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Culture-dependent Variation in Esterase Isozymes and Malathion Susceptibility of Diamondback Moth, *Plutella xylostella* L.

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William Can-Jen Maa and Sin-Chung Liao (2000) Culture-dependent variation in esterase isozymes and malathion susceptibility of diamondback moth, *Plutella xylostella* L. *Zoological Studies* **39**(4): 375-386. Five cultures of the Sheh-Tzu diamondback moth, *Plutella xylostella* L., showing different levels of malathion resistance, were successfully raised by the single male-female mating method. The LD_{50} of malathion to ST10 was 29- and 22-fold that to ST15 and ST12, respectively. ST26 and ST34 possessed 14- and 13-fold the resistance to malathion as did ST12. The difference in the adult emergence rate of treated larvae between ST10 and ST12 was 920 fold. Toxicity of malathion to ST26 larvae was mostly synergistically enhanced by S, S, S- tributyl phosphortrithioate and diethyl maleate. A zymogram study using 1-naphthyl acetate as a substrate on PAGE gel showed that ST10, 26, and 34 were possessed of significantly higher frequencies of esterases 8b/9b which were rarely found in ST12 larvae. Esterases 9 and 8null were found in most susceptible larvae. ST26 shared toxicological characteristics against malathion and in biochemical properties of the slow-moving esterase with ST34. Subcultures of ST26, which showed differential susceptibility to malathion, were significantly and linearly correlated with the differential frequency of esterases 8b/9b of the moth. Frequencies of esterases 3 and 4b of subcultures were either positively or negatively correlated with a low titer of resistance. The significance of the correlation between isozyme frequency and malathion resistance of DBM is discussed. The possibility of using the recessive alleles of esterases 4b and 9b as indicator proteins for monitoring malathion resistance of the DBM is also discussed.

Key words: *Plutella xylostella* L., Esterase isozymes, Resistance, Correlation.

Insect esterases perform both physiological and defensive functions and are found in both soluble and membrane-bound forms. Among insect species, carboxylesterases of the *Myzus persicae* aphid (Devonshire 1977), *Culex pipiens* mosquito (Georghiou and Pasteur 1980), house fly (Oppenoorth 1965), brown planthopper (Ozaki and Kassai 1970), and tobacco cutworm (Bull and Whiten 1972) have been studied extensively because of their involvement in resistance to organophosphates (OP) or other kinds of insecticides. Characteristics of indicator proteins or enzymes of an insect have already been used to distinguish clones of potato-peach aphids which differentially resist various synthetic pesticides (Devonshire and Moores 1982), and to study the *C. pipiens* complex from various geographic area which resists OP (Fournier et al. 1987). Enhanced esterase activities to malathion and phenthoate were also found in a resistant population of the diamondback moth (DBM), *Plutella xylostella* L. (Nuppon et al. 1987). A previous study on the larval esterase of the DBM (Maa et al. 1990) revealed that the total esterase activity to 1-naphthyl acetate (1-NA) of the 2 resistant populations was somehow lower than those of the 2 susceptible populations. The specific esterase activity of the major fraction, the supernatant of 100 000 g, of the larval homogenates of 4 populations was, however, at the same level. Nevertheless, the soluble esterase of the resistant larva was more tolerant to paraoxon, DDVP, mevinphos, and malaoxon than was that of the susceptible larvae. Four isozymes, namely ESTs 3, 4, 8,

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and 9, were prominent in the zymogram.

In general, isozymes were concerned with identification of allelic frequencies at even the basic level of strains. For those involved in resistance discrimination at much finer levels as subcultures may be of crucial importance. In this study, we attempt to explore the following questions. 1. What differences exist in the esterase isozymes of the soluble fraction in between the different strains? 2. Can these differences in allozymes or isozymes be exploited for discrimination of malathion resistance between different strains? 3. Are these esterase isozymes involved in malathion resistance in the DBM?

MATERIALS AND METHODS

Insect

Diamondback moth, *Plutella xylostella* L., collected from a vegetable farm at Sheh-Tzu (ST) near Taipei City, was reared under a constant temperature of 24 ± 1 °C and a photo regime of 14L:10D conditions. The larvae were fed with rape seedlings according to Koshihara and Yamada (1981). The last instar larva which was 84 h old was used for the insecticide susceptibility test, synergism test, and for esterase zymogram study according to Maa and Lin (1985). Since fast-moving esterase of adult DBM are usually faint, this necessitated the use of the larval stage for the zymogram study.

Chemicals

All chemicals and reagents are of analytical or reagent grade. Diazoblue, lauryl sulfate, fast blue RR, 1-naphthyl acetate (1-NA), eserine, para-hydroxymercuri-benzoate (PHMB), and diethyl maleate (DEM) were purchased from Sigma Chem. (St. Louis, MO). All chemical reagents for electrophoresis were purchased from Bio-Rad Lab. (Hercules, CA). Paraoxon, O,O-diethyl-o-p-nitro-phenylphosphate; malathion, O,O-dimethyl-S-(1, 2-dicarboethoxyethyl) phosphorodithioate, piperonyl butoxide (PB), and S, S, S-tributylphosphoro trithioate (TBPT) were purchased from Chemical Service (Pa.).

Selection of cultures

Selection of resistant or susceptible strains of DBM was initiated by mating 100 pairs of single males and females individually in isolated containers. Two factors should be considered in the process of selection: 1) the selected strain must have a sufficiently high fertility to produce enough offspring to carry on the susceptibility test, zymogram study, and further propagation; and 2) selection must be oriented to gain either resistant strains with a high frequency of esterases (ESTs) 8b/9b, or susceptible cultures with a high frequency of esterases (ESTs) 8null/9a. Esterase isozymes were authenticated by PAGE stained with diazoblue. The susceptibility test was done by topical application of 33 μg of malathion on each larva. All larvae used for the above-mentioned investigation were be sibling larvae from a brood descended from a single male-female pair. Those larvae, which were highly resistant or very vulnerable to malathion, were allowed to develop into virgin moths, were mated in groups, and produced progeny for further selection.

More than 300 individual larvae, descended from the same brood, were used for the LD_{50} assay. Batches of 30 larvae were treated topically with malathion in 2 series of concentrations: 0.5, 1.6, 4.0, 8.3, 16.5, and 33.0 μg/larva for the susceptible cultures, and 16.5, 33.0, 88.0, 132.0, and 176.0 μg/larva for the resistant cultures. The treated larvae were checked every 12 h until the insects developed into adults. Dosage-mortality curves of LD_{50} were calculated using the probit analysis method proposed by Finney (1971). The lethal dosages to adult emergence were also calculated by using Abbott's formula. Larvae which were significantly resistant to or susceptible to malathion were therefore maintained and reared as newly selected strains. Identification of the culture was based on the zymogram pattern of the larval homogenate. These cultures were either interbred or intrabred for 1, 2, or several generations depending on the purpose of the study. Single pairs of female-male adults were reared separately. Larvae descending from either resistant or susceptible cultures were tested with 66 μg or 6 μg malathion, respectively, for susceptibility. Hybrids of the resistant and susceptible cultures were treated with 8, 33, or 66 μg malathion depending upon the test. The frequency and the intensity of every esterase isozyme were carefully evaluated for hydrolytic activities to 1-NA in the zymogram. Tolerance of each isozyme to paraoxon was also evaluated for biochemical characteristic of the esterases.

Synergism test

For the synergism test, each larva was treated topically with 1 of 3 synergists 2 h before malathion was applied to the insect. Maximum sublethal dosages of the synergist, either 0.5 μg PB, 10 μg DEM, or 0.2 μg TBPT, were applied respectively to the

insect. Mortality of the larvae was counted accordingly.

Zymogram study

Samples from different cultures for the zymogram study were prepared for PAGE according to Davis (1964). Esterase isozymes were stained with 1-NA (Maa et al. 1990). Esterase inhibitors were used to characterize the isozyme in the gel according to Ogita and Kasai (1965).

Data analysis

Correlations between mortality rates of the treated larvae and the frequency of each esterase isozyme were assayed by linear regression. Correlations between isozyme frequency and the rate of aberrant immatures or the rate of adult enclosing, respectively, in presence or in absent of the inhibitor were analyzed according to Maa et al. (1992).

RESULTS AND DISCUSSION

Intoxication and resistance

It is difficult to examine the acute toxicity of malathion within 24 h after treatment. Malfunction of neuro-secretary systems was hypothesized to be involved during the intoxication of caterpillars, especially when organophosphorous insecticides were used (Maddrell and Rey 1972). Nevertheless, death of treated DBM larvae due to acute toxicity was determined when the immature ones became melanized and dehydrated into miniature carcasses. Such a phenomenon was found when high doses (> 16 μg/larva) of malathion were applied to the larvae of both resistant and susceptible strains. Similar results were obtained in susceptible individuals when low doses (< 1 μg/larva) of malathion were used. Larvae affected by the induced toxicity usually grew into aberrant forms. All aberrant immature caterpillars larvae died before pupation. On the other hand, no aberrant immature forms were found in larvae of the resistant strain when 6 μg malathion was applied to them. Still, resistant larvae of the pre-wandering phase (stage before pre-pupation) were, however, affected by low doses of malathion. Larvae of this stage either accelerated their development, emerged into miniature pupae, or delayed their maturation when they were exposed to malathion. Acceleration in development resulted in enclosed pupae losing approximately 20% of their body weight. Nevertheless, these lower-weight pupae emerged successfully into the adult stage. These evidences suggested that insensitivity of the target organ, possibly the neurosecretary or growth-regulating hormone system of the insect, was associated with the resistance mechanism of DBM. Details of the effect of malathion on DBM larvae in terms of disruption of the neurosecretary and endocrine systems were worthy of further investigation.

The mechanism involved with metabolic enzymes was investigated through the synergism test. Other resistance mechanisms were also certainly involved but were not explored in this study.

Esterase isozymes

Preliminary characteristics of larval esterase of the DBM were reported elsewhere (Maa et al. 1990). ST DBM, recognized as one of the most resistant population in Taiwan (Cheng 1981), had a high frequency of ESTs 8b/9b (Maa 1996). DBM of Jeou-fen (JF), a malathion-susceptible population, had a high frequency of ESTs 8n/9a. Different zymogram patterns evidently exist for larvae of different populations. In fact, the band coded for EST 9, found in many tissues, organs, and hemolymph (Maa et al. 1990), actually contains at least 2 isozymes with different chromatographic and biochemical properties. These isozymes, which are superimposed in the same electrophoretic site on the gel, can be characterized by an inhibitory assay with paraoxon. These isozymes can be separated by elution through hydrophobic chromatography. In absence of genetic data, it is difficult to attribute each isozyme to a given locus. It is known that EST 9b is tolerant to paraoxon and that it stains lightly with 1-NA; while EST 9 represents all other superimposed allozymes, which are sensitive to paraoxon and stain intensively with 1-NA. EST 8b represents the allozyme found in the zymogram of resistant larvae. EST 8n represents the allozyme of susceptible DBM. This allozyme was not detectable by function staining (Fig. 1). Partially purified slow-moving esterases, containing ESTs 8/9, are capable of hydrolyzing malathion as well as malaoxon (Maa, unpubl. data). EST 9b is hydrophobic, tolerant to paraoxon, and possibly acts as a binding protein to sequestrate malathion and/or malaoxon. Sequestration as a mechanism of moderate resistance is found not only in insects but also in higher animals (Stereo and Phoneme 1989, Chen and Sun 1994). However, unlike EST 4 of the potato-peach aphid (Denvonshire et al. 1981), EST 2 of the *Culex* mosquito (Geoughiuoi et al. 1981), or EST 1 of the rice brown planthopper (Chen and Sun

1994), EST 9b of the DBM is limited in quantity and hence can not function as a sequestering and degrading protein in malathion detoxification. Thus, EST 9b can possibly be a protein indicator for malathion resistance in the DBM.

Esterase 8b is possibly associated with aberrant growth of larvae. We found that treated larvae which died within 3 h after treatment contained a low frequency (lower than 30%) of EST 8. Others that survived for 4 h or longer contained a high frequency of EST 8 (about 80%). This isozyme exists in tissues of the central nervous system of the DBM. In addition, JF DBM, the most susceptible population found in this island, was also contained a low frequency of EST 8b, and these larvae become aberrant when they were exposed to low doses of malathion (Maa et al, 1987). EST 8 is therefore, presumed to be associated with target insensitivity to malathion or its derivatives.

ESTs 3/4, which move fast electrophoretically, are also polymorphous. Partially purified ESTs 3/4, which can split malathion, are also hydrophobic. EST 4 is tolerant to paraoxon. Its molecular weight is estimated to be 1/2 that of EST 9, and it shares a similar pI to EST 9. Since enhanced fast-moving isozymes were found in larvae descended from the intrabred susceptible strain these isozymes were thus considered to be associated with a low level of resistance. Other esterase isozymes were comparatively dim in 1-NA staining and hence were not explored.

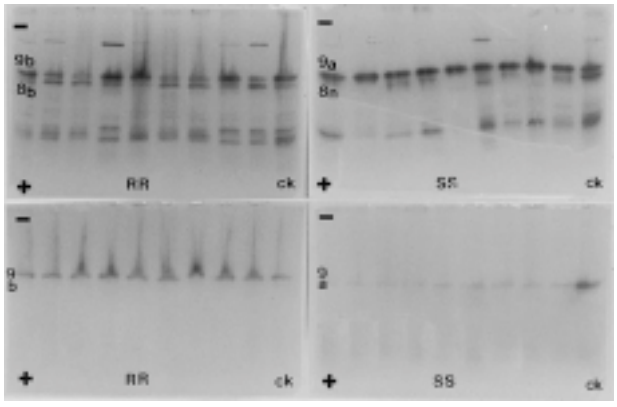

Fig. 1. Esterase isozyme pattern of resistant and susceptible cultures of the Sheh-Tzu population showing the biochemical differentiation of ESTs 8a/9n from ESTs 8b/9b. The 2 upper gels were stained with 1-NA. The 2 lower gels were pretreated with 10^{-6} M paraoxon before staining with 1-NA. All fast-moving bands of SS larvae were inhibited. RR represents a resistant strain; SS represents a susceptible strain. Ten SS and 10 RR larvae were used for this assay. A half-larva equivalent of homogenized sample was applied to each trough of the gel comb.

Strain resistance and isozymes

Table 1 shows that the larvae of ST10 were 22 times more resistant than those of ST12. The resistance ratio between ST26 (and ST34) and ST12 was about 14. A comparison study on dosage-mortality parameters (Table 1) and isozyme patterns of soluble fractions (Fig. 2) of these strains revealed that larvae of ST26 and ST34 shared many characteristics with one another, including: LD_{50} to malathion, slope of dosage-mortality curves, enclosing rate of the treated larvae, mobility and pattern of slow-moving isozymes (ESTs 8b/9b), and reaction of ESTs 8b/9b to substrate and inhibitors. In addition, the zymogram pattern of the slow-moving isozymes and the mortality rate of hybrids descended from a ST12 male/ST26 female were also like those of hybrids descended from a ST12 male/ST34 female (Table 2). However, ST26 differed from ST34 in the mobility and zymogram pattern of the fast-moving isozymes (ESTs 3/4) (Fig. 3). These results imply that mechanisms of major resistance are likely associated with the larvae of different strains even which had similar

Table 1. Culture-dependent variation in the susceptibility of diamondback moth larvae to malathion

ST strain	LD_{50} (μg/larva) M ± SD at 24 h	LD_{50} (μg/larva) M ± SD at enclosing	Slope at 24 h	Slope at enclosing
12	6.2 ± 2.3	0.06 ± 0.03	0.79	1.88
15	4.7 ± 1.8	0.17 ± 0.15	0.69	2.23
26	88.5 ± 9.3	18.30 ± 7.60	1.95	4.26
34	82.4 ± 11.6	16.98 ± 6.40	1.92	4.23
10	136.1 ± 23.7	55.23 ± 16.34	2.13	4.74

Larvae of ST12 and ST15 were treated with 5 doses ranging from 0.1 to 16.0 μg (per larva). Those of other strains were treated with doses ranging from 8.0 to 176 μg (per larva). Assays were done in triplicate.

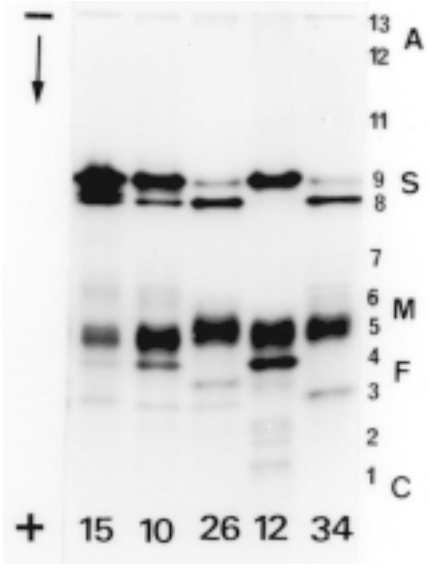

Fig. 2. Zymogram patterns of 5 strains of the Sheh-Tzu population showing the fast- and slow-moving esterase isozymes. One-larva equivalent was applied to each trough of the gel comb. A represents the anode end, and C represents the cathode end. Figures on the right side of the gel represent the coded number of the esterase isozymes.

slow-moving esterases. They also reflect that a major resistance mechanism is likely associated with the slow-moving esterases, and that these esterases are more important than the fast-moving ones in view of resistance monitoring. Zymogram studies revealed that most intrabred resistant larvae possess ESTs 8b/9b (Maa 1996). ST10 was obtained by interbreeding of 2 different resistant strains. It has dense ESTs 8/9b. Nevertheless, approximately 25% of resistant larvae had EST 4b in their offsprings (Fig. 4). ST15 was obtained by interbreeding 2 susceptible strains. A closed-triple band was detected in the position of EST 8 on the ST15 zymogram. However, for unknown reasons, subcultures like ST10 and ST15 could barely be maintained in the insectarium. Occasionally, approximately 25% of ST12 larvae had EST 8b in their offspring. This may hint that the gene coding for EST 8b is possibly incompletely dominant. In addition, hybrids descending from ST12 and ST26 are endowed with ESTs 8 and 9.

Table 2. Correlation between frequencies of larval ESTs 8/9b and the enclosing rate of treated F_1 larva of 3 cultures

Pairing mate m × f	Sex for assay	Enclosing rate (%)	Frequency of isozyme (%)	
			EST8	EST 9b
34 × 26	MS	70.3 ± 11.7	76	75
34 × 34	M	70.8 ± 9.4	76	73
26 × 26	M	83.3 ± 10.6	95	80
26 × 12	MS	61.7 ± 10.3	50	36
12 × 26	MS	49.5 ± 7.0	50	36
12 × 34	MS	34.8 ± 13.3	48	33
12 × 12	M	0	11	0

Fourteen larvae were used for each malathion (8 μg/each larva) treatment: 14 to 30 larvae were used for the frequency study.
M: male only; MS: mixed sexes; m: male; f: female.

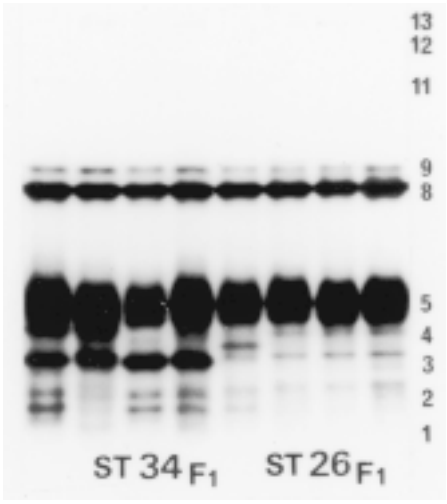

Fig. 3. Zymogram patterns showing the difference in fast-moving esterases between the F_1 generations of ST26 and ST34. The heavily stained EST 3 of ST34 may represent the difference from the lightly stained EST 3 of ST26 which showed a slight difference in malathion resistance between the 2 cultures.

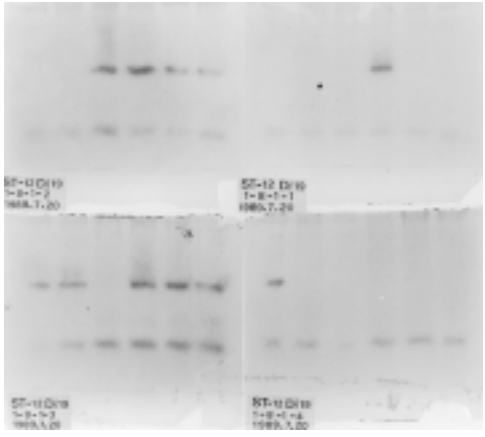

Fig. 4. Zymogram pattern of sequentially intrabreeding ST12 subcultures showing the paraoxon-tolerant EST 4b near the anode area in each individual offspring of these 4 isolated subcultures. All 4 gels were pretreated with paraoxon before staining. EST 9b, near the cathode area, was occasionally concomitantly shown with EST 4b. Other isozymes were inhibited.

The LD_{50} of malathion to adult enclosion of ST10 or ST26 (ST34) was 920- or 305-fold that of ST12. There is a 3-fold difference in resistance between ST10 and ST26 (or ST34). All this evidence suggests that ESTs 8b/9b of ST26 (or ST34) are likely usable as indicator proteins to monitor the malathion resistance of the DBM.

Synergism test

Table 3 shows that the STRR larvae were 10 times more resistant to malathion than were the STSS larvae irregardless of whether synergists were used or not. Synergism of malathion with TBPT/DEM against the DBM was significant, which suggests that hydrolases and/or glutathion-S-transferases might play important roles in malathion degradation in both strains. Nevertheless, TBPT showed a stronger synergistic effect with malathion against the larva of susceptible cultures indicating that hydrolases of the larvae were totally blocked by TBPT. Comparatively, DEM showed a weak effect on susceptible larvae. Meanwhile, TBPT and DEM showed the same level of synergistic effect with malathion against resistant larvae. Asperen and Oppenoorth (1960) suggested that an OP-resistant house fly was endowed with alterated esterase for enhanced hydrolysis activity. Phosphatases usually are not blocked by malaoxon or TBPT, as the larvae of both cultures of ST10 and ST26 contain same level of non-specific esterase activity (Maa et al. 1990). It is assumed that the enhanced activity of alkaline phosphatases is possibly involved in the resistance mechanism of the DBM, since we found that mid-gut alkaline phosphotases from the resistant larva was more active against naphatyl phosphate than were those from susceptible larva (Jeng et al. 1985).

It was noted that larvae pretreated with PB became more tolerant to malathion. Oppenoorth (1984) suggested that malathion is degraded into an acid derivative through oxidative hydrolysis. Aizawa (1982) indicated that malaoxon was determined to be the major metabolite (about 25% of total metabolites) and is produced mainly through enzymatic oxidation. PB, the inhibitor of monooxygenases in vivo, decreases either the rate of desulfuration of the dithiophosphoric structure or the rate of dealkylation of the carboxylester moiety of malathion. Since malaoxon is an anti-acetyl cholinesterase reagent, the reaction of blocking desulfuration of malathion by PB consequently releases acetyl cholinesterases and/or aliesterases of the DBM from inhibition and reduces the mortality of the insect. This would explain how malathion was antagonistically affected by PB against the DBM. Study on in vitro metabolism of malathion showed that partial purified ESTs 8/9 did split malathion, but in a minimum quantity (Maa, unpubl. data). It seems that the resistance mechanism of the DBM to malathion does not depend on hydrolysis alone. In addition, the specific enzymatic activity of soluble esterases of different cultures is not correlated with the frequency of ESTs 8b/9b (Maa et al. 1997). Multiple mechanisms of resistance are found in different populations of DBM (Cheng 1986). Carboxylesterases were, however,

Table 3. Effect of 3 insecticide synergists with malathion on larvae of the diamondback moth

		Parameters estimated						
Treatment	Strain	Chi-s	A	B	ED_{50}	95% limited		df
Malathion	RR	0.95	0.16	1.95	301.22	173.62	695.64	3
	SS	6.79	3.89	0.77	25.47	11.30	76.59	4
Malathion	RR	4.62	1.51	1.62	140.98	95.37	478.04	3
+TBPT	SS	3.23	4.83	1.95	1.49	0.29	3.96	5
Malathion	RR	5.84	0.26	2.41	153.38	114.43	303.84	3
+DEM	SS	0.59	3.97	1.03	9.93	3.76	22.67	3
Malathion	RR	3.96	2.09	1.15	333.09	160.71	669.71	3
+PB	SS	3.24	3.32	1.24	29.21	10.46	94.60	5

RR: ST26-1915, a resistant subculture intrabred from ST26.
SS: ST26-1008, a susceptible subculture interbred from ST26 x ST12.
TBPT: S, S, S, tributylphosphorus trithioate.
PB: piperonyl butoxide.
DEM: diethyl maleate.
A: interception of the linear regression.
B: slope of the linear regression.
df: degrees of freedom.

reported to be one of the major enzyme systems for malathion detoxification in DBM (Sun et al. 1986). Miyata et al. (1986) and Noppun et al. (1987) also have suggested that fenthoate is degraded possibly by enhanced carboxylesterase of a resistant DBM in Japan. On the other hand, it was reported that a fenvalerate-resistant DBM would become susceptible to fenvalerate when the selection pressure was released, and the reversional strain would regain its resistance to fenvalerate in 1 generation once malathion was used to screen the DBM. The reversion can also be detected in a resistant DBM released from selection of malathion (Motoyama et al. 1986). Yet, Yu and Nguyen (1992) and Yu (1993) found that monooxygenases of the DBM are the major enzyme system for degrading malathion and fenvalerate. Evidence mentioned above suggests that there is common ground for DBM to resist both malathion and fenvalerate. We also found that the malathion-selected DBM was highly resistant to fenvalerate. In fact, an in vitro study revealed that MFO is apparently important in cleavage of the ester bonds of malathion in the resistant DBM (Maa, unpubl. data). An in vivo study revealed that correlation between O-demethylation of MFO activity and EST 9b frequency in DBM larvae was positive ($p < 0.1$). Although it is insignificant statistically (Maa et al. 1997), we should not neglect that insects with chewing-type mouth parts would have complex biochemical defense mechanisms rather than hydrolysis and sequestration. And one of the major defenses would possibly be the metabolic activity of monooxygenases.

Slow-moving ESTs

Table 4 shows that correlation between frequencies of ESTs 8b/9b and malathion resistance of ST12 progenies are statistically significant at the positive fitness of $p < 0.01$. This suggests that genes for EST 8b and EST 9b of ST12RR are genetically closely linked ($r = 0.9695$) and are expressed concomitantly. It should also be noted that the gene for EST 9b is possibly more closely linked to the gene associated with malathion resistance ($r = 0.841$). Highly resistant individuals usually presented intensively stained EST 8b and lightly stained EST 9b in the zymogram. Resistant individuals endowed with EST 8b and heavily stained EST 9b were considered to be heterogeneous and to be less resistant against malathion. In fact, intensively stained ESTs 8 and 9 were found in the zymogram of ST10 DBM. However, these characteristics of ST10 were not heritable in their offspring for an unknown reason. On the other hand, those with EST 9b but without EST 8 showing off in their zymogram were possibly heterogeneous ones which are considered to be endowed with a null allele for EST 8 and are considered to be malathion susceptible.

Table 4 shows that, in total, fourteen pairs produced enough offspring, e.g., thirty-five larvae, to fulfill the requisites for the zymogram study, susceptibility test, and sequential propagation. Initially more than 60 pairs of intra- and inter-breeding subcultures were designed for the test. However, forty-six pairs not listed in table 4 were either sterile or produced only a few larvae. Currently, we can not determine whether the low fecundity of the STRR male is associated with the resistance mechanism of the DBM. In general, an insect equipped with enhanced activity of monooxygenases is likely to be disadvantaged in regard to propagation or reproduction. The 4 mated pairs with the STRR males had extremely low fecundity, possibly because of a sex-linked lethal factor in STRR males. On the other hand, mates of STSS males produced more offspring than did those

Table 4. Correlation[a] between frequencies of larval ESTs 8/9b and the rate of adult emergence of treated larvae of the ST12 subcultures

ST brood no.		Frequency EST 8	Frequency EST 9b	Rate of adult enclosing
ST12RR	no. 8	0.889	0.667	0.500
	no.15	0.556	0.500	0.429
ST12RR female x ST12SS male				
	no. 1	1.000	0.889	0.846
	no. 4	0.333	0.000	0.000
	no. 6	0.444	0.444	0.381
	no.14	1.000	1.000	0.667
	no.15	0.556	0.556	0.724
ST12SS female x ST12RR male				
	no. 4	1.000	1.000	0.833
	no.13	1.000	1.000	0.400
ST12SS				
	no. 5	0.222	0.000	0.500
	no. 6	0.222	0.000	0.111
	no. 7	0.222	0.000	0.000
	no.12	0.666	0.444	0.500
	no.18	0.300	0.000	0.143

[a]$Y = 0.031 + 0.665X$, $r = 0.835$, $p < 0.01$, for the linear regression fitness between frequency of EST 8 and adult enclosing rate of treated larvae.

[a]$Y = 0.116 + 0.624X$, $r = 0.841$, $p < 0.01$, for the linear regression fitness between frequency of EST 9b and adult enclosing rate of treated larvae.

$n = 18$, df = 16, treatment; 66 μg/larva, 15 larvae for each single brood, zymogram assay; 9 larvae for each single brood.

of STRR males irregardless of whether their mate was a susceptible female or not. ST12SS was selected to deprive the larvae of EST 9b. It is likely that if these larvae were treated with malathion, they would have a low enclosing rate to adulthood. This suggests a link between the frequency of EST 9b and malathion resistance. The larva of no. 12 of the ST12SS mate, however, had a medium level of ESTs 8/9 frequency as well as medium resistance to malathion. This hints that the try on to intrabreed a homologous susceptible culture was somehow hindered. Possibly the recognized zymogram pattern for susceptible stock, ESTs 8n/9a, is a phenotype rather than a genotype. It would not be feasible to pick up a homogeneous zygote of ESTs 8n or 9a for susceptibility monitoring because these allozymes are likely semi-dominant and would not fit the prerequisite of a monitoring protein. It is believed that a heterogeneous zygote will have a better chance to survive in ordinary conditions and will produce more offspring as well. The analysis for table 3 as a whole suggests that ESTs 8b/9b are a good example to illustrate how the correlation between frequencies of ESTs 8b/9b and resistance of the larvae were interpreted. The fitness of the correlation between an estimable constant (isozyme frequency) and a predictable variable (resistance) can be determined in a reciprocal interaction. The same analytical method can be applied to the bred progenies of susceptible strains treated with either low or high

Table 5. Correlation of linear regressive fitness between mortality rate, aberration rate, or adult enclosing rate of malathion-treated larvae of ST cultures and the frequency of EST 8b and 3 minor esterase isozymes of ST larvae

Strain and assay parameters	Coded isozyme	Aberration (%)	Mortality (%)	Adult enclosing (%)
assay 1	EST 3	---	no	no
ST12, 26, 34, ST12 x ST26, ST12 x ST34	EST 4	---	no	no
n, 7; df, 5	EST 8	---	no	no
8 μg/larva	EST 9b	---	–($p < 0.01$)	+($p < 0.01$)
assay 2	EST 3	no	no	no
ST12R	EST 4	no	+($p < 0.1$)	–($p < 0.05$)
n, 9; df, 6	EST 8	no	no	no
66 μg/larva	EST 9b	–($p < 0.1$)	–($p < 0.01$)	+($p < 0.02$)
assay 3	EST 3	no	no	no
ST12S	EST 4	no	no	no
n, 8; df, 6	EST 8	no	no+	no-
6 μg/larva	EST 9b	no	no	no
assay 4	EST 3	no	no	no
ST12S	EST 4	no+	no+	–($p < 0.1$)
n, 9; df, 7	EST 8	no	no	no
66 μg/larva	EST 9b	no	no	+($p < 0.1$)
assay 5				
ST12RR, SS	EST 3	–($p < 0.01$)	–($p < 0.01$)	+($p < 0.05$)
ST12RR m x	EST 4	no	no	no
ST12SS f	EST 8	no	no	no
n, 8; df, 6				
66 μg/larva	EST 9b	–($p < 0.1$)	–($p < 0.05$)	+($p < 0.05$)

+: positive correlation; –: negative correlation; ---: not detected; no: not significant correlation; no-: with the highest value in negative correlation but not significant; no+: with the highest value in positive correlation but not significant; n: number of assay, with 15 larvae used for each assay; df: degrees of freedom; ST12 x ST10: mating in single female-male pairing between ST12 and ST10 strains.

Rate of adult enclosing of the treated larva of the subculture.

doses of malathion. Effects of the low dose of malathion on the susceptible cultures were emphasized in the following bioassay in order to differentiate the effect of low doses of malathion to susceptible larvae in which a correlation between ESTs 3/4 frequency and susceptibility of the DBM can be made.

Fast-moving ESTs

Larvae that died within 48 h were considered to be poisoned by acute toxicity of malathion. Those with aberrant symptoms which ceased 48 h after treatment were considered to be affected by OP-induced neuro-secretary toxicity. Larvae with aberrant symptoms, usually without EST 8 in their zymogram, might have undergone pre-pupation or pupation, but failed to reach adulthood. Data in table 5 were used to justify whether frequencies of ESTs 3 and 4, the fast-moving isozymes, in the absence of ESTs 8b/9b, were correlated with each of the 3 measured parameters.

Results of sequential breeding between different cultures or subcultures were interesting in three respects. First, EST 9b, as expected, was positively correlated with malathion resistance in most cases studied except ST12S, a substrain selected to deprive the larvae of ESTs 8/9b (Table 5, assay 3). Second, EST 4b of ST12R was negatively correlated with resistance (Table 5, assays 2 and 4). Third, EST 3 was positively correlated with resistance (Table 5, assay 5).

EST 4b was negatively correlated with resistance of the larvae at different levels of significance: $p < 0.05$ for ST12R; $p < 0.10$ for ST12S, each treated with 66 μg; and non-significant for ST12S, treated with 6 μg of malathion. A non-significant correlation between EST 4b frequency and malathion resistance was found in interbred progenies descended from the paired mate of an ST12 male/ST26 female or from the mate of an ST12 male/ST34 female (Table 5, assay 1) and in interbred progenies from the paired mate of ST12R/ST12S (Table 5, assay 5). These results suggest that enhanced EST 4b can only be found in zymograms of intrabred progenies of susceptible cultures and can only be used as a recessive indicator protein for susceptible and homogeneous DBM. It is interesting to note that EST 4 is the only isozyme that can be used as a counterpart protein of EST 9b to justify the susceptibility of the DBM.

Larvae of ST12S were highly susceptible to 6 μg of malathion. Most of the treated ST12S larvae became larva-pupa intermediates, and less than 25% of treated ones were able to emerge into adulthood. On the other hand, no aberrant immatures were found in the resistant larvae of ST10, ST26, or ST34 when 6 μg malathion was applied to them. No ESTs 8/9b were detected simultaneously in zymograms of the intrabred ST12.

The frequency of EST 3 was significantly and positively correlated with malathion resistance of the 3rd generation hybrids of ST 12Rf1 and ST 12Sf1 only. In other words, the correlation between EST 3 frequency and malathion susceptibility could not be detected in any inter- or intrabreeding of the parent ST stocks, nor in the intrabreeding brood of ST12S and ST12R. This implies that EST 3 is possibly coded by a recessive gene of the susceptible DBM. This isozyme would not be recommended for monitoring the susceptibility of DBM in the field since the approach method is too tedious to be practical. Nevertheless, the above-mentioned approach enable one to distinguish the weak resistant factor associated with either EST 3 or 4 from the strong resistant factor associated with ESTs 8/9b. In other words, malathion-resistant DBM populations could be obtained either by intrabreeding the DBM stock in orientation to gain a strain with a high frequency of ESTs 8/9b, or by continuously selecting a wild population of DBM with high doses of malathion for generations until resistance is achieved. Susceptible DBM cultures could scarcely be obtained by selecting the population in orientation to gain a strain with enhanced EST 4 or just by completely releasing the DBM from pressures of selection. Thus, correlation between a specific isozyme and malathion susceptibility of DBM can be illustrated through a comprehensive study on strain selection and isolation. Study of esterase isozymes of DBM thus provide a feasible method for approach. Changes in the resistance status of a DBM population might be reflected through monitoring the fluctuation of a special esterase isozyme of the larvae in that population.

Monitoring OP- resistance with esterase isozymes

A previous report revealed that the titer of total esterase activity of a susceptible population of DBM (GF) is higher than that of a resistant population (ST). However, The soluble esterase of the ST DBM had a higher tolerance to eserine, and thiolo- or thiono-organophosphorous compounds (Maa et al. 1990). Campbell et al. (1997) revealed that aliesterase, OP hydrolase, and malathion carboxylesterase were found in *Lucilia cuprina*. He commented that cultures resistant to malathion but not diazinon have low aliesterase, intermediate OP

hydrolase, and high malathion carboxylesterase. Kao and Sun (1991) indicated that the enhanced carboxylesterase was the major concern with malathion resistance in the DBM. Chen and Sun (1994) reported that enhanced carboxylesterases (E_1) of rice brown planthopper could bind paraoxon or malaoxon to render them nontoxic.

There are more than 11 esterase isozymes capable of being detected in the PAGE zymogram of DBM larvae (Maa et al. 1990). ESTs 3, 4, 8, and 9 are the predominant ones, of which ESTs 4b and 9b are tolerant to paraoxon and are highly hydrophobic. Both isozymes are recessive in nature and are either negatively or positively associated with malathion resistance of the DBM. ESTs 3 and 8 are coded either by recessive or by incompletely dominant genes. Both isozymes are susceptible to paraoxon. ESTs 3, 8b, and 9b are widely distributed in field DBM, while EST 4b is rare (Maa et al. 2000). Although, these isozymes are limited in quantity and are hardly to be justified to function as major metabolic enzymes for degrading malathion or as major binding proteins for rendering malathion or its derivatives nontoxic.

The DBM was known for becoming resistant to different categories of insecticides, and the broad spectrum of insect resistance observed in field populations is due to multiple resistance mechanisms, including increased detoxification of these insecticides by microsomal oxidases, and enhanced carboxylesterase, glutathion-S-transferase, and target site insensitivity such as insensitive acetyl cholinesterase (Cheng 1986, Miyata et al. 1986, Sun 1986, Yu and Nguyen 1992, Maa et al. 1997). Currently, it is difficult to figure out which isozyme or allozyme of esterase is involved in malathion degradation since the biochemical properties of these esterases are not fully understood yet. However, results of regression analysis and synergism tests revealed that certain esterases are surely associated with malathion resistance of the DBM. We argue that it is possible to simplify the often complex arguments of resistance mechanisms and detoxification systems using desirable general results, and offer as proof, but without a powerful explanation, the actual patterns in nature. Most of all these isozymes are good monitoring proteins for malathion resistance for the following reasons: first, these isozymes are easily detected by 1-NA staining with a combination of paraoxon inhibition; and second, ESTs 4b/9b are coded by recessive genes. It is a good quality for a monitoring protein to be recessive for exercise since the recessive gene can be protected by a dominant or a incomplete gene, and thus be heritable even under extreme environmental stress. Further study is underway in this laboratory on frequency and distribution of ESTs 4b and 9b in association with insecticide resistance of field DBM populations. It will be interesting to know the expression and disappearance of these indicator proteins in Taiwan in the adaptation of the DBM to harsh man-made environments.

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小菜蛾不同品系其酯解異構酶及馬拉松抗藥性之相關性的差異研究

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吾人以單雌雄交配法，成功地由社子小菜蛾族群中分離出五個對馬拉松具不同抗藥性的品系。就幼蟲半致死劑量最高的社子十號而言，其抗藥性是感藥品系社子十五號的二十九倍、是十二號的二十二倍；抗性次高的社子二十六號及三十四號則為十二號的十三倍。如以羽化成功率之半致死劑量來估算，則十號為十二號的九百二十倍。協力劑毒效試驗結果證實社子二十六號者其抗馬拉松特性和酯解酶及谷胱甘肽硫轉移酶有關。從乙酸酯為受質去染由電泳分離之酯解酶譜，可看出十、二十六、三十四號品系的族群體內均含高頻率的第八乙及九乙號酯解酶譜，此正是十二號者所缺。反之十二號有的八及九號酯解酶，在抗藥族群身上也不易找到。社子十五及十號在抗藥性及酶譜的穩定度上無法掌控。社子二十六及三十四號兩品系，在抗藥性之特徵上，在酯解酶譜上皆具類似的表現，兩者所異在於快速游動居陰極區的酯解酶。以社子二十六號所生後代作單雌自交，得到含第八乙及九乙酯解酶發生頻率高低的各亞品系。各亞品系所具抗馬拉松的強弱與其所異之第八乙及九乙酯解酶發生頻率高低呈統計上的正相關。如將社子十二號的亞品系所生後代作單雌自交，可在其第二代測得第三號酯解酶發生頻率之高低，與其低抗藥性之強弱呈正相關；第三代可測得第四號酯解酶頻率之高低，卻與弱抗藥性之強弱呈負相關。諸由異構酯解酶發生頻率與抗藥性的相關性間呈現的生命意義，其將在本文討論，如何利用具隱性基因調控的第四乙及九乙號酯解酶，以作檢測田間小菜蛾族群抗馬拉松的可能性，亦一併討論之。

關鍵詞：小菜蛾(*Plutella xylostella* L.)，酯解酶，抗藥性，相互關聯。

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