GENETIC ASSOCIATION BETWEEN HIGHLY ACTIVE ESTERASES AND ORGANOPHOSPHATE RESISTANCE IN CULEX TARSALIS

NILIMA PRABHAKER, GEORGE P. GEORGHIOU AND NICOLE PASTEUR

ABSTRACT. The genetics of two highly active esterases, A3H and B3H, in a methyl parathion-resistant strain (MP-R) of Culex tarsalis was investigated in relation to organophosphate (OP) resistance. The increased activity of esterase A3 and of esterase B3 is under the control of two distinct and closely linked (~4.4 centimorgans) genes. Each gene possesses two forms, one controlling high activity (A3H or B3H, respectively) and one controlling low activity (A3L or B3L, respectively) of the enzymes. Organophosphate resistance in the MP-R strain is strongly associated with the presence of these highly active esterases. The results are discussed in relation to the present knowledge on the mechanisms responsible for high activity of esterases observed in other OP-resistant mosquitoes of the genus Culex.

INTRODUCTION

In Culex tarsalis Coquillett from California, two types of mechanisms of organophosphate (OP) resistance have been identified, i.e., increased detoxification and reduced penetration of the insecticide (Apperson and Georghiou 1975). In a study of esterases in various species and strains of mosquitoes, Georghiou and Pasteur (1978) reported the presence in OP-resistant strains of Cx. tarsalis of two esterases that display a high level of activity towards naphthyl acetate (NA) substrates and their absence in susceptible strains.

Since esterases with such high levels of activity have been shown to be responsible for OP resistance in a number of insect species (Georghiou et al. 1980, Pasteur et al. 1981, Devonshire 1977), the present study was undertaken to further clarify their role in the resistance of Cx. tarsalis.

MATERIALS AND METHODS

Two strains of Cx. tarsalis were used in this study: (a) MP-R, the OP-resistant strain studied by Apperson and Georghiou (1975) which has been maintained since then under constant selection pressure with methyl parathion, and (b) CAR, an OP susceptible strain. A mass cross between CAR female and MP-R male mosquitoes produced F1 hybrids, males of which were then backcrossed to females of the CAR strain.

One hundred mosquitoes of each sex were used in these crosses. Esterases in homogenates of single adult mosquitoes were analyzed by starch gel electrophoresis using the procedure described by Pasteur et al. (1981).

To detect possible associations between OP resistance and the genes coding for esterases, early 4th-instar larvae of the backcross 9CAR x δMP-R were exposed during 24 h to a methyl parathion dose that killed about 50% of the insects. Surviving larvae were reared to the adult stage for analysis of esterases.

RESULTS

Electrophoretic analysis showed that in the MP-R strain all adults tested (more than 500) possess two highly active esterases, A3H and B3H, which hydrolyze preferentially α-naphthyl acetate and β-naphthyl acetate, respectively. In the CAR strain, two esterases with the same electrophoretic mobility as A3H and B3H but with a low level of activity were detected in all the adult mosquitoes; they will be referred as esterases A3L and B3L.

The 180 adults obtained from the SMP-R x δCAR cross (i.e., from a cross between A3H B3H and A3L B3L phenotypes) displayed two esterases with an activity indistinguishable from that of the MP-R adults.

When these hybrids were backcrossed to the CAR parent (A3L B3L), four phenotypes were observed among the progeny: the parental phenotypes A3H B3H and A3L B3L as well as two new phenotypes A3H B3L and A3L B3H (Table 1). This observation indicates that the increased activity of esterase A3 and of esterase B3 is under the control of two distinct genes.

The distribution of the four phenotypes observed in the backcross progeny is significantly different (P < 0.01) from that which would be expected in the case of independent assortment of two genes (Table 1). The proportion of recombinants (A3H B3L and A3L B3H phenotypes) indicates that these two genes are on the same chromosome, approximately 4.4 centimorgans from one another.

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Table 1. Observed and expected segregation of esterase A3 and esterase B3 phenotypes among the F1 (9CAR x dMP-R) and backcross [9CAR x d(9CAR x dMP-R)] offspring of Culex tarsalis.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains</td>
<td>Phenotypes</td>
</tr>
<tr>
<td>CAR x MP-R</td>
<td>A3^L B3^L x A3^H B3^H</td>
</tr>
<tr>
<td>CAR x (CAR x MP-R)</td>
<td>A3^L B3^L x A3^H B3^H</td>
</tr>
<tr>
<td></td>
<td>CAR x MP-R</td>
</tr>
<tr>
<td></td>
<td>CAR x (CAR x MP-R)</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

* Expected values were calculated assuming independent assortment and taking into account the observed proportions of A3^H and B3^H phenotypes.

Table 2. Observed and expected esterase A3 and esterase B3 phenotypes among backcross adults of Culex tarsalis that had survived exposure to methyl parathion as larvae.

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Observed</th>
<th>Expected</th>
</tr>
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<tbody>
<tr>
<td>A3^H B3^H</td>
<td>247</td>
<td>177.59</td>
</tr>
<tr>
<td>A3^H B3^L</td>
<td>34</td>
<td>135.91</td>
</tr>
<tr>
<td>A3^L B3^H</td>
<td>23</td>
<td>9.06</td>
</tr>
<tr>
<td>A3^L B3^H</td>
<td>14</td>
<td>5.44</td>
</tr>
<tr>
<td>Total</td>
<td>328</td>
<td>—</td>
</tr>
</tbody>
</table>

χ² test for independence between esterase phenotypes and survival: 180.3, 3 d.f.
χ² test for excess of A3^H B3^L phenotypes: 65.3, 1 d.f.
χ² test for excess of A3^H B3^H phenotypes: 13.7, 1 d.f.
χ² test for excess of A3^L B3^H phenotypes: 59.2, 1 d.f.

* Expected frequencies were calculated from the data obtained in the untreated backcross (see Table 1).

DISCUSSION

Our investigation has shown that the high activity of esterases A3 and B3 (i.e., A3^H and B3^H phenotypes) observed in strain MP-R of Cx. tarsalis is encoded by two distinct loci that are closely linked (~4.4 centimorgans). In addition, we have shown that methyl parathion resistance does not assort independently of these highly active esterases. These results are best interpreted in the light of our present knowledge of the resistance mechanisms in Cx. tarsalis and of the highly active esterases observed in a number of OP-resistant strains of the genus Culex.

The methyl parathion resistance of the MP-R strain of Cx. tarsalis used in the present study was shown by Apperson and Georgiou (1975) to be due to more than one genetic factor and to involve at least two types of physiological mechanisms: increased detoxification, which is suppressible by the esterase inhibitor DEF* (S,S,S-tributyl phosphorotrithioate) and reduced penetration. This polyfactorial inheritance of methyl parathion resistance was confirmed in subsequent studies. Thus, our backcross offspring consisted of at least four resistant genotypes, resulting from the combination of the gene(s) coding the mechanism suppressed by DEF, and that coding reduced penetration. The methyl parathion dose that killed 50% of this backcross progeny removed all genotypes with no resistance alleles, and may also have affected genotypes with one or more resistance alleles in proportion to the resistance they confer. Therefore, the very large excess of insects with a highly active esterase A3^H, B3^H or both among the survivors, indicated that the increase in activity of these enzymes is either controlled by genes located in the vicinity of a "major" resistance gene or that they, themselves, are conferring resistance. This last conclusion is congruent with the fact that esterases A3^H and B3^H are inhibited in vitro by DEF as well as by methyl parathion and paraoxon.

Esterases A3 and B3 have, respectively, biochemical properties very similar to those of the highly active esterases A3 and B1 that are responsible for resistance to organophosphates in mosquitoes of the Culex pipiens complex (Georgiou and Pasteur 1978, Pasteur et al. 1981). Recent tests with antisera raised against partially purified esterases A1 and B1 have shown that the high activity of these esterases results from a 70- to >500-fold increase in their pro-

duction, respectively, in resistant mosquitoes (Mouchès et al. 1987). Anti-esterase A1 and anti-esterase B1 antisera were also found to recognize immunologically related proteins in *Cx. tarsalis*; these proteins are also produced in large quantities in mosquitoes of a strain possessing the A3Hβ3H phenotype (Mouchès et al. 1987).

Thus, all the available evidence points to a direct role of esterases A3 and B3 in OP-resistance of *Cx. tarsalis*, and it is very likely that these enzymes are overproduced in mosquitoes showing high esterase activity (A3Hβ3H phenotypes). It is now important to determine whether this overproduction of esterase is genetically controlled by amplification of the structural esterase genes A3 and B3, as is the case for esterase B1 of *Cx. quinquefasciatus* Say (Mouchès et al. 1986), or by some other mechanism.

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**REFERENCES CITED**


