Interactions Between Pesticide Genes: Model and Experiment

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ABSTRACT

In response to years of intense selection pressure by organophosphate insecticides, several different insecticide resistance mechanisms have evolved in natural populations of the mosquito Culex pipiens. We examined interactions between two of the most important mechanisms using a four-compartment model of insecticide pharmacokinetics. The joint effect of different mechanisms of resistance can be expressed in terms of epistasis at the physiological level in this model. The type of epistasis predicted by the model depends on the particular physiological mechanisms of resistance involved. Resistance due to a reduced penetration of the insecticide combines multiplicatively with other resistance factors, but resistance due to detoxorative processes and to insensitivity of the target site combines additively. How the pattern of epistasis at the physiological level is translated into fitness epistasis in natural populations of this mosquito depends on the intensity and pattern of insecticide selection in the field.

Given that gene expression exerts effects on the biochemical physiology of organisms, it is natural to ask whether an understanding of metabolic mechanisms permits prediction of patterns of gene action and interaction in vivo. As pointed out by Crow (1987), following Sewall Wright's pioneering approaches to this problem, this question was generally neglected. However, it has recently been taken up by workers such as Kacser and Burns (1981).

Much of the recent emphasis seems to be directed to interpreting naturally occurring genetic variation in enzyme loci from the point of view of its physiological effects on overall metabolic efficiency. A recurrent theme in much of this work is that a large genetic change in a protein's enzymatic properties may be buffered by the biochemical network in which it functions, to yield a much smaller genetic effect on overall physiological performance and perhaps even fitness. If this is a generally valid conclusion, it might imply that traditional selectionist arguments for the maintenance of genetic polymorphisms for "housekeeping" enzyme loci are weakened, and the importance of natural selection at the population level may not be clear.

On the other hand, there are cases in which natural selection has led to allelic substitution for genes with markedly different physiological effects (Endler 1986). In many cases it is clear that selection, and not some other agent, was responsible for the rapid evolutionary change, but the question still remains as to exactly how a physiological differential was translated into a fitness differential. The phenomenon of evolved resistance to insecticides offers many interesting examples of such cases, but it has received relatively little attention from population geneticists.

Our aim in this contribution is to provide a general theoretical framework for insecticide pharmacokinetics, and to use it to illustrate various ways in which different genes conferring insecticide resistance can act and interact. We believe that certain features common to evolutionary responses to toxic chemicals can lead to general and useful predictions about the types of epistasis exhibited by combinations of resistance genes.

There is an increasing number of cases where different genes, providing resistance to a same insecticide, are present at the same time in populations of treated insects. Thus several evolutionary responses have been elicited by the same challenges to survival in a toxic environment. For example, the mosquito Culex pipiens pipiens in southern France possesses at least seven different genes conferring resistance to organophosphate (OP) insecticides (Pasteur 1977; Raymond et al. 1986; Magnin 1986), the Australian sheep blowfly Lucilia cuprina possesses two unlinked diazinon-resistance genes (Whitten, Dearn and McKenzie 1980), the housefly Musca domestica possesses several resistance genes for all the major classes of insecticides (references in Scott 1985).

Only a few studies have measured the interaction between these genes in particular cases (Hoyer and Plapp 1966, 1968; Plapp and Hoyer 1968; Sadowski 1970), but, to our knowledge, no general model exists to predict or understand how these different genes may interact when they are in the same genome.
We will provide a reasonable candidate for such a model, and illustrate it with data on the interaction between two different genetic mechanisms of resistance to organophosphorus compounds in the mosquito *C. pipiens*.

THE MODEL

A mathematical model was constructed to represent the dynamics of insecticide concentrations as they change in time. Our purpose in constructing this model is twofold. The first is to represent the major processes the insecticide undergoes at the physiological level. The second is to represent mechanisms of resistance to the insecticide, in terms of changes in specific components of the pharmacokinetics model. Although more detailed models of this process could be derived, we believe this one represents the major physiological components of insecticide resistance adequately enough for useful quantitative and qualitative predictions to emerge.

Although some insecticides exert sublethal effects on organisms (e.g., behavioral effects reviewed by Haynes 1988), we shall concentrate on lethal effects only. From this perspective, the key effect of an insecticide is exerted at a specific and unique "target site," namely a specific molecule or physiological function. For example, the target site of organophosphate and carbamate insecticides is the enzyme acetylcholinesterase. In most cases, the effect of the insecticide on its target will be concentration-dependent, and for this purpose we define $C_T$ to represent the insecticide concentration in the vicinity of the target site. When the insecticide concentration reaches or exceeds a critical value $D$ ($C_T \geq D$), death occurs. Below this value ($C_T < D$), the insecticide is considered to have no effect on survivorship.

The variable $C_T$ is a function of several parameters: time since application of the insecticide ($t$), the environmental insecticide concentration ($C_0$), and different constants describing the distribution and the metabolism of the insecticide within the body (Figure 1). Our approach will be to represent this functional relationship in a simple mathematical form. From the point of view of insecticide pharmacokinetics, four compartments are considered. Compartment I represents the environment external to the organism, and its insecticide concentration is $C_0$. The three other compartments are within the insect body. II is a compartment in which the insecticide has entered from compartment I but cannot express its toxicity because of the local absence of the target; the insecticide concentration in this compartment is $C_I$. From compartment II, the insecticide can enter either compartment III which is the target compartment where the insecticide concentration is $C_T$, or compartment IV where the insecticide is no longer toxic, because it has been either metabolized, stored or excreted.

![Figure 1](https://academic.oup.com/genetics/article/123/3/543/5998722)

FIGURE 1.—Compartment model of insecticide flow in a larva. I, outer compartment, where the insecticide concentration is $C_0$. II, intermediate compartment within the larva, where the insecticide concentration is $C_I$. III, target site compartment, where the insecticide concentration is $C_T$. IV, compartment where the insecticide is no longer toxic. $k_1$, $k_2$ and $k_3$ are constants. See text for further explanation.

Note that the insecticide is also allowed to go from III to IV, meaning that detoxication occurs also in the III compartment. Moreover, the insecticide entering the organism is considered to be a negligible fraction of $C_0$ (i.e., the volume of I is far greater than the volumes of II + III + IV). This last assumption is generally true in a typical mosquito bioassay situation. For example, larval mosquito bioassays are done using 20 or 25 larvae per cup holding 100 or 250 ml of water with an insecticide dose. Under such circumstances the larval volume is less than $\frac{1}{1000}$ of the total assay volume.

In order to translate this general compartmental framework into specific equations, four cases were considered. The main distinction between the cases rests on whether the environmental concentration of insecticide is assumed to be constant or variable in time, and whether or not the detoxication mechanism is operating at its maximal rate. Although many other cases could be considered, the four cases generated by all combinations of these two factors are sufficient for interpretation of our data on *Culex*. For example, constancy of $C_0$ in time (case 1 and 3) is certainly true in typical mosquito bioassay situations where mortality is assessed after 24 hours exposure to an aqueous solution (most insecticides degrade in solution at longer times). But it is certainly not true in field conditions, or in certain other types of bioassay. Also, as we shall see, the nature of the gene interaction depends on whether the detoxication mechanism is saturated or not.

Our basic assumption concerning fluxes will be that they are unidirectional, and governed by first-order rate constants: the insecticide moves from one compartment to another (following the paths of Figure 1) at a rate proportional to its upstream concentration only (case 1 and 2). Apart from active excretion, the only relevant case where this assumption is violated is when the enzyme involved in a particular process is
saturated, operating at a rate near its maximum velocity. In this case, the rate of the reaction becomes a constant, and is no longer dependent on the upstream concentration of insecticide. Enzymes identified as resistance mechanisms are always detoxicative enzymes (review in Oppenoorth 1985), and they are described here with the $k_3$ parameter. The rate of the reactions described by $k_3$ was then set constant to study the effect of the saturation of a detoxication process (Cases 3 and 4).

Insecticide resistance can be represented by a change in any component in the compartmental model. Resistance mechanisms documented in insects include reduced penetration, reduced transport within the body, increased detoxication (internal sequestration of the insecticide away from the target, metabolism or excretion), or a reduced sensitivity of the target site (Oppenoorth 1985). We shall consider all of these in the model.

**Case 1: Constant environmental concentration of insecticide, detoxication mechanism not saturated.**

In this case we assume that $C_0$ is constant in time, and all reactions are first order. The dynamics are summarized by the following rate equations:

$$\frac{dC_t}{dt} = k_1 \cdot C_0 - (k_2 + k_3) \cdot C_t$$

$$\frac{dC_T}{dt} = k_2 \cdot C_t - k_3 \cdot C_T.$$  

(1)

(2)

Taking as initial conditions $C_T = C_T = 0$ when $t = 0$, integration of Equations 1 and 2 gives the following analytical expression of $C_T$ as a function of $C_0$, $k_1$, $k_2$, $k_3$ and $t$:

$$C_T = C_0 \cdot k_1 \cdot k_2 \cdot \frac{e^{-(k_2 + k_3) \cdot t} - e^{-k_3 \cdot t}}{(k_2 + k_3) \cdot k_3 - k_2 \cdot k_3}.$$  

(3)

$C_T$ is therefore a linear function of $C_0$ and $k_1$, and can be expressed as $C_T = C_0 \cdot k_1 \cdot f(k_2, k_3, t)$.

The trajectory of $C_T$ rises asymptotically to the value $V = k_1 \cdot k_2 \cdot C_0 / ((k_2 + k_3) \cdot k_3)$ (Figure 2A). Therefore, if death is to occur, the outer insecticide concentration ($C_0$) needs to be high enough to guarantee that $V > D$. If $V < D$ there is no lethal effect, whatever the length of time considered.

The presence of one or more resistance mechanisms in an insect can be simulated in this model by altering the parameters. A reduction of $k_1$ (or $k_2$) simulates reduced penetration (or transport within the body), an increase in $k_3$ simulates accelerated detoxication, and a higher value of $D$ simulates target insensitivity.

The effects of these simulated resistance mechanisms are assessed by the “resistance ratios” they provide. To motivate our definition of resistance ratio, first consider the trajectory of $C_T$ in a susceptible insect

![Graph A](image1)

**Figure 2—Representation of the variation of $C_T$ with time in a susceptible insect (A), and the effect of a reduced penetration resistance mechanism (B).** See text for further explanation.

(Figure 2A). When exposed to an external insecticide concentration $C_0$, death occurs at time $t_0$, where the trajectory crosses $D$. Now consider the trajectory of $C_T$ in a resistant insect (Figure 2B). No matter what the resistance mechanism, a greater concentration than $C_0$ is required if the trajectory is to cross $D$ at the same time $t_0$. We define this higher concentration as $C_{EF}$, and refer to it as the “concentration of equal effectiveness” for the resistant insect. Then the resistance ratio is defined as $C_{EF}/C_0$. This choice of definition measures the effects of resistance in terms of different effective concentrations at equal times. This is exactly the same situation as the mosquito bioassay described above.

**Effects of individual resistance mechanisms: Alteration of $k_1$:** The rate of penetration is described by $k_1$, and its alteration affects the level of the asymptote value $V$. A decrease of $k_1$ to $k_1'$ lowers the value of $V$ to $V'$ (with $V' > D$). Death would occur after a longer time ($t_r$) in insects resistant by this mechanism than in susceptible insects ($t_s$), if both were exposed to environmental concentration $C_0$ (Figure 2B). For the susceptible insect we have:

$$D = C_0 \cdot k_1 \cdot f(k_2, k_3, t_s).$$

and for the resistant one we have:

$$D = C_0 \cdot k_1' \cdot f(k_2, k_3, t_r).$$
The concentration of equal effectiveness $C_1$ (for the insect resistant by virtue of its lower $k_1'$) obeys the equation:

$$D = C_1 \cdot k_1' \cdot f(k_2, k_3, t).$$

Combining the first and third of these equations, we find that the $f$-term cancels and we are left with $C_1 = C_0 \cdot k_1/k_1'$. The resistance ratio $R_1$ provided by this mechanism is $C_1/C_0$, and thus we have $R_1 = k_1/k_1'$. In this case the resistance ratio is not only independent of $t$, but independent of $k_2$ and $k_3$ as well.

**Alteration of $k_2$:** Phenomena that modify the time required by the insecticide to reach the target site are represented by $k_2$. It will be referred as "transport" within the body, and its variations affect the asymptote $V$ and the shape of the curve. A resistance mechanism is generated when $k_2$ takes a lower value, $k_2' (k_2' < k_2)$. The resistance ratio $R_2$ provided by this mechanism is given by $R_2 = C_2/C_0$, where $C_2$ is the concentration of equal effectiveness for the mechanism of decreased internal transport. Then it follows that:

$$D = C_0 \cdot k_1 \cdot f(k_2, k_3, t)$$
$$= C_0 \cdot k_1 \cdot f(k_2', k_3, t)$$
$$= C_2 \cdot k_1 \cdot f(k_2', k_3, t),$$

and solving for the resistance ratio yields:

$$R_2 = f(k_2, k_3, t)/f(k_2', k_3, t).$$

Note in this case $R$ depends on the value of $t$, as well as the parameter $k_3$.

**Alteration of $k_3$:** The rate of the reaction that results in the detoxication of the insecticide either by storage, excretion or by degradation catalyzed by enzymes is represented by $k_3$. An increase of $k_3$ to $k_3'$ ($k_3' > k_3$) simulates a detoxication resistance mechanism. Again, the asymptote decreases and the shape of the curve is changed. The resistance ratio $R_3$ is equal to $C_3/C_0$, with $C_3$ defined similarly as $C_1$ and $C_2$. With the same calculations as above,

$$R_3 = C_3/C_0 = f(k_2, k_3, t)/f(k_2', k_3', t). \quad (4)$$

**Alteration of $D$:** The value of $D$ defines the sensitivity of the target to the insecticide. A higher value ($D'$) of $D$ simulates a reduced target sensitivity. Death occurs after a longer time or even not at all if $D' \gg V$. The resistance ratio $R_4 = C_4/C_0$ provided by this mechanism is calculated in a similar way to that of the other resistance mechanisms:

$$D = C_0 \cdot k_1 \cdot f(k_2, k_3, t),$$
$$D' = C_0 \cdot k_1 \cdot f(k_2, k_3, t),$$
$$D' = C_4 \cdot k_1 \cdot f(k_2, k_3, t),$$

yielding

$$R_4 = C_4/C_0 = D'/D. \quad (5)$$

**Effects of combinations of resistance mechanisms:** The joint effect of several resistance mechanisms can be simulated by simultaneously changing the corresponding parameters, and the resulting resistance ratio can be compared to the resistance ratios conferred by each mechanism acting alone.

For example, let us consider the combined effect of an increased detoxication process (increasing $k_3$ to $k_3'$) and a target site insensitivity (increasing $D$ to $D'$). The resistance ratios provided by each mechanism when alone are $R_2$ and $R_4$, respectively given by (4) and (5). When both mechanisms are present together, the insecticide concentration required to obtain death at time $t$ is $C_{3&4}$, and the resulting resistance ratio $R_{3&4}$ is $C_{3&4}/C_0$. Therefore:

$$D' = C_{3&4} \cdot k_1 \cdot f(k_2, k_3', t),$$

but since in susceptible insects

$$D = C_0 \cdot k_1 \cdot f(k_2, k_3, t) \quad (5)$$

and Equation 4 is true, then

$$D'/D = C_{3&4} \cdot C_0/(C_0 \cdot C_4) = C_{3&4}/C_3.$$

Then (from Equation 5) $C_{3&4} = C_3 \cdot C_4/C_0$, or

$$R_{3&4} = R_3 \cdot R_4. \quad (6)$$

Thus, in this example the resistance ratio resulting from the joint action of two resistance mechanisms is the product of the resistance ratios provided by each one acting alone. This result is true in the particular case considered above (increased detoxication and target site insensitivity), and it is easy to demonstrate that it holds for most pairs of resistance mechanisms.

The only exception is when both $k_2$ and $k_3$ are altered, i.e., when increased detoxication and a reduced transport within the body occur. We have been unable to find a simple formula for the resulting resistance ratio ($R_{3&4}$) in this case.

If three or four resistance mechanisms are simultaneously present, interaction is still multiplicative in most cases, and the following equations are true:

$$R_{1&2&4} = R_1 \cdot R_2 \cdot R_4$$
$$R_{1&3&4} = R_1 \cdot R_3 \cdot R_4$$
$$R_{1&2&3&4} = R_1 \cdot R_2 \cdot R_3 \cdot R_4.$$

**Case 2: Variable environmental concentration of insecticide, detoxication mechanism not saturated.**

Under field conditions, the external insecticide concentration $C_0$ is not constant with time and in this case we write $C_0(t)$ to make this time dependence explicit. In the majority of cases, the concentration decreases after the initial application due to factors such as photochemical decomposition, microbial degradation, dilution from rain, etc. . . . Let us consider that the
insecticide decreases at a rate proportional to its concentration \( C_0(t) \):

\[
dC_0(t)/dt = -a \cdot C_0(t),
\]

with \( a > 0 \). Then

\[
C_0(t) = K \cdot e^{-a \cdot t},
\]

where \( K \) is the insecticide concentration at time \( t = 0 \). Equation 1 now becomes:

\[
\frac{dC_t}{dt} = k_1 \cdot K \cdot e^{-a \cdot t} - (k_2 + k_3) \cdot C_t
\]

and solving the system (8) and (2) gives:

\[
C_t = K \cdot k_1 \cdot k_2 \left( \frac{e^{-a \cdot t}}{(k_2 + k_3 - a) \cdot (k_3 - a)} + \frac{e^{-(k_2+k_3) \cdot t}}{(k_2 + k_3 - a) \cdot k_2 - (k_2 \cdot (k_3 - a))} \right)
\]

The curve is bell-shaped, and \( C_t \) goes to zero as \( t \) goes to positive infinity. \( C_t \) being linear in \( K \) and \( k_1 \), the Equations 4 and 5 found above are still true in this case, as well as Equation 6.

If \( C_0(t) \) obeys a more complicated function than simple exponential decay, it is possible that \( C_0(t) \) will not be linear with respect to \( K \). In this case the relation (6) will not apply, except when reduced penetration is one of the two resistance mechanisms. This is because the dependence of \( C_t \) on \( k_1 \) will always be linear due to the construction of the model. Particular cases of the presumed function of \( C_0(t) \) would need to be worked out to determine the interactions between other resistance mechanisms.

**Case 3: Constant environmental concentration of insecticide, detoxication mechanism saturated.**

In this case, constant amount of insecticide \( (\alpha) \) is detoxified per unit of time. Equations 1 and 2 are rewritten as:

\[
\frac{dC_t}{dt} = k_1 \cdot C_0 - \alpha \cdot k_2 \cdot C_t
\]

\[
\frac{dC_r}{dt} = k_2 \cdot C_t - \alpha
\]

The system of Equations 9 and 10 gives:

\[
C_t = (k_1 \cdot C_0 - \alpha) \cdot g(k_2, t) - \alpha \cdot t,
\]

where \( g(k_2, t) = t + ((e^{-k_2 \cdot t} - 1) / k_2) \).

If one resistance mechanism is increased detoxication \( (\alpha' > \alpha) \), and the other is target insensitivity \( (D' > D) \), we have:

\[
D = (k_1 \cdot C_0 - \alpha) \cdot g(k_2, t_0) - \alpha \cdot t,
\]

\[
D' = (k_1 \cdot C_0 - \alpha) \cdot g(k_2, t_0) - \alpha' \cdot t,
\]

\[
D' = (k_1 \cdot C_0 - \alpha) \cdot g(k_2, t_0) - \alpha' \cdot t,
\]

\[
D' = (k_1 \cdot C_0 - \alpha') \cdot g(k_2, t_0) - \alpha' \cdot t,
\]

Solving this system gives:

\[
R_3 = C_0 + C_t - \alpha \cdot t,
\]

\[
R_4 = C_0 + C_t - \alpha' \cdot t,
\]

In this case the resistance ratios corresponding to the two separate mechanisms combine additively to produce the resistance ratio given by their joint action. If one resistance mechanism is increased detoxication as above and the other resistance mechanism is reduced penetration \( (R_1) \), it is easy to demonstrate that relaxation (11) is not valid and that relation (6) is true, i.e., \( R_1 \& R_3 = R_1 \cdot R_3 \). Unfortunately, as in the previous two cases, the interaction of \( R_2 \) with the other mechanisms is not of a simple form.

If the three resistance mechanisms, decreased penetration \( (R_1) \), increased detoxication \( (R_3) \) and target insensitivity \( (R_4) \) are present at the same time, and if we suppose that detoxication is saturated, then the resulting resistance ratio \( (R_1 \& R_3 \& R_4) \) is given by:

\[
R_1 \& R_3 \& R_4 = R_1 \cdot (R_3 + R_4 - 1)
\]

**Case 4: Variable environmental concentration of insecticide, detoxication mechanism saturated.**

If we allow \( C_0 \) to vary as in equation (7), under the hypothesis of Case 3, it is easy to demonstrate that

\[
C_t = k_1 \cdot K \cdot h(a, k_2, t) - \alpha \cdot i(k_2, t),
\]

where

\[
h(a, k_2, t) = e^{-k_2 \cdot t} / (k_2 - a) - k_2 \cdot e^{-a \cdot t} / (a \cdot (k_2 - a)) + 1 / a
\]

and

\[
i(k_2, t) = (e^{-k_2 \cdot t} - 1) / k_2 + 2 \cdot t,
\]

and that relations (11) and (12) are still true.

**MATERIALS AND METHODS**

Three strains of mosquitoes were used, all belonging to the \( C. \) \( pipiens \) complex. 1) \( S-Lab \), an OP-susceptible strain of \( C. \) \( p. \) \( quinquefasciatus \), was isolated by GEORGHIOU, METCALF and GLIDDEN (1966). 2) Tem-R, a highly OP-resistant strain of \( C. \) \( p. \) \( quinquefasciatus \), possesses a B1 esterase-mediated detoxication mechanism (GEORGHIOU, PASTEUR and HAWLEY 1980; PASTEUR, ISKJ and GEORGHIOU 1981) plus one or more minor factors not yet characterized (RANASINGHE 1976). 3) MSE, a strain of \( C. \) \( p. \) \( pipiens \) highly resistant to OPs and carbamates, has a type of acetylcholinesterase that is insensitive to inhibition by other insecticides (i.e., target site insensitivity). It is homozygous for the allele \( Ace^B \) at the structural locus for this enzyme (RAYMOND et al. 1985). MSE also possesses minor resistance factors, one of them being involved in oxidative detoxication (RAYMOND et al. 1986; RAYMOND, PASTEUR and GEORGHIOU 1987). Resistance genes in Tem-R and MSE strains are autosomal and approximately codominant.

Three mass crosses between the strains were performed as described in RAYMOND, PASTEUR and GEORGHIOU (1987). Noting the female parent first, they were:

\[
M-3: Tem-R \times S-Lab
\]

\[
M-4: S-Lab \times MSE
\]

\[
M-3\&4: Tem-R \times MSE
\]

Resistance characteristics of parental and offspring strains
were analyzed by performing bioassays on fourth instar larvae (Georgiou, Metcalf and Glidden 1966). Batches of larvae were exposed to an increasing series of insecticide concentrations, and mortality after 24 hr was scored. Two insecticides of technical or analytical grade were applied in ethanol solutions: chlorpyrifos (Dow Chemical, Midland, Michigan) and temephos (American Cyanamid, Princeton, New Jersey). To measure the effects of blocking the esterase-mediated detoxication mechanism on bioassay mortality, the specific esterase inhibitor DEF (S,S,S-trihexyl-phosphorothioate, Intercim) was added, when needed, at 0.08 ppm to the larvae four hours before the insecticide. In each bioassay, the final concentration of ethanol was adjusted to 1%, a level inducing no mortality in the control. Mortality data were analyzed using a log-probit microcomputer program (IBM version of Raymond 1985) based on Finney (1971). By assuming a linear relationship between the logarithm of insecticide dose and the probit of mortality, this analysis yielded estimates of the median lethal concentration (LC50) for each combination of insecticide and strain. Resistance ratios were defined as the LC50 for a resistant strain divided by the LC50 for the susceptible strain.

By comparing strains possessing two different resistance mechanisms with those having one or none, the hypothesis of multiplicative combination of resistance ratios could be tested. A generalized linear logit model (McCullagh 1983) as implemented by the program GLIM (Baker and Nelder 1985) was employed for this purpose. Presence or absence of each of two resistance mechanisms was represented by the level of a qualitative variable (G1 for one mechanism and G2 for the other), and the model was fitted with the log of the dose, the effect of G1 alone and the effect of G2 alone. Then, the effect of the interaction between G1 and G2 was introduced, and a statistically significant nonzero value for this interaction meant that the null hypothesis of multiplicative combination of resistance ratios was rejected.

THE EXPERIMENTS

Mosquito bioassays involve placing larvae into cups containing 100 ml of water with various doses of insecticide, and recording the numbers that survive and die after 24 hr. Under our laboratory conditions the insecticides used in this study are stable in aqueous solution over the entire time, corresponding to Cases 1 and 3 discussed above.

Percent mortality (probit scale) is plotted against the logarithm of dose for each mosquito strain, and from this the median lethal concentration (LC50) is estimated. This is the environmental concentration of insecticide observed to kill half the treated larvae of the strain.

We additionally interpret the LC50 as an estimate of the value of C0 required for C2 to attain the value D at time t = 24 hr. In order to make this interpretation consistent with actual bioassay data, we must assume that there is a certain amount of randomness associated with insecticide response. Of course, if every mosquito obeyed the same physiological model exactly, none would be dead before 24 hours and all would be dead after 24 hr. We therefore assume that there are random differences between individuals, providing some variability of response, but that this variation is nearly symmetrical such that the LC50 provides a nearly unbiased estimate of C0. A rigorous consideration of this assumption will not be attempted in this paper. However, we believe that in the case of the Culex strains discussed below, this variability of response within strains is much smaller than the differences in response among strains, and that using LC50 values to estimate concentrations of equal effectiveness is indeed useful in understanding interactions of resistance mechanisms.

The Tem-R and MSE strains were crossed (Cross M-3&4) in order to have the resistance genes from both in the same F1 individuals. The effect of each resistance gene from each parent was estimated from offspring of crosses M-3 and M-4, in order to compare the gene effect under the same conditions, i.e. heterozygous. Every strain (S-lab, M-3, M-4 and M-3&4) was bioassayed with two organophosphate insecticides (chlorpyrifos and temephos).

The use of a specific esterase inhibitor (DEF) blocks the resistance mediated by the B1 esterase in the M-3 or the M-3&4 strains. Then, it is possible to estimate, by comparison to the S-Lab or M-4 strain respectively (Table 1), the effect of other minor genes in this strain. This gives the following two estimates of the resistance ratio of the minor genes (Rm) in the M-3 strain for each insecticide:

<table>
<thead>
<tr>
<th>Estimtes</th>
<th>Chlorpyrifos + DEF</th>
<th>Temephos + DEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-3/S-Lab</td>
<td>3.29</td>
<td>1.55</td>
</tr>
<tr>
<td>M-3&amp;4/M-4</td>
<td>1.88</td>
<td>1.51</td>
</tr>
</tbody>
</table>

From these results, we may consider that these minor factors act mainly in a multiplicative way, their contribution to resistance being about the same at different doses. Effectively, they provide approximately a 1.53-fold resistance (i.e., the mean of 1.55 and 1.51) at about 0.0025 (S-Lab's LC50) or 0.011 (M-4's LC50) ppm of temephos. However, for chlorpyrifos, the contribution of these genes suggests a departure from strict multiplicity.

The nature of this small resistance is unknown. There is indirect evidence that it might be a reduced penetration mechanism (M. Raymond unpublished data). It is in agreement with its multiplicative contribution to overall resistance. The real contribution of the B1 esterase alone (Rb) is then estimated by dividing the resistance ratio observed in M-3 (Rm,M) by the contribution of the minor multiplicative genes (Rm):

\[ R_b = \frac{R_{M,M}}{R_m} \]

The resistance ratios of the different resistance genes present are then estimated in Table 2. Prediction of Cases 1 (detoxication mechanism not saturated) and 3 (detoxication mechanism saturated) for the resulting resistance can be tested against the observed total resistance (Table 2), and Case 1 is rejected (null hypothesis: \( R_{1\&3\&4} = R_1 \cdot R_3 \cdot R_4 \); GLIM statistics;
TABLE 1

Parameters of the probit lines of each strain or cross, with temephos or chlorpyrifos (OP insecticides), and with or without DEF (a specific blocker of esterases)

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Strains or crosses</th>
<th>LC50</th>
<th>95% Confidence interval</th>
<th>Slope</th>
<th>se</th>
<th>d.f.</th>
<th>$\chi^2$</th>
<th>$H^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos</td>
<td>S-LAB</td>
<td>0.00291</td>
<td>0.00260–0.00325</td>
<td>6.97</td>
<td>0.91</td>
<td>4</td>
<td>14.2**</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>M-3</td>
<td>0.118</td>
<td>0.109–0.126</td>
<td>5.83</td>
<td>0.62</td>
<td>3</td>
<td>5.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-4</td>
<td>0.170</td>
<td>0.162–0.178</td>
<td>6.97</td>
<td>0.49</td>
<td>4</td>
<td>7.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-3&amp;4</td>
<td>0.481</td>
<td>0.464–0.498</td>
<td>8.68</td>
<td>0.56</td>
<td>5</td>
<td>8.8</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos + DEF</td>
<td>S-LAB</td>
<td>0.00042</td>
<td>0.00020–0.00025</td>
<td>7.78</td>
<td>0.69</td>
<td>3</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-3</td>
<td>0.00138</td>
<td>0.00120–0.00159</td>
<td>4.51</td>
<td>0.48</td>
<td>5</td>
<td>12.2**</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>M-4</td>
<td>0.171</td>
<td>0.162–0.185</td>
<td>6.41</td>
<td>0.66</td>
<td>2</td>
<td>5.4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-3&amp;4</td>
<td>0.322</td>
<td>0.308–0.334</td>
<td>7.82</td>
<td>0.69</td>
<td>3</td>
<td>7.2</td>
<td>1</td>
</tr>
<tr>
<td>Temephos</td>
<td>S-LAB</td>
<td>0.00255</td>
<td>0.00245–0.00264</td>
<td>9.72</td>
<td>0.88</td>
<td>2</td>
<td>5.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-3</td>
<td>0.526</td>
<td>0.500–0.546</td>
<td>5.71</td>
<td>0.41</td>
<td>5</td>
<td>10.4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-4</td>
<td>0.0108</td>
<td>0.0103–0.0114</td>
<td>5.92</td>
<td>0.42</td>
<td>6</td>
<td>9.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-3&amp;4</td>
<td>0.0111</td>
<td>0.079–0.746</td>
<td>7.33</td>
<td>0.57</td>
<td>5</td>
<td>5.3</td>
<td>1</td>
</tr>
<tr>
<td>Temephos + DEF</td>
<td>S-LAB</td>
<td>0.000101</td>
<td>0.00096–0.00107</td>
<td>7.24</td>
<td>0.66</td>
<td>4</td>
<td>1.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-3</td>
<td>0.000155</td>
<td>0.00148–0.00162</td>
<td>7.60</td>
<td>0.53</td>
<td>2</td>
<td>4.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-4</td>
<td>0.0106</td>
<td>0.0101–0.0111</td>
<td>6.53</td>
<td>0.59</td>
<td>5</td>
<td>9.4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M-3&amp;4</td>
<td>0.0159</td>
<td>0.0149–0.0169</td>
<td>5.13</td>
<td>0.40</td>
<td>4</td>
<td>3.6</td>
<td>1</td>
</tr>
</tbody>
</table>

Heterogeneity factor (FINNEY 1972).

Significant at the 5% confidence level.

TABLE 2

Estimates of resistance ratio to chlorpyrifos or temephos provided by each resistance genes separately ($R_1$, $R_2$ and $R_4$) or together ($R_{1234}$), and the expected resistance under hypothesis of Case 1 and 3

<table>
<thead>
<tr>
<th>Case</th>
<th>Chlorpyrifos</th>
<th>Temephos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed $R_1$</td>
<td>1.88</td>
<td>1.53</td>
</tr>
<tr>
<td>Observed $R_3$</td>
<td>21.5</td>
<td>135</td>
</tr>
<tr>
<td>Observed $R_4$</td>
<td>58.4</td>
<td>4.24</td>
</tr>
<tr>
<td>Observed $R_{1234}$</td>
<td>165</td>
<td>279</td>
</tr>
<tr>
<td>Expected $R_{1234}$</td>
<td>2365 (0.07***$^a$)</td>
<td>873 (0.32***$^a$)</td>
</tr>
</tbody>
</table>

Case 3: $R_{1} (R_3 + R_4 - 1)$

| Case 3: $R_{1} (R_3 + R_4 - 1)$ | 149 (1.11)$^a$ | 211 (1.31)$^a$ |

$^a$ Ratio of observed resistance ratio/expected resistance ratio. *** Significantly different from 1, at the 0.001 confidence level.

for the interaction term: $\chi^2 = 986$, d.f. = 1, $P < 0.001$ for chlorpyrifos, and $\chi^2 = 342$, d.f. = 1, $P < 0.001$ for temephos). No tests are available to tentatively reject Case 3, but the predictions of this case are very close to the observed values (Table 2). This means that a model with saturated enzyme kinetics fits the data much better than one with unsaturated enzyme kinetics. The slight differences to expected values of Case 3 (Table 2) might be attributed to departure from strict multiplicativity of the effects of the minor genes or differences in genetic background between the strains. Effectively, because the model supposes the same genetic background between the strains, a slight shift of the level of tolerance of the susceptible strain (due to general vigor or other genetic traits indirectly related to resistance) change the value of the $R$ parameters so that Equations 6, 11 and 12 are no longer exactly true. Another possibility is that the time to reach saturation may not be negligible, and therefore, during some period of time, the esterase would detoxify at a rate first linear (no saturation), then intermediate, and finally constant (saturation). In this case, the possibility remains, then, for a slight non additive contribution of $R_3$ in formula (12).

DISCUSSION

Evolution of resistance genes in response to pesticide treatments is one of the best examples of Darwinian selection. Although there are still few cases of species not developing resistance, it is the general case for most species that have been intensively treated (GEORGHIOU 1987). Genes conferring resistance to xenobiotics have been identified in many species, especially insects (OPPENOORTH 1985) and plants (LE BARON and GRESSEL 1982).

Classical evolutionary theories account well for simple situations in which, a single resistance gene being present, the frequency of the resistant allele increases progressively until this allele becomes fixed if selection pressure is sufficiently strong and prolonged. However, when two or more genes conferring resistance to the same insecticide are present in a panmictic population, the result of selection depends on several genetic factors: dominance, linkage disequilibrium and epistasis. Dominance and, if recombination is allowed, linkage disequilibrium, will only alter the speed of the selection process. However, interaction between two or more genes at different loci (epistasis) may change the qualitative result of selection.

Genetic interaction among loci are classically either
additive or multiplicative (Crow and Kimura 1970; Falconer 1981). There is no real biological justification for such a way of combining individual gene effects, because in most of the cases, the identity or even the number of genes involved in a particular trait is unknown. Among the commutative operations, addition and multiplication are certainly the simplest, and this might explain their extensive use in quantitative or population genetics to combine gene effects or fitnesses. The model presented in this paper infers a function of interaction between resistance genes. Furthermore, our results demonstrate that the effect resulting from a combination of resistance genes depends not only on their identity but also on environmental factors. For example, a gene responsible for decreased penetration will contribute multiplicatively with any other resistance gene, and this is in accordance with experimental data (Hoyer and Plapp 1968; Plapp and Hoyer 1968; Sawicki 1970; Oppenorth and Welling 1976). An insensitive target site gene will combine multiplicatively with a detoxication gene during the first moments of intoxication process, and will combine additively after the saturation of the detoxication enzyme. It should be noted that outside the controlled conditions of the laboratory, it is difficult to predict the type of interaction that particular resistance genes will have. The insecticide concentration \( C_0 \) is not applied for only 24 hr as in a bioassay, and may vary through time in a complicated and unpredictable way. The result of the variations of \( C_0 \), time and of other environmental parameters on the interaction between resistance genes still needs to be worked out.

This model does not pretend to represent the complexity of the physiological process of intoxication. It may be possible, for example, to extend it to include the detailed steps of mixed function oxidase detoxication, which involves several cytochrome P-450 isozymes, cytochrome P-450 reductase and possibly cytochrome b5 (each one possibly coded for by at least one gene). Additional arrows or compartment numbers might also be added to Figure 1. The advantage to our model is that it is simple enough to pinpoint which parameters \( k_i, k_0, k_4, a \) and \( D \) need to be estimated to understand resistance gene interactions.

One point of clarification brought about by the model concerns the discrepancy found in literature between the presence of detoxicative enzymes (detected by metabolization studies) and their apparent noncontribution to resistance. As a consequence, the lack of effect of a specific blocker of a detoxicative enzyme does not mean that the resistance mechanism involved is not present (Hodgson 1983). This is actually true when interactions have an additive form. To understand this point, consider two resistance genes: one coding for increased detoxication and the other one for target site insensitivity (providing the resistance ratios \( R_3 \) and \( R_4 \), respectively). The resulting resistance \( R_{34} \), in a standard bioassay, is as described in (11):

\[
R_{34} = R_3 + R_4 - 1.
\]

The contribution of detoxication to the overall resistance, which is estimated as \( R_{34}/R_4 \), is then: \( 1 + (R_3 - 1)/R_4 \). Assuming that increased detoxication provides a low resistance level \( (R_3 = 4 \) for example) and that the insensitive target site confers a high level of protection \( (R_4 = 100) \), then this expression is not different from 1 in classical bioassays (1.05 with the given values of \( R_3 \) and \( R_4 \)). In this case, the increased detoxication mechanism does not contribute significantly to resistance, and the use of a specific blocker of the detoxication mechanism will not significantly decrease the overall resistance. However, it should be noted that even though not detectable, the small advantage of possessing this resistance mechanism (3%) is enough to allow its selection in a sufficiently large treated population, if this increase in resistance ratio corresponds also to an increase in fitness (see below).

The model presented in this paper deals mainly with resistance gene effects, i.e., the resistance level they confer. It also assumes for simplicity that the organism is haploid (the dominance level is not taken into account). If now we consider that there is a random dose of insecticide in the environment, following a uniform distribution, there is a tight correlation between resistance ratios and fitnesses of each resistance gene. In this case, it is possible to link the interaction of the resistance gene effects developed in this model with the interaction of the genes fitnesses. If we take the resistance ratio as a good estimate of fitnesses, then it is possible to calculate \( E_a \) and \( E_m \), the additive and multiplicative epistasis coefficient for the haploid case (Fisher 1918; Felsenstein 1965; Crow and Kimura 1970; Hedrick 1983):

\[
E_a = 1 - R_i - R_j + R_{ikj},
\]

\[
E_m = R_{ikj}/(R_i \cdot R_j),
\]

where \( R_i \) and \( R_j \) are the resistance ratio provided by each resistance gene. Absence of additive epistasis is when \( E_a = 0 \), i.e., when:

\[
R_{ikj} = R_i + R_j - 1,
\]

and absence of multiplicative epistasis is when \( E_m = 1 \), i.e. when:

\[
R_{ikj} = R_i \cdot R_j.
\]

Under the above conditions, additive (multiplicative) composition of fitnesses corresponds to the additive (multiplicative) form of combining the resistance ratio. Fitnesses should then be composed additively or multiplicatively according to the identity of each gene under study, and according to environmen-
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tal conditions to take into account an eventual satu-
ration of detoxicative enzymes.

But if the insecticide concentration does not follow a uniform distribution, the relationship between the resistance ratio and fitnesses will depend on the particular distribution that this concentration follows through time. Each particular case must then be worked out to establish how resistance gene fitnesses will combine.

This work was supported by PIREN/CNRS (ASP "Mécanismes de Résistance aux Insecticides") and INSERM (Contract "Fondation pour la Recherche Médicale" from May 87 to February 88. We are grateful to L. D. BROOKS, D. COUVET, G. P. GEORGHIOU, Y. Michalakis, N. PASTEUR and an anonymous reviewer for their helpful comments and suggestions, and to D. LEBRETON for his help and advice in GLIM statistics.

LITERATURE CITED


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