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Pleiotropy of adaptive changes in populations: comparisons among insecticide resistance genes in *Culex pipiens*

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Summary

Resistance to toxicants is a convenient model for investigating whether adaptive changes are associated with pleiotropic fitness costs. Despite the voluminous literature devoted to this subject, intraspecific comparisons among toxicant resistance genes are rare. We report here results on the pleiotropic effect on adult survival of *Culex pipiens* mutants involved in the same adaptation: the resistance to organophosphorus insecticides. This field study was performed in southern France where four resistance genes sequentially appeared and increased in frequency in response to intense insecticide control. By repeated sampling of overwintering females through winter, we analysed the impact of each of three resistance genes on adult survival. We showed that (i) the most recent gene seems to be of no disadvantage during winter, (ii) the oldest affects survival in some environmental conditions, and (iii) the third induces a constant, severe and dominant survival cost. Such variability is discussed in relation to the physiological changes involved in resistance.

1. Introduction

Genes responsible for an adaptation to a new environment (new parasite, climatic variation, chemical challenge, etc.) are usually assumed to have a fitness cost, i.e. to be at a disadvantage in the previous environment (e.g. Fisher, 1958; Lande, 1983; Holloway, 1990; Macnair, 1991; Orr & Coyne, 1992; Carrière *et al*., 1994). This is based on the general view that resource re-allocation occurs or that metabolic or developmental processes are affected (Uyenoyama, 1986; Davies *et al*., 1996), thus decreasing other fitness-enhancing characters. Few situations exist where both the environmental changes and the adaptive genes are clearly identified. Resistance to pesticides is one of them, although the existence of a cost of resistance genes lacks substantial support from field observations and has been questioned by some laboratory experiments (Roush & McKenzie, 1987; Roush & Daly, 1990). A notable exception is resistance to dieldrin and diazinon in the sheep blow fly *Lucilia cuprina* (e.g. McKenzie *et al*., 1982; McKenzie & Purvis, 1984; Clarke & McKenzie, 1987; McKenzie & Clarke, 1988; McKenzie, 1990, 1994; McKenzie & O'Farrell, 1993; McKenzie & Batterham, 1994; Davies *et al*., 1996). For each of these insecticides, field resistance has evolved through allelic substitution. At the *Rdl* locus, the allele involved in dieldrin resistance was found to be associated with a significant fitness cost. At the *Rop-1* locus, similar results were at first found for the allele involved in diazinon resistance (R_{1A}), but this cost subsequently decreased due to the occurrence of a modifier gene. This modifier gene has a dominant effect and it completely suppresses the cost of R_{1A}. The absence of evolution of a dieldrin-resistance cost modifier is attributed to the shorter life-span (2 years of dieldrin use compared with 10 years of diazinon use) to select for a modifier gene.

Resistance to organophosphate (OP) insecticides in *Culex pipiens* mosquitoes is another convenient model for investigating the pleiotropic effects of resistance genes directly in natural populations. Three loci are involved in OP resistance in *Culex pipiens*, and each resistant mutant can be characterized in single mosquitoes. Two tightly linked loci, *Est-2* and *Est-3*, code detoxifying esterases A and B, respectively. They confer OP resistance through overproduction of the esterase, achieved by the modification of gene regulation or by gene amplification (Rooker *et al*., 1996). In addition, the *Ace* locus codes the OP target acetylcholinesterase, variants of which are insensitive

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to OP inhibition (Raymond *et al.*, 1986; Bisset *et al.*, 1991; Khayrandish & Wood, 1993; Bourguet *et al.*, 1996).

In southern France, sites containing larvae have been subjected to OP insecticide treatment for the larger part of the breeding season since 1968. Four OP resistance mutants have sequentially appeared from 1972 to 1986: the overproduced esterases A1, A4-B4 and A2-B2, and the insensitive target Ace^R (Pasteur & Sinègre, 1975; Pasteur *et al.*, 1981 *a*, *b*; Raymond *et al.*, 1986; Poirié *et al.*, 1992; Chevillon *et al.*, 1995). The presence of OP insecticides is temporally and spatially limited. *Culex pipiens* has only been the target of OP control within a coastal area along the Mediterranean seashore approximately 20 km wide. In addition, OP are usually applied between mid-May and mid-September with little variation among years, but the breeding period is more extended in time (from April to November). As a consequence, at least the first and last generations of the reproductive season escape OP treatment, even within the treated area. During the autumn, mated females seek shelter in cellars and caves, where they survive for several months before laying eggs the following spring. Males are supposed to die before the overwintering period, and are not present in overwintering sites. The overwintering generation is thus composed of long-lived adult females and of sperm stored within the spermathecae of these overwintering females (Clements, 1963). Overwintering females may experience differential survival rate according to their OP resistance genotypes, but overwintering sperm would presumably preserve the allelic frequencies determined in the autumn.

The present study was undertaken to compare OP-resistant mutants for their impact on adult survival in the absence of OP insecticide. The characteristics of OP treatment in southern France and of *Culex pipiens* biology suggested that the overwintering period would be the best candidate for these purposes. Two complementary surveys were thus undertaken in the same restricted area. First, we followed the fate of mutants involved in OP resistance in overwintering females by repeated sampling of caves between November and March. Second, we compared the OP resistance composition of the first spring generation, i.e. the offspring of the overwintering generation, with the OP resistance composition of females that survived the winter. Such a comparison was aimed at verifying whether OP-resistant mutants effectively experience differential fates between overwintering adult females and overwintering sperm.

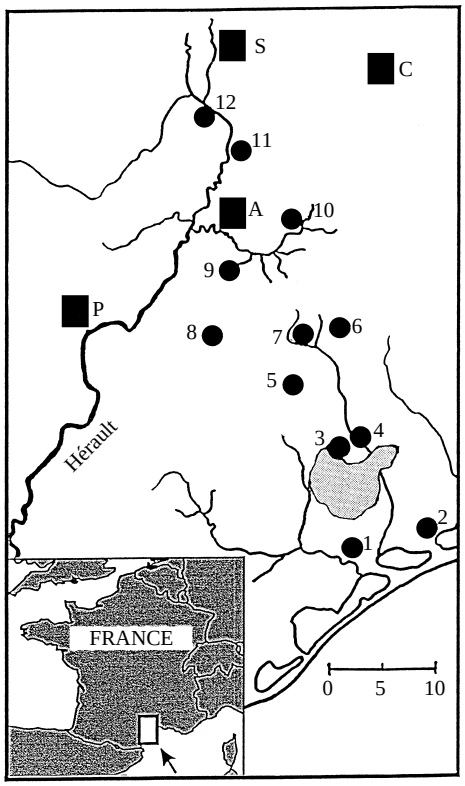

Fig. 1. Geography of sampling. Overwintering females and their putative offspring were sampled within a restricted area (scale in km) near the city of Montpellier (in grey). Nine caves were sampled within four localities indicated by their initial letters: C is for the cave CLA, P for the cave PIG, S for the caves SUM1 and SUM2, and A for the five ARC caves. See text for further details. Dots indicate the 12 sampling localities of the first spring generation.

2. Materials and methods

(i) *Mosquito samples*

Overwintering *Culex* sp. females were repeatedly sampled between November 1992 and March 1993 within nine caves from four localities near Montpellier (Fig. 1). These caves were located outside the coastal area where *Culex pipiens* control ended in September 1992 (Anonymous, 1992), so that it is very unlikely the sampled individuals would have experienced any OP contact. Within two localities, near the villages of Claris and Saint Guilhem le Désert, a single cave has been sampled (sampling code in parentheses): 'Claris' (CLA) and 'Cochon' (PIG), respectively. Near Sumène village, the two sampled caves are separated by less than 200 m: 'Trou d'Auguste' (SUM1) and 'Sumène' (SUM2). In the 'Ravin de l'Arc', five nearby caves (maximal distance around 800 m) were sampled: 'Nicole' (ARC1), 'Ermite' (ARC2), 'Eboulis' (ARC3), 'Grande' (ARC4) and 'CAF' (ARC5). Except in two caves (SUM1 and ARC3) where density in *Culex* sp. females was constantly low, between 60 and 80 females were collected at each sampling site. Each mosquito captured was identified to species, and deep-frozen for further analyses. Only *C. pipiens* mosquitoes were considered further.

In the same area, the offspring generation of overwintering females was collected as eggs and first-instar larvae within 12 breeding sites. These breeding

sites are close to the following localities: Faïsses (1), Maurin (2), Lavalette (3), CEFE (4), Saint Gely (5), Triadou (6), Les Matelles (7), Saint Martin (8), Notre Dame (9), Saint Bauzille (10), Ganges (11) and Sumène (12; see Fig. 1). Each breeding site was checked twice a week between the disappearance of overwintering females from caves (19 March 1993 in the 'Ravin de l'Arc') until the first occurrence of egg-rafts. These first spring egg-rafts were taken between 7 and 9 April 1993, i.e. about 1 month before any insecticide control was performed. These mosquitoes were reared under standard laboratory conditions (larval mortality below 5%), and deep-frozen in liquid nitrogen as adults.

(ii) *Identification of insecticide resistance genes*

For each adult, the head and thorax were used for establishing the genotype at the *Ace* locus as described by Raymond & Marquine (1994), and the abdomen was used for detecting the presence or absence of overproduced esterases by starch gel electrophoresis (Chevillon *et al.*, 1995). Acetylcholinesterase genotypes are recorded as Ace^{RR} and Ace^{SS} for resistant and susceptible homozygotes, and as Ace^{RS} for heterozygotes. Since overproduced esterases are dominant, esterase phenotypes are discriminated by their presence or absence. Five esterase phenotypes were recorded: the susceptible phenotype (Null) is defined by the absence of any overproduced esterase; the phenotypes A1, A4-B4 and A2-B2 by the detection of the corresponding overproduced esterases; and the phenotype (A1 + A4 − B4) corresponds to one heterozygote.

(iii) *Statistics*

Deviations from Hardy–Weinberg expectations were tested using the exact score test of Rousset & Raymond (1995), and its multisample extension, when the alternative hypothesis is heterozygote excess. Probabilities and their standard errors were estimated using Markov chains of 300000 steps long with a dememorization of 1000 steps (see Guo & Thompson, 1992). The power of the Hardy–Weinberg test towards a specific H1 alternative hypothesis was estimated according to Rousset & Raymond (1995), and computed from 1000 samples.

At the *Ace* locus, genotypic differentiation was tested using the G_a-based test described by Goudet *et al.* (1996) with a Markov chain method of 100000 steps. For esterases, phenotypic differentiation was tested by using the Fisher exact test on $R \times C$ contingency tables with R rows corresponding to esterase phenotypes and C columns to samples. The Markov chain used was of 200000 steps, with a dememorization of 1000 steps. All these computations of probabilities and their standard errors were performed with version 3.1 of GENEPOP (Raymond & Rousset, 1995).

Under the assumption of fitness cost, resistant phenotypes should decrease in proportion with time within the single overwintering generation. The change in each phenotype frequency was analysed using the GLIM computer package (Baker & Nelder, 1985). Each phenotype frequency was modelled as a variable with binomial error (Crawley, 1993). The interaction of the sampling location (factor $CAVE$) and the day of sampling (variable $DATE$) was dropped if not significant. The factor $CAVE$ being controlled, the evolution of each phenotype frequency with the variable $DATE$ was then tested according to Crawley (1993).

Relative viabilities associated with *Ace* genotypes (Ace^{RR}, Ace^{RS} and Ace^{SS}) were estimated by a log-linear model with Poisson error described by Manly (1985) using the GLIM computer package (Baker & Nelder, 1985). This model corresponds to a classical analysis of covariance. Female counts were fitted as follows: step (a) basic model $CAVE + GENO + REP$, step (b) addition of the interaction $GENO \cdot DATE$, and step (c) substitution of $GENO \cdot DATE$ by $GENO \cdot DATE \cdot CAVE$; where REP and $GENO$ are factors describing the sampling sequence and the *Ace* genotypes, respectively (Manly, 1985; Crawley, 1993). In this model, the regression slopes (W_{RR} and W_{RS} for Ace^{RR} and Ace^{RS}, respectively) estimate the daily viabilities associated with resistant genotypes when the daily viability associated with the susceptible genotype Ace^{SS} is taken as reference ($W_{SS} = 1$). The viability costs associated with Ace^{RR} and Ace^{RS} genotypes are thus defined as $(1 - W_{RR})$ and $(1 - W_{RS})$, respectively. Overdispersion was corrected according to Crawley (1993) before testing the adequacy of the model to explain the data. The same model was applied to esterase phenotypes.

3. Results

(i) *Evolution of resistance gene composition among overwintering females*

Among the resistance genes known to be present in southern France, the esterase phenotype A2-B2 is at low frequency in the Montpellier area (see Rivet *et al.*, 1993; Chevillon *et al.*, 1995). In the present survey, it was observed in only 6 females out of 588 (Table 1) and was not considered further in our analysis. Variations in frequencies of other esterase phenotypes and of *Ace* genotypes were separately tested using a logistic regression on the data set. Results are detailed in Table 2. The ratio (ρ) of residual variance of the full model ($CAVE + DATE + CAVE \cdot DATE$) to residual degrees of freedom was never larger than 1·3, indicating that the logistic regression accurately fitted the data. The interaction between the $CAVE$ factor and the $DATE$ variable was never significant ($P >$

Table 1. *Resistance gene composition of overwintering females*

Sampling			*Ace*			Esterases				
Cave	Date	*n*	*SS*	*RS*	*RR*	Null	A1	A4−B4	A2−B2	A1+A4−B4
ARC1	18-11-92	25	16	6	3	16	1	2	0	2
	15-01-93	18	13	5	0	12	1	5	0	0
	28-01-93	10	10	0	0	8	0	3	0	0
	05-02-93	13	11	2	0	9	0	4	0	0
	10-02-93	7	4	2	1	6	1	2	0	0
	18-02-93	16	14	2	0	13	1	2	0	0
	25-02-93	6	5	0	1	4	2	1	0	0
	04-03-93	14	10	3	1	11	0	3	0	0
	11-03-93	3	3	0	0	3	0	0	0	0
	18-03-93	7	7	0	0	5	1	0	0	1
ARC2	10-12-92	29	23	4	2	18	2	4	1	2
	28-01-93	15	15	0	0	11	0	4	0	0
	18-03-93	16	16	0	0	12	0	4	0	0
ARC3	10-12-92	39	28	10	1	26	2	6	0	2
	05-02-93	2	1	1	0	1	0	1	0	0
	25-02-93	4	4	0	0	5	0	0	0	0
ARC4	10-02-93	27	20	4	3	14	4	6	0	1
	18-02-93	13	10	1	2	11	0	2	0	0
	25-02-93	25	21	4	0	15	2	6	0	1
	04-03-93	23	15	7	1	10	3	7	0	1
ARC5	14-02-93	7	6	1	0	3	1	3	0	0
	18-02-93	6	5	0	1	5	0	1	0	0
	04-03-93	4	3	1	0	3	1	0	0	0
	11-03-93	26	20	6	0	13	4	6	0	1
CLA	29-11-92	43	29	11	2	23	7	10	3	0
	05-01-93	22	18	3	0	17	5	0	0	0
	27-01-93	38	28	7	1	25	2	10	1	0
PIG	15-11-92	20	8	11	1	10	3	2	0	2
	31-01-93	11	7	3	1	7	1	3	0	0
SUM1	15-11-92	19	15	2	2	13	3	3	0	0
	11-12-92	19	10	7	1	17	1	1	0	0
	25-01-93	10	8	2	0	7	1	2	0	0
SUM2	11-12-92	32	20	12	0	21	6	4	1	0
	25-01-93	24	21	2	1	17	3	4	0	0

For each sample, counts are detailed for *Ace* genotypes and for esterase phenotypes. *n* is sample size.

Table 2. *Variation of esterase phenotypes and* Ace *genotypes*

	P value		Logistic regression	
Phenotype	CAVE·*DATE*	*DATE*	Slope	SE
Null	0·92	**0·021**	+0·0070	0·0030
A1	0·54	**0·0005**	−0·015	0·0046
A4-B4	0·80	0·74		
(A1+A4−B4)	0·87	**0·0014**	−0·0023	0·0081
Ace^{SS}	0·45	**0·0001**	+0·0133	0·0035
Ace^{RS}	0·10	**0·042**	−0·0110	0·0037
Ace^{RR}	0·37	**0·0021**	−0·0141	0·0073

Homogeneity among caves of evolution in frequency with time (factor *CAVE·DATE*) and stability of frequency through time (variable *DATE*) were tested for each phenotypic (genotypic) distribution. The corresponding *P* values are indicated with significant values ($P < 0{\cdot}05$) in bold face. When variation in frequency with time was significant, slope and its standard error (SE) of the logistic regression is indicated.

0·05, Table 2), indicating a similar evolution in resistance gene frequencies among caves. This interaction term was therefore dropped. The *DATE* variable was then removed (the *CAVE* factor still being present) in order to test whether phenotype frequency varies with time. Homogeneity in frequency

Table 3. *Estimates of the survival cost induced by* Ace^R

	Cost estimates (%)		
	Model 1		Model 2
Time period	Ace^{RS}	Ace^{RR}	Ace^R
Day	0·97 (0·70–1·24)	0·80 (0·27–1·33)	0·94 (0·69–1·19)
Winter	71 (58–79)	63 (29–81)	69 (58–77)

Viability estimates were computed for the three *Ace* genotypes without any assumption of their rank orders (model 1) or by assuming complete dominance of the viability cost of Ace^R (model 2). Estimates of survival costs are given with their confidence interval (in parentheses). See text for explanation.

through time was rejected for all phenotypes ($P < 0{\cdot}05$, Table 2) except for A4-B4 ($P = 0{\cdot}74$). As indicated by the logistic regression slopes (Table 2), significant effects of time corresponded to a decrease in frequency of OP-resistant phenotypes and to a corresponding increase in frequency of the Null phenotype and of the Ace^{SS} genotype.

(ii) *Estimates of survival cost*

Variation in viabilities associated with *Ace* genotypes was analysed within the single overwintering generation by a log-linear model with Poisson errors (Manly, 1985; Crawley, 1993). The *Ace* composition was found to vary significantly with the variable *DATE* ($P = 0{\cdot}017$) but not with the interaction *DATE·CAVE* ($P = 0{\cdot}54$), indicating that viabilities did not differ among caves but only among genotypes. Viability costs were significantly higher than the null value and did not differ significantly between homozygotes and heterozygotes (Table 3). Due to this similar evolution of two resistant genotypes, the model was simplified according to Crawley (1993): the Ace^{RS} and Ace^{RR} classes were pooled. Such simplification assumes a complete dominance of the viability cost. This simplified model presented a lower overdispersion ($\rho = 1{\cdot}6$ instead of $\rho = 2{\cdot}5$) and provided a more informative estimate of viability cost (see confidence intervals in Table 3). Results are consistent with a dominant survival cost of Ace^R, slightly lower than 1 % per day.

For esterases, the interaction between phenotypes and sampling location was significant ($P = 0{\cdot}0058$), indicating that viability estimates associated with esterase should be computed separately for each cave. The individual estimates are very inaccurate and this analysis was not developed further.

(iii) *Resistance gene composition in offspring of overwintering females*

Only mated *C. pipiens* females overwinter (e.g. Clements, 1963; Sulaiman & Service, 1983; Oda, 1992). No dependence between sex and Ace^R has been found during the breeding season (Raymond & Marquine, 1994; Chevillon *et al*., 1995); thus when overwintering begins the Ace^R frequency is expected to be identical among sperm cells and among mated females. Since the *Ace* gene does not seem to be expressed in sperm cells (at least in Diptera: see review by Toutant, 1989), it should be neutral in the male gametes that are present in the spermathecae of these overwintering females. The survival cost associated with Ace^R in overwintering females (but not in sperm cells) is thus expected to induce differences in Ace^R frequency between male and female gametes in early spring when surviving females lay eggs. We then expect to detect (a) an excess of Ace^{RS} genotype (compared with Hardy–Weinberg expectations) in the offspring of the overwintering generation, and (b) an increase in mean Ace^R frequency between overwintering mothers and their offspring. Expectations of the evolution of overproduced esterases between overwintering and first-spring generation larvae are difficult to perform since genotypes are generally unknown and phenotypic frequencies can differ between sexes (Raymond & Marquine, 1994; Chevillon *et al*., 1995).

Egg-rafts and larvae of the first spring generation were collected in 12 distinct breeding sites (Fig. 1). The excess of Ace^{RS} heterozygotes was generally not significant within each sample (Table 4), but the power of the test, in the presence of the most likely alternative hypothesis, was low: the probability of detecting a significant heterozygote excess was 0·06 for the sample size achieved ($n = 29$), assuming that the true F_{is} value is $-0{\cdot}088$ and that the true Ace^R and Ace^S frequencies are 0·327 and 0·673 (i.e. the multisample estimates, Table 4). Nevertheless, a significant ($P = 0{\cdot}0447$, SE $= 0{\cdot}0013$) tendency for heterozygote excess was found from the multisample test.

Direct mother–offspring relationships are not available in the present survey. However, mark–capture–recapture experiments on *Culex pipiens* indicate that offspring can be collected only within 10 km around the original location of their mother (see, for instance, Reisen *et al*., 1992). In March, overwintering females were collected in the 'Ravin de l'Arc' (Table 1). Larvae from breeding sites 9–11 appear likely to be their offspring (Fig. 1). This assumption allows qualitative comparisons of Ace^R frequency between generations. No differentiation among these three breeding sites was detected at the *Ace* locus ($\hat{F}_{st} = -0{\cdot}0046$, $P = 0{\cdot}68$) or at the esterase loci ($P = 0{\cdot}50$) – a result congruent with the assumption that they are indeed offspring of the same population. Ace^R frequency is on average 0·22 (range 0·19–0·22; Table 4) among this offspring generation, and is about half

Table 4. *Resistance gene composition of the first spring generation*

	Ace					Esterases				
Site no.	*RR*	*RS*	*SS*	$\hat{F}_{is}$	*P* value	Null	A1	A4−B4	A2−B2	A1+A4−B4
1	6	14	9	+0·042	0·7272	10	11	3	1	3
2	3	13	5	+0·059	0·7613	13	3	5	0	9
3	2	21	6	−0·463	**0·0159**	11	10	7	0	2
4	3	13	13	−0·000	0·6700	14	9	7	0	0
5	1	17	11	−0·315	0·0973	15	7	7	0	1
6	1	12	16	−0·113	0·4816	18	4	6	1	1
7	1	10	18	−0·033	0·6753	17	2	9	0	1
8	5	13	11	+0·081	0·7975	14	5	10	0	1
9	0	11	18	−0·217	0·3112	12	4	13	0	0
10	0	13	16	−0·273	0·1762	16	7	6	0	1
11	3	9	17	+0·208	0·9452	16	4	9	0	0
12	2	13	14	−0·064	0·5522	13	0	17	0	0
All				−0·088	**0·0447**					

At the *Ace* locus, are detailed for each sample: genotypic counts; $\hat{F}_{is}$, which was computed according to Weir & Cockerham (1984); and the *P* value of the exact score test for Hardy–Weinberg equilibrium, when the alternative hypothesis is heterozygote excess. Significant departures ($P < 0{\cdot}05$) are indicated in bold characters. Global tendency among samples of deviation from Hardy–Weinberg equilibrium toward heterozygote excess was tested according to Rousset & Raymond (1995), and results are presented in 'All'. In the last five columns, counts for each esterase phenotype are detailed within samples.

this value (0·11 on average, range 0–0·18; Table 1) among overwintering females collected during March in the 'Ravin de l'Arc'. The overall pattern observed at the *Ace* locus is consistent with a differential selection between sexes acting on Ace^R during winter, such that this allele is strongly selected against in females but neutral within the male sperm that they carry.

At the esterase loci, the number of individuals with resistant phenotypes is also higher in breeding sites 9–11 (mean frequency 0·50, range 0·45–0·59; Table 4) than in overwintering females collected in March in the 'Ravin de l'Arc' (mean 0·33, range 0·26–0·38; Table 1). Similar patterns are observed when A1 and A4-B4 are separately considered. A1 exhibits a mean frequency of 0·17 (range 0·13–0·23; Table 4) among breeding sites 9–11 and of 0·11 (range 0·04–0·19; Table 1) among overwintering females. A4-B4 exhibits a mean frequency of 0·32 (range 0·20–0·45; Table 4) among breeding sites 9–11 and of 0·18 (range 0·17–0·26; Table 1) among overwintering females.

4. Discussion

(i) *Insecticide survival cost during overwintering period*

The overwintering period has previously attracted attention concerning insecticide resistance costs, because it is a time of high mortality in insects (Roush & Hoy, 1981; Daly & Fitt, 1990; McKenzie, 1990, 1994). Heterogeneous results have been reported: minor effects in the mite *Metaseiulus occidentalis* (Roush & Hoy, 1981) and in the lepidopteran *Helicoverpa armigera* (Daly & Fitt, 1990), and strong effects in the blowfly *Lucilia cuprina* (McKenzie, 1990, 1994). In *L. cuprina*, the *Rdl* allele involved in dieldrin resistance was found to induce a survival cost that was severe and dominant during the overwintering period, mild and additive otherwise (McKenzie, 1990); in absence of the modifier, similar results were found for the carboxylesterase R_{1A} involved in diazinon resistance (McKenzie, 1994).

The present study also reports heterogeneous results among *Culex pipiens* OP resistance genes. No variation in A4-B4 frequency was observed in the overwintering generation, indicating an absence of strong selection against this phenotype in winter. A decrease in A1 frequency was observed among females from the overwintering generation, which can be attributed only to differential survival since there is no migration during winter (M. Raymond & C. Chevillon, unpublished data). These results were obtained when considering these phenotypes separately, although A1 and A4 do not evolve independently from one another since they are alleles at the same locus (*Est-3*). When they were considered together for survival estimation purposes, a significant change in esterase composition with time was revealed. However, this latter effect seems cave-dependent, suggesting that a precise ecological monitoring of the caves is required to better understand the variation of survival with esterase phenotypes. In the same individuals, the modified acetylcholinesterase gene Ace^R was found to induce a survival cost that was severe, dominant and not detectably different between caves. The mortality attributable to Ace^R during the whole overwintering period was estimated to be 60%, i.e. similar to the survival cost associated with R_{1A} in *Lucilia cuprina* during the overwintering period (McKenzie, 1994). Such severe dominant costs are higher than the values

usually considered in most theoretical models (Comins, 1977; Tabashnik & Croft, 1982; Mani, 1989; Follett *et al*., 1993). However, in the present study its overall impact on the population seems to be sex limited during winter (only expressed in females; males overwinter as sperm). This further emphasizes the need to obtain field estimates of cost in various life stages and to reconsider predictive models.

(ii) *Variability of fitness costs with the nature of adaptive changes*

The literature on fitness costs associated with adaptive changes reports few comparisons of mutants involved in the same adaptation within a single species. Studies in antibiotic resistance in *Escherichia coli* (Schrag & Perrot, 1996) and in *Bacillus subtilis* (Cohan *et al*., 1994) have found a variation in associated fitness cost among adaptive mutants, but no physiological explanation is available for this observation. In the present study, known differences in the nature of resistance genes could partly explain the observed differences revealed.

The change in frequency of resistant genotypes during winter was observed to be either cave-independent (*Ace* locus) or cave-dependent (esterase loci). Ace^R corresponds to a qualitative change in the acetylcholinesterase (Raymond *et al*., 1986; Fournier *et al*., 1993) of the central nervous system, and affects an essential function required life-long, even for adult females hibernating in caves, where (for example) the risk of predation is high due to the presence of numerous predatory spiders (Sulaiman & Service, 1983). As spiders that capture and eat mosquitoes (e.g. *Meta bourneti* and *Tegenaria parietina*) were found in all caves inspected, these predators could represent a cave-independent selection. This hypothesis has been tested for another mosquito, *Anopheles gambiae*: individuals resistant to γHCH/dieldrin are less successful at predator avoidance and in mating competition, due to various subtle behavioural changes correlated with the presence of an insensitive target in the central nervous system (Rowland, 1991*a*, *b*). In contrast, A1 or A4-B4 correspond to a protein overproduction (Mouchès *et al*., 1987; Poirié *et al*., 1992) that takes place mainly during pre-imaginal stages (Ferrari & Georghiou, 1990). These overproduced esterases are expected to affect resource allocation, such that associated cost is expected to depend greatly on environmental conditions, which could vary among caves and create a cave-dependent fitness cost.

This large difference in the expression of the associated costs of different resistance genes might explain some of the conflicting results reported in the literature concerning the existence of a cost under laboratory conditions (Roush & McKenzie, 1987; Roush & Daly, 1990). Furthermore, it is possible that the insensitivity of the target located in the nervous system leads to a behavioural cost (e.g. Rowland, 1991*b*) that cannot easily be detected in most laboratory settings, thus causing further discrepancies between laboratory and field results.

All the mutants surveyed in this study have been strongly selected for by intense OP control of *Culex pipiens* populations since their appearance. A1 appeared in 1972 (Pasteur & Sinègre, 1975), Ace^R in 1977 (Raymond *et al*., 1985) and A4-B4 in 1986 (Poirié *et al*., 1992). It is worthy of note that the most recent (A4-B4) mutant did not display any detectable fitness cost, contrary to older ones. Further investigations are needed to understand this phenomenon.

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