Fitness Cost in Laboratory Selected Strain of the Potential Mosquito Vector of West Nile Virus (*Culex pipiens*) Associated with Resistance to the Insecticide Temephos

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Abstract: The fitness cost associated with resistance genes was investigated on mosquito's viability. We studied four fitness parameters in a laboratory-selected strain of *Culex pipiens* associated with resistance to the insecticide temephos. S-Lab was the susceptible strain used for comparison. Two genes are involved in resistance to organophosphate (OP) insecticides: *Ace-1* and *Ester*. After 5 generations of pressure, the temephos resistance ratio increased to 60.51 at RR95, exhibited a deficiency in the following two parameters: female fecundity ($\chi 2$=infini; dl=1; P<<0.05) and mortality rate (P<<0.05). Characterizations of resistance mechanisms indicate that resistance *Ace-1* alleles coding for a modified acetylcholinesterase were associated with a higher mortality rate and lower fecundity. Several previous studies were used to compare and discuss our results.

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1. Introduction:

The existence of a cost associated with resistance genes has already been demonstrated in natural populations of mosquitoes due to the existence of a frequency cline of resistance alleles between treated and untreated zones (Crow, 1957; Roush and McKenzie, 1987; Carriere et al., 1994; McKenzie, 2000; Shi et al., 2004).

Laboratory studies have shown that resistant genotypes of mosquitoes have a longer duration of larval development and decreased female fertility in relation to susceptible genotypes. More recent approaches showed a cost associated with *Ester1*, *Ester4* alleles and *Ace-1R* which would exert a significant cost on male reproduction (Berticat et al., 2002) and the exhaust to predation (Berticat et al., 2004). In nature, experiments have established costs, associated with these three alleles, on the survival of females during the hibernation period (Clarke and McKenzie, 1987; Rowland, 1991a, 1991b; Minkoff and Wilson, 1992; Boivin et al., 2001; Boivin et al., 2003; Foster et. al., 2003; Bourguet et al., 2004; Liu and Han, 2006). In addition, subsequent studies (Berticat et al., 2002) have shown that the level of infection by *wolbachia* is significantly higher in resistant strains than in the sensitive reference strain.

The different alleles of resistance seem to be associated with different costs. Indeed, the overproduced esterases appear to be associated with a lower cost than that associated with the modified acetylcholinesterase (Lenormand & al., 1998; Lenormand et al., 1999; Lenormand & Raymond, 2000). Other studies have shown that the *Ester4* allele may confer a lower cost than the *Ester1* allele (Guillemaud et al., 1998).

Although the cost of adaptation has often been shown to be associated with resistance to various pesticides, predators and pathogens, in plants (herbicides, pathogens and herbivores) (Simms & Rausher, 1987, Bergelson & Purrington, 1996), as well as in bacteria (antibiotics and viruses) (Lenski, 1988; Levin et al., 2000) or insects (pesticides and parasites) (Coustau et al., 2000), negative effects remain poorly understood and commonly interpreted as altering metabolic and developmental processes (Davies & al., 1996).

The aim of this study is to look for the existence of an adaptive disadvantage associated with resistance genes, by comparing the performance of a selected strain with temephos to a sensitive strain S-Lab for different biological parameters.

2. Materials and Methods:

2.1. Mosquitoes

Two colonies of *Culex pipiens* were used in this study. A susceptible strain (S-Lab) which have been maintained in the insectarium of Unit of Genetics, the University of Monastir for many years and have not been exposed to any insecticide and/or biological control agent. The resistant colony (Bou.tem5) was started from the Bou.nat (field population) colony and was subjected to continuous selection pressure with insecticide temephos. The selection procedure consisted of exposing a large

number (10,000-20,000) of young 4th-stage larvae to temephos at concentrations ranging from the median lethal concentration to the 95 % lethal concentration for 5 generations.

2.2. *Culex pipiens* Laboratory Rearing

To synchronize development, eggs were allowed to hatch and groups of 1,000 first instar larvae were then transferred to plastic basins containing 1 liter dechlorinated water and 1 gram rabbit crop and were maintained in a laboratory. Both would have been maintained on sugar water. Only females then blood fed on birds. Adult mosquitoes were maintained in an insectary at 26 ± 1ºC. The cycle is repeated after obtained eggs.

2.3. Chemical Insecticides

Two technical grade insecticides were used for selection and bioassay: the organophosphates temephos (9l%o; American Cyanamid, Princeton, NJ) and the carbamate propoxur (997o; Mobay). We have a stock solution having a concentration of 10,000 ppm from which is prepared a series of solutions to 1000; 100; 10; 1; 0.1; 0.01; 0.001 ppm by diluting in each case to 1/10 a quantity of 10 ml of the solution 10 times more concentrated in 90 ml of undenatured 95 ° alcohol.

2.4. Bioassays

Temephos resistance levels were evaluated in larvae from both populations through dose-response bioassays (WHO, 1963). In each assay, five insecticide concentrations prepared with Temephos were tested. For each concentration, there were five replicates, each with 20 third instar larvae in 100 mL solution. Lethal concentrations (LCs) were calculated via probit analysis (Raymond et al., 1985). Resistance ratios (RR50 and RR95) were obtained by dividing the LC of the field population (Bou.tem5) by the equivalent LC from the S-Lab strain.

2.5. Characterization of the Resistance

This test was similar to the bioassay tests except that 0.5 ml of the maximum sub-lethal concentration of an esterase inhibitor, S, S, S-tributylphosphorotrithioate, (0.5 µg/ml) was added to each cup with 0.5 ml of insecticide and piperonyl butoxide (pb), an inhibitor of mixed function oxidases. Esterase phenotypes were established by starch electrophoresis (TME 7.4 buffer system) as described by Pasteur et al. (1981, 1988) using homogenates of thorax and abdomen.

2.6. Fitness Cost Assessment

The parameters fecundity, fertility, development time and preadult survivorship were compared between the two colonies (S-Lab and Bou.tem5) to determine whether resistance to temephos was associated with any reproductive disadvantage.

Egg rafts were taken from female mosquitoes that had not been exposed to temephos during their larval stage. Fully blood-fed females were selected randomly from each S-Lab and Bou.tem5 colony and allowed to lay eggs. Fecundity was then measured by using egg rafts from each colony and determining the average number of eggs per raft at the first gonotrophic cycle.

Fertility was assessed as the mean number of first stage larvae (L1) and the percentage of eggs that hatched within 24 and 48 h after oviposition. Egg rafts were used from the S-Lab and Bou.tem5 colonies. Each egg raft was placed individually in a plastic cup containing 200 ml of distilled water. We have agreed to quantify a spawning of Big if it gives a number of larvae greater than 150; Average if the number of larvae is between 150 and 100; small if it gives less than 100 larvae.

Preadult development time and survivorship were assessed by accompanying larvae from egg rafts of each susceptible and resistant colony. Larvae from each egg raft were reared in a plastic pan filled with dechlorinated water and fed ground rabbit crop. To neutralize the effect of density, we conducted an environmental stress gradient through the establishment of three density ranges: Low density (50 larvae/500ml), Average density (100 larvae/500ml) and High density (200 larvae/500ml). The pupae were transferred daily to a 200-ml cup and placed in screen cages for adult emergence.

2.7. Statistical Analysis

Comparison of the different traits studied between the resistant and the susceptible strains was carried out using the Statistical software. The comparison of the fecundity of females and eggs fertility was carried out by the test t. While the mortality rate and development times were compared using the Chi 2 test.

3. Results:

3.1. Temephos susceptibility and characterization of the resistance

Culex pipiens was placed under selection pressure and the resistant strain (Bou.tem5) was tested for susceptibility to temephos (Table 1). Under selective pressure, the resistance ratio was approximately a 4-fold increase in the LD95 (60.51 at LD95). Our results showed that neither esterases (or GST) inhibited by DEF nor P450 cytochrome mediated monooxygenases inhibited by PB played a role in the observed resistance of Bou.tem5. This conclusion was confirmed by Starch gel electrophoresis that did not disclose any overproduced known esterase in the resistant strain (Bou.tem5). C*ulex pipiens* of selection temephos showed resistance to Propoxur wich indicates an acetylcholinesterase insensitive (*Ace-1R*).

Table 1: *Insecticide resistance of resistant (Bou.tem5), reference (S-Lab) and original (Bou.nat) strains*.

Name of population	LD_{50} (a)	LD_{95} (a)	Slope (b)	H (df)	RR_{50} (c)	RR_{95} (c)
S-Lab.T	0.0012 (0.0011-0.0014)	0.0062 (0.0047-0.0094)	2.34± (0.22)	1 (3)	-	-
Bou.nat.T	0.0266 (0.0237-0.0301)	0.0934 (0.0741-0.1283)	3.02± (0.27)	1 (2)	21.45 (17.63-26.10)	14.90 (9.15-24.28)
Bou.tem5.T	0.0202 (0.0058-0.0738)	0.3792 (0.0188-10.3333)	1.29± (0.24)	3.96 (2)	16.29 (10.44-25.43)	60.51 (19.93-183.76)

Bou: Boussalem; nat: natural population; tem: temephos
(a) In mg/liter, 95% CI in parentheses.
(b) Standard errors in parentheses.
H: Heterogeneity, (df): testing linearity of the probit mortality/log dose response.
(c) RR, resistance ratio (LC50 of the population considered / LC50 of S-Lab); 95% CI in parentheses.

3.2.Life History Traits

The observations for egg fertility, female fecundity, mortality rate and egg-to-adult development time are shown in Figure 1 and Table 2, 3, 4. There was a significant difference within the resistant/susceptible groups between the different populations for the two parameters examined: female fecundity ($\chi 2$=infini; dl=1; P<<0.05; Table 2) and mortality rate (P<<0.05; Tables 3, 4). Despite egg fertility ($\chi 2$=0.03; dl=1 ; P>>0.05; Table 2) and development time (P>0.05; Tables 3, 4) did not differ between resistant and susceptible populations. Moreover, we note that the number of eggs given by the S-Lab females (61) is significantly higher than that given by Bou.tem5 (29). Also, we note that the two strains tend to give more small eggs (number of larvae <100) than big larvae (number of larvae> 150) and medium (150 <number of larvae <100). Development time seems to be affected by density. The emergence of mosquitoes from low-density larvae is faster than those of high densities showing longer development. All high densities have a high mortality rate compared to average and low densities. It should be noted that, on average, resistant individuals have a shorter development time compared to susceptible individuals. The high mortality rates of Bou.tem5 explain the difference in development time between the two strains. Whatever the resistance status (resistant or sensitive strain), males developed faster than females: compared to males, the development of females is slowed from one to three days, but this difference is not significant (P> 0.05, Tables 3 and 4). On the other hand, within each strain, the sex ratio is unbalanced, ie the number of females emerged is greater than that of males. This deficiency in males is significant only for Bou.tem5 (Bou.tem5: P <0.05, S-Lab: P> 0.05).

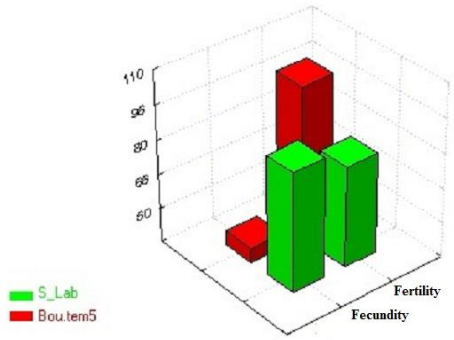

Figure 1. Female fecundity and eggs fertility of sensitive (S-Lab) and resistant (Bou.tem5) strains

Table 2: *Female fecundity and egg fertility of sensitive (S-Lab) and resistant (Bou.tem5) strains*

	Number of eggs	Egg size (a)	Bridges by female (%)	Big eggs (%)	Average eggs (%)	Small eggs (%)
S-Lab	61	79.03 ± (40.36)	87.14	3.27	32.78	63.93
Bou.tem5	29	101.24 ± (70.45)	41.42	24.13	20.68	55.17

(a) Standard errors in parentheses.

4. Discussion

In this study, authors did not observe any significant difference in egg fertility and development time between the resistant and the susceptible populations. However, resistance seems to increase the mortality rate of individuals and decrease the fecundity of females. Similar trends have been observed in many previous studies (Guillemaud et al., 1998; Lenormand et al., 1998). The results suggested that temephos resistance was not associated with monooxygenase and esterases or (GST). However, evidence was found of insensitive acetylcholinesterase in the resistant strain (Bou.tem5).

Authors noted that the increase of mortality rate and the decrease of fecundity females were probably due to the modified acetylcholinesterase that appears associated with a higher cost than that associated with overproduced esterase (Lenormand et al., 1998; Lenormand et al., 1999; Lenormand & Raymond, 2000).

In addition, results showed that individuals selected to temephos grow faster than S-Lab. These differences

may be due to the influence of mortality rates on development time.

Table 3: *Average number of larvae, percentage of emerged adults, mortality rate and development time of resistant strain (Bou.tem5)*

Density	Larvaes	Adults	Males	Females	Mortality rate	Development time (h)	
						Males	Females
ALDR	50± (0.00)	50± (6.92)	16.66± (1.15)	33.33± (7.57)	50.00± (6.92)	282.66± (15.14)	
						268.00± (18.33)	285.00± (15.02)
AADR	100± (0.00)	41.33± (12.89)	15± (4.58)	26.33± (11.06)	58.66± (12.89)	375.55± (7.34)	
						394.47± (53.88)	381.66± (12.50)
AHDR	200± (0.00)	29.66± (14.02)	13.16± (7.21)	16.5± (7.54)	71.33± (14.02)	419.66± (14.79)	
						372.00± (16.80)	430.86± (15.41)
AA	116.66± (76.37)	40.33± (10.20)	14.94± (1.75)	25.38± (8.45)	59.99± (10.72)	359.29± (69.93)	
						344.82± (67.47)	365.88± (74.13)

h : hours; Standard errors in parentheses; ALDR: Average of the three Low-Density Repetitions; AADR: Average of the three Average Density Repetitions; AHDR: Average of the three High-Density Repetitions; AA: Average of Averages

Table 4: *Average number of larvae, percentage of emerged adults, mortality rate and development time of sensitive strain (S-Lab)*

Density	Larvaes	Adults	Males	Females	Mortality rate	development time (h)	
						Male	Female
ALDR	50± (0.00)	90.66± (6.42)	46± (3.46)	44.66± (8.32)	9.33 ± (6.42)	302.85± (12.98)	
						292.00± (6.92)	316.22± (14.20)
AADR	100± (0.00)	87.33± (4.50)	38.66± (3.21)	48.66± (2.08)	12.66± (4.50)	375.46± (15.10)	
						370.18± (17.61)	409.04± (38.39)
AHDR	200± (0.00)	38± (9.64)	10± (2.50)	28± (7.85)	62± (9.64)	485.07± (13.41)	
						463.88± (20.41)	526.66± (24.11)
AA	116.66± (76.37)	71.99± (29.49)	31.55± (19.02)	40.44± (10.96)	27.99± (29.49)	387.80± (91.73)	
						408.68± (49.03)	417.31± (99.46)

h : hours; Standard errors in parentheses; ALDR: Average of the three Low-Density Repetitions; AADR: Average of the three Average Density Repetitions; AHDR: Average of the three High-Density Repetitions; AA: Average of Averages

Indeed, within each repetition, when the density was low, the competition between larvae was less and the development time was faster. This density-dependent character showed, on the one hand, the impact of the stressful conditions applied during the experiment and on the other hand the impact of high mortality rates of the resistant individuals which explain the shortening of their development times in relation to the sensitive strain.

Developmental time varied according to the sex. Indeed, slowing of the development of female could be explained probably by their need to accumulate resources for reproduction (Clements, 1992).

How then can we explain the heterogeneity of development times for the same sex? Effects related to uncontrolled environmental parameters or intra-strain genetic variability was involved.

Such genetic factors are known in *Culex pipiens*, which can induce a bias in favor of one sex or the other depending on the population (Clements, 1992). This confirms our studies which revealed a male deficiency of the resistant and sensitive strain. This deficit may also be due to a specific mortality of males.

5. Conclusion

In this study, we analyzed the details of the phenotypic expression of resistance genes. Indeed, overall measurements indicated that a cost is observable in the laboratory by increasing the mortality rate of individuals and decrease the fecundity of females. In this context, authors proposed to study the resistance of *Culex pipiens* further by integrating field studies, laboratory strains and molecular studies in order to better estimate the share of each of the different alleles of cost resistance on the physiology of resistant individuals.

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Conflicts of Interest:

Authors declared no conflicts of interest.

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