Analysis of Clines with Variable Selection and Variable Migration

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ABSTRACT: We report a likelihood-based method that estimates both dispersal and natural selection using the rate of change of the shape of a cline when selection and migration are not constant through time. We have investigated the case of local adaptation of the mosquito *Culex pipiens* to organophosphate insecticides in the Montpellier area in France. We have analyzed the modification of the clinal patterns at two resistance loci during the period from breeding to overwintering. We show that mosquitoes migrate extensively from breeding to overwintering sites at a rate that is markedly different from previous estimates made during the breeding season only. This migration is also strongly asymmetrical, which can be explained by different geographical distributions of breeding and overwintering sites, by variation in mosquito density along the transect, or by behavioral biases. We found that the starting time of overwintering is likely to vary between northern and southern populations and that substantial fitness costs are associated with resistance alleles at the two loci during overwintering. These results illustrate how demography and adaptive microevolution can be studied using selected markers. The method provides a framework to use population genetics and statistical models to reveal ecological and evolutionary processes.

Keywords: asymmetrical gene flow, variable gene flow, insecticide resistance, selection, cline, fitness cost.

Migration, by homogenizing the genetic composition of populations, plays a central role in evolutionary processes such as local adaptation and speciation (Barton and Hewitt 1989). However, there is an important and revealing gap between the current direct and indirect measurements of gene flow. The former corresponds to the measure of a life-history trait, while the latter reveals the history of migration as recorded in the genetic structure of population (Slatkin 1985). As a consequence, migration is often envisioned in a dichotomous way, being either a process driving evolution or a trait subject to evolution. Life-history theory has emphasized various aspects of dispersal: age and sex differences, spatial and temporal variability, seasonal and directional migration (from wintering to breeding grounds, philopatric behavior, etc.; see, e.g., Greenwood and Harvey 1982; Stinner et al. 1983; Swingland 1983; Dingle 1996). Although dispersal is a key element in ecological genetics, the microecology of migration is often not considered (Feldman 1989) because a technical challenge has to be resolved: by analyzing population dynamics as well as the interplay between demographic and genetic structures of natural populations (Richardson 1970; Slatkin 1985).

Classical indirect estimates of gene flow that use neutral markers are averaged over a long period of time and incorporate all kinds of dispersal. Thus, they can provide incorrect estimates of the present dispersal rate (Bossart and Prowell 1998; but see Rousset 1997), and they cannot estimate variability in migration except in some specific situations (e.g., gender-based differences in migration behavior detected by comparing mtDNA and nuclear DNA; Avise 1994, pp. 227–230). In addition, they require estimates of population densities to infer dispersal rates (Slatkin and Barton 1989; Rousset 1997).

Thus, direct measurements are generally used to determine specific features of contemporary dispersal in an ecological context. Direct measurements by mark-release-recapture can detect certain details of dispersal, but they are limited in scale and examine the movement of individuals rather than that of genes. Assignation methods or paternity assessment might become an efficient alternative to direct computation of migration distances (Smouse and Chevillon 1998; Waser and Strobeck 1998).

A better strategy is to use strongly selected markers. This method requires identifying such markers (this may not be trivial) and, as with other indirect methods, de-

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veloping a mathematical model of the interaction of gene flow and other forces (selection in this case; see Mallet and Barton 1989) to predict how much gene flow is required to fit the observed patterns. This method has some desirable properties of both direct methods, such as measuring dispersal over a specific timescale, and indirect methods, such as measuring the effective dispersal of genes and avoiding direct observation of individuals. However, this method requires that the factors governing changes in allele frequencies have been well specified.

Gradients of allele frequencies and linkage disequilibriums provide information about both selection coefficients and the migration distance distribution. This linkage-disequilibrium method has been used for bats (Barton 1982), toads (Szymura and Barton 1986), butterflies (Mallet et al. 1990; Porter et al. 1997), lizards (Sites et al. 1995), and mosquitoes (Lenormand et al. 1998*b*).

Another approach that allows one to determine the magnitude of gene flow is the repeated measurement of a cline through time. This method has been implemented for *Drosophila simulans*, using the speed of a wave of advance of *Wolbachia* infection (Turelli and Hoffman 1991), and is related to the analysis of range expansions (Shigesada and Kawasaki 1997). In this study, we develop a new method to estimate dispersal that uses the rate of modification of the cline shape when selection and migration vary through time.

We have investigated the case of local adaptation of the mosquito *Culex pipiens* to the presence of organophosphate (OP) insecticides in the Montpellier area in France. This adaptation is conferred by resistance alleles at two major loci. We have analyzed the modification of the clinal patterns from the breeding seasons to overwintering, first, to estimate dispersal from breeding to overwintering sites and to compare it to the dispersal estimate that was previously obtained using the linkage disequilibrium method, second, to analyze the dynamics of populations through the overwintering season, and, third, to determine the influence of overwintering on the distribution of resistance genes.

Material and Methods

Genetics of Resistance

Two main loci are responsible for OP resistance in *Culex pipiens*. They correspond to different mechanisms of resistance. The first locus, *Ace.1*, codes for an acetylcholinesterase (AChE1), the OP target (Bourguet et al. 1996*c*; Malcolm et al. 1998). In France, there are three alleles at this locus: $Ace.1^R$, which codes for an insensitive AChE1; $Ace.1^S$, which codes for a sensitive AChE1; and $Ace.1^{RS}$, which corresponds to a duplication of *Ace.1* that codes for both enzymes (Raymond et al. 1986; Bourguet et al. 1996*b*; Lenormand et al. 1998*a*). The second locus, *Ester*, corresponds to a "superlocus" (i.e., to two closely linked loci, *Est-3* and *Est-2*, coding for esterases A and B, respectively; de Stordeur 1976; Pasteur et al. 1981*a*, 1981*b*). Resistance alleles at these two esterase loci induce an overproduction of enzyme, owing to either gene amplification or gene regulation (Rooker et al. 1996). In southern France, three resistance alleles have been identified at this superlocus: $Ester^1$, $Ester^2$, and $Ester^4$, corresponding to overproduction of esterases A1, A2-B2, and A4-B4, respectively. The nomenclature used in this article is indicated in table 1.

Culex pipiens *and Its Environment*

We will focus on the period ranging from the end of the breeding season to the end of the overwintering. In the Montpellier area (southern France), the breeding season ends approximately in late October when the last larvae can be observed in the field. Insecticide treatments are restricted to a 20-km coastal belt but are not applied during this period (applications are performed from May to Sep-

Table 1: Nomenclature and coding rules

Genotypes	Genotypes	Phenotypes	Class
$Ester^4$ $Ester^4$	(44)	[4]	{E}
$Ester^4$ $Ester^0$	(40)	[4]	{E}
$Ester^1$ $Ester^1$	(11)	[1]	{E}
$Ester^1$ $Ester^0$	(10)	[1]	{E}
$Ester^2$ $Ester^2$	(22)	[2]	{E}
$Ester^2$ $Ester^0$	(20)	[2]	{E}
$Ester^4$ $Ester^1$	(41)	[41]	{E}
$Ester^4$ $Ester^2$	(42)	[42]	{E}
$Ester^2$ $Ester^1$	(21)	[21]	{E}
$Ester^0$ $Ester^0$	(00)	[0]	{O}
$Ace.1^R$ $Ace.1^R$	(RR)	[RR]	{R}
$Ace.1^R$ $Ace.1^S$	(RS)	[RS]	{R}
$Ace.1^{RS}$ $Ace.1^S$	(RSS)	[RS]	{R}
$Ace.1^{RS}$ $Ace.1^R$	(RSR)	[RS]	{R}
$Ace.1^{RS}$ $Ace.1^{RS}$	(RSRS)	[RS]	{R}
$Ace.1^S$ $Ace.1^S$	(SS)	[SS]	{S}

Note: The resistance allele $Ester^1$ corresponds to overproduced esterase A1; alleles $Ester^2$ and $Ester^4$ correspond to overproduced esterases A2-B2 and A4-B4, respectively. $Ace.1^S$ codes for a sensitive AChE1, $Ace.1^R$ for an insensitive AChE1, and $Ace.1^{RS}$ for both acetylcholinesterases. Coding rules are the simplified codes for each genotype and phenotype corresponding to the genotype indicated in the first column. "Phenotypes" corresponds to the electrophoretic phenotype (*Ester* locus) or to the TPP phenotype (*Ace.1* locus). "Class" groups genotypes with at least one resistance allele. {E}/{O} and {R}/{S} indicate the presence/absence of at least one overproduced esterase or one insensitive AChE1, respectively. This class is used in figures to present the data.

tember in larval breeding sites; "Rapport d'activité technique et scientifique" 1990–1995). We will therefore not consider their effect further in this study.

During the breeding season, *C. pipiens* larvae develop mainly in anthropogenic pools. The females are presumably fertilized at emergence (Subra 1972; Weidhaas et al. 1973) and then search for a blood meal and a site to lay about 150 eggs. The standard deviation of the axial parent-offspring distance distribution was estimated to be 6.6 km generation$^{-1/2}$ during summer (using the linkage disequilibrium method; Lenormand et al. 1998*b*).

During winter, nulliparous inseminated females enter diapause and overwinter in caves or similar temperature-buffered places. Photoperiod might induce diapause (Oda 1992). Movements between overwintering sites have been frequently inferred (Ishii et al. 1964; Service 1968; Hayes 1973). Mortality over the winter is high, reaching about 90% (Minar and Ryba 1971; Sulaiman and Service 1983).

Data Collection

Pupae and fourth instar larvae were sampled on October 19–21, 1996 (set 1, $N_1 = 1{,}056$), along the 50-km northwest/southeast transect studied previously (Chevillon et al. 1995; Guillemaud et al. 1998; Lenormand et al. 1998*a*, 1998*b*), which spans both the treated and nontreated areas. They were reared in the laboratory until emergence, and adults were stored at –80°C. The names and locations of the sampling sites are indicated in figure 1.

Overwintering *Culex* sp. females were captured in caves along the same geographic transect. They were identified to species using a microscope, and only *C. pipiens* were kept and frozen (−80°C) for further analyses. The first sampling of caves was done on October 19–25, 1996 (set 2, $N_2 = 384$); the second on December 5, 1996 (set 3, $N_3 = 464$); and the third on March 5–7, 1997 (set 4, $N_4 = 308$).

Resistance alleles at the *Ester* and *Ace.1* loci were determined as follows. The thorax and the abdomen were used to detect overproduced esterases using starch-gel electrophoresis (Tris-Maleate-EDTA 7.4 buffer; Pasteur et al. 1988). Set 2 was not analyzed for the *Ester* locus. The head was used to characterize the form of AChE1, using the Témoin-Propoxur-Propoxur (TPP) test described by Bourguet et al. (1996*a*). Overproduced esterases are dominant markers under our electrophoretic conditions, and the TPP test determines individuals displaying sensitive, resistant, or both types of acetylcholinesterases. Thus, these methods do not allow complete genotypic identification (table 1).

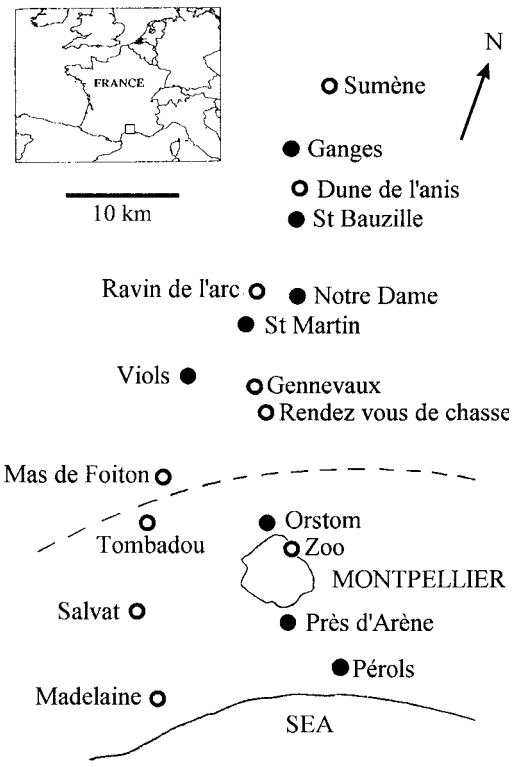

Figure 1: Sampling locations. The dashed line represents the limit between the treated (near the coast) and nontreated areas. Filled circles indicate the position of larval sites, whereas open circles indicate the position of caves.

Descriptive Statistics

To test for the presence of a frequency gradient for each resistance allele along the transect and to provide a picture of seasonal variation, data were first fitted to a purely descriptive cline function. A cline for resistance allele i at locus j and time k was fitted according to a scaled negative exponential (Guillemaud et al. 1998; Lenormand et al. 1998*a*, 1998*b*).

$$h_{ijk} \times \exp\left[-(a_{ijk}x + b_{ijk}x^2)\right], \tag{1}$$

where x is the distance from the coast and h_{ijk} is the frequency of resistance allele i at locus j and time k at $x = 0$ (i.e., on the coast). Parameters a_{ijk} and b_{ijk} are rates of decline in allele frequency with distance and with the square of distance from the coast, respectively. We will denote the vector of parameters $\{h_{ijk}, a_{ijk}, b_{ijk}\}$ at time k by $\boldsymbol{V}_k$. The shape of the cline of a locally adapted allele at migration-selection equilibrium in a semi-infinite environment can be approximated by equation (1) with $a =$

0 (Lenormand et al. 1998*a*; see Nagylaki 1975, eq. [32], for the diffusion approximation). We added the parameter *a* to avoid overconstraining the shape of clines that are not at equilibrium. Finally, this descriptive model allows one to fit simultaneously clines of several alleles at the same locus (see Lenormand et al. 1998*a*, 1998*b*). Phenotypic distributions were computed using allelic distributions and assuming each locus to be at Hardy-Weinberg equilibrium at each location. The phenotype was considered to be a three- or seven-state random variable for the *Ace.1* and *Ester* loci, respectively (see table 1). The log-likelihood of a sample was computed from the phenotypic multinomial distribution, and $\boldsymbol{V}_{1-4}$ was estimated by maximum likelihood.

Departure from Hardy-Weinberg proportions was tested in each population at the *Ester* locus by a likelihood ratio test. For an overall test, *P* values of each test were combined across populations using Fisher's method (Manly 1985). At the *Ace.1* locus, departure from Hardy-Weinberg cannot be evaluated since only three phenotypes are identified for three alleles. The presence of the $Ace.1^{RS}$ allele creates an apparent excess of [RS] if only $Ace.1^{R}$ and $Ace.1^{S}$ alleles are considered. If Hardy-Weinberg proportions are assumed at this locus, $Ace.1^{RS}$ frequencies can be computed from this apparent excess of [RS], and an additional cline of allele frequency can be fitted (Lenormand et al. 1998*a*).

Migration Selection Model

To estimate the migration and selection intensities that cause the frequency gradients of resistance alleles along the transect and their variations, data were then fitted to the migration-selection model, described next.

Migration. We used a linear stepping-stone model of length λ, which was taken to be sufficiently large to simulate a semi-infinite environment (i.e., λ was increased until the migration barrier at λ had no detectable effect on clines). Let us denote d_{ij} and d'_{ij} as the densities of genotype i (i varying from 1 to γ) in deme j before and after migration, respectively, and $m_{jj'}$ as the probability that an individual in deme j migrates to deme j'. Letting

$$\mathbf{M} \equiv (m_{jj'})_{\lambda\cdot\lambda}, \mathbf{D} \equiv (d_{ij})_{\gamma\cdot\lambda}, \text{and } \mathbf{D}' \equiv (d'_{ij})_{\gamma\cdot\lambda} \qquad (2)$$

for the migration (**M**) and relative density matrices (**D** and **D**′), we have $\mathbf{D}' = \mathbf{D} \times \mathbf{M}$, where

$$\mathbf{M} = \begin{bmatrix} 1-\varphi^2 & \varphi^2 & 0 & & & & & \\ \phi^2 & 2\phi\varphi & \varphi^2 & 0 & & & & \\ 0 & \phi^2 & 2\phi\varphi & \varphi^2 & 0 & & & \\ & \cdots & \cdots & \cdots & \cdots & \cdots & & \\ & & \cdots & \cdots & \cdots & \cdots & \cdots & \\ & & & 0 & \phi^2 & 2\phi\varphi & \varphi^2 & 0 \\ & & & & 0 & \phi^2 & 2\phi\varphi & \varphi^2 \\ & & & & & 0 & \phi^2 & 1-\phi^2 \end{bmatrix}^n. \qquad (3)$$

Parameter $\phi = 1 - \varphi$ is the probability of migrating to the right (ignoring border effect) and indicates to what extent the migration is symmetrical, and parameter n is proportional to the variance of the migration distribution. For individuals in demes whose position is greater than n from the sea, the migration distribution is not affected by the barrier effect of the sea and is a centered binomial distribution $B(2n, \phi)$. In this situation, the centered variance of the migration distribution σ^2 equals $\epsilon^2 2n\phi\varphi$, where ϵ is an arbitrary scaling factor corresponding to the distance between demes (we used $\epsilon = 1$). Even though the migration distribution depends on the original position (since mosquitoes were assumed not to migrate into the sea), $\sigma^2 = \epsilon^2 2n\phi\varphi$ was taken as a global measure of its variance and $\mu = \epsilon n(2\phi - 1)$ as a global measure of its mean. For instance, if $\phi = 1/2$, then the migration distribution is symmetrical ($\mu = 0$, ignoring border effects), and its variance equals $n/2$.

Finally, we let g_{ij} and g'_{ij} be the frequency of genotype i in deme j before and after migration, respectively. We then have

$$g_{ij} = d_{ij} / \sum_i d_{ij} \text{ and } g'_{ij} = d'_{ij} / \sum_i d'_{ij}. \qquad (4)$$

Selection. Let the fitness of a susceptible and a resistant allele i at locus j be 1 and $1 - c_{ji}$, respectively, in the absence of insecticide, where c represents any fitness cost of resistance. For each resistance locus *Ace.1* and *Ester*, the fitness of each individual was computed assuming multiplicative effects of alleles. We assumed that for each locus, either each resistance allele had a specific effect on fitness or all resistance alleles had the same effect on fitness. Five parameters were used to describe selection in the first case (c_{aR} and c_{aRS} for the *Ace.1* locus and c_{e1}, c_{e2}, c_{e4} for the *Ester* locus) but only two in the second case (c_a for the *Ace.1* locus and c_e for the *Ester* locus). We neglect interactions between alleles and between loci in the selection coefficients, which could not have been estimated given the

incomplete genotype identification (see table 1). The time unit for the selection coefficients is the period between successive samples. Finally, if we let g''_{ij} equal the frequency of genotype i in deme j after both migration and selection (if this order is assumed), we have

$$g''_{ij} = g'_{ij} w_i / w_{.j} , \quad (5)$$

where w_i is the fitness of genotype i and $w_{.j}$ is the mean fitness in deme j.

Estimation

Let n_{ijk} equal the number of individuals having phenotype i in deme j at time k, and let f_{ijk} equal the expected frequencies of phenotype i in deme j at time k (f_{ijk} is the sum of the g_{ijk}, corresponding to the different genotypes with the same phenotype; see table 1). Transitions in allele frequencies between the successive samples were studied sequentially to estimate the relevant set of parameters: descriptive models were used to describe the initial frequency distribution over space (for, e.g., sample set s); expected frequencies for sample set $s + 1$ were then deduced using the sequence of events occurring during the transition period and the corresponding parameter set $\boldsymbol{p} = \{c_{ar}, c_{aRS}, c_e, c_{e2}, c_{e4}, \sigma, \mu\}$ of selection and migration parameters. The corresponding log-likelihood (L) of the data (the n) for a given set of parameter values ($\boldsymbol{p}$, $\boldsymbol{V}$) was computed as

$$L(n_{ijs}, n_{ij(s+1)};\ \boldsymbol{p}, \boldsymbol{V}_s) = \sum_{k=s}^{s+1} \sum_i \sum_j n_{ijk} \times Ln(f_{ijk}) , \quad (6)$$

where f_{ijs} is computed from the g_{ij}, which depends on the value of the parameters in $\boldsymbol{V}_s$ (note that $\hat{\boldsymbol{V}}_s$ here may not correspond exactly to $\hat{\boldsymbol{V}}_s$ previously estimated using purely descriptive models as they are reestimated as part of the migration-selection analysis), and $f_{ij(s+1)}$ is computed from the g''_{ij} (i.e., after migration and selection). This model was reduced by dropping elements of $\boldsymbol{p}$ and $\boldsymbol{V}_s$ that were not significantly different from 0 and simplified for parameters that were not significantly different between alleles or between loci. (Note that this method does not use the information given by the variation of linkage disequilibriums between the loci, although this could be included given a proper description of their spatial distribution.) Finally, we compared this model to descriptive models in which clines were fitted independently for each period $L(n_{ijs}, n_{ij(s+1)};\ \boldsymbol{V}_s,\ \boldsymbol{V}_{(s+1)})$, or constrained to be identical, $L(n_{ijs}, n_{ij(s+1)};\ \boldsymbol{V}_s = \boldsymbol{V}_{(s+1)})$.

The First Overwintering Populations. We compared the clines of diapausing mosquitoes from October (set 2) and December (set 3). If mosquitoes do not differ in their diapause behavior over the transect, and if there is no selection during this period, we do not expect the frequencies of the resistance alleles to depend on the starting date of diapause. In this case, we expect the two clines to be almost identical. If this is not true, then diapausing might depend on the resistance genotype and/or on environmental conditions.

From Larval Sites to Overwintering Grounds. Larvae present in the breeding sites in late October can be assumed to be the last generation before overwintering. In other words, females emerging at this period seek nectar to store nutrients and then look for overwintering sites for entering diapause. Thus, migration and, eventually, selection as a result of the cost of resistance, but not selection by insecticides, occurred between October (set 1) and December (set 3) when almost all mosquitoes are in diapause.

We computed the maximum likelihood estimates of $\boldsymbol{p}$ (migration and selection parameters), assuming a uniform density across the transect or a step function for density across the transect in October:

$$\sum_i d_{ij} = K \text{ for } j \le x^* \text{ and } \sum_i d_{ij} = 1 \text{ for } j > x^*, \quad (7)$$

where x^* describes the position where density changes from K to 1 (relative density units). We described the g_{ij1} array (October) using phenotypic cline models. We considered the effect of selection as occurring before entering diapause (i.e., before females reached an overwintering site) and assumed the order of processes to be selection migration accordingly. We computed the log-likelihood over this period as $L(n_{ij1}, n_{ij3};\ \boldsymbol{p}, \boldsymbol{V}_1)$.

Overwintering. During the overwintering period, there is no reproduction. Thus, as in the previous case, only migration and differential adult survival were included in the model. We considered the effect of selection occurring during diapause (i.e., after females settled in an overwintering site) and assumed the order of processes to be migration to selection accordingly. We computed the log likelihood over this December–March period as $L(n_{ij3}, n_{ij4};\ \boldsymbol{p}, \boldsymbol{V}_3)$.

Model Comparisons and Tests

Maximum likelihood estimates of parameters were computed conjointly using the Metropolis algorithm adapted from N. H. Barton (see Szymura and Barton 1986 for a detailed description). *G*-tests were computed between related models and scaled to the dispersion of residual deviance (Crawley 1993). Model selection was performed

using an Akaike Information Criterion scaled to the dispersion of residual deviance (QAIC), a heuristic measure that can be compared between unrelated models fitted on the same data (QAIC = scaled deviance + 2 × df; Akaike 1974; Lebreton et al. 1992; Anderson et al. 1994).

Results

Descriptive Models

Clinal patterns were detected for *Ace.1* and *Ester* loci for each sample set (table 2) and were found to differ significantly from one another (*G*-test, $P < .05$ for every pair). They were best fitted by an $\exp(-x^2)$ shape, with the exception of clines in set 1 (samples collected in larval sites in October), which were best described by an $\exp(-x)$ shape. This last case is due to the Pérols population (the one nearest to the sea) where the frequency of resistant individuals was very high (see fig. 2). A similar situation had been observed in previous samples from 1995 (Lenormand et al. 1998*b*), and it is possible that OP residues are present at high concentration at this site. The overall trend from October 1996 to March 1997 is that clines became less and less steep and that resistance allele frequencies decreased at the sites where they had been most common (fig. 3). However, the cline of set 2 (samples collected in caves in October) did not fit this trend. Resistance alleles at the *Ace.1* locus were found to be markedly lower in frequency in this sample set than in sets 1 and 3 (fig. 4; table 2).

From Larval Sites to Overwintering Grounds

We assumed that in early December migration from larval to overwintering sites had ended and that resistance allele frequencies before this migration were well described by the late October set of samples collected from larval sites. Thus, we used the difference between clines of data sets 1 and 3 to compute migration and selection during this period. The best model describing frequency change during this period involves only migration (table 3, model D). The migration distribution mean and variance over this period were both significantly different from 0 (μ = 12.7 km, support limits 9.0–16.7; σ = 14.6 km, support limits 12.5–17.4). A very asymmetrical and extensive migration toward the north is therefore inferred between larval sites and overwintering grounds. Both loci support this model, as the migration distributions fitted independently at each locus did not differ significantly (model G).

Table 2: Descriptive models of resistance allele clines

	Ace.1^R	*P*	*Ace.1*^{RS}	*P*	*Ester*^{4}	*P*	*Ester*^{1}	*P*	*Ester*^{2}	*P*
Set 1 (October, larval sites):[a]										
f(0)	.61	…	.16	$<10^{-4}$	.47	…	.11	…	.01	…
a	.098	$<10^{-4}$	.032	.035	.051	$<10^{-4}$	.080	$<10^{-4}$	.023	.63
b	0	…	0	…	0	…	0	…	0	…
Set 2 (October, caves):[b]										
f(0)	.35	…	.09	.29	…	…	…	…	…	…
a	0	…	0	…	…	…	…	…	…	…
b	.0024	$<10^{-4}$	.011	.61	…	…	…	…	…	…
Set 3 (December, caves):[c]										
f(0)	.38	…	.17	$<10^{-4}$	.42	…	.08	…	.02	…
a	0	…	0	…	0	…	0	…	0	…
b	.0011	.0006	.0046	.14	.0011	$<10^{-4}$	.0019	.008	.004	.056
Set 4 (March, caves):[d]										
f(0)	.31	…	.10	.003	.39	…	.04	…	.01	…
a	0	…	0	…	0	…	0	…	0	…
b	.0023	.0128	0	1	.0009	.001	.0009	.34	.009	0.16

Note: Estimated parameters for each allelic cline, where the gene frequency is a function of distance to the sea (eq. [1]), with f(0) being the maximum frequency and *a* and *b* being rates of decrease of allele frequency with distance and square of the distance from the coast, respectively. *P* indicates whether the corresponding estimates differ significantly from 0 (when tested).

[a] Overall part of the total deviance explained = 89%, N = 1,056.

[b] Overall part of the total deviance explained = 66%, N = 384.

[c] Overall part of the total deviance explained = 48%, N = 464.

[d] Overall part of the total deviance explained = 50%, N = 308.

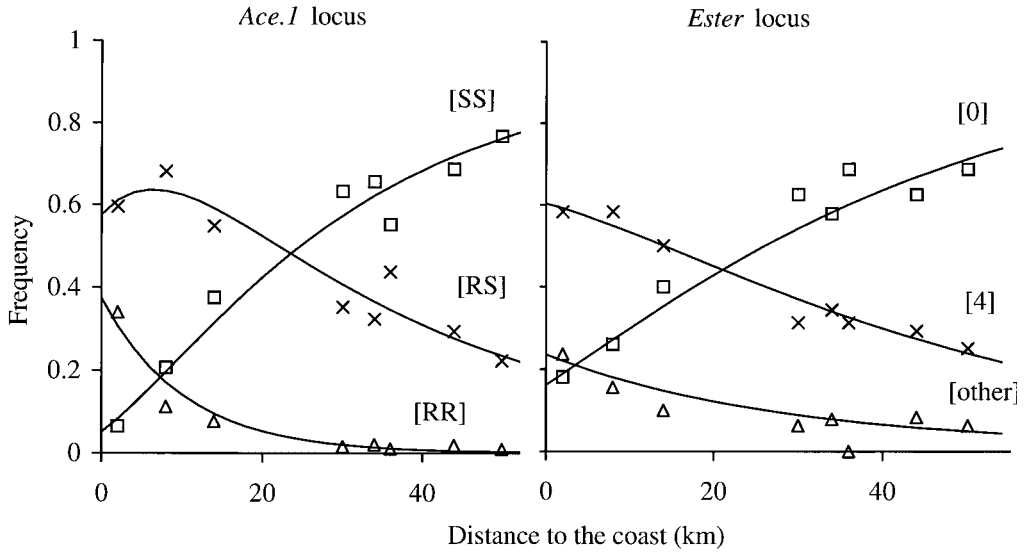

Figure 2: Observed and fitted frequencies of the different phenotypes for both *Ace.1* and *Ester* loci for sample set 1 (mosquitoes collected in October in larval sites) as a function of distance to the coast. At *Ester* locus, phenotypes other than [4] and [0] (i.e., [1], [2], [21], [41], and [42]) were pooled for clarity.

This migration distribution is consistently different from the migration distribution estimated from the breeding season ($\mu = 0$, $\sigma = 6.6$ km generation$^{-1/2}$ support limits 4.8–8.7; see model H). This asymmetrical migration explains why resistance allele frequencies increased at intermediate distances from the coast while decreasing at short distances from the coast (fig. 3). This asymmetrical migration could equally be due to a difference in density along the transect in October. We found that a step function for the density (see eq. [7]) provided an equally likely explanation for the data, where $x^* = 23$ km (i.e., approximately the limit between treated and nontreated zones), $K = 3.33$ (i.e., mosquitoes are three times denser in the treated area), and a symmetrical migration ($\mu = 0$ and $\sigma = 14.3$ km, model J). Selection was not detected during this period, but weak selection cannot be excluded (see models B, C).

During Overwintering

The period from early December to March corresponds to overwintering. Mosquitoes are found in diapause during this period, and some movement has been recorded during winter both within and between caves. We used the difference between clines of sets 3 and 4 to estimate migration and selection occurring during this period. The best model explaining frequency variation during this period involves only differential survival of genotypes (table 4, model P). Movement between overwintering sites was not detected (models K, L, M, N). We found significant fitness costs associated with resistance alleles at both *Ace.1* and *Ester* loci ($c_a = 0.33$ and $c_e = 0.16$, model O). Fitness costs between the different resistance alleles within each locus were not significantly different (model Q). These fitness costs explain the decrease in frequency of resistance alleles throughout the winter (fig. 3). Fitness costs associated with resistance at *Ester* locus were previously estimated during the breeding season to be approximately half the cost associated with resistance at *Ace.1* locus (Lenormand et al. 1998*b*), which corresponds well to our estimates. Model P, which considers a priori that $c_e = 0.54c_a$, is the best model among tested models, according to the QAIC criterion.

The First Overwintering Populations

Since we found that migration only occurred between larval sites in October (set 1) and caves in December (set 3), we expected that frequency clines of the early (set 2, October) and late (set 3, December) overwintering mosquitoes should not be different. By contrast, we found low frequencies of the resistance alleles at *Ace.1* locus throughout the transect in sample set 2 (i.e., lower than in both sample sets 1 and 3; see fig. 4). An explanation for this observation is that susceptible mosquitoes tend to enter the caves before resistant mosquitoes. This can be the consequence of two different causes: either genotype at the *Ace.1* locus influences the date at which mosquitoes enter

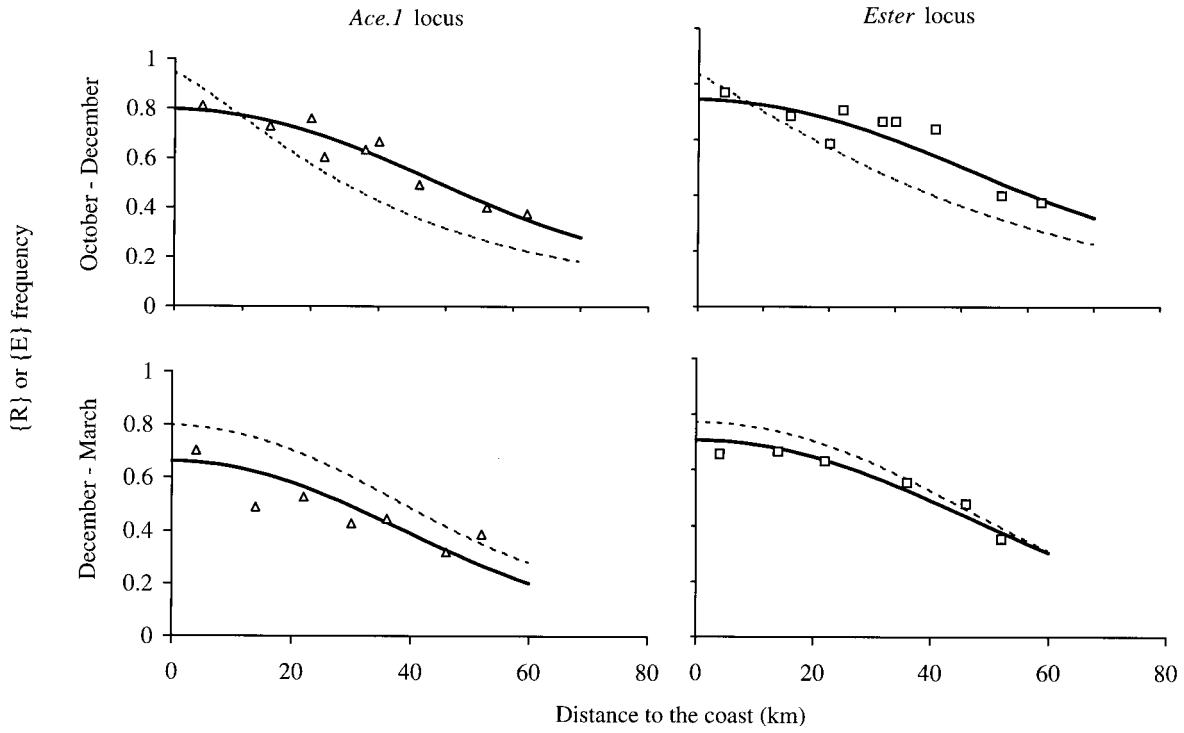

Figure 3: Illustration of migration-selection models. Transition from October to December corresponds to the period during which female mosquitoes seek overwintering sites (model D of table 3 is illustrated). Transition from December to March reflects the overwintering period (model P of table 4 is illustrated). For each transition, the descriptive model of the initial cline is indicated by a dashed line and the resulting cline by a thick line. Observed frequencies corresponding to the resulting cline are also indicated (*triangles* for *Ace.1* locus and *squares* for *Ester* locus). For legibility, only {R} and {E} frequency are represented (see table 1).

caves directly, or the northern populations (containing a high frequency of susceptible mosquitoes) enter the caves before the southern population (containing a high frequency of resistant mosquitoes), owing to different environmental conditions such as temperature gradient. In the first case, resistant mosquitoes might take a longer time to store nutrients or to find a proper overwintering site, and this delay is likely to be associated with some fitness cost. Since fitness costs were not detected between sets 1 and 3, we tentatively concluded that the frequency pattern found in sample set 2 was the signature of a difference between northern and southern populations in the timing of entering caves. There is a gradient of temperature along the transect of about 3°C (computed on average temperature from 1961 to 1995; Richard 1996). Temperature decreases from October to December, and a 3°C difference corresponds approximately to a 3-wk difference between the two ends of the transect. This difference might explain why northern populations enter the caves before the southern ones. According to this timing hypothesis, *Ester* should display the same signature as *Ace.1* locus. Even if we lack the data on *Ester* locus for this set 2 to demonstrate it, field experiments conducted during fall of 1997 have confirmed that susceptible mosquitoes enter the caves first and are quickly followed by more resistant ones (E. Gazave, unpublished data).

Discussion

The sequential analyses of clines in resistance allele frequencies allowed us to decipher the relative contributions of selection and gene flow occurring throughout the overwintering period. We will first discuss the estimates of migration and selection to demonstrate how demographic processes and adaptive microevolution can be studied using selected markers. We will finally discuss the results in the context of local adaptation to pesticides, emphasizing the interest of the method for ecological genetics studies.

Migration

The migration distribution estimated from larval sites to overwintering grounds ($\mu = 12.7$ and $\sigma = 14.6$ km) differs markedly from the migration distribution that was pre-

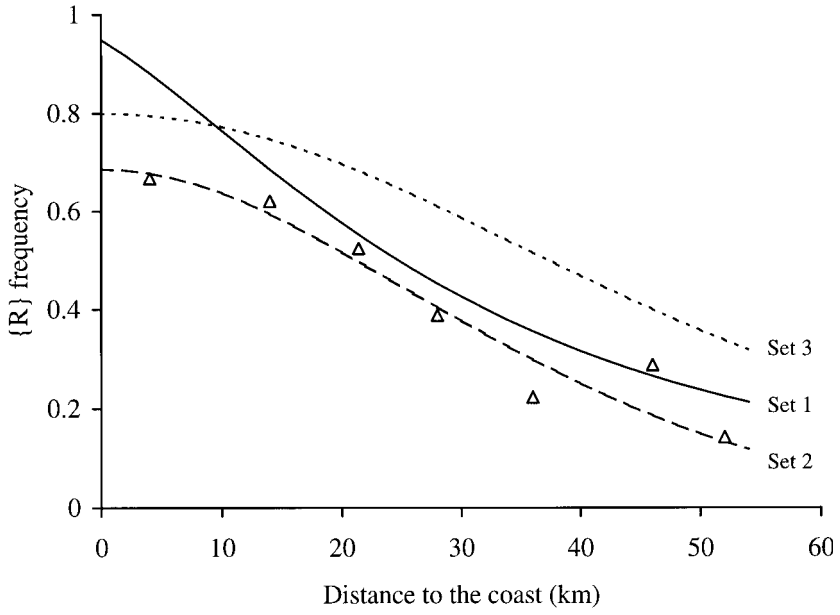

Figure 4: Descriptive models. Resistance allele frequencies in the overwintering populations measured in October (*set 2*) are lower than in the larval population at the same date (*set 1*) and lower than in the overwintering populations measured in December (*set 3*). Only the fitted frequencies of {*R*} individuals (see table 1) for sample *sets 1*, *2*, and *3* are presented for clarity. Observed frequencies of {*R*} individuals for sample *set 2* are represented by triangles.

viously estimated for the breeding season ($\mu = 0$ and $\sigma = 6.6$ km generation$^{-1/2}$). This difference could result from differences in foraging behavior between the two periods: searching for a blood meal and a site to lay eggs during the breeding season (Reisen et al. 1991) and seeking food to store nutrients and an overwintering site afterward. This difference could also result from differences in the spatial distribution of breeding and overwintering sites: overwintering sites such as caves are nearly absent in the first 10 km from the sea, whereas their density is very high in the karstic areas of the northern part of the transect. By contrast, breeding sites (the presence of which depends mainly on human activities) are more evenly distributed along the transect. This asymmetry in the distribution of caves is likely to contribute to the observed asymmetry in migration, although density variation may cause this asymmetry as well (the treated zone is probably a more suitable environment for *Culex pipiens* breeding than is the countryside; it is a warmer, more human-modified area with more larval sites and hosts for blood feeding). Asymmetrical migration is unlikely to be due to the direction of the predominant winds, which are in the opposite direction in the Montpellier area (70% of November winds are sector north, northeast, and northwest on average from 1977 to 1992; P. Cour, personal communication). Movement during the winter (i.e., between overwintering sites) was not detected using our method. Movements that have been inferred from field experiments (Ishii et al. 1964; Service 1968; Hayes 1973; E. Gazave, unpublished data) may therefore correspond to short spatial scale events.

Natural Selection

We documented a lower survival of resistant genotypes between December and March for both *Ace.1* and *Ester* loci. At both loci, the intensity of fitness costs over the overwintering period was much higher than fitness costs per generation during the breeding season (about seven times higher than estimates from Lenormand et al. 1998*b*). However, when scaled to the number of days, both estimates of fitness costs are comparable (i.e., 0.87% and 0.83% d^{-1}, respectively, for *Ace.1* locus, assuming a 2-wk generation time in the breeding season; recall that $c_e = 0.54c_a$ in both seasons).

The fitness cost estimated for (RR) individuals (55% from December 5, 1996, to March 6, 1997) is close to that estimated by Chevillon et al. (1997) in the same area but over a slightly longer period (63% from November 15, 1992, to March 11, 1993). Long-term studies have revealed that the $Ester^{A}$ allele and the $Ace.1^{RS}$ haplotype suffer lower fitness costs than $Ester^{I}$ and $Ace.1^{R}$ alleles, respectively (the differences in fitness costs may not be greater than a few percentage points in each case; Guillemaud et al. 1998; Lenormand et al. 1998*a*). There are undoubtedly some

Table 3: Migration-selection model, transition October–December

Model	$\boldsymbol{p}$					$\boldsymbol{V}_1$	df	%TD	%ICD	Residual deviance	Residual df	Dispersion of residuals	QAIC
	σ	μ	c	K	x^*								
A	+	+	*all*			*all*	17	81	95	125.8	119	1.06	155.0
B	+	+	*loc*			*all*	14	81	92	127.2	122	1.04	150.3
C	+	+	*ap*			*all*	13	80	90	128.6	123	1.05	149.6
D	+	+				*all*	12	80	89	129.3	124	1.04	*148.4*
E	+					*all*	11	75	34	162.8	125	1.30	178.5
F			*loc*			*all*	12	72	0	183.2	124	1.48	200.2
G	*loc*	*loc*				*all*	14	81	92	127.3	122	1.04	150.4
H	*ap*		*ap*			*all*	11	74	14	172.6	125	1.38	188.0
I	+		*loc*			*all*	13	75	34	162.7	123	1.32	182.5
J	+			+	+	*all*	13	80	89	129.0	123	1.05	150.0

Note: Simplified table of analysis of deviance for the transition between sample sets 1 and 3. The different models are depicted by their parameters. Plus sign indicates that the parameter (or parameter set) is constrained to be identical for both loci, whereas "*loc*" or "*all*" indicates that it has been fitted for each locus or each allele, respectively; "*ap*" indicates that an a priori value corresponding to the summer estimates was used for the parameter (σ = 6.6 km generation$^{-1/2}$ or $c_e = 0.54c_a$); and an absence of symbol indicates that the parameter was not included (i.e., set to its null hypothesis). The column $\boldsymbol{V}$ corresponds to the vector of descriptive parameters, whereas $\boldsymbol{p}$ corresponds to the vector of the migration and selection parameters. Among the latter, σ and μ are the standard deviation and mean of the migration distribution, respectively, and c is the fitness costs associated with resistance alleles. K and x^* are descriptions of the density before migration when nonuniform (see text for details). %TD refers to the part of the total deviance explained and %ICD to the part of the intercline deviance explained by the model. "QAIC" is the scaled Akaike Information Criterion, respectively. The lowest QAIC value is indicated in italics.

differences between these resistance alleles since slow allele replacements are currently observed when studying frequency variations at a 10-yr timescale, but we did not detect significant differences between alleles within each locus at the short-term scale of this study.

Adaptation

Our data indicate that the overwintering period starts with an extensive mixing of populations. This high level of gene flow disrupts considerably the migration-selection balance established during the breeding season and flattens the clines for resistance alleles. During overwintering, natural selection acts against the resistance alleles. Their overall frequency decreases, and, at the end of winter, the frequency gradients have been strongly reduced. In 4 mo and a single generation, the local adaptation has been nearly swamped out. A large part of frequency variation is due to environmental (spatial distribution of overwintering sites) and demographic (migration and its timing) effects unrelated to natural selection. As demonstrated in other studies on insects (Roush and Hoy 1981; Daly and Pitt 1990; McKenzie 1990, 1994), the overwintering period appears to select against resistance alleles. However, it is possible in our case that during the period ranging from the end of treatments to overwintering, resistance alleles have

Table 4: Migration-selection model, transition December–March

Model	$\boldsymbol{p}$			$\boldsymbol{V}_3$	df	%TD	%ICD	Residual deviance	Residual df	Dispersion of residuals	QAIC
	σ	μ	c								
K	+	+	*loc*	*all*	14	50	62	114.8	90	1.28	118.0
L	+		*loc*	*all*	13	50	62	114.9	91	1.26	116.0
M	+	+		*all*	12	48	44	118.9	92	1.29	117.2
N	+			*all*	11	48	43	119.2	93	1.28	115.5
O			+	*all*	12	49	60	115.4	92	1.25	114.4
P			*ap*	*all*	11	49	60	115.5	93	1.24	*112.4*
Q			*all*	*all*	15	52	82	110.4	89	1.24	116.5

Note: Simplified table of analysis of deviance for transition between sample sets 3 and 4. The different models are depicted as in table 3.

the indirect benefit of being on average more frequent in the most favorable environments for ecological reasons (see Nagylaki 1978). This hypothesis would be especially relevant if the observed asymmetrical migration were due to a density ratio (K) as high as 3 between treated and nontreated areas.

Fitness costs of resistance alleles have often been inferred in the field from the decline in resistance after the end of treatment, while ignoring how much gene flow existed. This is the first study that deciphers the contribution of both selection and migration to the change in frequency over time and space. We have provided evidence that there are deleterious pleiotropic effects of resistance alleles in the absence of insecticide. Nevertheless, we emphasize that a large part of frequency change is not due to natural selection. Both selection and migration must be analyzed simultaneously.

Selected Markers: A Tool for Population Dynamics Analysis

We used selected markers to analyze the dynamics of populations at a very precise scale for both time (in months) and space (about 50 km). In particular, the strong differentiation between populations created by selection enables detection and quantification of detailed features of the movement of populations such as the mean and variance of dispersal distance or its timing. This indirect method does not require assumptions concerning neutrality of markers (although it ignores selection on tightly linked unknown loci), equilibrium, or external estimates of some parameters (e.g., population size). It can also take into account specific features of the environment (e.g., presence of geographical barriers). Finally, instead of averaging over a long period of time and over large geographical areas, it provides current measures of dispersal and selection that can be compared for different periods and whose variability can therefore be estimated. This method links population genetic models, ecological context, and statistical analysis and is thus particularly well suited for ecological genetic studies.

Conclusion

Cline models have been extensively studied theoretically (see Felsenstein 1976 for review), even for specific situations such as variable or asymmetrical migrations (Jain and Bradshaw 1966; Slatkin 1973; May et al. 1975; Nagylaki 1976, 1978; Fife and Peletier 1981), but have received only limited attention from field biologists. We have illustrated a situation in which gene frequency clines and their variations are close to expectations based on simple migration-selection models. In addition, we demonstrated that this framework could be a very efficient indirect method to quantify migration and natural selection, their combined effect, and their variability through time and space.

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