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Resistance Gene Replacement in the Mosquito *Culex pipiens*: Fitness Estimation From Long-Term Cline Series

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ABSTRACT

How adaptation appears and is later refined by natural selection has been the object of intense theoretical work. However, the testing of these theories is limited by our ability to estimate the strength of natural selection in nature. Using a long-term cline series, we estimate the selection coefficients acting on different alleles at the same locus to analyze the allele replacement observed in the insecticide resistance gene *Ester* in the mosquito *Culex pipiens* in the Montpellier area, southern France. Our method allows us to accurately account for the resistance allele replacement observed in this area since 1986. A first resistance allele appeared early, which was replaced by a second resistance allele providing the same advantage but at a lower cost, itself being replaced by a third resistance allele with both higher advantage and cost. It shows that amelioration of the adaptation (here resistance to insecticide) through allele replacement was successively achieved by selection of first a generalist allele (*i.e.*, with a low fitness variance across environments) and later a specialist allele (*i.e.*, with a large fitness variance across environments). More generally, we discuss how precise estimates of the strength of selection obtained from field data help us understand the process of amelioration of adaptation.

"WITHOUT extensive knowledge of natural selection in the wild, we have no idea how relevant experiment or theory are to the evolution of natural populations" (Endler 1986, p. xi). Twenty years after Endler's famous monograph, many advances have been made in the theory of adaptation. Some predictions made by this theory have been tested in the lab, but little insight has been gained into the process in nature. The main advance has been the shift from a strict micromutationnal view to one when mutations of larger effect also played a role. Several experimental studies support this view (see review in Orr 2005). When adaptation to a new habitat is like a sequential approach toward a phenotypic optimum, mutations of large effect will tend to occur early and be followed by mutations of smaller effects "refining" the adaptation (Hartl and Taubes 1996; Barton 1998; Orr 1998, 2000; Barton and Keightley 2002).

Adaptation to a new environment involves two types of traits: (i) traits that change to match the new environmental challenge and (ii) traits that are not involved in this environmental change and have to remain unchanged. "Amelioration" of adaptation (*sensu* Cohan *et al.* 1994) occurs therefore in two directions: improving traits from set 1 and correcting the correlated (or "pleiotropic") changes that may have occurred on traits from set 2 because of changes that occurred on traits in set 1. We designate below these two modes of amelioration as being "direct" or "compensatory," respectively. Most new adaptive mutations affect these two sets of traits together, by producing traits that better match the environment but also modifying some traits that should not change, generating conflicting selection pressures and impeding the rate of adaptation (Caspari 1952; Wright 1969; Carrière *et al.* 1994). It is, however, difficult to disentangle these different selection pressures in nature. Moreover, this process of amelioration can take several routes at the genetic level: it can involve either several loci (*e.g.*, a modifier gene with a compensatory effect) or only a single locus repeatedly (allele replacement) (Cohan *et al.* 1994). Additionally, although less commonly appreciated, more complex molecular processes such as gene duplication may also be involved on a short timescale (Labbé *et al.* 2007). While several examples of these processes have been described in laboratory studies (*e.g.*, Lenski 1988a,b; Cohan *et al.* 1994), it is difficult to study them in nature without precise methods to estimate selection coefficients and without the knowledge of the genetic bases of the adaptation.

In this article, we developed an approach that allowed us to precisely measure selection coefficients to study

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this process of amelioration in nature. We used a well-known case study, the evolution of organophosphate insecticide (OP) resistance in the mosquito *Culex pipiens* (vector of West Nile encephalitis, filariasis, etc.). In particular, we focused on the allele replacement that occurred at the *Ester* locus in the Montpellier area (southern France) during the 1990s and 2000s (Guillemaud *et al.* 1998; Labbé *et al.* 2005). The *Ester* superlocus codes for detoxifying carboxylester hydrolases (or esterases). The overproduction of these esterases is one of the major resistance mechanisms to OP in *C. pipiens* (see for review Raymond *et al.* 2001). Several resistance alleles, each corresponding to a distinct overproduced esterase, have been described. They are selected for in insecticide-treated areas (*i.e.*, a selective advantage as they survive better in this environment), but they are costly (*i.e.*, confer a fitness disadvantage such as lower mating success, lower survival, etc., in absence of treatment) and thus selected against in nontreated areas (Lenormand *et al.* 1998). No estimates of the relative fitness of these three resistance alleles are available so far.

We are still largely ignorant as to the precise mechanisms of amelioration operating in this example and wish to know if these allele replacements involved alleles increasing their advantage in a treated area (s), decreasing their cost (c), or both. It is important to stress at this point that whether one allele replaces another does not simply follow from s and c values, but also depends in a nontrivial way on gene flow and habitat spatial structure (Nagylaki and Lou 2001). Although observing gene replacement allows qualitative inferences about the relative fitness of the competing alleles, only quantifying differences in fitness and their components (s and c) will enable us to discriminate between distinct scenarios of amelioration (direct or compensatory) and measure the net fitness change occurring during the process of amelioration.

The problem is that identifying such a subtle process requires precise methods to disentangle the relative values of s and c among adaptive alleles: while it is relatively easy to measure large fitness differences between two alleles (*e.g.*, between susceptible and resistance alleles), it is more difficult when the differences are slight (*e.g.*, between two resistance alleles) and a greater number of alleles are involved. In laboratory and model organism (especially microbes) studies it is possible to measure a selection coefficient with a precision of $<1\%$ (*e.g.*, De Gelder *et al.* 2004; de Visser and Rozen 2006). However, despite a long history of estimating the strength of natural selection in nature (see Manly 1985; Endler 1986; Hoekstra *et al.* 2001, for review), this precision has never been reached with field data. The study of spatial and temporal frequency variation of adapted alleles has been one of the most accurate methods of estimating natural selection (*e.g.*, Haldane 1948; Kettlewell and Berry 1961; May *et al.* 1975; Mallet and Barton 1989; Mallet *et al.* 1990; Mani 1990; Mani and Majerus 1993; Lenormand *et al.* 1998; Cook 2003; Hoekstra *et al.* 2004). However, at least two issues are crucial to obtaining accurate estimates with this method. The first issue is disentangling the effects of migration and selection on frequency variation (*e.g.*, Brakefield and Liebert 1990). This problem can be overcome by estimating migration directly [*e.g.*, using mark-recapture (Brakefield and Liebert 1990) or neutral genetic markers (Hoekstra *et al.* 2004)] or by estimating migration from patterns of linkage disequilibria among locally adapted alleles (Lenormand *et al.* 1998), an approach similar to that used to study hybrid zones (Barton and Hewitt 1985; Mallet and Barton 1989). The second difficulty is that inference of selection depends on the validity of the underlying migration–selection model. In most cases it is necessary to assume that the observed spatial frequency pattern is at migration–selection equilibrium, precluding the study of transient allele replacement.

In this article, we developed a powerful approach that does not assume migration–selection equilibrium and that comes close to the precision obtained under laboratory conditions. We used this approach to estimate the selection coefficients of the *Ester* alleles in *C. pipiens* from the pattern of resistant allele replacement at this locus in the Montpellier area. We used the 15-year *Ester* allele frequency series to estimate the magnitude of the two fitness components, cost (c) and selective advantage (s), conferred by each resistance *Ester* allele, by combining the information of both spatial and temporal frequency variation. We also investigated the effect of dominance among the different alleles on the quality of our estimates. In addition we looked for evidence that the selection regime had changed or that compensatory modifiers had spread in these populations. Finally, we discuss what this case study tells us about the processes of amelioration in natural populations.

MATERIALS AND METHODS

In the Montpellier area, the mosquito *C. pipiens* is treated with OP insecticides on a coastal belt delimiting two areas (Figure 1): a treated area close to the Mediterranean Sea, where resistant alleles tend to be frequent due to their selective advantage (s), and a nontreated area more inland where resistant alleles are less frequent due to their cost (c). The frequency of resistant alleles thus displays a clinal shape along a transect from the sea (treated) to the inland (untreated). At equilibrium between migration and selection, the rate of decline in the frequency of resistance is proportional to the intensity of migration (σ) and depends on the magnitude of selection in the two areas.

In this study, we thus present a model using spatial information from clines to estimate selective advantages and costs, but also temporal information from a long-term survey ($\sim$15 years) to estimate the selection coefficients of each allele in each environment. With this approach, we focused on

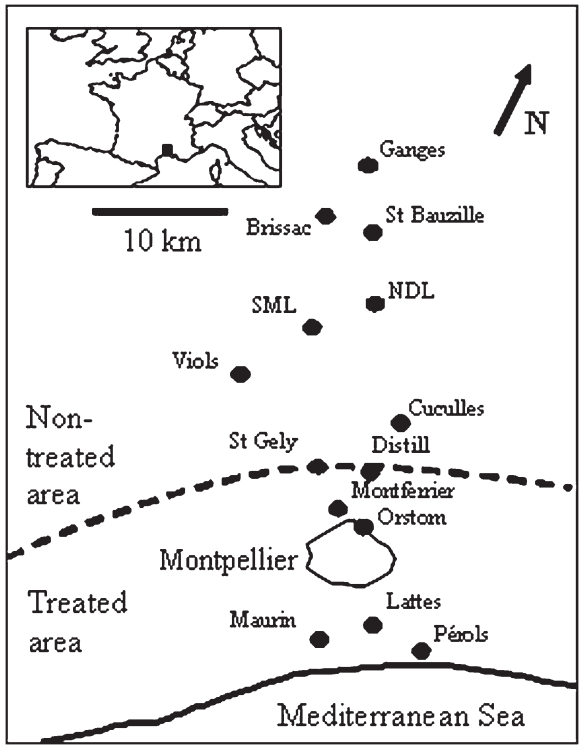

FIGURE 1.—Sample site locations in the northwest–southeast transect in the Montpellier area. Samples are indicated with solid circles. The dashed line represents approximately the border between treated and untreated areas (LABBÉ *et al.* 2005). *C. pipiens* is present in the whole area.

the long-term trend of allele replacement. For a proper comparison among years, we used data from only one season (summer), ignoring within-years variations that are not directly relevant to the long-term trend. Importantly, this approach does not make any equilibrium assumption: the initial situation is fitted by a description of the clines of the first year in the series (1986), and all the clines in the following years depend on these initial frequencies, the migration rate, and selection coefficients of the different alleles (see supporting information, File S1, for a review of previous models).

Data collection: The data set is a compilation of published *Ester* starch-gel electrophoresis phenotype frequencies from samples collected in the Montpellier area from 1986 to 2002 (GUILLEMAUD *et al.* 1998; LABBÉ *et al.* 2005). We used samples from years 1986 (nine populations, $N = 354$), 1987 (three populations, $N = 193$), 1991 (nine populations, $N = 217$), 1993 (two populations, $N = 110$), 1995 (eight populations, $N = 1203$), 1996 (nine populations, $N = 512$), 1999 (nine populations, $N = 582$), 2001 (nine populations, $N = 736$) and 2002 (nine populations, $N = 521$) to perform our analysis, considering only samples collected during the summer. A total of 4428 individuals were analyzed.

Insecticide treatment: The size of the treated area is pivotal to estimating the selection parameters from cline analysis (LENORMAND *et al.* 1998). To obtain good estimation of this size, we used data from total insecticide quantities per district provided by the local insecticide treatment agency (Entente Interdépartementale de Démoustication, EID) from 1990 to 2002. In previous years (1986–1989), treatment applications (*i.e.*, treated area size and quantities used) did not change significantly (EID 1992; GUILLEMAUD *et al.* 1998). We used Geographic Information Systems data to estimate the quantities of insecticide applied in the Montpellier area. The total area analyzed was 40 km wide (20 km each side of the sampling transect) and 35 km long, perpendicularly to the coast. We divided this area in 2-km-wide stripes, parallel to the coast. The OP quantity used in each stripe, q_j, expressed in liters per square kilometer, was computed as

$$q_j = \sum_i \frac{A_{ij}}{A_i} q_i \Big/ \sum_i A_{ij}, \qquad (1)$$

where A_{ij} is the area of each district i within the stripe j, and A_i and q_i are the total area and the quantity of insecticide used in this district i, respectively. We determined the width of the treated area as the distance from the sea where the quantity of insecticide treatments used drops for most years to 0 liter/km^2.

Migration–selection model: To estimate the relative costs and selective advantages of each of the three alleles at the *Ester* locus we used a deterministic stepping-stone-like model to follow frequency changes. The order of the life cycle was assumed to be reproduction–selection–migration. We assumed 13 generations per year according to LENORMAND *et al.* (1999) and a constant population density across treated and nontreated areas (we discuss this last hypothesis below). Algorithms of migration and selection were checked using analytical results for one locus provided by Equations 32 and 33 in NAGYLAKI (1975).

Reproduction: Each generation was computed from the previous one assuming Hardy–Weinberg proportions in each deme independently (each deme being considered as an infinite population), as it has been shown that data are congruent with this assumption (LABBÉ *et al.* 2005).

Selection: The fitnesses of the *Ester* alleles were computed as follows: let s_i and c_i be the fitness advantage conferred by resistance alleles in the treated area and the fitness cost of the allele i, respectively; let h_{ij}^s be the dominance of the benefit for resistance and h_{ij}^c be the dominance of the cost of the allele i over the allele j. The fitness w_{ij} of the diploid genotype (ij) was computed as

$$w_{ij} = 1 + \gamma[s_i + h_{ij}^s(s_j - s_i)] - [c_i + h_{ij}^c(c_j - c_i)], \qquad (2)$$

with $\gamma = 1$ in the treated area and $\gamma = 0$ in the nontreated area and where the fitness of a susceptible homozygote $w_{00} = 1$ (*i.e.*, $s_0 = c_0 = 0$). For $\gamma = 1$, $[s_i + h_{ij}^s(s_j - s_i)]$ represents the overall advantage of the resistance genotype in the treated area. $[c_i + h_{ij}^c(c_j - c_i)]$ represents its cost in both the treated and the untreated area. h_{ij}^s ranges from 0 (total dominance of i over j) to 1 (total dominance of j over i), with 0.5 representing codominance. h_{ij}^c ranges from 0 to a value >1 (overdominance), providing that $c_j > c_i$. As there are three resistance alleles at the *Ester* locus, 18 parameters were used to describe selection at this locus: the costs, c_1, c_2, c_4; the advantage of resistance, s_1, s_2, s_4 (for *Ester*^{1}, *Ester*^{2}, and *Ester*^{4}, respectively); and the 6 dominance parameters for the cost and the advantage of resistance of the four alleles (*Ester*^{0}, *Ester*^{1}, *Ester*^{2}, and *Ester*^{4}).

To consider indirect selection due to genetic association of *Ester* alleles with the other *C. pipiens* main OP resistance locus, *ace-1*, we used fitness parameters estimated for *ace-1* in a previous study [$s_R = 0.33$ and $c_R = 0.11$ (LENORMAND *et al.* 1999), ignoring possible fitness differences between *ace-1* resistance alleles] and assumed codominance at this locus. We also assumed multiplicative fitness between the *ace-1* and the *Ester* loci and used the recombination rate between the two loci ($r = 14.5\%$) estimated from laboratory crosses (LENORMAND *et al.* 1998).

The frequency of genotype k in deme l after selection, g'_{kl}, was computed from its frequency before selection, g_{kl}, as

$$g'_{kl} = g_{kl} w_{kl} / W_l, \quad (3)$$

where w_{kl} is the fitness of genotype k in deme l and W_l is the mean fitness in deme l. We first computed selection coefficients by assuming that all alleles were codominant (*i.e.*, $h^s_{ij} = h^c_{ij} = 0.5$ for all alleles): this is the codominant (COD) model. We then relaxed this hypothesis by allowing dominance parameters to differ from 0.5: this is the noncodominant (NOCOD) model.

Migration: One-dimensional clines were simulated by a series of demes connected by migration (1 deme every 2 km, 35 demes in total). The migration distribution was reflected at one edge of the stepping stone, to simulate a semi-infinite environment and take into account the presence of the sea (Lenormand *et al.* 1998). We used an approximately Gaussian dispersal kernel with a parent–offspring distance standard deviation $\sigma = 6.6$ km · generation$^{-1/2}$; this value has been estimated by Lenormand *et al.* (1998), using the spatial pattern of linkage disequilibrium between resistance loci. This method does not require assuming migration–selection equilibrium at the selected loci as long as the linkage disequilibrium equilibrates faster than frequency changes. This latter situation will be easily met if the resistance alleles are not too far from an equilibrium or if their frequency changes slowly, provided that the loci are not too tightly linked [this in the well-studied quasi-linkage equilibrium situation (Barton and Gale 1993; Nagylaki 1993)]. This situation is very likely to be met in our case as the recombination rate between resistance loci is quite large ($r \sim 15\%$) and the important frequency changes at these loci occur at the scale of hundreds of generations.

Initial conditions: Only phenotype data are available due to the dominance of overproduced esterases in the identification method (starch-gel electrophoresis, see *Parameters estimations*). Thus, to obtain initial conditions, we estimated (simultaneously with the selection coefficients) the distribution of the frequency of each allele in 1986 (first year of sampling available), using a descriptive model as in previous studies (Lenormand and Raymond 2000; Labbé *et al.* 2005). In 1986, only *Ester*^{1} and *Ester*^{4} alleles were present, as *Ester*^{2} was detected for the first time in 1990 in the Montpellier area [one heterozygote individual found among all populations sampled (Rivet *et al.* 1993)]. Frequency clines for the two first resistance alleles i ($i = 1$ or 4 for *Ester*^{1} and *Ester*^{4}, respectively) were simultaneously fitted to a scaled negative exponential

$$p_i = k_i \exp[-(a_i x^2 + b_i x)], \quad (4)$$

where x is the distance from the coast, and k_i, b_i, and a_i are the estimated parameters. k_i is the frequency of resistance allele i for $x = 0$ (*i.e.*, at the coast). As the *Ester*^{2} allele was not yet present in 1986, we introduced it t^* generations after 1986, at a frequency of 0.001 in all demes. t^* was estimated together with the other parameters. As all the parameters (initial conditions, selection coefficients, and t^*) are fitted simultaneously, the model is not particularly susceptible to the initial conditions and choosing another year to start the estimation would not change them significantly (of course, the longer the period considered, the better the estimation).

Parameters estimations: The method of estimation is a standard maximum-likelihood approach. Deterministic recursions described above generate the predicted clines of each allele at any point in time for a given set of parameter values. It is then straightforward to compute the probability of observing a sample at any location in any year given this prediction. Assuming that the different samples are independent, the likelihood of a full 15-year scenario can then readily be obtained. The only difficulty, however, is the computer time needed to maximize this likelihood given that a single 15-year prediction requires simulating ~200 generations in a relatively large stepping stone. Recursions and likelihood-maximization algorithms were written and compiled using Delphi v. 7 (Borland Software).

The *Ester* phenotype of each mosquito was obtained using starch-gel electrophoresis. This technique does not allow complete identification of genotype (Guillemaud *et al.* 1998; Lenormand *et al.* 1998; Labbé *et al.* 2005) because the presence of a susceptible allele cannot reliably be detected in an individual with an overproduced resistance allele. The phenotype was thus considered to be a seven-state random variable ([0], [1], [2], [4], [12], [14], [24], where phenotype [i] corresponds to genotypes *Ester*^{i}/*Ester*^{0} or *Ester*^{i}/*Ester*^{i}, and phenotype [ij] correspond to genotype *Ester*^{i}/*Ester*^{j}; see Lenormand *et al.* 1998). The log-likelihood of a sample was computed from the phenotypic multinomial distribution. Let n_{ij} and f_{ij} be the observed number and the expected frequency of individuals having phenotype i in population j, respectively. The log-likelihood L of observing all the data is proportional to

$$L = \sum_i \sum_j n_{ij} \ln(f_{ij}). \quad (5)$$

It was maximized for parameters joint estimation, using a simulated annealing method (using a Metropolis algorithm, see Lenormand and Raymond 2000).

Model comparison and tests: For the complete COD model (model COD-A), a total of 13 parameters needed to be estimated: (i) s_1, s_2, s_4, the selective advantages of *Ester*^{1}, *Ester*^{2}, and *Ester*^{4}, respectively; (ii) c_1, c_2, c_4, the selective costs of *Ester*^{1}, *Ester*^{2}, and *Ester*^{4}, respectively; (iii) k_1, k_4, a_1, a_4, b_1, and b_4, the parameters of the initial frequency clines in 1986 of *Ester*^{1} and *Ester*^{4}, respectively; and (iv) t^*, the date of apparition of *Ester*^{2}. When the codominance hypothesis is relaxed (NOCOD model), 12 additional parameters are needed: (vi) h^s_{10}, h^s_{20}, h^s_{40}, h^s_{21}, h^s_{41}, and h^s_{24}, for the dominance of the resistance fitness benefit; and (vii) h^s_{10}, h^s_{20}, h^s_{40}, h^s_{21}, h^s_{41}, and h^s_{24}, for the dominance of the cost (where *0*, *1*, *2*, and *4* represent *Ester*^{0}, *Ester*^{1}, *Ester*^{2}, and *Ester*^{4}, respectively).

Model COD-A was then simplified using likelihood-ratio tests corrected for overdispersion (*F*-test) (Lebreton *et al.* 1992; Anderson *et al.* 1994) to find the best adequate model. We first determined whether the selective advantages s_i and s_j of alleles i and j were significantly different by setting $s_1 = s_2$, $s_1 = s_4$, $s_2 = s_4$ in models COD-B_1, COD-B_2, and COD-B_3, respectively, all other parameters being freely estimated. Similarly, we then determined whether costs c_i and c_j of alleles i and j were significantly different. We computed the models COD-C_1, COD-C_2, and COD-C_3 by setting $c_1 = c_2$, $c_1 = c_4$, $c_2 = c_4$, respectively, with all other parameters being freely estimated. Models combining more complex hypotheses (*i.e.*, constraining both s and c values to be identical among some alleles) were then computed as models COD-D, with all other parameters again being freely estimated.

Overdispersion was computed from model COD-A as the ratio of residual deviance (the deviance equals $-2 \times \ln$[likelihood]) over residual degrees of freedom. We computed the percentage of total deviance explained by a model (%TD) as

$$\%\text{TD} = (D_{\max} - D_{\text{model}})/(D_{\max} - D_{\min}), \quad (6)$$

where the maximal deviance ($D_{\max}$) is obtained by fitting a minimal model in which the frequency of each allele in each population is set to its average along the transect and over years, and the minimal deviance ($D_{\min}$) obtained by fitting a

TABLE 1

Model selection

Model	Simplification	Dev[a]	d.f.[b]	%TD[c]	*F*-test	*P*-value	
COD-A	Complete model	6215.98	7	0.705	—	—	
COD-B1	$s_1 = s_2$	6230.86	6	0.694	14.88	0.000	***
COD-B2	$s_1 = s_4$	6216.38	6	0.704	0.40	0.526	NS
COD-B3	$s_2 = s_4$	6238.40	6	0.689	22.43	0.000	***
COD-C1	$c_1 = c_2$	6217.86	6	0.703	1.88	0.171	NS
COD-C2	$c_1 = c_4$	6233.88	6	0.692	17.90	0.000	***
COD-C3	$c_2 = c_4$	6222.20	6	0.700	6.22	0.013	*
COD-D	$s_1 = s_4$ and $c_1 = c_2$	6218.48	5	0.703	1.25	0.288	NS
NOCOD-A	Complete model	6179.95	19	0.730	—	—	
NOCOD-B1	$s_1 = s_2$	6193.16	18	0.720	13.20	0.000	***
NOCOD-B2	$s_1 = s_4$	6179.95	18	0.730	0.00	1.000	NS
NOCOD-B3	$s_2 = s_4$	6187.23	18	0.725	7.28	0.007	**
NOCOD-C1	$c_1 = c_2$	6183.88	18	0.727	3.93	0.048	*
NOCOD-C2	$c_1 = c_4$	6180.00	18	0.730	0.05	0.830	NS
NOCOD-C3	$c_2 = c_4$	6184.57	18	0.726	4.62	0.032	*
NOCOD-D	$s_1 = s_4$ and $c_1 = c_4$	6179.96	17	0.730	0.00	0.997	NS

The models are described in the text. They correspond to different simplifications of the complete model (COD-A and NOCOD-A, respectively, with and without the codominance hypothesis), indicated in the "Simplification" column. *F*-test statistic values and *P*-values indicate whether the deviance of the model considered is significantly different from that of the complete model A (NS, *P*-value >0.05; *P*-value <0.05; **P*-value <0.01; ***P*-value <0.001). Each dominance parameter was set to 0.5 (COD) or freely estimated (NOCOD).

[a] The residual deviance of each model is scaled to the overdispersion of model A.

[b] The number of degrees of freedom.

[c] The part of the total deviance explained by each model.

maximal model in which the frequency of each allele in each population is set to its observed frequency. Models were compared using *F*-tests to correct for overdispersion. All deviances were also corrected for overdispersion to estimate the support limits (SL) of each parameter p. These were computed by maximizing or minimizing the value of p, for upper limit ($p_{\max}$) and lower limit ($p_{\min}$), respectively. All other parameters were allowed to change, ensuring that we find the actual $p_{\max}$ and $p_{\min}$ in the range of the multidimensional parameter landscape where likelihood is not significantly different from the maximum likelihood of the model, using the same simulated annealing method.

We used the same process when relaxing the overdominance hypothesis (NOCOD models) and simply tested whether each dominance parameter was significantly different from 0.5 (codominance) or not, all other parameters being again freely estimated.

RESULTS

Insecticide treatment: The treatment practices for the period 1990–2002 are presented in Figure S1. Over the 12 years analyzed, the treated area size is roughly constant and runs 16 km inland from the sea, although treatment extension and intensity were higher in the first years analyzed (Figure S1). The total amount of insecticides used is variable over the years, from 124.3 to 733.1 liters · km^{-2} (Figure S2). The treated area is not evenly treated: immediately close to the sea, treatments are less intense, due to less suitable breeding sites for *C. pipiens*. This mosquito needs freshwater to reproduce and brackish lagoons near the sea largely reduce the area with potential breeding sites. However, for simplicity we considered the treated area as an approximately uniformly treated surface in the model, as we have no clue about how the dose of insecticide relates to fitness in the field.

Model comparison and tests: Model selection was performed using likelihood-ratio tests corrected for overdispersion (*F*-test) (Lebreton *et al.* 1992; Anderson *et al.* 1994) to determine whether the selection coefficients were different among alleles. Results of each comparison are detailed in Table 1. Under the codominance hypothesis, in the simplest adequate model (model COD-D) the selective advantages of *Ester*^{1} and *Ester*^{4} and the costs of *Ester*^{1} and *Ester*^{2} are not significantly different (*i.e.*, $s_1 = s_4$ and $c_1 = c_2$, resp.), but the cost is lower for *Ester*^{4} (*i.e.*, $c_4 < c_2 = c_1$). Relaxing the codominance hypothesis modifies the results, such that neither the selective advantages nor the costs of *Ester*^{1} and *Ester*^{4} are significantly different (*i.e.*, $s_1 = s_4$ and $c_1 = c_4$, model NOCOD-D).

Parameters estimations: The first six parameters of our models are those describing the initial clines (1986): the initial maximum frequencies (k_1 and k_4) and the rates of decline (a_1, a_4, b_1, and b_4) of each allele present (*Ester*^{1} and *Ester*^{4}). This initial cline is best described by an $\exp(-x^2)$ shape, where x is the distance from the coast ($k_1 = 0.461$, $k_4 = 0.233$, $a_1 = 0.095$, $a_4 = 0.071$, $b_1 = b_4 = 0$).

Under the codominance hypothesis, the simplest model (COD-D) is built with four other parameters describing selective advantage and cost: $s_1 = s_4$, s_2, $c_1 = c_2$, and c_4. The best value of each of these and corresponding support limits are indicated in Table 2A. This model explains 70.3% of the total deviance with low overdispersion (∼1.52).

Relaxing the codominance hypothesis introduces 12 additional parameters of dominance associated with s and c, h^s_{ij} and h^c_{ij}. Among these only one, h^s_{20}, the advantage dominance of *Ester*^{2} over *Ester*^{0}, is significantly different from 0.5 (codominance) (h^s_{20} = 0.076, Table 2B). Under this hypothesis, the simplest model (NOCOD-D) is built with four parameters describing selective advantage and cost: $s_1 = s_4$, s_2, $c_1 = c_4$, and c_2, the differences in cost and advantage dominances, h^c and h^s, alone explaining the allele replacement. The best value of each of these and corresponding supporting limits are indicated in Table 2B. This model explains 72.9% of the total deviance with low overdispersion (∼1.43).

The last parameter, the time to *Ester*^{2} appearance t*, is estimated to 45 and 48 generations (with and without the codominance hypothesis, respectively), which corresponds to ∼1989 with support limits being 30–60 generations, *i.e.*, 1988–1990.

The predicted cline of each allele (spatial frequencies variation) is presented in Figure 2, for each year for which samples are available, under the NOCOD-A model. The computed variation over the period 1986–2002 (temporal frequencies variation) of the maximal frequency of each allele is presented in Figure 3 (NOCOD-A model). It shows the replacement of *Ester*^{1} by *Ester*^{4} that occurred during the 1990s. It also predicts the replacement of *Ester*^{4} by *Ester*^{2} over the period 2002–2024 (Figure 3), providing that treatment practices will not change during this period.

To assess whether there was a consistent variation of selection coefficients over time, we used two estimators of the goodness of fit, overdispersion and percentage of deviance explained by the model, for each sampling year. The variability over the 1986–2002 period of these two measures is presented in Figure S3. Each sampled year independently is well fitted with >50% and up to 82% of the total deviance explained and an overdispersion inferior to 2 for each of them.

TABLE 2

Best fitted parameters

Parameter	Best value	Support limits
A. Codominance hypothesis (COD-A model)		
s_1	0.19	0.15–0.24
s_2	0.37	0.28–0.53
s_4	0.19	0.16–0.21
c_1	0.078	0.058–0.10
c_2	0.12	0.050–0.28
c_4	0.036	0.027–0.045
t*	45	30–60
B. Dominance parameters freely estimated (NOCOD-A model)		
s_1	0.17	0.13–0.21
s_2	0.51	0.35–0.72
s_4	0.18	0.15–0.24
c_1	0.057	0.040–0.079
c_2	0.20	0.084–0.52
c_4	0.059	0.027–0.11
t*	48	30–60
h^s_{10}	0.19	0.00–0.57
h^s_{40}	0.29	0.00–0.59
h^s_{20}	**0.076**	**0.00–0.29**
h^s_{14}	1.00	0.00–1.00
h^s_{12}	1.00	0.44–1.00
h^s_{24}	0.54	0.095–1.00
h^c_{10}	0.65	0.44–1.02
h^c_{40}	0.21	0.00–0.52
h^c_{20}	0.36	0.00–0.86
h^c_{14}	2.86	0.00–3.00
h^c_{12}	0.00	0.00–0.88
h^c_{24}	0.64	0.00–1.92

For each parameter, the best value fitted is indicated, associated with the corresponding support limits (see text). In the last case, the estimated values of the dominance parameters for selection, h^s_{ij}, or for cost, h^c_{ij}, are also indicated (values significantly different from 0.5, *i.e.*, codominance, are in boldface type).

DISCUSSION

Natural selection is notoriously difficult to measure in the wild, and this imposes an important limit on our ability to study evolution and to test *in natura* theories of adaptation. However, the study of cline series is a particularly informative situation since temporal and spatial frequency variation can be combined to estimate selection. We illustrate this approach with the evolution of insecticide resistance in the mosquito *C. pipiens*: the resistance allele replacement observed at the *Ester* locus in the Montpellier area since 1986 allows us to analyze the process of amelioration with a degree of precision more typical of laboratory than field studies (down to a precision of ∼2% for some selection coefficients). In addition, this is the first time, to our knowledge, that estimation of the relative fitness of more than two alleles at a single locus has been performed using field data (our model is nevertheless in accordance with previous average measures of fitness on this system, see *Comparison with previous estimations*, File S1). This approach enabled us to study in more detail the process of allele replacement in the field and to determine whether it was driven by direct or compensatory amelioration. It also enables us to quantify the amount of fitness variation occurring at different stages of the process.

Selection coefficients: The selection coefficient estimations provided for the various resistance alleles (Table 1) accurately explain the allele replacements

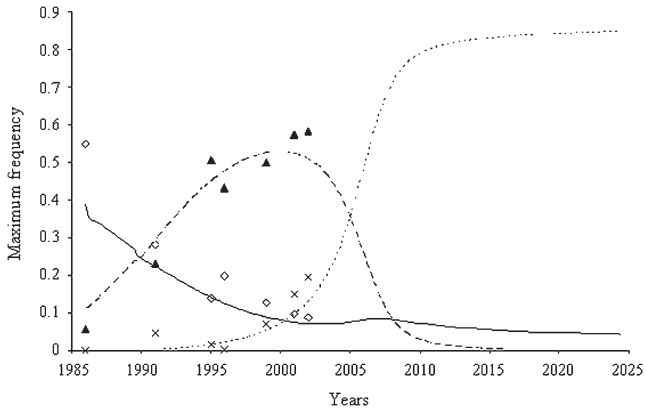

FIGURE 2.—Cline fitting of the best model. The expected patterns of frequency variation along the sampling transect are presented for each year of sampling. Sample frequencies of each allele are represented: open diamonds, *Ester*^{1}; solid triangles, *Ester*^{4}; and x's, *Ester*^{2}. Lines represent the expected clines under the NOCOD-A model (see MATERIALS AND METHODS): solid line, *Ester*^{1}; dashed line, *Ester*^{4}; and dotted line, *Ester*^{2}. The susceptible allele is not represented as its frequency is equal to $1 - \Sigma$(resistant allele frequencies).

observed in the Montpellier area (70% of the total deviance is explained). With the susceptible allele fitness being $w_0 = 1$, the overall resistance allele fitness orders are *Ester*^{2} ($w_2 = 1.250$) > *Ester*^{4} ($w_4 = 1.154$) > *Ester*^{1} ($w_1 = 1.112$) in the treated area and *Ester*^{4} ($w_2 = 0.964$) > *Ester*^{1} ($w_4 = 0.922$) > *Ester*^{2} ($w_1 = 0.880$) in the nontreated area (COD-A model, Table 2). The fitness differences detected between resistant alleles can thus be relatively small compared to the differences between susceptible and resistant (*e.g.*, in the treated area *Ester*^{4} fitness is $w_4 = 1.038$ relative to *Ester*^{1}; *i.e.*, if $w_1 = 1$, although *Ester*^{2} differences with *Ester*^{4} and *Ester*^{1} are still quite large, *e.g.*, in the treated area $w_2 = 1.124$ relative to *Ester*^{1}).

During the 1990s, *Ester*^{4} replaced *Ester*^{1} (Figure 3) (GUILLEMAUD *et al.* 1998; LABBÉ *et al.* 2005). We show here that *Ester*^{1}'s selective advantage is not significantly different from that of *Ester*^{4} (relative to s_1, $s_4 = 0$, SL = −0.026–0.019), but that its cost is higher under the codominance hypothesis (relative to c_1, $c_4 = -0.045$, SL = −0.055 – −0.035). Thus, the lower cost of *Ester*^{4} could be pivotal in explaining the replacement of *Ester*^{1} by *Ester*^{4}. However, if dominances are different from 0.5, the estimated costs of *Ester*^{1} and *Ester*^{4} are not distinguishable (relative to c_1, $c_4 = -0.002$, SL = −0.032–0.059). In this case, the allele replacement is due to the cost being more dominant for *Ester*^{1} than for *Ester*^{4} [the cost of (*Ester*^{1}/*Ester*^{0}) heterozygotes is closer to that of (*Ester*^{1}/*Ester*^{1}) than (*Ester*^{0}/*Ester*^{0}) homozygotes, whereas the cost of (*Ester*^{4}/*Ester*^{0}) heterozygotes is closer to that of (*Ester*^{0}/*Ester*^{0}) than (*Ester*^{4}/*Ester*^{4}) homozygotes]. However, dominances remain difficult to precisely estimate using these data (Table 2B). Fitting dominances only marginally increases the overall goodness of fit, explaining no more than 3% more of the deviance than the codominance model. Finally, laboratory data provide support for *Ester*^{1} having a higher cost than *Ester*^{4} (see *Comparison with laboratory experiments*, File S1), suggesting a compensatory amelioration in this first allele replacement.

Ester^2 was first detected in southern France near Marseille (∼150 km from Montpellier) in 1986, but it was first detected at a very low frequency in the Montpellier area in 1990 (RIVET *et al.* 1993). Our study estimates its first occurrence near Montpellier during the year 1989, which indicates a quite fast spread of this allele in the south of France (∼50 km per year in ∼3 years). This alone suggests a strong fitness advantage for this resistance allele. It remained at low frequency (coastal frequency <0.1) until 1999 (GUILLEMAUD *et al.* 1998; GAZAVE *et al.* 2001; LABBÉ *et al.* 2005) and then progressively increased in frequency (coastal frequency ∼0.2 in 2002), leading to a decrease in the frequency of *Ester*^{1} and the stabilization of the frequency of *Ester*^{4} (LABBÉ *et al.* 2005). As shown in this study, this seems to be explained by a strong selective advantage leading to a higher fitness of *Ester*^{2} in the treated area compared to the previous alleles (relative to s_1, $s_2 = 0.158$, SL = 0.072–0.282; relative to s_4, $s_2 = 0.158$, SL = 0.077–0.288), despite a higher cost (relative to c_1, $c_2 = 0.188$, SL = 0.118–0.329; relative to c_4, $c_2 = 0.155$, SL = 0.082–0.302; see Table 2). Thus, the increased advantage of *Ester*^{2} in the treated area compensates for its increased cost in the nontreated area, indicating that this second allele replacement is most probably due to a direct amelioration (see also *Comparison with laboratory experiments*, File S1). Finally, our estimations (Figure 3) indicate that without any modification in the insecticide treatment practices, *Ester*^{2} would eventually replace both alleles in ∼20 years, the approximate time from the appearance of *Ester*^{4} until now.

Limit of the approach: In this study, we used two main assumptions by neglecting the intra-annual variation and the density variation between treated and nontreated areas (other parameters, including the initial conditions, are fitted in the model or were obtained independently; see *Migration–selection model*, MATERIALS AND METHODS). For practical reasons of computational time, intra-annual variations of selection coefficients were not taken into account in our model. Because we ignored them, we cannot determine if the different resistance alleles are selected differently in different seasons [*e.g.*, if differences in the fitness cost are due to differences in mortality during female overwintering or to differences of larval development time during summer (LENORMAND *et al.* 1999; LENORMAND and RAYMOND 2000; GAZAVE *et al.* 2001)]. Thus, the estimates we give have to be understood as annual averages. However, the cline observed in summer is relatively independent of what happened during the rest of the year, so that the hypothesis of a temporary migration–selection equilibrium reached each year at the end of

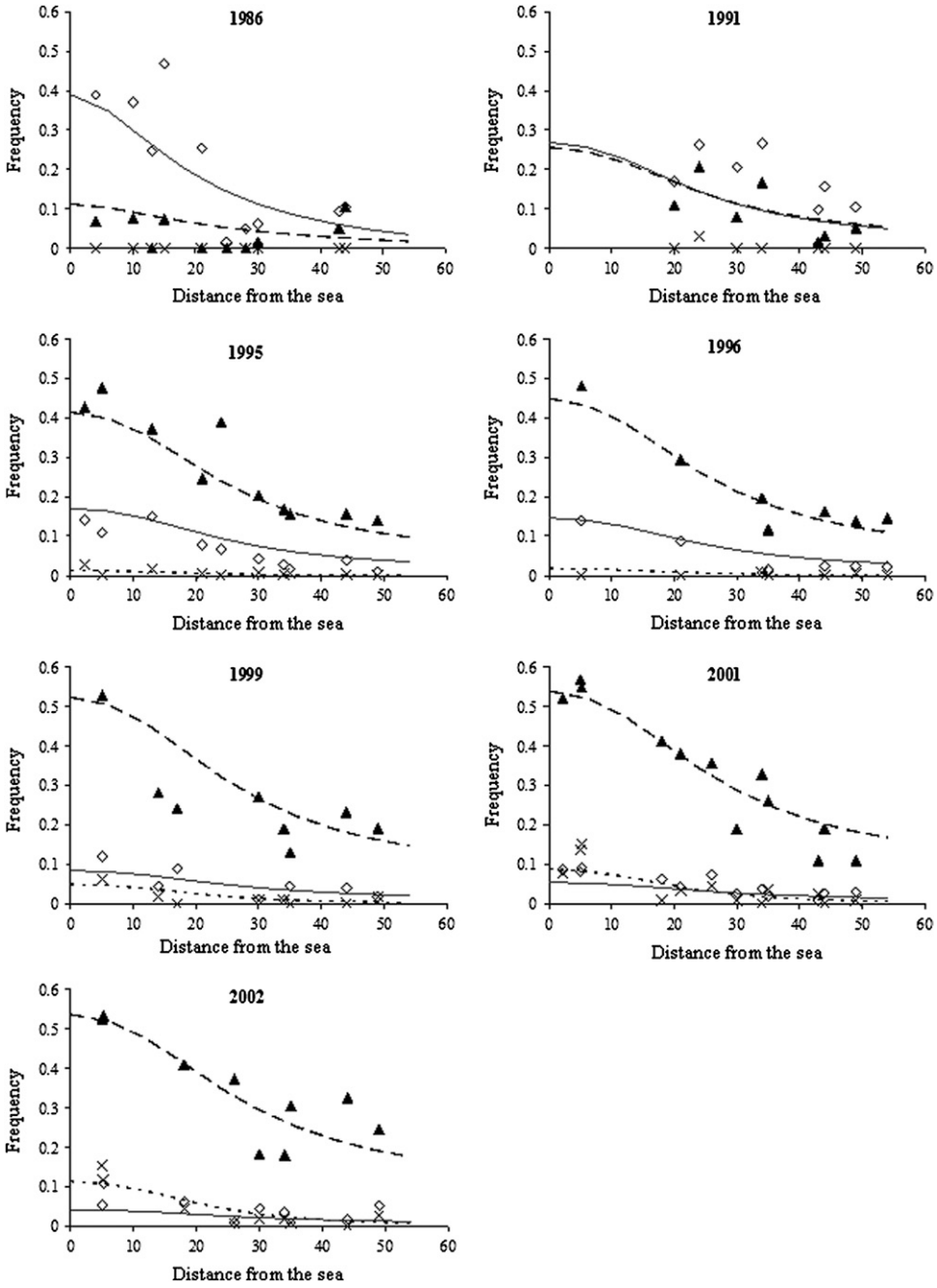

FIGURE 3.—Interannual variation of *Ester* alleles frequencies. The frequencies presented here are the maximum frequencies, *i.e.*, at the coast. The first allele to appear was *Ester*^{1} (with open diamonds), which was then replaced by *Ester*^{4} (solid triangles) (GUILLEMAUD *et al.* 1998). Recently, a third allele, *Ester*^{2}, invaded the Montpellier area (x's) (LABBÉ *et al.* 2005). We used the estimation of coastal frequencies of the different alleles provided by LABBÉ *et al.* (2005). Lines represent the fitted values according to the NOCOD-A model (see MATERIALS AND METHODS). The susceptible allele is not represented as its frequency is equal to $1 - \Sigma$(resistant allele frequencies).

summer is reasonably accurate (see *Comparison with previous estimations* in File S1), although the allele replacement modifies this equilibrium from one year to another. Density differences between treated and nontreated areas may bias our estimates of selection by causing an asymmetrical gene flow between the two habitats (NAGYLAKI 1978). This is true in particular for the relative selection coefficients between susceptible and resistance alleles. Like for selection, the density pattern may also vary seasonally or among years. Thus, the estimates we give have to be understood as if density was constant. For instance, if density is lower in the treated area [which is not necessarily the case (LENORMAND *et al.* 1998)], larger s would be required to maintain the same clines. However, it is important to underline that because the different resistance alleles experience the same density variation across treated and nontreated areas and because they have very similar spatial distribution (see Figure 2), this bias is minimal as long as resistance alleles are compared to one another, as is the case in our study (*i.e.*, the values of s and c relative to the susceptible allele might be biased, but the differences between s_1, s_2, and s_4 on one hand and between c_1, c_2, and c_4 on the other hand cannot be strongly affected by density effects). The same arguments hold for demographic, topological, or treatment intensity variations that could occur perpendicular to our transect (although we are unaware of such variation over a few tens

of kilometers east or west of our transect). Globally, our model explains >70% of the total deviance (TD) observed in the evolution of *Ester* resistance genes in the Montpellier area (with a low global overdispersion, ~1.43). There is no indication of a trend toward increase or decrease of the percentage of TD or overdispersion during allele replacement (Figure S3), as would be the case if selection changes with time. This would be the case, for example, if a modifier gene appeared at another locus during the course of the replacement, as was seen in *Lucilia cuprina* (for a review see McKenzie 1996). Such a modifier could increase the fitness of resistant *Ester* alleles by reducing their deleterious side effects. Although our power to detect a compensatory modifier was limited, especially if it had a weak effect, a modifier gene is not necessary to explain the trends observed in natural populations through this long-term study.

The process of amelioration: This work enables us to understand the causes of the resistance allele replacement observed at the *Ester* locus in the Montpellier area. Our model and previous laboratory experiments (see File S1) suggest that $Ester^1$ has most probably been replaced by $Ester^4$ because $Ester^4$ is less costly (compensatory amelioration). Currently, a second replacement is occurring: $Ester^2$ is replacing $Ester^4$ despite a higher fitness cost, due to a higher selective advantage in the treated area (direct amelioration). Thus, contrary to the first replacement during which a more "generalist" allele was selected, the second replacement involves an allele that is more "specialist" to treated areas, leading to the reinforcement of local adaptation. There are two ways by which evolution may proceed ultimately if the insecticide treatments are maintained. The first option would involve further evolution toward specialist alleles that confer high resistance but with strong pleiotropic effects, such as $Ester^2$. This type of situation occurs when there is a strong trade-off between conflicting selection pressures in the different habitats. In such a situation a stable polymorphism is likely to be maintained and may lead to the evolution of distinct niches. The second option would involve further evolution toward generalist alleles that confer resistance with weak pleiotropic effects, such as $Ester^4$. This type of situation occurs when there is a weaker trade-off between the conflicting selection pressures in the different habitats. In this scenario the polymorphism is likely to be lost rapidly, with the fixation of a "cost-free" resistance allele, which corresponds to an extension of the niche. It is interesting to note that in our case both options have occurred successively in combination with a changing environment. The evolution at the *Ester* locus is certainly influenced by treatment practices, and it is possible that if insecticide treatment intensity decreases, $Ester^4$ may be favored again due to its lower cost, with the stronger resistance of $Ester^2$ then being less advantageous. This emphasizes the role of local treatment practices, which have been shown to be crucial in the competition of *Ester* alleles on a worldwide scale (Labbé *et al.* 2005).

The theory of adaptation: From a more general point of view, the evolution of insecticide resistance at the *Ester* gene represents a first step to study theories of adaptation (Orr 2005) in nature. Although amelioration has been reported from field studies (*e.g.*, McKenzie 1993), our study allows quantitative estimation of the selection coefficient involved with a clear knowledge of the history and genetic bases of the adaptive changes. Viewed at first as a slow process of accumulation of small mutations toward a fitness optimum (Fisher 1928; Hartl and Taubes 1996), the process of adaptation is now thought to imply larger fitness-effect mutations, occurring early in the process, and smaller mutations occurring subsequently to refine the adaptation (Cohan *et al.* 1994; Barton 1998; Orr 1998, 2000, 2005; Barton and Keightley 2002). This theory has been supported by laboratory studies (*e.g.*, Cohan *et al.* 1994; Oxman *et al.* 2008), but ours gives new support from field data: the first mutation, $Ester^1$, was indeed a mutation of large fitness effect (relatively to ancestral susceptible alleles); the second, $Ester^4$, had a smaller fitness effect and refined the adaptation by lowering the cost; and the third, $Ester^2$, had again a large effect on fitness (Table 2). Albeit representing few steps, this field study is consistent with the theory formalized by Orr (1998). Interestingly, this study also provides some insights into the fact that adaptation is local, and thus that several strategies are possible in the course of adaptation to a heterogeneous environment. It is difficult to predict whether local adaptation will reinforce itself and lead to the evolution of specialist strategies or whether a generalist strategy that can exploit all habitats will emerge. The course of events obviously depends on the selective properties of the new resistance alleles that occur (and the underlying trade-off). Our study illustrates this point precisely: $Ester^4$ was more generalist than $Ester^1$ (low c), but it is now being replaced by $Ester^2$, a more specialist allele (high s, high c). The theory of adaptation tends to focus primarily on evolution in a single population. It will benefit from taking into account the spatial and temporal variability of the environment.

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Resistance Gene Replacement in the Mosquito *Culex pipiens*: Fitness Estimation From Long-Term Cline Series

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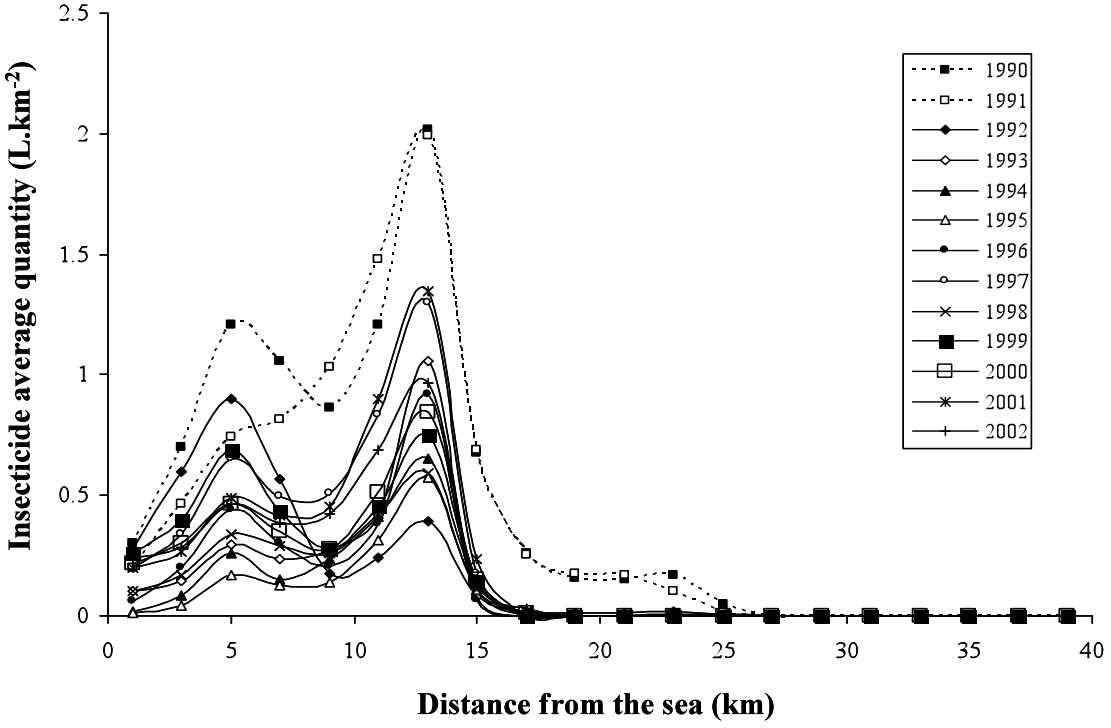

FIGURE S1.—Insecticide treatment distribution along the sampling transect. The variation of the average quantity of insecticide (L.km^{-2}; see text for calculation) used along a transect from the coast inland is indicated for all years from 1990 to 2002.

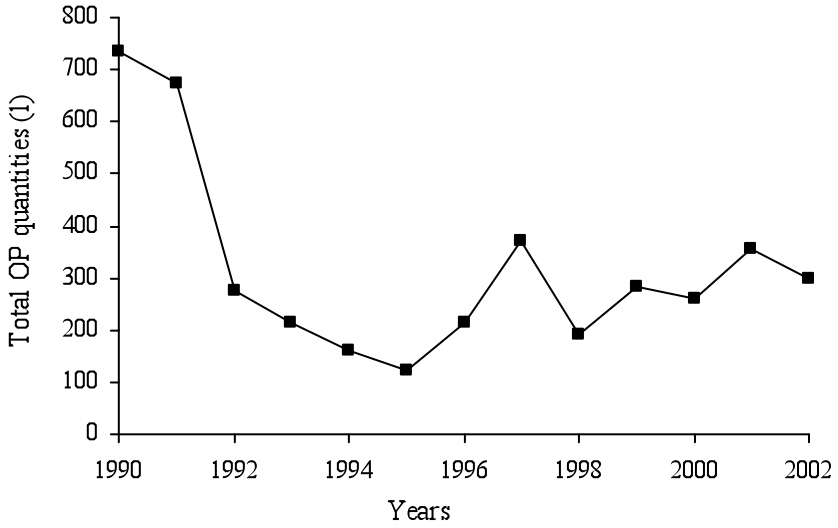

FIGURE S2.—Evolution of OP insecticide quantities applied in Montpellier area. The variation of the total quantity of insecticide (l) used in the treated area is indicated for all years from 1990 to 2002.

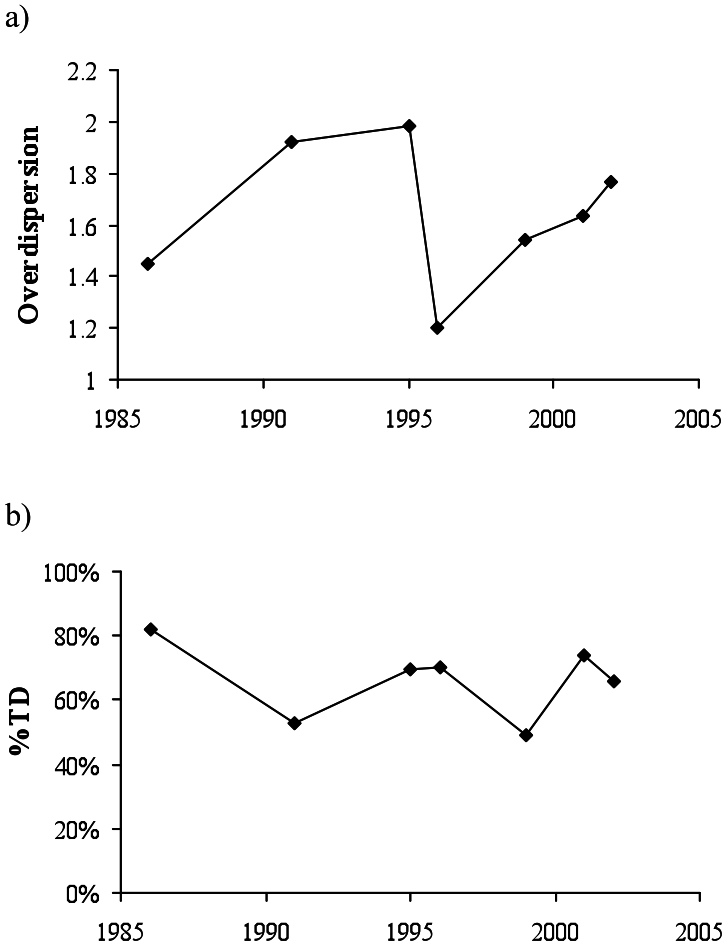

FIGURE S3.—Interannual variation of %TD and overdispersion. The percentage of deviance explained by the model (%TD; a) and the overdispersion (b) of the model are presented for each year of sampling (see calculation in text). Note that %TD and overdispersion of each sampling year are calculated by fitting each cline independently but using the best values of each parameter (COD-D model) and are thus different from the global %TD and overdispersion calculated for all clines simultaneously.

FILE S1

Resistance Gene Replacement in the Mosquito *Culex pipiens*: Fitness Estimation From Long-term Cline Series

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Previous models: Using data from one cline from summer 1995, LENORMAND *et al.* (1998) estimated the three parameters, *s* (selective advantage), *c* (cost) and *s* (migration rate)). They hypothesised that, during this season, the frequencies of the different alleles reach an equilibrium between selection and migration, and used measures of linkage disequilibrium between the *ace-1* and *Ester* loci to estimate the migration. Later, LENORMAND *et al.* (1999) showed that the cline shape differs between seasons but is consistent between the same seasons of different years, thus following an annual cycle. Using the assumption of cyclic equilibrium they estimated variation in the parameters (migration and selection) within a year. However, in both these studies, migration-selection equilibrium was assumed, precluding study of the transient dynamic of allele replacement, and only average selection coefficients of resistance alleles were estimated (i.e. they considered only two alleles, resistant vs. susceptible). LENORMAND AND RAYMOND (2000) relaxed this hypothesis of equilibrium and analyzed sequential time variation of clines on a small scale period, during fall and winter 1996-1997. With this sequential approach, descriptive models were used to describe the initial frequency distribution over space, and migration and selection were inferred from the time variation of clinal patterns. However, due to the small time scale, they were unable to estimate fitness differences between alleles at the same locus.

Comparison with previous estimations: The first determination of fitness parameters for the *C. pipiens* resistance gene in the Montpellier area used a transect of populations collected in summer 1995 and assumed an equilibrium between selection and migration (LENORMAND *et al.* 1998). A second estimate was made using data collected over a two year period from July 1995 to October 1997 and assumed a seasonal cyclic equilibrium of frequency patterns each year (LENORMAND *et al.* 1999). Both studies took advantage of the treatment patterns in the Montpellier area, which allow the detection of clinal patterns in the frequencies of resistance alleles. But their assumptions of equilibrium lead the authors to not consider differences in fitness among alleles at *Ester* (and at *ace-1* loci): they considered only two classes of alleles (resistance vs. susceptible, for each locus). This assumption was later relaxed to estimate variations of selection coefficients for each allele and variation of gene flow during the winter 1996-1997 (LENORMAND and RAYMOND 2000). However, at this short time scale, there is almost no allele replacement so that there is little information to reliably estimate differences among alleles. In the present study, we also study transient dynamics, but on a much longer time scale, which enables us to distinguish selective effect of each resistance allele. Because our approach does not depend on the assumption that equilibrium has been reached, it provides an interesting comparison to estimates that used this assumption. If we pool together the individuals carrying two resistance alleles, we can roughly estimate the fitness w_{rr} of an average resistant homozygote

as $w_{rr} = \sum_i \sum_j f_{ij} \times w_{ij}$ where f_{ij} is the frequency of (*Ester*^{i}/ *Ester*^{j}) genotype and w_{ij} the fitness of this genotype. The average fitness w_{rs} of a heterozygote carrying one susceptible and one resistance alleles is given by the same equation where j is *Ester*^{0}. Finally the fitness w_{ss} of a susceptible homozygote is directly the fitness of individuals carrying (*Ester*^{0}/ *Ester*^{0}) genotype. By setting w_{rs} = 1, such transformations allow us to compare our results with previous published estimates. We found a good agreement, as we estimated the fitness of a resistant homozygote, heterozygote and susceptible homozygote, in the treated area, for summer 1995, to be ~1.058, 1, ~0.945 respectively, where LENORMAND *et al.* (1998) estimated it to ~1.056, 1, ~0.944, respectively (considering a treated area of 20 km as these authors). This demonstrates that the hypothesis of equilibrium between selection and migration being reached at the end of summer is reasonably accurate. The same conclusion was reached when studying in more details the seasonal cyclic variations of clinal patterns (see LENORMAND *et al.* 1999). Of course, this is expected since the approach to a migration-selection equilibrium is fast when both migration and selection are large (as in our case) compared to when they are both small. Moreover, due to the slow change in resistance allele frequencies from one year to another it is possible to estimate average selection coefficients for each allele and each year independently: using one or two years of sampling, it is possible to ignore the "background" allele replacement, which is too slow at this scale to significantly influence selection coefficients estimates.

Comparison with laboratory experiments: Considering the selective advantage (s), *Ester*^{4} is known to confer approximately the same OP resistance level as *Ester*^{1}: the LC_{50} resistance ratio is 2.06 and 1.6 for temephos and 5.7 and 5.57 for chlorpyrifos, for *Ester*^{1} and *Ester*^{4} respectively (RAYMOND *et al.* 1986; POIRIÉ *et al.* 1992). Individuals bearing *Ester*^{2} display levels of resistance about two to three times higher than *Ester*^{1} and *Ester*^{4} (temephos LC50 resistance ratio = 7.3 and chlorpyrifos LC50 resistance ratio = 10, WIRTH *et al.* 1990; see also RAYMOND *et al.* 1993), consistent with our model which shows that selective advantages s are similar between *Ester*^{1} and *Ester*^{4}, but much higher for *Ester*^{2} (Table 2).

To evaluate the cost (c) of the different *Ester* alleles, life history traits associated with fitness have previously been measured in several laboratory and field experiments: males mating competition (BERTICAT *et al.* 2002a; DURON *et al.* 2006), development time, fluctuating asymmetry (BOURGUET *et al.* 2004), larval mortality, female size, fecundity, predation, intracellular bacteria *Wolbachia* density (BERTICAT *et al.* 2002b, DURON, *et al.* 2006). These studies were generally in agreement with a fitness cost associated with resistance compared to susceptible individuals: all *Ester* resistance alleles display a cost for at least one, generally several, traits studied. Although it is not known how costs associated to these individual life-history traits combine in the life cycle, these laboratory studies suggest that the overall fitness cost is substantial. This has been confirmed by field studies: when sampled on a transect across treated and non-treated areas, all resistance *Ester* alleles display frequency clines in summer, when insecticide treatments are applied, whereas those clines are shallower in winter, when there is no insecticide (LENORMAND *et al.* 1999; GUILLEMAUD *et al.* 1998; LABBÉ *et al.* 2005, this study).

Comparing the cost of different alleles is difficult. Several laboratory experiments examining various traits point to a higher fitness cost of *Ester*^{1} than to *Ester*^{4} ($c_4 < c_1$) (BERTICAT *et al.* 2002b, DURON, *et al.* 2006). *Ester* resistance alleles like

Ester² are known to be highly variable in resistance and in amplification level (WIRTH *et al.* 1990; RAYMOND *et al.* 1993; CALLAGHAN *et al.* 1998), which could lead to variability in the fitness advantage-cost balance (BERTICAT 2001). Laboratory strains isolation (BERTICAT *et al.* 2002a) and the absence of selection could have modified the amplification level of *Ester²* and *Ester⁴*. Alternatively, selection coefficients may show some plasticity from the laboratory to the field. Laboratory results from standardized environments can not always be extended to the field, where the environment is more variable and generally different from that of the laboratory (MCKENZIE 1996 and e.g. DE FOUNTAIN and HOPKIN 2004; STOOPS 2005; FICETOLA and BERNARDI 2005). Measures from laboratory studies on a particular trait may prevent any extrapolation to fitness in natural environment, which is not, in fact, very surprising.

Supporting Information Bibliography

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