Esterases A5-B5 in organophosphate-resistant *Culex pipiens* from Italy

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**Abstract.** *Culex pipiens* mosquitoes from Lignano city, Udine province, northeast Italy, were found to carry over-produced non-specific esterases A1, A2-B2 and A4-B4 or A5-B5, detected by starch gel electrophoresis, giving multiple resistance to organophosphorous insecticides. In order to differentiate between A4-B4 and A5-B5 esterases, the latter known only from Cyprus whereas the former is widespread in Italy and elsewhere, restriction fragment length polymorphism (RFLP) analysis was performed at the esterase B locus. Both B4 and B5 haplotypes were found. This is the first record of A5-B5 esterase-mediated resistance in continental Europe.

**Key words.** *Culex pipiens*, chlorpyrifos, temephos, organophosphate resistance, insecticide resistance, RFLP analysis, esterases, Italy.

Introduction

The evolution of insecticide resistance genes in the mosquitoes of the *Culex pipiens* L. complex has been the subject of many studies by different workers in various geographic areas (see review by Pastoré & Raymond, 1996). World-wide surveys of resistance to organophosphorous (OP) insecticides have disclosed only three loci that have developed major resistance alleles (Villani et al., 1983, 1987; Pastoré et al., 1992; Georgiou, 1992; Pastoré & Raymond, 1996). Two loci, known as *Eve* 2 (or *esterase B*) and *Eve* 3 (or *esterase A*) that code for detrimming carboxylester hydrolyses, have resistant alleles giving esterase over-production (Mouchlis et al., 1987; Fournier et al., 1987; Pastoré et al., 1992). So far, five distinct electromorphs have been described at the *esterase B* locus and three at the *esterase A* locus (Pastoré et al., 1992; Georgiou, 1992; Pastoré & Raymond, 1996). The over-production of esterases is the result of gene amplification (Mouchlis et al., 1986; Pastoré et al., 1992; Vaughan et al., 1995) and/or gene regulation (Rooker et al., 1996). In some situations, both *esterase A* and *B* loci are co-amplified, which explains the tight statistical association (close genetic linkage) of some electromorphs, e.g. A2 and B2 (Rooker et al., 1996). The third locus, Ace, codes the acetylcholinesterase (AChE, wildtype); insensitive *Ace* alleles have been reported in various localities, e.g. Cuba (Bisset et al., 1991), Italy (Bomming et al., 1991; Villani & Henningsvær, 1987) and Provence (Bourgerot et al., 1996), but it is not known if multiple resistant *Ace* alleles occur.

Some over-produced esterase alleles seem to migrate easily through mosquito populations, becoming geographically widespread in both *C. pipiens* and *C. quasiquasifasciatus Say, a super-species pair of allopatric sister species (White, 1979). The best example is probably the associated A2 and B2 (A2-B2) esterases found on four continents, apparently spread intercontinentally via stowaway mosquitoes on aircraft (Curtis & White, 1984) and followed during local invasions (Robert et al., 1993). Other esterases, e.g. A5-B5 previously described only from Cyprus (Pastoré et al., 1992), have a more restricted distribution. In Italy, A1, A2-B2 and A4-B4 esterases have been reported from various field populations (Villani et al., 1982; Bonning et al., 1991; Marchi & Addis, 1990; Severini et al., 1994), although A2-B2 has probably disappeared from the Lucca area (Severini et al., 1993) where it was first described by Villani et al. (1986). This article reports findings in Lignano Sabbiadoro area of northeast Italy, near the Adriatic coast, where *C. pipiens* field populations showed unusually high resistance to the OP compound temephos in 1989-90 (Zambonini & Belloni, 1993). The same location was sampled in 1995 and, from the investigations described below, we attributed organophosphate-resistance to four different factors: A1, A2-B2, A4-B4 and A5-B5 esterases, the last being found for the first time in *C. pipiens* from continental Europe.

**Materials and Methods**

*Culex pipiens* samples were collected during summer 1995 in the sewage station of Lignano city, Udine Province, Friuli-Venezia

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Color pupae larvae from Lignano were found to be highly resistant to chlorpyrifos (0.0164) and temephos (0.6451) (Table 1). The temephos LC50 is not significantly different from the value reported from the same area in 1989–90 by Zambonini & Bellantone (1993), suggesting that the OP resistance observed has stabilized for at least 5–6 years. This high resistance level cannot be readily explained by specific OP resistance genes usually found in European Culex pipiens populations, most of which provide only low resistance to temephos (see Table 3 of Severini et al., 1993).

**Results and Discussion**

Color pupae larvae from Lignano were found to be resistant to chlorpyrifos (0.0164) and temephos (0.6451) (Table 1). The temephos LC50 is not significantly different from the value reported from the same area in 1989–90 by Zambonini & Bellantone (1993), suggesting that the OP resistance observed has stabilized for at least 5–6 years. This high resistance level cannot be readily explained by specific OP resistance genes usually found in European Culex pipiens populations, most of which provide only low resistance to temephos (see Table 3 of Severini et al., 1993).

### Table 1. Larval mortality rates and resistance ratios (RR) of Culex pipiens from Lignano with 24-hour exposure to chlorpyrifos or temephos compared to the susceptible reference strain, ISS.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Sample</th>
<th>LD50 (mg/l)</th>
<th>Slope</th>
<th>χ²</th>
<th>DF</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temephos</td>
<td>ISS</td>
<td>0.15</td>
<td>1.001</td>
<td>2</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Lignano Subbadio</td>
<td>0.15</td>
<td>1.001</td>
<td>2</td>
<td>203</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlordane</td>
<td>ISS</td>
<td>0.43</td>
<td>0.636</td>
<td>1</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Lignano Subbadio</td>
<td>0.43</td>
<td>0.636</td>
<td>1</td>
<td>67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Oviposition rates in Culex pipiens from Lignano, detected by RFLP of first cell discrimination. α = Sample size.

<table>
<thead>
<tr>
<th>Electrophorosis</th>
<th>RFLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>α A-B 4 A-B 3</td>
<td>54</td>
</tr>
<tr>
<td>α A-B 4 A-B 3</td>
<td>57</td>
</tr>
</tbody>
</table>

mosquitoes analysed among our Lignano samples (Table 2). However, A4-B4 cannot be distinguished from A5-B5 electrophoretically (Point et al., 1992). Two mosquitoes displayed an electrophoretic pattern consistent with the presence of both A2-B2 and A4-B4 or A5-B5 (Fig. 2a), and one specimen had an highly active esterase C (Fig. 2b), probably similar to the esterase C1 reported from Corsica in 1988 (Raymond & Marquise, 1994). A1 esterase was found only in nine specimens, indicating a minor role of this esterase in the resistance. The distinction between A4-B4 and A5-B5 is important because they provide very different levels of OP resistance (Point et al., 1992). Only RFLP can unambiguously identify the A or B haplotype of these esterases (Point et al., 1992; Rooker et al., 1996). Using EcoRI, BamHI or PstI restriction enzymes for RFLP analysis (Fig. 3), the amplified haplotype of A5-B5 was identified in 51% (36/70) and A4-B4 in 7% (4/57) of mosquitoes from Lignano. Two mosquitoes showed the A2-B2 amplified haplotype.

The arrival of A5-B5 in Italian Cx. pipiens is probably recent, since A5-B5 was not detected in 1993 at Padova, which is only 120 km from Lignano, nor in Cx. pipiens from other parts of Italy (Severini et al., 1994). The presence of A5-B5 esterases in about half the Cx. pipiens larvae probably explains their high degree of OP resistance in the Lignano area. By analogy with the low level of OP resistance caused by A2-B2 esterases at Lucena (Villani et al., 1987), the presence of A5-B5 in Lignano Cx. pipiens apparently provides less OP resistance than A5-B5. This first record of A5-B5 amplified haplotype in Cx. pipiens from outside Cyprus, c. 2000 km to the southeast, indicates that
these estuaries may be migrating toward the Mediterranean. Alternatively, A5-B5 could have arisen de novo in Ca piperis by homoplastic but independent local mutation at Liguino—but, as this has not occurred elsewhere, it seems more parsimonious to assume introgression from Cyprus. If so, this resembles the geographically widespread other amplified estuaries, such as A2-B2 which are now distributed almost worldwide (Raymond et al., 1991; Rotz et al., 1993), or B1 in America and Asia (qiao & Raymond, 1995). It is not known why these over-produced estuaries migrate so extensively whereas others (A1, A4-B4 or A5-B5) seem to have a more restricted distribution in the Mediterranean area. The limited occurrence of A5-B5 in Italy currently provides a good opportunity to document and study the processes involved in such genetic invasions, and to better understand the migration and interaction of resistant alleles in Culex piperis mosquito populations.

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References