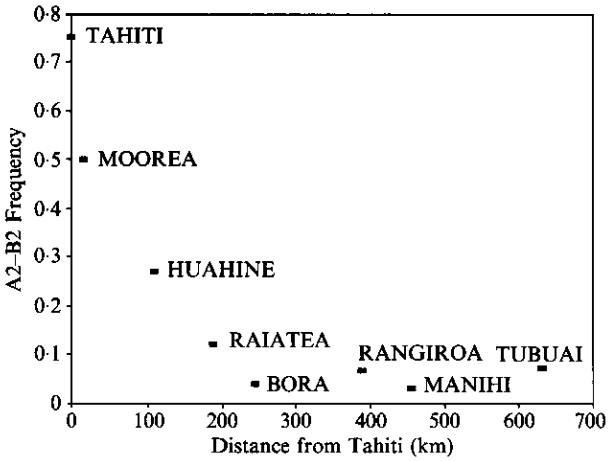

Fig. 2. Variability of esterases A2–B2 mean frequencies in French Polynesian islands in relation to their distance from Tahiti.

The associated esterases A2–B2 were observed in samples from all islands. The electrophoretic identification of A2–B2, which involves mobility comparisons with the esterases present in the SELAX reference strain, was confirmed by analysing the RFLP pattern of the amplified DNA region encompassing esterase B2 structural gene (Raymond *et al.* in preparation). A strict RFLP similarity was obtained with 13 restriction enzymes in mosquitoes of the SELAX and MOOREA strains (the MOOREA strain derived from the Economie collection by selecting larvae of each generation with temephos until all insects contained esterases A2–B2).

Esterases A2–B2 frequency was high in both Tahiti and Moorea in 1990 and in 1992. In Tahiti, A2–B2 frequencies were not significantly different between samples collected in the same or different years (sequential Bonferroni procedure: $P > 0{\cdot}05$ for five samples both in 1990 and in 1992, Holm, 1979), indicating an overall homogeneity and a stability over a 2-year period. In contrast, in Moorea A2–B2 frequencies displayed significant differences between samples in 1990 ($P < 0{\cdot}01$), but not ($P > 0{\cdot}05$) in 1992. In the other islands (surveyed only in 1992), large and significant ($P < 0{\cdot}05$) variations of A2–B2 frequencies were observed between samples from Huahine, Raiatea, and Rangiroa. In addition, the mean esterase A2–B2 frequency was highest in Tahiti (0·75), and, within the Society archipelagoes, it displayed a significant (Spearman rank correlation, $r_s = -0{\cdot}83$, $n = 8$, two-tailed test $P = 0{\cdot}02$) decrease with increasing distance of the islands from Tahiti (Fig. 2).

(iii) *Genes non-selected by insecticides*

Among the 12 loci investigated, two (*Idh2* and *Gpd*) were monomorphic for the same allele, and six (*Hk1*, *Mdh1*, *Mdh2*, *Mdh3*, *Gpi* and *Ihd1*) had the same allele at a frequency of 0·98 or higher in all samples. The last four loci (*Aat1*, *Aat2*, *Pgm* and *Mpi*) displayed a high level of polymorphism in most samples (Table 2). Possible non-random genotypic associations between pairs of loci (considering sex, *Aat1*, *Aat2*, *Pgm* and *Mpi*) were tested using Fisher's exact test on contingency tables. No association was significant ($P > 0{\cdot}05$) taking into account multiple tests. Significant ($P < 0{\cdot}05$) heterozygote deficits were observed in nine cases, but none remained significant when considering multiple tests (sequential Bonferroni test, Holm, 1979). Over all samples, *Aat1* and *Mpi* displayed a significant heterozygote deficit ($P = 0{\cdot}01$ and $P = 0{\cdot}4$, respectively). Over all samples and all loci, there was a low ($F_{is} = 0{\cdot}022$) but significant ($P = 0{\cdot}02$) heterozygote deficit.

Population differentiation was analysed by comparing the allelic composition of different sets of samples combining independent probabilities (Fisher, 1970). Only *Aat1*, *Aat2*, *Pgm* and *Mpi* loci were considered since little or no information is provided by the other loci due to their almost or complete lack of polymorphism. Significant heterogeneity ($P < 0{\cdot}01$) was found between (i) all samples or (ii) samples collected within each island, and after grouping samples between (iii) all islands or (iv) islands within each archipelago (Table 3). Results were similar for any combination of three of the four loci (data not shown), indicating that heterogeneity was not due to a single locus.

Importance of genetic exchanges between populations and groups of populations was assessed by estimating F_{st} according to Weir & Cockerham (1984), and deriving from it the number of effective migrants (Table 3). Between samples from the same island, F_{st} varied between 0·012 (Moorea) and 0·084 (Huahine) which corresponds to Nm values between 20·1 and 2·7, respectively. Between islands, F_{st} was equal to 0·025 in the Society archipelago ($Nm = 9{\cdot}7$) and to 0·16 in Tuamutu archipelago ($Nm = 1{\cdot}3$). Finally, F_{st} was equal to 0·12 between the three archipelagoes ($Nm = 1{\cdot}8$). These results indicate that gene flow between *C. p. quinquefasciatus* populations from different French Polynesian islands is highly heterogeneous. The most striking results concern the large difference observed between islands of the Society and of the Tuamutu archipelagoes.

To better understand the forces controlling genetic exchanges, we examined the variation of the F_{st} values in relation to various parameters. The relation between F_{st} and geographic distances can be expressed by the slope of the regression of log *Nm* on log distance (Slatkin, 1993), which was −0·731. Both F_{st} and geographic distance being correlated with each of the commercial traffic measures, partial Mantel tests were used to determine whether F_{st} was still significantly correlated to either geographic distance, air traffic or boat traffic, once effects of the two other variables were removed. F_{st} was found significantly correlated

Table 2. *Allelic frequencies of genes non-selected by insecticide from islands in French Polynesia. Samples are numbered as in Table 1. The following loci where either monomorphic* (Idh2 *and* Gpdh) *or polymorphic with a single rare allele at a frequency of 0.02 (i.e.* Hk *in samples nos 9, 24 and 27,* Mdh1 *in samples no 9 and 18,* Mdh2 *in sample 21,* Mdh3 *in sample no. 9,* Pgi *in sample no. 15,* Idh1 *in sample 15).* Idh1 *and* Idh2 *were not studied in samples no. 9, 10, 21 and 26, and* Gpdh *in sample no. 16. Significant heterozygote deficits are indicated by *(P < 0·05), **(P < 0·01) and ***(P < 0·001).*

		Aat1			*Aat2*			*Mpi*					*Pgm*				All
Sample	Size	A	B	F_{is}	A	B	F_{is}	A	B	C	D	F_{is}	A	B	C	F_{is}	F_{is}
9	60	0·97	0·03	−0·018	0·67	0·33	−0·184	0·35	0·40	0·25	0	+0·303*	0·72	0·08	0·20	+0·182	+0·123*
10	65	1·00	0	—	0·64	0·36	+0·081	0·41	0·27	0·32	0	+0·101	0·88	0	0·12	+0·205	+0·108
11	56	1·00	0	—	0·83	0·17	−0·021	0·37	0·41	0·22	0	−0·019	0·66	0·18	0·16	−0·086	−0·047
12	28	0·68	0·32	−0·293	0·79	0·21	−0·256	0·45	0·34	0·21	0	+0·180	0·55	0·20	0·25	−0·127	−0·086
13	43	0·97	0·03	−0·016	0·77	0·23	+0·034	0·37	0·34	0·27	0·02	+0·048	0·61	0·19	0·20	−0·052	+0·008
14	61	0·92	0·08	+0·360	0·70	0·30	−0·254	0·50	0·28	0·22	0	−0·088	0·56	0·23	0·21	−0·084	−0·087
15	59	0·97	0·03	+1**	0·60	0·40	+0·321*	0·48	0·28	0·24	0	+0·253*	0·69	0·12	0·19	+0·144	+0·273***
16	30	0·75	0·25	+0·039	0·68	0·32	−0·294	0·30	0·40	0·30	0	+0·100	0·65	0·15	0·20	+0·239*	+0·038
17	30	0·98	0·02	—	1·00	0	—	0·50	0·31	0·19	0	−0·608	0·60	0	0·40	+0·183	−0·245
18	31	0·98	0·02	—	0·60	0·40	−0·063	0·35	0·50	0·15	0	+0·530***	0·79	0·05	0·16	−0·006	+0·204
19	29	0·96	0·04	−0·020	0·74	0·26	−0·333	0·52	0·41	0·07	0	−0·458	0·81	0·06	0·13	−0·153	−0·328
20	54	0·96	0·04	+0·661*	0·73	0·27	−0·006	0·42	0·40	0·18	0	−0·159	0·74	0·03	0·23	+0·169	+0·019
21	30	0·93	0·07	+0·055	0·72	0·28	−0·215	0·36	0·37	0·27	0	+0·206	0·72	0·03	0·25	−0·087	−0·002
22	31	0·97	0·03	+0·017	0·47	0·53	+0·302	0·48	0·37	0·15	0	+0·166	0·66	0·02	0·32	−0·181	+0·104
23	55	0·96	0·04	−0·035	0·86	0·14	+0·022	0·26	0·34	0·40	0	+0·045	0·84	0·03	0·13	+0·039	+0·034
24	30	0·48	0·52	+0·051	0·79	0·21	+0·176	0·43	0·36	0·21	0	−0·149	0·59	0·07	0·34	+0·240*	+0·060
25	30	0·95	0·05	−0·036	1·00	0	—	0·28	0·02	0·70	0	+0·284	0·38	0	0·62	−0·041	+0·096
26	30	0·78	0·22	−0·261	0·88	0·12	−0·115	0·32	0·15	0·53	0	+0·117	0·27	0·08	0·65	−0·252	−0·103
27	30	0·97	0·03	−0·018	0·93	0·07	−0·055	0·39	0	0·61	0	+0·416*	0·83	0	0·17	+0·296*	+0·285
28	28	1·00	0	—	0·68	0·2	+0·361	0·27	0·46	0·27	0	−0·096	0·25	0	0·75	−0·125	+0·034

Table 3. *Population structure in* C. p. quinquefasciatus *from French Polynesia*

Comparisons	N^a	F_{st} [b] *Aat1*	*Aat2*	*Pgm*	*Mpi*	Total	Nm
Between samples							
All samples	20	0·1963***	0·0829***	0·1161***	0·0543***	0·0922***	2·5
Tahiti samples	5	0·2494***	0·0209*	0·0295**	−0·006	0·0305***	7·9
Moorea samples	3	0·0969**	−0·003	−0·005	0·0085	0·0125*	20·1
Huahine samples	3	−0·011	0·2176***	0·0711**	0·0197	0·0837***	2·7
Raiatea samples	4	−0·010	0·1066***	0·0180*	0·0230*	0·0428***	5·6
Rangiroa samples	2	0·1016**	0·1034**	0·0077	0·0267	0·0395***	6·3
Between islands							
All islands	8	0·1505***	0·0389***	0·1158***	0·0550***	0·0785***	2·9
Society islands	5	0·1835***	0·0062	0·0163***	0·0022	0·0251***	9·7
Tuamutu islands	2	0·0435*	−0·011	0·3551***	−0·001	0·1631***	1·3
Between archipelagoes							
All archipelagoes	3	0·0085**	0·0862***	0·1749***	0·1305***	0·1243***	1·8

[a] Number of samples considered. [b] Probability of homogeneity is indicated by stars after each Fst value.
$*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

to geographic distance after removing the effects of air and sea traffic ($\rho = 0·55$; $P < 0·01$), but not to air traffic after removing the effects of geographic distance and sea traffic ($\rho = -0·01$; $P = 0·54$), sea traffic after removing the effects of geographic distance and air traffic ($\rho = -0·0004$; $P = 0·53$), or air plus sea traffic after removing the effect of geographic distance ($\rho = -0·22$; $P = 0·16$). It thus appears that commercial sea and/or air traffics have no significant effect on genetic differentiation at these loci which are not expected to be under insecticidal selection.

Discussion

Culex p. quinquefasciatus populations have developed resistance to organophosphate (temephos and chlorpyrifos) and pyrethroid (permethrin) insecticides in French Polynesia. For the three insecticides, resistance ratios did not exceed 25−fold in 1992, and they were below 5-fold in all islands except Tahiti and Moorea. In these two islands, resistance to temephos had increased between 1990 and 1992 by about 2-fold. Two genes known to confer resistance to organophosphates in many parts of the world were observed: esterase B1 present in California since the mid 70s (Georghiou & Pasteur, 1978) and recently described in China (Qiao & Raymond, 1995; Xu *et al.* 1994), and the associated esterases A2–B2 also reported in California (Raymond *et al.* 1987, 1991) and in southeast Asia (Xu *et al.* 1994, and unpublished results). Esterase B1 was observed only in samples collected in 1990 at a low frequency, and it may have disappeared between 1990 and 1992. Esterases A2–B2 displayed three notable features. (1) They were observed in all the islands surveyed. (2) In 1990, their frequency was homogeneous in samples from Tahiti but not in samples from Moorea, whereas in 1992 it was homogeneous in Tahiti as well as in Moorea, but not in other islands (Huahine, Raiatea, Rangiroa). (3) In 1992, A2–B2 frequency was highest in Tahiti and displayed a striking decrease with increasing distance from Tahiti.

In French Polynesia, no organized control is specifically aimed at *C. p. quinquefasciatus*. Insecticide pressures on the species is therefore indirect and depends on intensity of control of the dengue vector *Aedes aegypti* with which it shares many breeding sites, agricultural pest control, and domestic use of insecticides (see Failloux *et al.* 1994). *Aedes aegypti* control is intense in Tahiti where potential carriers of dengue virus arrive via the international airport or seaport; agriculture is developed mainly in Tahiti, Moorea, Huahine and Raiatea, and to a lesser extent in Tubuai; and domestic insecticides are mostly used in urban areas (i.e. mainly on Tahiti) and near tourist hotels which are most abundant in Tahiti and Moorea, and less common in other islands. Overall, A2–B2 frequencies and resistance ratios are reflecting the intensity of insecticide use. An heterogeneous distribution of A2–B2 within islands (as in Moorea in 1990 and in 1992 in Huahine, Raiatea, Bora Bora and Rangiroa) could indicate a recent introduction or heterogeneous selection. Thus A2–B2, which are frequent on both sides of the Pacific, can have been introduced to French Polynesia only through Tahiti, the only island on which international flights and cargoes land. Their dispersal from Tahiti will then depend on the intensity of gene flow between Tahiti and other islands, and ultimately between these other islands.

Genetic differentiation (measured by F_{st} estimates for genes other than those concerned with insecticide resistance) between islands significantly increased with geographic distances and the confounding factor of amount of commercial air and sea traffic. This situation contrasts with the findings of Chevillon *et al.*

(1995) who observed no isolation by distance between *C. pipiens* populations colonizing the Mediterranean coast from Valencia in Spain to Nice (near the Italian border) in France. Along the coast there are no barriers to mosquito flight and innumerable opportunities for transport by man. By contrast in Polynesia, the ocean between islands (with relatively little commercial traffic) is a strong geographic barrier to gene flow. We know that *Culex pipiens* mosquitoes are regularly found in aircrafts (e.g. Highton & Van Someren, 1970; Curtis & White, 1984), and can also travel in ships (e.g. Craven *et al.* 1988), as exemplified by its colonization of Hawaii in 1826 (Van Dine, 1904). However, the effect on genetic differentiation due to either commercial air or boat traffic (or both) was confounded with that of geographical distance.

In conclusion, at least two genes conferring resistance to organophosphates, both present east and west of the Pacific Ocean, have been imported into French Polynesia: esterase B1 and the associated esterases A2–B2. It is likely that they arrived through the international airport or seaport of Tahiti, as do *Aedes aegypti* carrying new variants of dengue virus which have been responsible for recent dengue epidemics (Chungue *et al.* 1991). There is no doubt that the insecticidal control of *Aedes aegypti* around the Tahiti airport and seaport has selected for A2–B2 resistance genes in *C. p. quinquefasciatus*. The extent of insecticide resistance due to A2-B2 has two origins: it depends on the frequency of the gene, but also on the amplification level of the esterase B2 gene. Between 1990 and 1992, A1–B2 frequency has remained fairly constant, so that an increase in amplification level (due to the selection of insects with the highest number of gene copies) may be responsible for the slight (2-fold) increase in temephos resistance observed. Dispersal of the A2–B2 resistance gene most probably proceeded from Tahiti. Moorea, the island which is both the nearest and the most closely connected with Tahiti through commercial traffic, was likely to be the first island invaded by A2–B2. There may have been a single introduction of the resistance gene into each of the islands, and the gene presumably then increased in frequency according to the selection pressures existing there.

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