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PRELIMINARY CHARACTERIZATION OF THE LARVAL ESTERASES AND THE ISOZYMES OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.)[1]

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C. J. William Maa, Wen-Pin Tseng and Ing-Chieh Huan (1990) Preliminary characterization of the larval esterases and the isozymes of diamondback moth, *Plutella xylostella* (L.). *Bull. Inst. Zool., Academia Sinica* **29**(3): 181-194. The esterase activities and the esterase isozymes of malathion-resistant (Sheh-Tzu) and -susceptible (Geou-Fang) populations of diamond-back moths, *Plutella xylostella* L., were compared in last instar larvae. The Sheh-Tzu larvae was about 6 times as resist to malathion as the Geou-Fang larvae. Membranous esterase activities of the Sheh-Tzu larvae were about twice the activities of the Geou-Fang larvae. Variations in esterase activities in the presence of eserine, and four kinds of OP-compounds between two populations were also found. Although both populations' soluble esterases demonstrated the same titer of activities, both populations exhibited multiple forms of the esterases with as many as 13 isozymes being detected. These isozymes were grouped into four categories: alkaline phosphatase, arylesterase, aliesterase and acetylesterase. Of these esterases bands 3, 4, 8 and band 9 were the most prominent. Bands 4 and 8 were able to split all the esters tested. Band 9, being tolerant to paraxon, was found in most tissues and organs examined. This band of Sheh-Tzu larvae was also more tolerant to paraoxon then the Geou-Fang larvae. Quantitative differences between the two populations of esterase isoenzymes in responding to paraoxon, PHMB and eserine were also discovered, although the differences were not significant. Analysis of the frequency of the esterase isozymes showed that the Sheh-Tzu population possessed an extra high frequency on band 8.

Key words: *Plutella xylostella*, Esterases, Isozymes, Characteristics.

The esterases of insects perform a wide variety of functions in growth and development. This is evident from their role in the hydrolysis of various chemicals that contain ester linkages. In fact, esterases were well known for their detoxifying capability of degrading various pesticides with carboxyl- or amide-groups. These pesticides include organophosphorous compounds (OP), carbamates, synthetic pyrethroids and insect growth regulators (Krueger and O'Brien, 1959; Miyamoto and Suzuki, 1973; Motoyama and Dauterman, 1974; Yu and Terriere, 1977).

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Diamondback moth (DBM), *Plutella xylostella* L., is one of the most notorious pests of cruciferous vegetables and has been known to develope resistance to various pesticides including malathion (Georghiou, 1981; Hama, 1983; Liu *et al.*, 1982; Maa and Guh, 1986; Sun *et al.*, 1986). Multiple mechanisms including: enhanced detoxifying enzyme systems, insensitive target organs and differential penetration have been postulated to cause resistance of DBM larvae to OP compounds (Liu *et al.*, 1981; Cheng *et al.*, 1984; Noppun *et al.*, 1984; Feng, 1984; Maa and Chung, 1986; Maa and Guh, 1988).

Synergists like S, S, S,-tributyl phosphorotrithioate (DEF), triphenyl phosphate (TPP) and ethyl *p*-nitrophenyl phenylphosphonothionate (EPN) were treated with OP insecticides and introduced into DBM larvae to justify whether this insect's resistant mechanism to OP-insecticides is due to the enhanced hydrolase activity of the insect. Sun *et al.* (1986) reported that DEF showed a low synergistic effect to either dichlorvos, mevinphos or carbofuran on the larva. Nevertheless, Feng (1984) pointed out that EPN shows a different synergistic effect when the DBM larvae were treated with different OP insecticides. He found that the synergistic effect of EPN ranged unaccountably from strongly synergistic, weakly synergistic, no effect, weakly antagonistic to strongly antagonistic. Noppun *et al.* (1987) studied the effect of TPP on the toxicity of phenthoate in DBM. Results of their studies hinted that an enhanced carboxyl-esterase might be involved as one mechanism of phenthoate resistance.

In the study "*In vivo* degradation of malathion in the larva of DBM" Maa and Guh (1988) detected more malaoxon monoacid in the larvae of malathion resistant strains than in the larvae of susceptible strain. Doichuanngam and Thornhill (1989) found that malathion resistant larvae hydrolysed more malathion into more polar products than the susceptible larvae. Information about the similarities and the differences in the activity and the properties of the larval esterase and the esterase isozymes of DBM larvae from different populations would be helpful in explaining population differences in the development of more selective methods of insect control. The work reported here was undertaken in an effort to obtain such information about the soluble esterases and the esterase isozymes.

MATERIALS AND METHODS

Insect

Diamondback moth (DBM), *Plutella xylostella* L., collected from vegetable farms in Lu-Chu (LC), Kaohsiung County and Shehtzu (ST), Taipei City were reared in insectarium according to Koshihara and Yamada (1976) begining in October, 1982. Populations collected in 1983 from I-lan County (IL) and Geou-Fang (GF) of Taipei County were also reared accordingly. LC and ST diamondback moths were selected by heavy pesticide applications and were, thus, resistant to various kinds of pesticides (Cheng, 1981). I-Lan County is a farming community southeast of Taipei City. Geou-Fang is a mountain village, northeast of Taipei City, which has not used commercial pesticides of any kind in the last five years. Geou-Fang and I-Lan larvae were introduced into this laboratory in 1983. The LC and the ST larvae were obtained in 1982. The DBM larva of the LC and ST populations were selected with 132 μg malathion per larva for two consequent generations in November 1984 and with 176 μg malathion per larva for additional generation in December 1984. Since that

time the selected populations were established in the insectarium since then. GF DBM was also selected with 176 μg malathion, not one individual larve treated with the pesticide successfully reached adulthood.

Chemicals

Nine esters including: 1-naphthyl-acetate (1-NA), alpha-naphthyl phosphate (1-NP), beta-naphthyl acetate (2-NA), alpha-naphthyl butyrate (1-NB), 4-methyl-umbelliferyl acetate (4-MA), 4-methyl-umbelliferyl butyrate (4-MB), 4-methyl-umbelliferyl propionate (4-mp), fluorscein diacetate (FD) and tributyrin were used as substrates.

All of the chemicals and reagents are of analytical grade or reagent grade. Diazoblue, lauryl sulfate, EDTA, Brilliant Blue G, eserine, para-hydroxyl-mercuriobenzoate (PHMB) and the other chemicals were purchased from Sigma Chemical Co., USA. All of the chemical reagents for electrophoresis were purchased from Bio-Rad Lab., USA. DEF: S, S, S-tributyl phosphorothiolate, piperonyl butoxide (PB): 3, 4-methylene-dioxy-6-propylbenzyl *n*-diethylene glycol ether, paraoxon: *O*, *O*-dithyl-*o*-*p*-nitrophenyl phosphate, mevinphos: 2-carbomethoxy-1-methylvinyl dimethyl phosphate, malathion: *O*-dimethyl-*S*-(1, 2-dicarboethoxyethyl) phosphorodithioate were purchased from Chemical Service Co., USA. Malaoxon: *O*, *O*-dimethy-*S*-(1, 2-dicarboethoxyethyl) phosphorothiolate, was a gift from American Cyanamid Company, USA. All of these substrates, inhibitors and insecticides were used to characterize the esterase isozymes of the DBM larva.

Toxicity and Synergistic Assays

Batches of thirty larvae were treated topically with malathion in a series of concentrations; 0.25, 0.5, 1.6, 4.0, 8.5, 16.5, 33.0, 66.0 and 132.0 μg per larva. After treatment, each batch of the larvae was confined in a petridish, 6.0 cm in diameter and 1.5 cm in height, with a cover of 7.0 cm in diameter, and kept at 24±2°C for 24 hours. The mortality rate was determined at the end of the test. Each treatment was replicated three times. Dosage-mortality curves were calculated using the probit analysis method as proposed by Finney (1971).

Enzyme Assay and Electrophoresis

The major method of verifying the substrate-specificity of esterase isozymes was carried out by means of PAGE according to Davis (1964). The Pharmacia Apparatus GE-2/4 with 2 mm-thick gel was used for isozyme separation.

Nine kinds of substrates including 1-naphthyl acetate were used to react with the isozymes in the gel in order to specify each isozyme of interest. Methods for staining the isozyme with substrates of arylesters, which were more polar, followed Ogita and Kasai (1965). For the less polar substrates like tributyrin and methylumbelliferyl compounds the following method was used. One tenth gram of the ester was dissolved into acetone. The solution was evenly coasted over a piece of Whatman no. 1 filter paper. The paper was dried before use. The gel to be stained was carefully and closely placed between two pieces of the filter paper treated with the ester solution. A few drops of pH 7.5, 0.04 M phosphate buffer were applied to the filter paper to keep it moist in the room temperature Twenty-five minutes after this preincubation the gel was washed with tap water. The materials stained with naphthyl moiety were developed with FBRR solution (Davis, 1964) for 15 minutes and fixed in 7% acidic acid solution for investigation. The materials stained with methylumbelliferyl or with fluorscein moiety were first investigated

for band identification and location under UV light and were recorded accordingly. The results of these assays revealed that 1-NA is the best substrate for an esterase assay.

General esterase activity to NA was spectrophotometrically determined by the method proposed by Van Asperen (1962). Diamondback moth populations were compared with one another for subcellular distribution of the NAase activity and general esterase (NAase) responded to OP insecticides and other chemical reagents.

In the isozyme study, a single larva was homogenized in 70 μl of 0.1 M pH 7.5 phosphate buffer according to Maa and Chuang (1983). The homogenate was centrifuged at 1,000 g for 15 minutes in 4°C. The supernatant was used for the NAase activity assay by spectrophotometer and esterase-isozyme separation through PAGE. The method used to determine the inhibitory effects of various inhibitors and insecticides followed Stephen and Cheldelkin (1971) and Bigly and Plapp (1965). EDTA was used to discriminate the alkaline phosphotase from esterase (Stadtman, 1961).

In the study on isozyme distribution in different tissues and organs, the larva was dissected in icy-cold Ringer's solution into various tissues and organs. The reproductive organ of the individual male larva was used. The haemolymph of the larva was pooled according to Maa and Terriere (1983). Haemolymph and the 1,000 g supernatant of the tissues and organs were applied separately to the electrophoresis set for isozyme separation.

The migrating and biochemical characteristics of each esterase isozyme and the frequency of every defined isozyme in the gel of the larvae preparations were investigated and counted accordingly.

RESULTS AND DISCUSSION

Toxicity Study

The LD_{50} of DBM larva to malathion was estimated 24 hours after topical treatment. It is 177.9±63.1 μg/larva for ST larvae, 96.9±9.7 for LC, 76.2±11.3 for IL and 30.8±11.7 for GF. ST larva was the most resistant and the GF larva, the most susceptible. The resistance ratio to malathion between TS and GF is 5.78 favoring to ST. The ratio between ST and IL is 2.3. Thus, GF and ST larvae were chosen as the two counterparts for the comparison study.

Synergistics Assay

Since oxidation and hydrolysis were recognized as the primary phases involved in the detoxification mechanism of xenobiotics in organisms (Williams, 1959), DEF and PB were used to verify whether or not the toxicity of malathion would be enhanced by these synergists. In general, it is postulated that malathion, a ester-link compound, would be greatly synergised by DEF or by other organophosphorous compounds. The synergistic assay revealed that 70% of the larvae pretreated with DEF died off 12 hours after the treatment of malathion, while the larvae which were pretreated with PB would have a mortality rate less than 30% of the total treated larvae (data not shown). This suggests that the detoxification of OP-compounds was at least partially, through non-oxidative degradation in the DBM larvae. In fact, the *in vivo* study of malathion metabolism in DBM larvae showed that larvae treated with DEF would produce less hydrolytic product; monoacid of malathion (Maa and Guh, 1986).

General Esterase Assay

Preliminary studies conducted on esterase isozymes in DBM larvae through

the use of nine different substrates showed that 1-NA produced the best result (see Table 1). Hence, 1-NA was used as the substrate in locating the esterase bands in the zymogram study as well as to quantitate the esterase activity in spectrophotometric assay. Activities of larval esterases were biochemically determined either in an activity per larva basis or in an activity per mg of protein basis.

The esterase activities of different DBM populations are tabulated in Table 2. This table is interesting in three perspectives. First, the upper part of the first column of the table shows that the GF larva had a higher titer of total NAase activity and the ST larva had a lower titer of activity. On the other hand, the lower part of the column shows that the ST larva had a higher titer of enzymatic specific activity than the GF larva. Deviation of the two measurements for the esterase activities of the ST and GF larvae were due partially to the body weight of these larvae. GF larva, which is considered as susceptible population, usually gained more body weight than ST larva. The variation of the esterase activities of these two DBM populations were possibly a result of differentiated esterases either in quantity or in quality. Sometimes the higher non-specific NAase activity of a insect did not correspond at all to the enhanced degradative activity to OP-compounds in the insect (Oppenoorth and Van Asperene, 1965; Townsend and Bosvine 1969; Maa and Terriere, 1983).

The second interesting aspect of this study concerns the subcellular distribution of NAase activity. The fifth column of Table 2 shows that 71.4% of the total NAase activity was present in the 100,000 g supernatant and 28.6% in the membranous fractions of the GF larval preparation. The case of ST produced 62.5% of NAase activity in supernatant and 37.5% in the three membranous fractions. The ratio of soluble esterase activity to membraneous esterase actvity in the ST was approximately 6 to 4 and 7 to 3 in GF. This fact again assured the variation between the two DBM populations in esterase composition.

Table 1
Response of esterase isozymes to nine ester compounds

Ester	Coded number of the esterase of the zymogram												
	1	2	3	4	5	6	7	8	9	10	11	12	13
tributyrin	+	−	+	##	−	−	−	+	−	+	##	+	+
1-NP	+	−	+	##	−	−	−	+	+	−	+	+	+
2-NA	+	+	##	##	##	+	+	##	##	−	+	−	+
1-NB	+	##	+	##	##	−	−	+	+	−	+	−	+
1-NA	+	+	##	##	##	+	##	##	##	+	+	+	##
4-MA	+	−	+	##	−	−	−	+	+	−	−	−	+
4-MB	+	−	+	##	+	−	−	+	+	−	+	+	+
4-MP	+	−	+	+	+	−	+	+	+	−	−	+	+
FD	+	+	##	##	+	−	−	+	+	−	+	+	+

ST larvae were used for this study
1-NP: alpha-naphthyl phosphate, 2-NA: beta-naphthyl acetate, 1-NB: alpha-naphthyl butyrate, 1-NA: alpha-naphthyl acetate, 4-MA: 4-methylumbelliferyl acetate, 4-methylumbelliferyl propionate, FD: Fluorscein diacetate. The responses of esterase to substrates are shown in a sequence of ##, indicating the strongest stain, to −, indicating no effect. See content for staining method of the esterase isozymes.

Table 2
Comparison in NAase activity of five different subcellular fractions of the larvae

Strain	Subcellular fractions average of enzyme activity (10^{-5} M of product/0.5 larva/15 mins)				
	1,000 g Sup.	1,000 g Pelt.	10,000 g Pelt.	100,500 g Sup.	100,500 g Pelt.
I-Lan	18.16	1.23	1.58	12.4	1.87
Geou-Fang	18.91	1.43	1.68	13.5	2.17
Lu-Chu	17.53	0.93	1.56	10.4	2.13
Sheh-Tzu	16.42	1.32	2.04	10.25	2.27
	Average of specific enzyme activity (10^{-4} M of product/mg protein/15 min)				
I-Lan	2.68	1.75	2.55	2.42	3.05
Geou-Fang	2.29	1.82	2.26	2.44	2.22
Lu-Chu	2.77	1.22	4.27	2.33	3.45
Sheh-Tzu	3.26	1.98	4.50	2.46	4.80

One hundred and fifty 4th-instar larvae of each population were used. Each fraction was diluted to one-fourth of larval equivalent for each assay. Each assay was repeated three times.

The third interesting aspect of this study is that enzyme specific activities in the 100,500 g supernatant of larva homogenate in all four populations were about the same. Meanwhile, the esterase specific activity of membranous fractions of the ST larvae were significantly higher than those of the GF larvae; more than twice the difference, favoring to the ST larvae. The high NAase-activity found in membranous fractions of the ST larvae was possibly a physiological and/or a biochemical compensation for the low titer of the total soluble fraction in the larvae.

The biochemical properties of the general esterases of the DBM larvae can also be characterized by using esterase inhibitors. Results of an inhibition assay were tabulated in Table 3. It shows that the general esterases of the ST larvae were significantly more tolerant to thiono- (malathion) or thiolo-phosphorous (mevinphos, malaoxon and paraoxon) insecticides than the GF larvae. The difference in the level of tolerance between the ST and GF larval esterases was about two folds favoring to the ST larvae. Nevertheless, in the esterase zymogram study we found that 10^{-4} M paraoxon inhibited all isozymes in the GF larvae. Bands 4, 8 and 9 of the ST zymogram were partially inhibited. However, 2.5×10^{-4} M paraoxon would strongly depress all the esterases in both the GF and ST larvae. Yet, on the other hand, 10^{-4} M eserine or 10^{-4} M PHMB, showed weak inhibitory effects only on esterases in the GF larvae (data not shown). Doichuanngam and Thornhill (1989) also found that resistant strains of DBM larvae achieved higher levels of activity of arylesterase in the presence of PHMB or DDVP. This evidence hints that quantitative differences in esterases are most likely presented between the resistant and the susceptible larvae.

Characteristics of Esterase Isozymes

In this study only the zymograms of the ST and GF populations were compared to each other as two counterparts

Table 3
Response of soluble esterases of DBM larvae to four organophosphorous insecticides and two inhibitory reagents

Insecticide (percentage of inhibition)	DBM populations		
	Geou-Fang	Lu-Chu	Sheh-Tzu
	Average of inhibitors (M in concentrations)		
Malathion (I_{10})	2.67×10^{-5}	6.04×10^{-5}	9.50×10^{-5}
Malaoxon (I_{50})	4.50×10^{-6}	7.57×10^{-6}	8.67×10^{-6}
Mevinphos (I_{50})	3.33×10^{-6}	2.33×10^{-6}	6.33×10^{-6}
Paraoxon (I_{50})	$\ll10^{-6}$	$\ll10^{-6}$	$\ll6.0\times10^{-6}$
Eserine (I_{50})	4.66×10^{-5}	$>4.66\times10^{-5}$	$>4.66\times10^{-5}$
PHMB (I_{50})	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$

One hundred and fifty 4th-instar larvae were used. The 100,500 supernatant of larval homogenate was diluted to one-fourth of the larval equivalent for the biochemical assay. Each assay was repeated three times.

to justify the variation of the esterase isozyme between the resistant and the susceptible DBM populations. A soluble fraction of the larval homogenate was examined electrophoretically to determine the number and migrating characteristics of the isozymes present in the acrylamide gel. Thirteen esterase bands were detected in the zymograms stained with 1-NA (Fig. 1). These bands were divided into five groups and deignated as: anode (remaining at the original end of the gel), slow moving, medium moving, fast moving and the cathode bands. The isozymes, which migrated midway or far away from the anode end, were prominent.

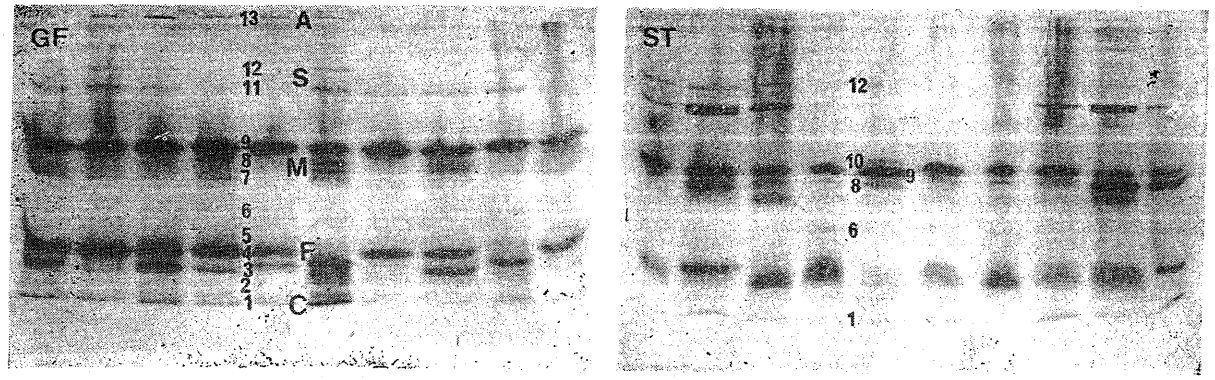

Fig. 1. Zymograms of 1-naphthyl acetate esterases of diamondback moth larvae of Geou-fang population showing polymorphism of general esterase. Each pattern represents one single larval homogenate. Zymogram code: A, anode bands; S, slow-moving bands; M, medium-moving bands; F, fast-moving bands; C, cathode bands.

The responses of the esterase isozymes to nine substrates mentioned above are tabulaed in Table 1. Five bands, namely bands 1, 3, 4, 8 and 13, can be detected in the zymograms stained with each of the nine substrates respectively. Band 9 split all esters except tributyrin. During the separation of the larval esterases by chromatography, we were able to collect at least two different isozymes from the band 8 area. Another two different isozymes were also obtained from the band 9 position. The individual isozymes with overlapping R_f values, either from the band 8 and 9 areas, had different hydrophobic properties (C. J. W. Maa, Y. L. Lin, and M. H. Tang, unpublished data). This fact might explain why the isozymes in the bands mentioned above produced a wide-range spectrum of hydrolytic activity. Band 4 was the most potential hydrolyase among the five. This band split all nine of the esters. As of yet, we are still unable to fractionate band 4. Band 6 and band 11 were also found to have more than one isozymes. This fact suggests the possibility that more than seventeen isozymes were present in the soluble fraction of larvae homogenate. It is postulated that certain isozymes, with overlapping R_f values, were possibly differentiated from different tissues or organs, as we found that much of the tissues contained isozyemes with the same R_f values (see Fig. 2).

Bands 2, 5, 6, 7 and 11 were able to hydrolyze arylesters. Bands 6 and 7 which only produced a faint detection in naphthyle acetate staining were not detectable by any other staining reagents. Band 12 should be grouped into B-type esterases since this band reacted only with aliesters. Band 10 was seldom found in zymograms of both populations (Table 4). Properties of the esterase bands could be further characterized by the inhibition assay in PAGE gels which have been described.

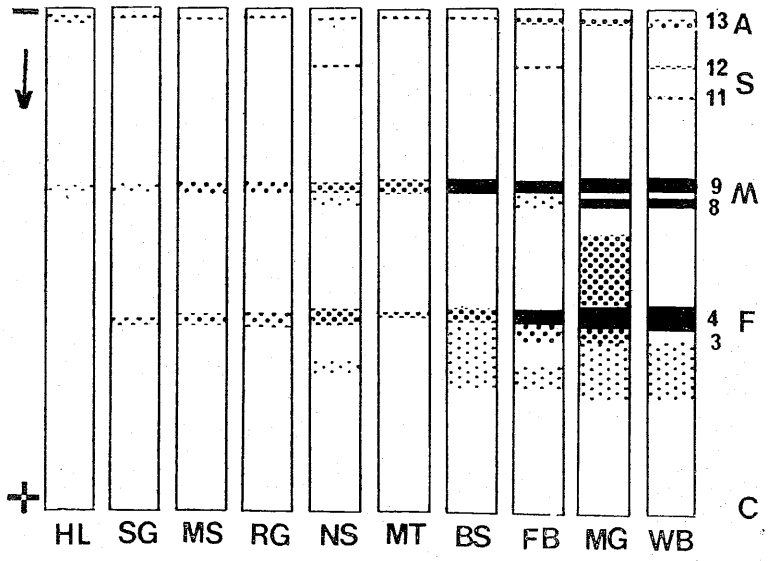

Fig. 2. Zymgrams of 1-naphthyl acetate esterases from different tissues and organs of the diamondback moth larvae showing the esterase isozymes. Each pattern represents the equivalent of 8 organs. WB, whole body homogenate; MG, mid-gut; FB, fat-body; BS, body section; MT, malphigian tubules; NS, brain and ganglia; RG, male reproductive glands; MS, muscle; SG, silk gland; HL, haemolymph. Zymogram code, same as in Figure 1.

Table 4
The migrating and biochemical characteristics of each isozyme and the frequency of isozymes distributed in individual larva of two diamondback moth populations

Code no. of band	R_f value	Paraoxon 10^{-5} M		Eserine 10^{-2} M		PHMB 10^{-2} M		EDTA 10^{-2} M		Frequency (%) of band	
		GF	ST	GF	ST	GF	ST	GF	ST	GF	ST
1	0.92	±	±	○	○	○	○	−	−	100.0	100.0
2	0.86	−	−	−	−	○	○	○	○	41.4	82.8
3	0.75	−	−	−	−	−	−	○	○	90.0	65.7
4	0.71	−	±	○	○	○	○	−	−	100.0	100.0
5	0.65	−	−	○	○	−	−	○	○	60.1	32.8
6	0.55	−	−	○	○	−	−	○	○	74.3	68.6
7	0.49	−	−	+	+	○	○	○	○	80.1	92.8
8	0.45	−	±	−	−	±	±	○	○	58.6	92.8
9	0.41	−	±	○	○	−	−	+	+	100.0	100.0
10	0.36	−	−	○	○	○	○	○	○	14.3	17.1
11	0.32	−	±	○	○	○	○	+	+	85.7	70.0
12	0.26	±	±	○	○	○	○	+	+	68.6	72.8
13	0.16	±	±	○	○	○	○	−	−	100.0	100.0

Seventy larvae per population were used for this frequency study. Bands which had a frequency of isozymes less than 10% are not counted, listed or described in this content. See content for the concentrations of inhibitors. Twenty eight larvae from each population were used for each inhibition assay. +: activated, ±: partially inhibited, −: inhibited, ○: not affected.

Table 4 demonstrates that the majority of the esterase isozymes of GF larvae were susceptible to paraoxon. The table also demonstrates that bands 1, 4, 8, 9, 12 and 13 of ST larvae were partially tolerant to 10^{-5} M paraoxon. This explains why the homogenate esterases of the ST larvae were more tolerant to paraoxon or the other three OP-compounds (see Table 3). Nevertheless, we believe that the variation of tolerance level is likely due to the quantitative differences in the esterases. The table also shows that the isozymes in bands 3, 5, 6, 8 and 9, in a portion or in the total, were A-type esterases (arylesterases) since these bands could be partially or totally blocked by 10^{-2} M PHMB (Aldridge, 1953). Bands 2 and 8 were somehow sensitive to eserine inhibition, indicating that they are acetyl esterases in nature (Aldridge, 1953). In fact, studies on tissue distributions of esterase isozyme (Fig. 2) showed that bands 2, 4, 8, 9 and 11 were all found in the zymograms of the brain homogenate of the insect. The slow moving bands coded 11 and 12 were tolelant to eserine, PHMB or EDTA, but were more or less sensitive to paraoxon. Band 2 and 6 were faint and not easily detectible. Bands 4 and 8, along with bands 1, 11 and 13 were distinguished by tributyrin stains, indicating the nature of aliesterase. Band 7, the cathode band (13) and the anode band (1) were complex in color. These three bands were found to be tinted with a grey color in cases when the gel was pretreated with paraoxon and stained with 1-NA. The band at both terminals were more or less tolerant to paraoxon but were all susceptible to EDTA. Band 4 was also susceptible to EDTA. It is likely that these bands are alkaline phosphotases in nature (Stadtman, 1961). Band

7, however, was not inhibited by EDTA.

Tissue and Organ Distribution of Esterase

The origins of each isozyme from the larval homogenate were also investigated. Mid-gut, malpighian tubules, haemolymph, brain and ganglia, muscle, fat body, silk glands, reproductive glands and body sections were all dissected off from the fresh insect larvae and were homogenated for isozyme investigation.

Figure 2 shows that bands 4 and 9 were detected in six of the nine zymogram samples prepared from various tissues and organs, indicating that these isozymes were probably associated with the juvenile state of the insect since they gradually faded away as the larva pupated and matured into an adult through metamorphosis (I. C. Huan and Maa, C. J. W. unpublised data). It is also possible that bands 4 and 9 were associated with degradative activities of OP-compounds in the larva.

Figure 2 also shows that PAGE zymograms prepared from mid-gut and fat body contained many isozymes with identical R_f values. The density of band 9, originating from different sources, could be visually scored in the order of mid-gut, body section, fat-body, brain and ganglia, malpighian tubules, reproductive glands, muscle, haemolymph, and silk glands. In the presence of 10^{-4} M paraoxon, all the isozymes except band 9 were wiped from the zymograms. The order of the density in band 9 in different tissues shifted to mid-gut, body section, malpighian tubules and the other tissues or organs (see Fig. 2). It is obvious that the mid-gut and the body section were composed of the major portion of the esterase.

OP-compounds were known to be inhibitory to esterases. It is known that both parathion and mevinphos are highly toxic to mammals and insects. Paraoxon, the thiolo derivative of parathion, is even more toxic (Eto, 1974). Mevinphos was considered to be an effective insecticide for DBM control in Taiwan (Chou and Cheng, 1983). Yet, band 8 from the mid-gut was not susceptible to mevinphos and malaoxon. On the other hand, band 9, either from mid-gut or body section was completely depressed by 10^{-5} M mevinphos (see Fig. 3).

Comparison of Esterase Isozyme Between Two Populations

The variation of the isozyme of DBM larval NAase between individual larva was observed (see Fig. 1). It is, therefore, difficult to clarify the biochemical properties of a single isozyme in each individual larva with one inhibition assay. Since the defined isozyme to be clarified could be present or absent from the individual larva investigated. Thus, the frequence of a defined isozyme or a band in the zymograms of either DBM populations were counted as additional parameters to monitor the vaiation on isozyme composition between two DBM populations.

Table 4 shows that the isozyme compositions of the two DBM populations are similar to each other. However, there were less bands detected in the zymogram of the ST larvae than in the GF larvae. Bands 1, 3, 4, 7, 8, 9, 11 and 13 were easily detected in the zymograms of both the GF and ST larvae. This shows that bands 1, 4, 6, 7, 9, 10, 12 and 13 of either populations had minor differences in band frequency with variation no more than 10%. The major differences between the two populations in band frequency were evident in bands 2, 4, 8 and 11.

Bands 2, 3, 5 and 11 of the GF zymogram were also superior in percentage of band frequency to those of the ST zymogram. A significant difference was found

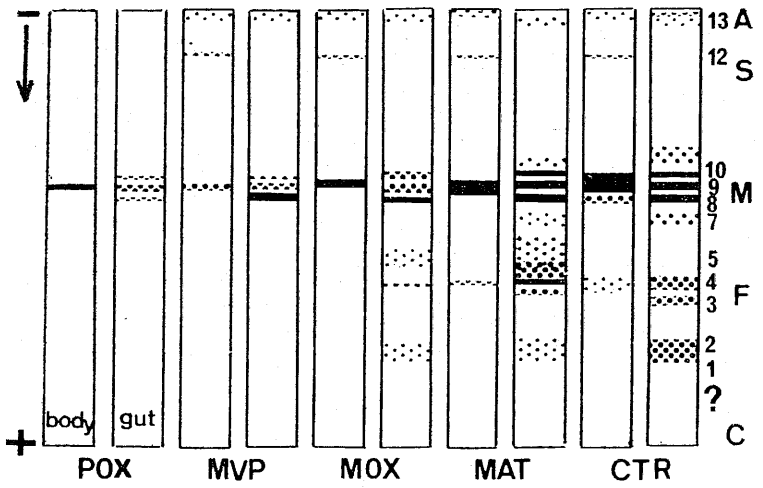

Fig. 3. Zymograms of 1-naphthyl acetate eterases of mid-body section in right, and mid-gut in left, showing that the inhibitory effect of four OP-compounds on the esterase isozymes. POX, paraoxon; MVP, mevinphos; MOX, malaoxon; MAT, malathion; CTR, blank with 5% acetone. 10^{-5} M of each of the four OP-compounds were used for inhibition. Zymogram code, same as in Figure 1.

in band 2; about 40% or more frequency in the GF larvae than the ST larvae.

Band 8 of the ST larvae had a frequency of 90%, while band 8 of the GF had 60%; a 30% difference between the two favoring to the ST larvae. It seems that all of the ST larvae had band 8. Band 9 of the ST larvae was denser in the NA stain than in the GF larvae, indicating that the ST larvae had a higher titer of aliesterase as well as arylesterase. In addition, these bands were tolerant to paraoxon, malathion and other OP-compounds. Since these bands were prominant, non-specific and were distributed in tissues and many organs, it was expected that bands 8 and 9 be associated with malathion resistance of the DBM.

Matsumura (1975) concluded that OP-resistant insects had a high content of paraoxon hydrolase, and the hydrolyase was able to split OP-compounds as a mechanism for insecticide resistance. Doichuanngam and Thornhill (1989) indicated that enhanced esterase activities were found in malathion-resistant strains of DBM. They found that the ratios of arylesterase and aliesterase activities of the larval homogenate between the resistant and the susceptible strains were 1.8 and 1.4 respectively, favoring to the resistant strains. Based on the zymogram study and the inhibition assay, Sun *et al.* (1978) claimed that qualitative differences in esterase isozymes were found between the diazinon-resistant- and the susceptible-populations. Although, minor differences in the density of the larval isozymes were found between the zymograms of the two populations when 10^{-4} M or less concentrated eserine or PHMB were used for enzyme pre-incubation, no significant qualitative differences in esterase isozyme was found between the GF and ST larvae. Miyata (1986) also indicated that no qualitative differences of esterase isozymes were found between the phethioate-resistant and the -susceptible DBM larvae. Recently, two evidences showed that amplification of esterase genes were responsible for OP-resistant insects (Field *et al.*, 1988; Mouches *et al.*,

1986). In our laboratory we also found that amplified or increased esterase activity was involved malathion-resistance of DBM larvae (Maa *et al.*, 1989).

It was known that, in some cases, an enhanced detoxification activity is usually associated with target-insensitivity with the resistant insect. Sun *et al.* (1978) also pointed out that cholinesterase in the resistant-DBM larvae was less sensitive to diazinon than in the susceptible ones. Doichuanngam and Thornhill (1989), however, found that the susceptible larvae had a higher titer of acetyl cholinesterase titer than the resistant larvae.

In the study on lethal dosages of malathion on the GF and ST larvae we found that more than 30% of the larvae treated with malathion died because of OP-compound-induced-neurosecretory-toxicity (C. J. W. Maa, San Lee and S. H. Guh, 1990). However, we could not justify whether the variation of mortality between the two populations was associated with the qualitative differences of the two DBM populations. Nevertheless, we noted that bands 4, 8 and 9 of the ST larvae were more tolerant to paraoxon than the GF larvae. Besides, esterases of the ST larvae were more tolerant to either PHMB, eserine or other OP-compounds than the GF larvae (Table 3).

The differences in esterase activities and esterase isozymes between the ST and GF larvae were obviously due to a higher titer of membranous esterases in the ST larvae and more frequency of band 8 in the ST larval zymograms. In addition, esterases of the ST larvae were more tolerant to OP-compounds and the other inhibitors than the GF larvae. This information reveals that the ST larvae were equipped with more capable esterases to degradate malathion and possibly less sensitive target organs to tolerant malathion toxicity compared to the GF larvae. These facts may explain the differentiation in the malathion resistance between the ST and GF larvae.

It is the authors' hope to sort out, at least one protein, possibly, band 8 or 9 from the isozyme study as an indicator for monitoring the resistant level in the field DBM population with more accuracy, while at the same time requiring less effort and time.

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小菜蛾幼蟲酯酶生化特性之探討

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本研究旨在比較抗馬拉松之社子小菜蛾族羣及感藥性之九份族羣其四齡幼蟲酯酶之生化活性及酯酶之同功酶之電泳特性在族羣間之異同。研究得知社子族羣幼蟲對馬拉松之抗性約六倍於九份者，其半致死量各爲每蟲三十一毫克及每蟲一百七十八毫克。協力劑試驗結果顯示酯酶確實參與幼蟲對馬拉松之解毒機制。社子幼蟲酯酶較九份者對不同的有機磷殺蟲劑具有較强的忍受性，這種情形也可在同功酶譜的實驗結果中偵測到。社子幼蟲酯酶水解醋酸萘酯之能力較九份者弱，然其膜質酯酶則較九份者强，絕大部份之酯酶活性係存在水溶性酯酶中，而兩族羣幼蟲水溶性酯酶之比活性則相等。兩族羣幼蟲皆爲同功酶多態型，數可十三。依生化特性可分屬於鹼性磷酸酶，甲、丙及丁型酯酶四類，其中第三、四、八、九號染色帶上的酯酶活性最强，而九號者能抗 10^{-4} M 對氧磷巴拉松之抑制。九號酶可在所有的組織及器官中偵測到。第八號染色帶上的酯酶可能超過兩個同功酶，具中之一對美文松有强抗性。所有十三個染色帶皆可在兩族羣幼蟲體中發現，唯發生頻度九份者高，社子者低。然而第八號染色帶發生在社子幼蟲者則遠高於發生在九份幼蟲者，引申出第八號染色帶之特殊性。