

## EVOLUTION OF RESISTANCE GENES IN ABSENCE OF INSECTICIDE SELECTION IN A HYPOGEOUS POPULATION OF *CULEX PIPIENS* FROM THE FRENCH ALPS

YANNICK RIVET AND NICOLE PASTEUR<sup>1</sup>

*Laboratoire de Génétique et Environnement, Institut des Sciences de l'Evolution (URA CNRS no. 327), Université de Montpellier II (case courrier 65), 34095 Montpellier, France*

**ABSTRACT.** A monthly survey of resistance gene frequencies was conducted in a larval population of *Culex pipiens* breeding in an hypogeous site ("molestus" form) between July 1989 and September 1991, after interrupting mosquito control. Several significant variations in resistance gene frequency were observed. Some variations are explained by fitness differences between resistant and susceptible insects, others by an immigration of insects from the surrounding epigeous populations ("pipiens" form), which are highly resistant.

### INTRODUCTION

A mosquito control program has been underway in the French Alps (Rhône-Alpes Region) since 1975, using mainly organophosphorous insecticides (OP). *Culex pipiens* Linn. control consists of treating all potential breeding sites 3-4 times during July and August with chlorpyrifos (Piridur 1G<sup>®</sup>) or temephos (Abate 1G<sup>®</sup>). Recently, four OP resistance genes have been identified in open air (epigeous) sites, namely insensitive acetylcholinesterase *Ace*<sup>R</sup>, and overproduced esterases A1, A2-B2 and A4-B4 (Rivet 1992).<sup>2</sup>

We report here the evolution of these genes in an hypogeous population (CHYE) collected in the Chambéry area over a period of 2 years. The CHYE population was localized in a flooded basement of a water-processing plant. This basement connects with the exterior by a metal door that is occasionally opened for maintenance, and through a network of underground sewage channels. During the winter, temperature remains mild due to the heat generated by hydraulic turbines, and larvae are continuously found although they are less numerous than during the summer. Adults (both males and females) are always present on the walls and ceiling, and it was shown that larvae collected from the site and reared until adulthood in the laboratory were autogenous, i.e., they do not require a blood meal to produce their first batch of eggs.

The above ecological and biological characteristics are typical of the so-called "molestus" form of *Cx. pipiens*. They contrast from those of the

"pipiens" form, which breeds in epigeous (open air) sites. In epigeous sites, larvae are found only from late spring to early autumn, and they produce females that are anautogenous (unable to produce their first batch of eggs in absence of a blood meal). In addition, these females hibernate during the cold season (Roubaud 1933, Barr 1982).

As all potential epigeous and hypogeous breeding sites of the Chambéry area, the CHYE breeding site has been subject to OP control for many years. However, during the period of our study (July 1989 to September 1991) no insecticide treatment was applied to the CHYE site. Our results are discussed in view of the biological characteristics of hypogeous populations and our knowledge of resistance gene fitness.

### MATERIAL AND METHODS

*Culex pipiens* samples were collected as larvae and/or pupae in the hypogeous CHYE breeding site. Larvae were reared under standard laboratory conditions until the imaginal stage, and 2-day-old adults were stored in liquid nitrogen until further processing.

Resistance genes were identified by biochemical techniques. Starch gel electrophoresis (Pasteur et al. 1988) was used to analyze overproduced detoxifying esterases, and microtiter plate assay (Raymond et al. 1985) to investigate insensitive acetylcholinesterase (AChE). Several laboratory strains of known resistance were used as controls: the susceptible S-LAB strain (Georghiou et al. 1966), which contains a normal AChE and no overproduced esterases, and OP-resistant strains S54 (Pasteur et al. 1981), SE-LAX (Wirth et al. 1990), VIM (Poirié et al. 1992), and MSE (Raymond et al. 1986). Strains S54, SELAX, VIM and MSE are OP-resistant due to the presence of esterase A1, esterases A2-B2, esterases A4-B4, and insensitive AChE, respectively. Insensitive AChE and overproduced

<sup>1</sup> To whom correspondence should be sent.

<sup>2</sup> Rivet, Y. 1992. Les populations de *Culex pipiens* (Diptera: Culicidae) vues au travers des gènes de résistance aux insecticides organophosphorés. Etude dans la région Rhône-Alpes. Thèse de doctorat, Université Claude Bernard, Lyon.

esterases were analyzed on the same mosquitoes, using homogenates of head and thorax for AChE and of abdomen for esterases. Contingency tables ( $2 \times 2$ ) were compared using the Fisher exact probability test.

## RESULTS

*Resistance genes in CHYE population in July 1989:* Among the 116 mosquitoes analyzed from the July 1989 collection, three resistance genes were observed: insensitive AChE (*Ace<sup>R</sup>* gene), and 2 sets of overproduced detoxifying esterases (esterase A1 and the associated esterases A4 and B4). *Ace<sup>R</sup>* allele frequency was 0.513, and frequencies of mosquitoes with esterases A1 and A4-B4 were 0.22 and 0.09, respectively. These frequencies were within the range of those observed in 10 epigeous populations localized within 10 km of the CHYE site. In these epigeous populations (Rivet 1992)<sup>2</sup>, *Ace<sup>R</sup>* frequencies ranged from 0.186 to 0.707 (mean = 0.368,  $\sigma = 0.181$ ), esterase A1 from 0.034 to 0.517 (mean = 0.233,  $\sigma = 0.161$ ), and esterases A4-B4 from 0.017 to 0.106 (mean = 0.057,  $\sigma = 0.031$ ).

*Evolution of overproduced esterases from July 1989 to September 1991:* Detoxifying esterases A1 and A4-B4 disappeared from the site between July 1989 and April 1990 (this variation is highly significant:  $P < 0.00001$  for A1 and  $P < 0.01$  for A4-B4). From April 1990 to July 1991, these esterases were either absent or present at a very low frequency (Table 1). In September 1991, the

2 esterases recovered a frequency which was not significantly different ( $P > 0.05$ ) from that of July 1989. In the collections of October 1990 and September 1991, two other detoxifying esterases (the associated esterases A2 and B2) were observed at a low frequency (0.009 and 0.026, respectively). Esterases A2-B2 are present in many countries of Europe, Africa, Asia and America; and there is evidence that they acquired their present geographic distribution through migration (Raymond et al. 1991). Rivet et al. (1993) have described their progression in France, and it seems that they reached the Rhône-Alpes Region in 1990.

*Evolution of insensitive AChE (*Ace<sup>R</sup>* allele) from July 1989 to September 1991:* Unlike detoxifying esterases, *Ace<sup>R</sup>* allele frequency displayed several significant variations after the initial decrease observed between July 1989 and April 1990 (Table 1). Four periods correspond to significant ( $P < 0.001$ ) changes: 1) a decrease of 0.513 to 0.103 between July 1989 and April 1990 associated with a similar decrease of overproduced esterases, 2) an increase of 0.017 to 0.199 between August and September 1990, 3) a decrease of 0.199 to 0.034 between September and October 1990, and 4) an increase of 0.018 to 0.231 between July and September 1991.

## DISCUSSION

Three important characteristics were revealed in the CHYE hypogeous population during the 2-year monthly survey described in the present

Table 1. Evolution of the frequencies of mosquitoes with overproduced esterase and of the frequency of the *Ace<sup>R</sup>* allele in the CHYE population between July 1989 and September 1991.

Date of collection	Sample	Over-produced esterases			<i>Ace<sup>R</sup></i>
		A1	A4-B4	A2-B2	
July 1989	116	0.223	0.095	0	0.513
April 1990	58	0	0	0	0.103 **1
May 1990	57	0	0	0	0.114 NS <sup>1</sup>
June 1990	58	0.017	0.034	0	0.098 NS <sup>1</sup>
July 1990	58	0	0	0	0.043 NS <sup>1</sup>
August 1990	58	0	0	0	0.017 NS <sup>1</sup>
September 1990	113	0.009	0.035	0	0.199 **1
October 1990	58	0	0.017	0.009	0.034 **1
November 1990	58	0	0	0	0.017 NS <sup>1</sup>
December 1990	58	0	0	0	0.026 NS <sup>1</sup>
January 1991	58	0	0	0	0.034 NS <sup>1</sup>
February 1991	44	0	0	0	0.011 NS <sup>1</sup>
March 1991	58	0	0.017	0	0.017 NS <sup>1</sup>
April 1991	58	0	0	0	0.026 NS <sup>1</sup>
May 1991	29	ND <sup>2</sup>	ND	ND	0.017 NS <sup>1</sup>
June 1991	58	ND	ND	ND	0.009 NS <sup>1</sup>
July 1991	57	ND	ND	ND	0.018 NS <sup>1</sup>
September 1991	48	0.231	0.077	0.026	0.231 **1

<sup>1</sup> Fisher exact probability testing the difference in *Ace<sup>R</sup>* frequency with the preceding sample: \*\* =  $P < 0.001$ .

<sup>2</sup> ND = no data.

study. 1) In 1989, at the beginning of our study, the frequencies of all resistance genes were comparable to those observed in epigeous populations of the same area. 2) These resistance genes almost disappeared in the following months as larval insecticide control was interrupted. 3) *Ace<sup>R</sup>* allele frequency displayed a significant increase in September of 2 consecutive years; this increase was immediately followed by a decrease in 1990 (there are no data after September 1991).

Changes in gene frequencies may have one or several of 4 origins: mutation, selection, migration or genetic drift. It seems reasonable to reject the mutation hypothesis to explain the large variations observed: 1) mutations giving rise to resistance genes are extremely rare (Raymond et al. 1991, 1992); and 2) resistance is known to remain stable in homozygous laboratory strains (e.g., Raymond et al. 1993), indicating that reverse mutations are unlikely. Likewise, genetic drift is also unlikely to have had a major impact, especially on the variations that occurred in September, a period when mosquito populations are very abundant. Thus, the observed variations in resistance gene frequencies are probably the result of either selection or migration, or an interaction of both.

Selection will have opposite effects depending on the presence or absence of insecticides in the environment, due to differences in the relative fitness of resistant and susceptible insects (Ferrari and Georghiou 1981, Raymond et al. 1993). Thus, insecticide treatments will induce an increase in resistance genes, whereas discontinuing control is likely to correspond to a decrease. Similarly, the 2 migration components (emigration and immigration) may have different effects on resistance genes frequencies. Emigration should not induce variations, since there is no reason to believe that resistant and susceptible insects have a different propensity to leave the site. The impact of immigration will depend both on its intensity and on the amplitude of the differences in resistance gene frequencies between immigrants and indigenous insects. The most likely immigrants are adult mosquitoes produced by breeding sites in the vicinity of CHYE. These sites are epigeous and present populations with high frequencies of resistant genes; unlike the CHYE hypogeous site, they were continuously treated during the 1990 and 1991 summers.

Taking these considerations into account, it is possible to explain the evolution of resistance genes frequencies in the CHYE site as follows. Interrupting mosquito control in 1989 induced the selection of susceptible insects and the correlated decrease in resistance gene frequencies.

The low *Ace<sup>R</sup>* frequency observed from April to August 1990 and from October 1990 to July 1991 may indicate a low rate of immigration from epigeous populations. Since to our knowledge no insecticide was applied directly to the CHYE site, the large increase in resistance genes between August and September in both 1990 and 1991 can only be attributed to immigration of resistant insects from surrounding epigeous populations. Thus, it seems that epigeous females massively enter hypogeous sites in September to lay egg rafts. If correct, this conclusion may clarify the relationship between hypogeous (colonized by the "molestus" form) and epigeous (colonized by the "pipiens" form) populations (Barr 1982). Further studies are needed to determine whether epigeous female offspring in hypogeous sites enter hibernation and leave the site the following spring, or whether they freely breed with the indigenous population. Our study gives no indication since the sharp decrease in resistance gene frequencies between September and October 1990 can either indicate absence of interbreeding or a counter-selection of resistant insects.

Knowledge of how mosquito populations are structured, and how and when migration occurs, are important aspects for understanding the evolution of insecticide resistance.

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