International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
www.entomologyjournals.com
Volume 2; Issue 3; May 2017; Page No. 37-41

Diagnosis and characterization of insensitive acetylcholinesterase and over-produced esterases associated with used organophosphate insecticide control in *Culex pipiens pipiens* (Diptera: Culicidae) from Tunisia

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Abstract
The polymorphism of AChE1 and over-produced esterases were analyzed in 18 field samples collected from Tunisia between 2002 and 2005. An AChE1 insensitive to propoxur inhibition was detected in all samples, with the exception of the sample # 18. The highest frequencies of the resistant phenotypes ([RS] and [RR]) (>0.83) were detected in the samples # 2, 3, 5, 6, 9, 13, 15, and 17. The highest frequencies of [RR] phenotype were recorded in samples # 9 (0.83) and 6 (0.75). This phenotype was absent in three samples (# 7, 14, and 18) and had a frequency ranged from 0.03 to 0.3 in the rest of the samples. The [RS] phenotype was present in all the samples, with the exception of the sample # 18, with a frequency ranged from 0.08 to 0.86, and > 0.75 in the samples # 3, 5, 15, and 17. The frequency of [SS] phenotype ranged from 0 in the sample (# 9) to 1 in the sample (# 18). Five esterases of high activity were observed in studied field samples. The esterase C1 encoded by the *Est-1* locus and four esterases encoded by the *Ester* super locus: A1, A2-B2, A4-B4 (or A5-B5, which has the same electrophoretic mobility) and B12. One or several esterases were detected in all the studied samples, with the exception of the sample # 18. Results were discussed in relation to resistance mechanisms and *Culex pipiens pipiens* control.

Keywords: *culex pipiens pipiens*, resistance mechanisms, control, ester locus, insensitive AChE 1, Tunisia

1. Introduction
For decades, the insecticides have been used to control pests and vectors of agricultural and public health importance. Among the most used insecticides in the control of mosquitoes, the most important insects involved in the transmission of vector-borne human diseases, we find the organophosphates (OPs). These insecticides inhibit the acetylcholinesterase (AChE), responsible for neurotransmitter degradation at the cholinergic nerve synapse, inducing lethal conditions. Two *ace* genes coding for AChE proteins are present in *Culex pipiens*: *ace1*, coding for AChE 1, involved in OPs and carbamates resistance (Weill *et al*., 2002) [25], and *ace2* (Malcolm *et al*., 1998) [15], coding for AChE 2, with a still unknown function. However, the extensive use of OPs had led to OPs resistance development in many mosquitoes, causing operational problems for control programs of these insects. OPs resistance mechanisms in mosquitoes and other insects include the selection of modified AChE 1 less sensitive to these insecticides (Oppenoorth, 1985; Weill *et al*., 2003; Hemingway *et al*., 2004; Russel *et al*., 2004; Alout *et al*., 2007) [1, 16, 26,], and increased metabolic detoxication of the insecticides (Rooker *et al*., 1996; Hemingway *et al*., 1998, 2004; Karunaratne and Hemingway, 2001; Ranson *et al*., 2002) [20, 7, 18] .

In Tunisia, *Culex pipiens pipiens* is very spread. This mosquito, although, does not actually transmit diseases, it is strongly fought, especially by the use of insecticides because of the nuisance that it causes. For years, the OPs and synthetic pyrethroids have been widely used in the mosquito control programs. Currently, in addition to pyrethroid insecticides (permethrin and deltamethnin), four OPs, the chlorpyrifos, temephos, pirimiphos methyl, and fenitrothion were largely used in *Culex pipiens pipiens* control. The previous studies realized over *Culex pipiens pipiens* populations of some Tunisian areas showed that these mosquitoes have developed high chlorpyrifos resistance levels (Ben Cheikh *et al*., 1995, 1998) [2,3]. These authors have also reported that the Tunisian populations of *Culex pipiens pipiens* possess an AChE insensitive to propoxur inhibition and over-produced esterases known to be involved in OP insecticides. The current study was realized to investigate the polymorphism of over-produced esterases and AChE 1 in Tunisian populations of *Culex pipiens pipiens* submitted to agricultural and public health control.

2. Materials and methods
2.1 Mosquitoes
Eighteen *Culex pipiens pipiens* samples were collected at preimaginal stages from breeding sites in 18 localities between March 2002 and November 2005 (Table1; Figure 1). Larvae and pupae were reared to imago under laboratory conditions. Two to three days after their emergence, adults from each collection were stored in liquid nitrogen for biochemical investigations. Reference strains included S-Lab, an insecticide-susceptible strain without any known resistance genes (Georghiou *et al*., 1966) [6], and two OPs resistant

strains: SA2, a resistant strain homozygous for Ester^2, displaying overproduced esterases A2-B2, and SA5, a resistant strain homozygous for Ester^5, displaying overproduced esterases A5-B5 (Berticat *et al.*, 2002) [4].

2.2 Over-produced esterases

Esterases of high activity were characterized on homogenates of adult thorax and abdomen by studying esterase activity in the presence of α-and-β-naphtyl acetate after protein separation by starch-gel electrophoresis (TME 7,4 buffer system) as described by Pasteur *et al*. (1988) [17] and were identified by comparing their electrophoretic mobility to that of known over-produced esterases.

2.3 Insensitive AChE1

AChE1 polymorphism was analyzed according to the method described by Bourguet *et al*. (1996a) comparing AChE1 activity of homogenates of adult heads in the absence or presence of propoxur. Each mosquito head was homogenized in 400 µl of 0, 25 M sodium phosphate buffer (PH 7, 0) containing 1% of triton X100. Enzymatic reactions were conducted in microtiter plate wells. For each adult head, aliquot of 100 µl were incubated for 15 min with 10 µl of alcohol, or of 10^{-3}M, and 10^{-1}M propoxur in alcohol. AChE activity was measured by adding 100 µl of a solution containing 15 mg of acetylthiocholine iodide (Sigma) and 15 ml of revelation solution (100 ml of 0.25 M phosphate buffer, 900ml H20, 91 mg DTNB, 30 mg $NaHCO_3$), using a spectrophotometer (Thermo Labsystems). This enzyme bioassay allows to discriminate between individuals expressing only the susceptible (ACHE1S, phenotype [SS]), only the resistant (ACHE1R, phenotype [RR]), or both types (phenotype [RS]) of ACHE1.

2.4 Data analysis

Statistical analyses of AChE1 polymorphism were performed using GENEPOP software, version 3.3 (Rousset, 2001) [21]. Conformity with Hardy–Weinberg was tested at *ace1* locus, using the exact U-score test of the alternative hypothesis of heterozygote excess and deficits (Rousset and Raymond, 1995) [22]. Estimate of Fis for each population was proposed by Weir and Cockerham (1984) [27]. Bonferroni procedure was used for taking into account multiple tests of significance (Hockberg, 1988).

3. Results

3.1 Overproduced esterases

Five esterases of high activity were observed in studied field samples (Table 2). The esterase C1 encoded by the *Est-1* locus and four esterases encoded by the *Ester* super locus: A1, A2-B2, A4-B4 (or A5-B5, which has the same electrophoretic mobility) and B12. One or several esterases were detected in all the studied samples, with the exception of the sample # 18. The A1 esterases were observed only in samples # 4 and 6, with a low phenotypic frequency of 0.03. The A2-B2 esterases were revealed in 14 samples with a frequency ranged from 0.02 in sample # 1 to 0.33 in sample # 9. The highest frequencies of these esterases (>0.31) were recorded in samples 9 and 15. The A4-B4 (and / or A5-B5) esterases were present in 15 samples with a frequency ranged from 0.03 in sample # 7 to 0.70 in sample # 17. The frequencies of these esterases were ≥0.45 in all the samples collected from in or near Tunis and from Cap Bon, in addition to the sample # 17 (South East). The B12 esterases were observed in 12 samples, with a frequency ranged from 0.03 in sample # 17 to 0.19 in samples # 8 and 10. The C1 esterases were found in 9 samples, with a frequency ranged from 0.03 in samples # 10 and 11 to 0.28 in sample # 16.

3.2 Insensitive AChE1

The polymorphism of AChE1 was analyzed in 18 field samples collected between 2002 and 2005 (Table 2). An AChE1 insensitive to propoxur inhibition was detected in all samples, with the exception of the sample # 18 located in the extreme south where no OPs insecticides were used for mosquitoes control. The highest frequencies of the resistant phenotypes ([RS] and [RR]) (>0.83) were detected in the samples # 2, 3, 5, 6, 9, 13, 15, and 17 where different OPs insecticides were used for control. The highest frequencies of [RR] phenotype were recorded in samples # 9 (0.83) and 6 (0.75). This phenotype was absent in the most susceptible samples (# 7, 14, and 18) and had a frequency ranged from 0.03 to 0.3 in the rest of the samples. The [RS] phenotype was present in all the samples, with the exception of the sample # 18, with a frequency ranged from 0.08 to 0.86, and > 0.75 in the samples # 3, 5, 15, and 17. When considering the presence of two alleles (*ace-1^R* and *ace-1^S*), an excess of [RS] phenotype was observed in samples # 1, 3, 5, and 15 with values of Fis of -0.464, -0.614, -0.612, and -0.717, respectively, and a deficit of this phenotype was noticed in sample # 6 (Fis=0.752). The frequency of [SS] phenotype ranged from 0 in the probably most resistant sample (# 9) to 1 in the probably most susceptible sample (# 18) and was significantly correlated to propoxur mortality (Spearman rank correlation, r=0.87, $p < 0.01$).

4. Discussion

Several overproduced esterases, known to be involved in the OPs resistance, were detected in all the resistant field samples. For example, the overproduced esterases A1, A2-B2, A4-B4 (and / or A5-B5) and B12, detected in the probably most resistant samples (# 6 and 9), with a phenotypic frequency of 0.67 and 0.85, respectively. Our results are in agreement with previous studies on the role of the EST and the GST in the OPs resistance (Ben Cheikh *et al.*, 1998; Liu *et al*., 2005) [2-13]. However, our results are not in agreement with other previous studies. The importance of esterases in the OPs resistance has been reported in *Culex* mosquitoes (Whyard *et al.*, 1994; Tomita *et al.*, 1996; Hemingway and Karunaratne., 1998; Hemingway *et al.*, 1998; Bisset *et al*., 1999) [28, 24, 7] and *Aedes aegypti* (Rodriguez *et al*., 2001; Macoris *et al*., 2003) [19-14].

The inhibition study of AChE 1 by propoxur showed the presence of sensitive ([SS]) and resistant ([RR] and [RS]) phenotypes of AChE 1 in Tunisian *Culex pipiens* samples. The [RR] phenotype was absent in the probably most susceptible samples not subjected to OPs insecticide control (# 7, 14, and 18). These samples have the highest [SS] phenotype frequencies (0.50-1) and a [RS] phenotype frequency equal to zero or ranged from 0.31 to 0.50. The highest [RR] phenotype frequencies (≥0.75) were observed in the probably most resistant samples subject to many OPs insecticides control (# 6, and 9). In these samples, the [RS] and [SS] phenotypes frequency was low (≤0.17), and equal to

0 or low (0.17), respectively. The highest frequencies of [RS] phenotype (0.75-0.86) were detected in probably very resistant samples (# 3, 5, 15, and 17), in the presence of low [RR] and [SS] phenotypes frequencies, not exceeding 0.18 and 0.10, respectively. These samples were not in H-W equilibrium because of an excess in heterozygote. The most susceptible samples were not fought by the use of OP insecticides. In contrast, the very resistant samples were subjected to very frequent or frequent applications of OP insecticides with the exception of sample # 15. This sample, rarely fought by the use of OP insecticides, was collected from a locality where the utilization of insecticides in agriculture was frequent (Table 1). The frequencies of different AChE 1 phenotypes observed can be the result of the insecticide selection pressure and the fitness costs associated with *ace-1*^{R} and *ace-1*^{S} alleles, and duplicated haplotype. The main frequency of [SS], [RS] and [RR] phenotypes in *Culex pipiens quinquefasciatus* from Martinique was 0.51, 0.49, and less than 0.01, respectively, with the frequency of [RS] reaching 0.76 in some populations (Yebakima *et al*., 2004) [29]. This corresponds to a very large departure from H-W equilibrium (Labbé *et al*., 2007) [11] and is certainly due to the high frequency of the duplicated haplotype (Lenormand *et al.*, 1998) [12]. The overall fitness advantage of the duplicated haplotype may result from a much lower fitness cost, but this hypothesis remains to be tested (Labbé *et al*., 2007) [11]. These results indicate the very particular interest of *Culex pipiens pipiens* control programs in Tunisia which integrate alternative methods (dredging of ditches and rivers, use of predator fish...), the mobilization and active participation of human populations, and a reasonable insecticides application. These integrated management plans of the *Culex pipiens pipiens* populations are especially necessary in the localities where this mosquito developed a high resistance level to the all used insecticides (areas of Tunis, Cap Bon). For an efficient use of insecticides in Tunisia, the stable zones strategy of Lenormand *et al*. (1998) [12], based on the antagonist role of selection and migration on the resistance evolution, might be suitable. The available data on the geographical distribution of resistance levels to the all used OP and pyrethrinoïd insecticides in the *Culex pipiens pipiens* control in Tunisia and genes involved in the observed resistances, the continuous monitoring of insecticides resistance and the possibility of using other products (bioinsecticides of *Bacillus thuringiensis israelensis* basis…) can help to conceive some protocols to apply the foresaid strategy.

Table 1: Geographic origin of Tunisian populations, breeding site characteristics, and insecticide control

Code	Locality	Breeding site	Date of collection	Mosquito control (used insecticides)	Agricultural pest control
1	Ousja	Ditch	July 2003	Occasional (F)	Yes
2	Sidi Thabet	Ditch	Aug. 2004	Rare (C,P)	Yes
3	Sokra	Canal	June 2003	Very frequent (C, Pm, F, P, D)	Yes
4	Mannouba	River	June 2005	Occasional (P,D)	Yes
5	Ouardia	Ditch	Aug. 2005	Very frequent (C, F, P, D)	None
6	Ezzahra	Ditch	Nov 2005	Very frequent (C, F, P, D, T)	None
7	Krib	River	0ct. 2005	Occasional (P)	Yes
8	Belli	River	Aug. 2003	Rare (C,D)	Yes
9	Tazarka	River	May 2005	Very frequent (C, T, Pm, F, P, D)	Yes
10	Sidi khalifa	Waste water pond	July 2004	None	None
11	kalaa Kebira	River	July 2003	Occasional (F, Pm, P, D)	None
12	Monastir	Ditch	Aug. 2003	Rare (C,F)	Yes
13	Moknine	Waste water pond	Aug. 2003	Very frequent (C)	Yes
14	Hajeb laayoun	River	July 2004	None	Yes
15	Sbiba	River	Sept. 2004	Rare (Pm, P)	Yes
16	Tozeur	Ditch	Oct. 2005	Frequent (C, Pm, F, P, D)	None
17	Gabes	Drain	June 2005	Frequent (C, Pm, P, D)	None
18	Bordj El Khadra	Water pond	March 2002	Occasional (P)	None

C: Chlorpyrifos; **T:** Temephos; **Pm:** Pirimiphos methyl; **F:** Fenitrothion; **P:** Permethrin; **D:** Deltamethrin

Table 2: Frequencies of insensitive acetylcholinesterase and over-produced esterases phenotypes in Tunisian populations of *Culex pipiens pipiens*

Population	*N*	*Ester* Locus								*Est-1* Locus		*ace-1* Locus	
		[1]	[2]	[4]	[12]	[24]	[212]	[412]	[0]	[C1]	[SS]	[RS]	[RR]
1-Ousja	42	-	0.02	0.07	-	-	-	-	0.91	-	0.36	0.64	-
2-Sidi thabet	34	-	0.09	0.44	-	0.03	-	0.06	0.38	0.06	0.15	0.59	0.26
3-Sokra	34	-	0.03	0.47	0.12	0.12	-	0.06	0.20	-	0.03	0.79	0.18
4-Mannouba	34	0.03	0.06	0.47	0.03	0.03	0.03	-	0.35	-	0.26	0.71	0.03
5-Ouardia	42	-	0.02	0.34	0.02	0.07	-	0.07	0.48	-	0.10	0.76	0.14
6-Ezzahra	36	0.03	0.06	0.36	0.06	0.08	-	0.03	0.33	0.11	0.17	0.08	0.75
7-Krib	36	-	-	0.03	-	-	-	-	0.97	-	0.69	0.31	-
8-Belli	36	-	0.03	0.42	0.19	0.03	-	-	0.28	0.11	0.36	0.58	0.06
9-Tazarka	48	-	0.15	0.50	-	0.16	0.02	0.02	0.15	0.04	-	0.17	0.83
10-Sidi khalifa	36	-	0.06	0.19	0.14	0.06	-	0.05	0.50	0.03	0.56	0.33	0.11
11-kalaa Kebira	35	-	0.14	0.23	0.11	0.03	-	0.03	0.46	0.03	0.51	0.46	0.03
12-Monastir	36	-	0.11	0.22	-	-	-	-	0.67	-	0.39	0.56	0.05
13-Moknine	36	-	0.11	0.28	0.03	0.03	-	0.03	0.52	0.06	0.11	0.69	0.20
14-Hajeb laayoun	18	-	-	-	0.11	-	-	-	0.89	-	0.50	0.50	-
15-Sbiba	36	-	0.31	-	-	-	-	-	0.69	0.11	0.08	0.86	0.06

16-Tozeur	36	-	-	0.33	-	-	-	-	0.44	0.28	0.42	0.44	0.14
17-Gabès	36	-	0.11	0.56	-	0.11	-	0.03	0.19	-	0.08	0.75	0.17
18- Bordj El Khadra	36	-	-	-	-	-	-	-	1	-	1	-	-

Phenotype [i] corresponds to genotypes *Ester*^{i} / *Ester*^{0} or *Ester*^{i} / *Ester*^{i}, and phenotype [ij] correspond to genotype *Ester*^{i} / *Ester*^{j}.
N represents the total number of mosquitoes analyzed for each sampling site.

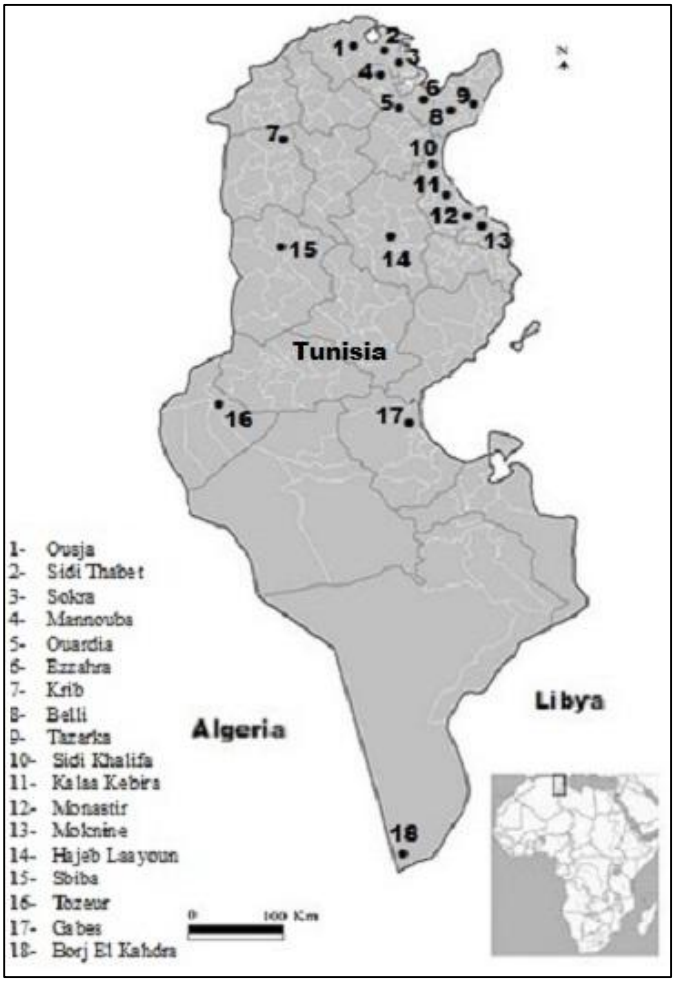

Fig 1: Geographic origin of Tunisian populations

5. Acknowledgements
This work was kindly supported by the Ministry of Higher Education and Scientific Research of Tunisia by funds allocated to the Research Unit (Génétique 02/UR/08-03) and by DHMPE of the Minister of Public Health of Tunisia. We are very grateful to S. Ouanes, for technical assistance, A. Ben Haj Ayed and I. Mkada for help in bioassays, S. Saïdi, Tunisian hygienist technicians for help in mosquito collecting, and M. Nedhif and M. Rebhi for their kind interest and help.

6. References
1. Alout H, Berthomieu A, Hadjivassilis A, Weill M. A new amino-acid substitution in acetylcholinesterase 1 confers insecticide resistance to *Culex pipiens* mosquitoes from Cyprus. Insect Biochem. Mol. 2007; 37:41-47.
2. Ben Cheikh H, Haouas-Ben Ali Z, Marquine M, Pasteur N. Resistance to organophosphorus and pyrethroid insecticides in *Culex pipiens* (Diptera: Culicidae) from Tunisia. J. Med. Entomol. 1998; 35:251-260.
3. Ben Cheikh H, Marrakchi, M, Pasteur N. Mise en évidence d'une très forte résistance au chlorpyrifos et à la perméthrine chez les moustiques *Culex pipiens* de Tunisie. Arch. Institut Pasteur Tunis. 1995 ; 72 :7-12.
4. Berticat C, Boquien G, Raymond M, Chevillon C. Insecticide resistance genes induce a mating competition cost in Culex pipiens mosquitoes. Genet. 2002 ; 83:189-196.
5. Bisset JA, Rodriguez MM, Diaz C, Soca A. Characterization of resistance to organophosphate insecticides, carbamates, and pyrethroids in *Culex quinquefasciatus* from the state of Miranda, Venezuela. Rev. Cubana Med. Trop. 1999; 51:89-94.
6. Georghiou GP, Metcalf RL, Gidden FE. Carbamate resistance in mosquitoes. Selection of *Culex pipiens fatigans* Wied. For resistance to Baygon. Bull. Word Health Org. 1966; 35:691-708.
7. Hemingway J, Karunaratne SH. Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance in mechanism. Med. Vet. Entomol. 1998; 12:1-12.
8. Hemingway J, Hawkes N, McCarroll I, Ranson H. The molecular basis of insecticide resistance in mosquitoes. Insect Biochem. Mol. Biol. 2004; 34:653-665.
9. Hochberg Y. A sharper Bonferroni Procedure for multiple tests of significance. Biometrika. 1988; 75:800-802.
10. Karunarathne SH, Hemingway J. Malathion resistance and prevalence of the malathion carboxylesterase mechanism in populations of mosquito vectors of disease in Sri Lanka. Bull. World Health Org. 2001; 79:1060-1064.
11. Labbé P, Berthomieu A, Berticat C, Alout H, Raymond M, Lenormand T, Weill M. Independent duplications of the acetylcholinesterase gene conferring insecticide resistance in the mosquito *Culex pipiens*. Mol. Biol. Evol. 2007; 24: 1056-1067.
12. Lenormand T, Guillemaud T, Bourguet D, Raymond M. Appearance and sweep of a gene duplication: adaptive response and potential for a new function in the mosquito Culex pipiens. Evolution. 1998; 52:1705-1712.
13. Liu H, Xu Q, Zhang L, Liu N. Chlorpyrifos resistance in mosquito *Culex quinquefasciatus*. J. Med. Entomol. 2005; 42:815-820.
14. Macoris MLG, Andrighetti MTM, Takaku L, Glasser CM, Garbeloto VC, Bracco JE. Mem. Inst. Oswaldo Cruz, Rio de Janeiro. 2003; 98:703-708.
15. Malcolm CA, Bourguet D, Ascolillo A, Rooker SJ, Garvey CF, Hall LMC, *et al*. A sex-linked *Ace* gene, not linked to insensitive acetylcholinesterase-mediated insecticide resistance in *Culex pipiens*. Insect Mol. Biol. 1998; 7:107-120.
16. Oppenoorth FJ. Biochemistry and genetics of insecticide resistance, In Kerkut, G.A., Gilbert, L.I.(Eds.), Comprehensive insect physiology, Biochemistry and

pharmacology, Pergamon, Oxford, England. 1985; 12: 731-773.
17. Pasteur N, Pasteur G, Catalan J, Bonhomme F, Britton-Davidian J. Practical isozyme genetics. Ellis Horwood, Chichester, United Kingdom, 1988.
18. Ranson H, Claudianos C, Ortelli F, Abgrall C, Hemingway J, Sharakhova MV, *et al*. Evolution of supergene families associated with insecticide resistance. Science (Wash.DC). 2002; 298:179-181.
19. Rodriguez MM, Bisset J, Fernandez DMD, Lauzan L, Soca A. Detection of insecticide resistance in Aedes aegypti (Diptera: Culicidae) from Cuba and Venezuela. J. Med. Entomol. 2001 ; 38:623-628.
20. Rooker S, Guillemaud T, Berge J, Pasteur N, Raymond M. Co-amplification of esterase A and B genes as a single unit in *Culex pipiens* mosquitoes. Heredity. 1996; 77:555-561.
21. Rousset F. Inferences from spatial population genetics. In: Balding, D., Bishop, M., Cannings, C. (Eds.), Handbook of statistical genetics. John Wiley & Sons. 2001, 239-269.
22. Rousset F, Raymond M, Testing heterozygote excess and deficiency. Genetics. 1995; 140:1413-1419.
23. Russel RJ, Claudianos C, Campbell PM, Horne I, Sutherland TD, Oakeshott JG. Two major classes of target site insensitivity mutations confer resistance to organophosphate and carbamate insecticides. Pestic. Biochem. physiol. 2004; 79:84-93.
24. Tomita T, Kono Y, Shimada T. Chromosomal localization of amplified esterase genes in insecticide resistant *Culex* mosquitoes. Insect Biochem. Mol. Biol. 1996; 26:853-857.
25. Weill M, Fort P, Berthomieu A, Dubois MP, Pasteur N, Raymond M, A novel acetylcholinesterase gene in mosquitoes codes for the insecticide target and is non-homologous to the *ace* gene in *Drosophila*. Proc. Roy. Soc. Lond. (Biol. Sci). 2007-2016, 2002 ; 269.
26. Weill M, Lutfalla G, Mogensen K, Chandre F, Berthomieu A, Berticat C,et *et al*. Insecticide resistance in mosquito vectors. Nature (Lond.). 2003; 423:136-137.
27. Weir BS, Cockerham CC. Estimating F-statistics for the analysis of population structure. Evolution. 1984; 38: 1358-1370.
28. Whyard S, Downe AFR, Walker VK. Isolation of an esterase conferring insecticide resistance in the mosquito *Culex tarsalis*. Insect Biochem. Mol. Biol. 1994; 24:819-827.
29. Yebakima A, Marquine M, Rosine J, Yp-tcha MM, Pasteur, N. Evolution of resistance under insecticide selection pressure in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) from Martinique. J. Med. Entomol. 2004; 41:718-725.