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DETOXIFYING ESTERASES IN *CULEX PIPIENS QUINQUEFASCIATUS* FROM THE CARIBBEAN COUNTRIES

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ABSTRACT. Several over-produced esterases confer resistance to organophosphorus insecticides in the *Culex pipiens* complex. We describe their distribution in islands and countries of the Caribbean region based on new collections and previous studies, and discuss the need to: 1) undertake DNA studies to correctly identify the esterase B alleles that are amplified in different regions, and 2) investigate the variability among gene copies within each amplification system in order to fully understand their origin and their evolution through time.

The involvement in resistance to organophosphorus (OP) insecticides of 2 types of esterases (A and B), displaying an increased activity toward naphthyl acetate, is now well documented in the *Culex pipiens* complex. A third type of esterases (esterases C) has recently been observed in Europe and Martinique. They are believed to also confer resistance to OP insecticides because their frequency increases in mosquitoes that survive exposures to OP insecticides (Yébakima et al. 1995). Esterases A, B, and C are encoded by distinct loci, namely *Est-3, Est-2,* and *Est-1.*

Increased activity of esterases A and B is due to enzyme over-production. In the case of esterases B, this over-production is due to amplification of the DNA sequences (at least 30 kb) encompassing the structural 2.1-kb gene (Mouchès et al. 1990). Several esterase B alleles have been amplified independently (Raymond et al. 1991, Poirié et al. 1992, Xu et al. 1994, Vaughan et al. 1995), and it has been suggested that the amplification of each allele was a unique event (Raymond et al. 1991). Thus, it follows that the present geographic distribution of each amplified esterase B allele was acquired by passive or active migration of the species.

We report here the nature of over-produced esterases observed in *Culex pipiens quinquefasciatus* Say[5] from different islands in the Caribbean Sea and surrounding countries, and summarize the findings of previous investigations.

Esterases were studied on single adult mosquito homogenates submitted to starch gel electrophoresis (TME 7.4 buffer systems) and revealed in the presence of equal quantities of alpha- and beta-naphthyl acetates (Pasteur et al. 1988). These mosquitoes were collected as larvae between 1988 and 1994 in Barbados, Cuba, Guadeloupe, Haiti, Martinique, Puerto Rico, St. Lucia, St. Martin, Brazil, French Guiana, and Venezuela (Table 1). Larvae were reared to the adult stage under standard laboratory conditions, and adults were stored in liquid nitrogen until processed. Four esterases of high activity previously observed in the American region were detected: esterase C2, esterase B1, and the associated esterases A2–B2. These esterases were present in all the studied Caribbean islands and in French Guiana; esterase B1 was not observed in the sample from Brazil and esterases A2–B2 were found in a single individual from one of the Venezuela samples (Table 1). A new over-produced esterase, hydrolyzing preferentially beta-naphthyl acetate as do esterases B, was observed in 2 mosquitoes from Venezuela (Table 1). This new esterase B may correspond to the new amplified esterase B gene observed in a DNA study by Qiao and Raymond (1995) in the same population.

Esterases B1, A2–B2, and C2 have been previously described in the American region. Esterase B1 was first reported in a laboratory colony (TEM-R strain) derived from mosquitoes collected in 1974 in California. It was later reported in other U.S. states: Florida, Illinois, Louisiana, Michigan, New Jersey, Texas, and Utah

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[5] We are using the name *Culex pipiens quinquefasciatus* rather than *Cx. quinquefasciatus* to indicate that *Cx. pipiens pipiens* from temperate areas and *Cx. pipiens quinquefasciatus* are subspecies and not distinct species. This is evident from the studies on genetic exchanges in areas where the 2 subspecies are sympatric.

Table 1. Percentages of mosquitoes with highly active esterases in Caribbean populations of *Culex pipiens quinquefasciatus*.

Country (locality)	Date of collection	Sample size	Esterase C		Esterases B				
			C2	None	B2[1]	B1	B2/B1	New	None
Barbados									
(Bridgetown)	Aug. 1991	34	31	3	1	28	0	0	5
Cuba									
(La Havana)	Oct. 1993	60	20	40	1	35	0	0	24
Guadeloupe									
(Pte à Pitre)	Feb. 1992	86	55	31	50	13	3	0	20
Haiti									
(Haiti I)	Oct. 1990	29	1	28	0	2	0	0	27
(Haiti II)	Oct. 1990	29	0	29	0	0	0	0	29
(Gonaïves)	Jul. 1991	92	40	52	0	25	0	0	67
(St Marc)	Jul. 1991	30	13	17	1	24	0	0	5
Martinique									
(Fort de France)	Jun. 1988	30	29	1	22	2	4	0	2
(—)[2]	May 1990	433	286	147	259	9	0	0	165
Puerto Rico									
(San Juan–1)	Feb. 1992	67	59	8	0	9	0	0	58
(San Juan–2)	Feb. 1992	8	6	2	1	0	0	0	7
(San Juan–3)	Jul. 1994	30	30	0	0	7	0	0	23
St. Lucia									
(Castries)	May 1992	90	54	36	16	25	0	0	49
(Gros îlet)	Oct. 1994	60	39	21	7	51	0	0	2
(Donnery)	Oct. 1994	60	40	20	4	33	0	0	23
(Soufrière)	Oct. 1994	60	38	22	5	41	0	0	14
St. Martin									
(Sandy Ground)	Jun. 1993	60	14	46	4	10	0	0	46
Brazil									
(Fortaleza)	Nov. 1993	24	22	2	19	0	0	0	5
French Guiana									
(Cayenne–1)	Jun. 1991	163	157	6	51	40	27	0	45
(Cayenne–2)	Nov. 1992	26	14	12	4	7	7	0	8
Venezuela									
(Punta del Monte)	Mar. 1991	58	25	23	0	30	0	2[3]	26
(Eriberto)	Mar. 1992	36	2[4]	34	1	0	0	0	33

[1] All mosquitoes with esterase B2 had also esterase A2, except in Martinique where one mosquito had no esterase A2 and in Cayenne–2 where 2 mosquitoes with both B1 and B2 had no A2.
[2] Sum of the data published in Yébakima et al. (1995).
[3] Highly active esterase never observed previously, see text.
[4] One of these mosquitoes had two esterases C (C2 and a band with lower mobility).

(see Raymond et al. 1991). Bisset et al. (1990) observed esterase B1 in Cuba.

Esterases A2–B2 were first described in Tanzania. In the American region, they were first observed in California in 1984 (SELAX strain), then in Louisiana, New Jersey, and Texas (see Raymond et al. 1991). These esterases have also been found in Cuba (Bisset et al. 1990).

Our and previous studies indicate that overproduced esterases are widely distributed in the Americas. In some cases, electrophoretic studies cannot discriminate different amplified esterase B alleles. This was the case with the B4 and B5 esterases from Mediterranean countries (Poirié et al. 1992), which can only be distinguished from one another by the restriction pattern of the amplified region. Similarly, Vaughan et al. (1995) reported that, in the Cuban MRES strain, the amplified esterase that has the same electrophoretic mobility as the TEM-R esterase B1 from California displays a different restriction pattern and amino acid sequence. Among the samples studied here, it was shown that the restriction pattern of the amplified esterase B re-

gion of mosquitoes 1) with an esterase B1 is identical to that of TEM-R in Puerto Rico, French Guiana, and Venezuela (Qiao and Raymond 1995), and 2) with an esterase B2 is identical to that observed in other regions by Raymond et al. (1991) in French Guiana (Qiao and Raymond 1995) and Martinique (Yébakima et al. 1995). Thus, in the American region, at least 3 esterase B amplified alleles are present ($B1^{TEM\text{-}R}$, $B1^{MRES}$, and *B2*), and 2 of these alleles can be correctly identified only by DNA investigations.

A hypothesis by Raymond et al. (1991) that each amplification derives from a unique event recently has been opposed by Hemingway et al. (1993) on the basis of variations observed in the kinetics of partially purified esterases and in the resistance spectrum of insects with the same electrophoretically identified esterase B2. There is evidence that the high level of amplification observed in some populations or laboratory strains (more than 250 copies) has not been reached in a single step, but has occurred gradually (Pasteur et al. 1980, Devonshire and Field 1991). Raymond et al. (1991) claim as unique the first event of amplification only, that is, the step that first increased the number of gene copies of a particular allele. This step has undoubtedly been followed by multiple secondary events that further increase gene copies up to the numbers presently observed. In the Caribbean islands investigated here, the levels of resistance to organophosphates are low compared to those observed in the reference laboratory strains with an amplified esterase B1 (TEM-R strain) or with the associated esterases A2–B2 (SELAX strain). In 1990, these strains presented a temephos resistance ratio of 1,230- and 32-fold, respectively (Raymond et al. 1993). For example, the 2 Haiti samples with a high frequency of esterase B1 had temephos resistance ratios of 5.4- and 6.0-fold (Yébakima 1991[6]) and the Martinique samples with high frequency of the associated esterases A2–B2, had temephos resistance ratios between 6.9- and 10-fold (Yébakima et al. 1995). These low levels of resistance are associated with esterases that display a much lower activity than that observed in the reference strains, indicating that esterase over-production and gene amplification are still at a low level. In countries where OP insecticides are used extensively, it is likely that both the frequency of carriers and the number of esterase gene copies will increase. As esterase gene copy number increases, the resistance characteristics will change in intensity and possibly in quality due to eventual mutation within some copies of the esterase gene coding sequences within the amplification.

In conclusion, the present study points out the need to carry out DNA studies in order to correctly identify esterase B alleles because several alleles may present the same electrophoretic mobility. In addition, understanding of the evolution of each amplified system requires the investigation of the variability of gene copies, both in their number and coding sequence.

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