Characterization and comparison of general esterases from two field populations of the grasshopper Oxya chinensis (Thunberg) (Orthoptera: Acridoidea)

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Abstract: Malathion susceptibility in the two populations of the grasshopper Oxya chinensis, collected from Linyi of Shanxi Province and Xuzhou of Jiangsu Province, China, was determined. General esterases from the two populations were characterized and compared. LD₅₀ of the Xuzhou population (13.00 μg/g body weight) was 2.80-fold higher than that of the Linyi population (4.64 μg/g body weight). Inhibition studies of general esterases using four inhibitors, including paraoxon, malaoxon, eserine, and carbaryl, indicated that most general esterases in the two populations were B-type. Kinetic studies showed that the Michaelis-Menten constant (Kₘ) and the maximal velocity (Vₘₙₐₓ) of general esterases from the Xuzhou population were higher than that from the Linyi population, using α-naphthyl acetate (α-NA), α-naphthyl butyrate (α-NB), β-naphthyl acetate (β-NA) as substrates. The esterase activities in females of the Xuzhou population were 2.02, 1.58, and 1.28-fold higher than those in the Linyi population, using α-NA, α-NB and β-NA as substrates, respectively, and in males they were 2.71, 1.67, and 1.33-fold higher in the Xuzhou population than in the Linyi population. The spectrum of esterase activities showed that O. chinensis individuals with high esterase activities were more in the Xuzhou population than those in the Linyi population using the three selected substrates. We speculated that esterases in the Xuzhou population may be biochemically different from those in the Linyi population and it might be attributed to the different geographic distributions, ecological environment and nutrition resources in the two localities. In addition, the biochemical differences might also be due to the difference in insecticides selective pressure on the two populations of O. chinensis.

Key words: Oxya chinensis; general esterases; malathion susceptibility; enzyme kinetics; esterase inhibition

1 INTRODUCTION

The grasshopper Oxya chinensis (Thunberg) (Orthoptera: Acridoidea) is distributed over a broad range of latitude from Far East coastal region of Russia, Korea, China to Japan and Vietnam. The insect is a prominent agricultural pest in China and represents a most widespread pest, which is commonly and abundantly found in rice paddies, sugar cane, maize, gramineous plants and other crop fields (Zheng, 1993) and brings about great losses for agricultural production.

Due to different ecological environment and geographic distribution, Oxya chinensis in different regions have some remarkable variations in growth, development and propagation (Ji, 1999), which may accompany by different genetic structures. Furthermore, a moderate geographical barrier might significantly restrict the gene exchange among populations, which may result in the accumulation of local genetic diversity within a population and the development of genetic differentiation among various populations (Li et al., 2004).

Synthetic insecticides, especially organophosphate (OP) insecticides, are often used in management programs to control O. chinensis in China owning to their properties of ready degradation and low residues (Shen et al., 1988; Sun and Peng, 1991). Recently, it has been noticed by pesticide applicators that control of O. chinensis populations in some localities in China has become increasingly difficult with the organophosphates. Numerous studies have demonstrated that esterases play important roles in conferring or contributing to insecticides resistance in insect and other arthropod species (Qiao et al., 2003; Li et al., 2003). Esterases cause insecticide resistance primarily via sequestration of insecticides by large amounts of esterases present in resistant insects (Devonshire and Moores, 1982; Hemingway and Karunatne, 1998).

Understanding population’s genetic background can shed light on effective control of O. chinensis. The
2.2 Chemicals

whereas the habitat of the Linyi population (LY) consists of a reservoir-shore field, whereas the habitat of the Linyi population (LY) consists of flood river desert sand. All collected specimens were stored at —20°C for short time stock.

2.3 Insecticide bioassay

In 2003. The habitat of the Xuzhou population (XZ) consists of a reservoir-shore field, whereas the habitat of the Linyi population (LY) consists of flood river desert sand. All collected specimens were stored at —20°C for short time stock.

2 MATERIALS AND METHODS

2.1 Insects

The fifth-instar nymphs of O. chinensis were collected from Xuzhou of Jiangsu, an coastal province in East China, and Linyi of Shanxi, an inland province in North China; in 2003. The habitat of the Xuzhou population (XZ) consists of a reservoir-shore field, whereas the habitat of the Linyi population (LY) consists of flood river desert sand. All collected specimens were stored at —20°C for short time stock.

2.2 Chemicals

Bicinchoninic acid solution (BCA), eserine (hemisulfate salt), fast blue B salt (O-dianisidine, tetrazotized), α-naphthol, β-naphthol, α-naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), α-naphthyl butyrate (α-NB) were purchased from Sigma Chemical Co. (St. Louis, MO). Malathion (99.5% pure), paraoxon (90% pure), malaoxon and carbaryl (99% pure) were purchased from Chem Service (West Chester, PA). Bovine serum albumin (BSA) was purchased from Sangon.

2.4 Assay of general esterase activity

The fifth-instar nymphs of O. chinensis were collected from Xuzhou of Jiangsu, an coastal province in East China, and Linyi of Shanxi, an inland province in North China; in 2003. The habitat of the Xuzhou population (XZ) consists of a reservoir-shore field, whereas the habitat of the Linyi population (LY) consists of flood river desert sand. All collected specimens were stored at —20°C for short time stock.

2.5 Kinetic analysis of general esterases

Kinetic parameters of general esterases were determined using three selected substrates, i.e. α-NA, α-NB and β-NA, as previously described (Zhu and He, 2000). 15 μL of appropriately diluted enzyme preparation, as previously described in the assay of general esterase activity, was used in each assay. Final concentrations for all three substrates were 6.25, 12.5, 25, 50, 100, 200 and 400 mmol/L. The Michaelis constant (K_m) and the maximal velocity (V_max) were estimated by Hanes transformations (Bell and Bell, 1988).

2.6 In vitro inhibition of general esterases

Inhibition of general esterases by paraoxon, malaoxon, carbaryl and eserine was studied in the female and male from the two populations. The inhibition reaction was started by incubating 10 μL of the enzyme preparation with 10 μL of each inhibitor at approximately 24°C for 5 min. The remaining esterase activity was determined immediately using α-NA as a substrate as previously described (Zhu and He, 2000).

2.7 Protein assay

Protein contents of enzyme preparations were determined according to Smith et al. (1985), using BSA as a standard. Measurements were performed with the microplate reader at 560 nm (Zhu and Clark, 1994).

3 RESULTS

3.1 Comparison of malathion susceptibility

Comparison of malathion susceptibility of O. chinensis in two populations was presented in Table 1. Although the ratio calculated with dividing the LD_50 of the Xuzhou population by that of the Linyi population was only 2.8, there was significantly difference in LD_50 between the Xuzhou and Linyi population. The 95% confidence limits (CL) of LD_50 were not overlapping between the two populations. The Xuzhou population was 2.8-fold less susceptible to malathion than the Linyi population.

3.2 Assay of general esterases

There were significant differences in esterase specific activities between the Xuzhou and Linyi population (Table 2). General esterase specific activities in females of the Xuzhou population were 2.02, 1.58 and 1.28-fold, and in males were 2.71, 1.67 and 1.33-fold higher than those in the females and males of the Linyi population, when α-NA, α-NB or β-NA was used as a substrate, respectively.
Table 1 Comparison of malathion susceptibility of the fifth-instar nymphs of Oxya chinensis collected from Linyi and Xuzhou populations

<table>
<thead>
<tr>
<th>Population</th>
<th>N*</th>
<th>Slope ± SE</th>
<th>χ²</th>
<th>P**</th>
<th>LD₅₀ (µg/g body weight) (95% CL)</th>
<th>LD₅₀ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xuzhou</td>
<td>390</td>
<td>1.57 ± 0.07</td>
<td>4.14</td>
<td>0.99</td>
<td>13.00 (10.11 - 16.41)</td>
<td>2.8</td>
</tr>
<tr>
<td>Linyi</td>
<td>487</td>
<td>2.43 ± 0.06</td>
<td>8.09</td>
<td>0.95</td>
<td>4.64 (4.05 - 5.41)</td>
<td>-</td>
</tr>
</tbody>
</table>

* Number of the O. chinensis nymphs tested in each bioassay. ** P ≥ 0.05 indicates a significant fit between the observed and expected regression lines in a probit analysis.

Table 2 Comparisons of general esterase activities [µmol/(min*mg)] using α-NA, α-NB and β-NA as substrates in Linyi and Xuzhou populations of Oxya chinensis

<table>
<thead>
<tr>
<th>Sex</th>
<th>LY</th>
<th>α-NA</th>
<th>XZ</th>
<th>α-NB</th>
<th>XZ</th>
<th>β-NA</th>
<th>XZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>♀</td>
<td>= 0.151 ± 0.036 a</td>
<td>0.304 ± 0.074 a*</td>
<td>0.174 ± 0.055 a</td>
<td>0.277 ± 0.060 a*</td>
<td>0.204 ± 0.044 a</td>
<td>0.262 ± 0.065 a*</td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>0.119 ± 0.031 b</td>
<td>0.321 ± 0.065 a*</td>
<td>0.170 ± 0.055 a</td>
<td>0.284 ± 0.062 a*</td>
<td>0.191 ± 0.041 a</td>
<td>0.254 ± 0.051 a*</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as the mean ± SD (n = 32). Means within columns followed by the same letter are not significantly different (P > 0.05) using Student’s t-test. * Means within rows are significantly different (P < 0.05) using Student’s t-test.

3.3 Esterase activity spectrum

Fig. 1 showed the different esterase activities spectrum between the two populations using α-NA, α-NB and β-NA as substrates. There were more individuals containing high esterase specific activity in the Xuzhou population than those in the Linyi population for both females and males using the three selected substrates.

3.4 General esterase kinetics

Fig. 2 showed the effects of the concentrations of the three substrates, α-NA, α-NB and β-NA on the activities and Hanes plots (inserted) for kinetics of esterases of Xuzhou and Linyi populations. Enzyme kinetics studies indicated that esterases from the two populations have significant differences in $K_m$ value and $V_{max}$ value (Table 3 and 4). $K_m$ value of general esterases hydrolyzing α-NA, α-NB, β-NA in the females of the Xuzhou population were 1.2, 1.5, 1.3-fold, and in males were 1.1, 1.4, 0.8-fold, respectively, higher than those in the Linyi population. $V_{max}$ values of general esterases in the females of the Xuzhou population were 2.0, 1.6, 1.6-fold, and in male were 1.9, 1.7, 1.2-fold, respectively, higher than those in the females and males of the Linyi population. Among three substrates tested, α-NA appeared to be the most favorable substrate for general esterases of O. chinensis, having the lowest $K_m$ values in both populations. In contrast, α-NB is not a preferred substrate for esterases, having the highest $K_m$ values in both populations.

3.5 In vitro inhibition of general esterases

Two organophosphates (paraoxon and malaoxon) and two carbamates (eserine and carbaryl) were used for in vitro inhibition of general esterases (Fig. 3). Pherocon was the most potent inhibitor of the esterases. Pherocon at 10⁻⁵ mol/L inhibited 91.1% and 93.9% of the esterase activities in females and 93.1% and 92.6% in males within the Xuzhou and Linyi population, respectively. For malaoxon, only 64.6% and 50.5% of general esterase activities in females and 53.5% and 57.8% in males within the Xuzhou and Linyi population, respectively, were inhibited at the same concentration. Carbaryl at the same concentration
inhibited 33.7% and 28.8% of general esterase activities in females and 34.7% and 28.6% in males for the Xuzhou and Linyi populations. Eserine was the least potent inhibitor of the esterases. Eserine at $10^{-5}$ mol/L inhibited nothing of general esterase activities in the Linyi population and in females for the Xuzhou population and only 3.8% of general esterase were inhibited in males for the Xuzhou population (Fig. 3). There were significant differences in the $p_L$ of the medium inhibition values for malaoxon, carbaryl and eserine in females, paraoxon, carbaryl and eserine in males between the two populations (Table 5) by Student's $t$-test.

![Graphs showing effect of substrate concentration on the hydrolysis of α-NA, α-NB, and β-NA by esterases from Xuzhou (XZ) and Linyi (LY) populations.](image)

**Fig. 2** Effect of substrate concentration on the hydrolysis of α-NA, α-NB, and β-NA by esterases from Xuzhou (XZ) and Linyi (LY) populations. Each point represents the mean of four determinations ($n = 4$). Vertical bars indicate SD of the mean. The secondary plots (inserted) are Hanes plots of $[S]/v$ for esterase hydrolyzing α-NA, α-NB or β-NA.

**Table 3** $K_m$ values (μmol/L) using α-NA, α-NB, β-NA as substrates in the Linyi and Xuzhou populations of *Oxya chinensis*

<table>
<thead>
<tr>
<th>Sex</th>
<th>α-NA</th>
<th></th>
<th>α-NB</th>
<th></th>
<th>β-NA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LY</td>
<td>XZ</td>
<td>LY</td>
<td>XZ</td>
<td>LY</td>
<td>XZ</td>
</tr>
<tr>
<td>♀</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>81.7 ± 17.3 a</td>
<td>101.8 ± 27.1 a</td>
<td>167.1± 8.1 a</td>
<td>249.6 ± 33.1 a a</td>
<td>93.1 ± 9.8 a</td>
<td>120.0 ± 15.3 a a</td>
</tr>
<tr>
<td>♂</td>
<td>73.5 ± 6.4 a</td>
<td>84.0 ± 5.6 a *</td>
<td>131.8 ± 2.6 b</td>
<td>182.5 ± 14.2 b *</td>
<td>95.4 ± 4.9 a</td>
<td>75.6 ± 7.2 b *</td>
</tr>
</tbody>
</table>

Results are presented as the mean ± SD ($n = 4$). Means within columns followed by the same letter are not significantly different ($P > 0.05$) using Student's $t$-test. * Means within rows are significantly different ($P < 0.05$) using Student's $t$-test. The same for Table 4 and 5.
Table 4  \( V_{\text{max}} \) values [\( \mu \text{mol/(min*mg)} \)] using \( \alpha\)-NA, \( \alpha\)-NB, \( \beta\)-NA as substrates in the Linyi and Xuzhou populations of \textit{Oxya chinensis}

<table>
<thead>
<tr>
<th>Sex</th>
<th>( \alpha)-NA</th>
<th>( \alpha)-NB</th>
<th>( \beta)-NA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LY</td>
<td>XZ</td>
<td>LY</td>
</tr>
<tr>
<td>♀</td>
<td>0.19 ± 0.05 a</td>
<td>0.37 ± 0.08 a*</td>
<td>0.20 ± 0.02 a</td>
</tr>
<tr>
<td>♂</td>
<td>0.18 ± 0.01 a</td>
<td>0.33 ± 0.03 a*</td>
<td>0.18 ± 0.02 a</td>
</tr>
</tbody>
</table>

**4 DISCUSSION**

General esterases are commonly classified into three types based on their interactions with organophosphates (Aldridge, 1953). A-type esterases are not inhibited by organophosphates but degrade organophosphates as their substrates, whereas B-type esterases are readily inhibited by organophosphates. In contrast, C-type esterases do not interact with organophosphates. Based on this classification, most of general esterases from both the Xuzhou and Linyi populations of \textit{O. chinensis} were B-type because they were very sensitive to inhibition by organophosphate compounds, especially paraoxon. Based on that \( 1 \times 10^{-5} \) mol/L paraoxon almost completely inhibited the esterase activities (Fig. 3), but did not
cause inhibition to arylesterases (A-type) (de Malkenson et al., 1984), we estimated that about 91.1% and 93.9% of general esterases in the females and 93.1% and 92.6% in the males for the Xuzhou and Linyi populations, respectively, were B-type esterases. Because B-type esterases can be further classified into carboxylesterases and cholinesterases based on their different responses to inhibition by eserine, our studies also suggested that carboxylesterases were predominant in the composition of general esterases in the two populations of *O. chinensis*. It had been reported that 1 × 10⁻⁷ mol/L eserine blocked cholinesterase activity completely in *Myzus persicae* (Sudderuddin, 1973) and *Musca domestica* (van Asperen, 1962), and it also partially inhibited carboxylesterase activity at higher concentrations (Sudderuddin, 1973). Based upon the criteria that cholinesterases can be completely inhibited by 10⁻⁷ mol/L eserine; it is suggested that almost all of B-type esterases were carboxylesterases for the Xuzhou and Linyi populations (Fig. 3).

Table 5  p<sub>50</sub> values for paraoxon, malaoxon, carbaryl and eserine in *in vitro* inhibition to esterases of Linyi (LY) and Xuzhou (XZ) populations of *Oxya chinensis*

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Population</th>
<th>p&lt;sub&gt;50&lt;/sub&gt; Female</th>
<th>p&lt;sub&gt;50&lt;/sub&gt; Male</th>
<th>Regression coefficient, r Female</th>
<th>r significance, P Female</th>
<th>Regression coefficient, r Male</th>
<th>r significance, P Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraoxon</td>
<td>LY</td>
<td>10.34 ± 0.98 a</td>
<td>10.12 ± 0.90 a</td>
<td>&lt; -0.97</td>
<td>&lt; 0.0001</td>
<td>&lt; -0.99</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>9.39 ± 0.38 a</td>
<td>7.75 ± 0.17 b</td>
<td>&lt; -0.98</td>
<td>&lt; 0.0001</td>
<td>&lt; -0.98</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Malaoxon</td>
<td>LY</td>
<td>4.47 ± 0.05 b</td>
<td>4.94 ± 0.32 a</td>
<td>&lt; -0.97</td>
<td>&lt; 0.001</td>
<td>&lt; -0.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>6.20 ± 0.30 a</td>
<td>5.20 ± 0.36 a</td>
<td>&lt; -0.97</td>
<td>&lt; 0.0001</td>
<td>&lt; -0.98</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>LY</td>
<td>3.73 ± 0.08 b</td>
<td>3.72 ± 0.07 b</td>
<td>&lt; -0.99</td>
<td>&lt; 0.001</td>
<td>&lt; -0.98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>3.97 ± 0.09 a</td>
<td>4.02 ± 0.20 a</td>
<td>&lt; -0.98</td>
<td>&lt; 0.001</td>
<td>&lt; -0.98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Eserine</td>
<td>LY</td>
<td>1.49 ± 0.11 b</td>
<td>1.49 ± 0.12 b</td>
<td>&lt; -0.98</td>
<td>&lt; 0.01</td>
<td>&lt; -0.98</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>XZ</td>
<td>1.75 ± 0.09 a</td>
<td>2.03 ± 0.18 a</td>
<td>&lt; -0.98</td>
<td>&lt; 0.01</td>
<td>&lt; -0.96</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Xuzhou and Linyi populations belong to the different distribution areas of *O. chinensis*. The Xuzhou population is distributed in the reservoir-shore area which consist of a maize field and a weed field, where *O. chinensis* is feeding mainly on maize and gramineous plants and insecticides, mainly organophosphates, were applied in recent years for protecting crop. However, the Linyi population belongs to a river flood area, which is a desolate sand; some weed including primarily gramineous plants are abundant food supply for *O. chinensis*, and insecticides were seldom used for *O. chinensis* control. Furthermore, the higher temperature and humidity in Xuzhou are more favorable to the survival of *O. chinensis* than in Linyi. The distance between the two localities is about 1 200 km. In addition, *O. chinensis* has low migratory capabilities and they often fly in a small scale, which make genetic differentiation among various populations easier to occur. However, in morphology there are no significant differences between the two populations.

Our bioassay results revealed only 2.8-fold decreased susceptibility to malathion in the Xuzhou population as compared with the Linyi population. The marginally decreased malathion susceptibility in the Xuzhou population is likely due to the different ecological breeding habitat, climate and nutrition resources. The significantly different *K<sub>m</sub>* values on esterase activity from the two populations suggested that their catalytic abilities toward the same substrate were different and that the different esterase activities in *O. chinensis* based on comparisons of general esterases activity and spectrum in two populations may be caused by the differentiation of the two populations. Higher *V<sub>max</sub>* values in the Xuzhou population also showed its esterase activity was different from the Linyi population. Consequently, we speculated that esterases in the Xuzhou population may be biochemically different from those in the Linyi population and it might be attributed to the different geographic distributions, ecological environment and nutrition resources in the two localities. In addition, that the biochemical differences might also be due to the different selective pressures of insecticides on Xuzhou and Linyi populations. Therefore, improving ecological environment plays an important role in effective control of *O. chinensis*.

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References


Devonshire AL; Moore GDA; 1982. carboxylesterase with broad substrate


