

Esterase Isozyme of Diamondback Moth

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Abstract

Electrophoresis is currently the best technique for quantifying inherited variation which has contributed to our knowledge of population biology in revealing population structure. Considerable variation in isozymes of diamondback moth, *Plutella xylostella* (L.), was detected. Esterase zymograms were prepared from 3rd and 4th instar larvae, pupae and adults. There were two groups of esterase bands, which appeared to be independent. The faster band was simple to analyze genetically and involved at least six alleles. In a preliminary study, esterase zymograms of four populations, collected in Shimane and Osaka prefectures of Japan, were compared using these alleles. Gene frequency was different between the four populations, suggesting that esterase isozymes could be useful in analyzing the population biology of diamondback moth.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a serious insect pest of crucifers. DBM has developed high levels of resistance to various insecticides (Hama 1987). Tanaka and Kimura (1990) reported a decrease in susceptibility to *Bacillus thuringiensis* Berliner insecticide (Toarow CT wettable powder). Hama et al. (1990) found that the *B. thuringiensis* resistance in DBM is genetic. Ecology, occurrence and control of DBM in Japan has been widely studied (Sakai 1988). The migration of DBM is known in Europe (Williams 1958), however, there is no evidence of DBM migration in Japan. Honda and Miyahara (1987) suggested that DBM may migrate from southern areas to northern Japan. It is not clear, however, that DBM do, in fact, migrate. Isozyme analysis could be used to identify local populations and monitor any large-scale migration.

Isozyme analysis has contributed not only to discrimination of insect species but also analysis of population biology in many insect species (Loxdale and Hollander 1989). In this paper, isozymes of DBM were studied as a means of analyzing population structure and insecticide resistance.

Materials and Methods

Insect source

The origins of four populations of DBM used in this study are shown in Table 1. These populations were reared on Japanese radish seeds cultured in plastic vessels in a chamber at $23 \pm 1^\circ\text{C}$ and 16L:8D. Adult moths were stored at -30°C before being homogenized.

Table 1. Origins of DBM.

Population	Locality	Date collected	Resistance type ^a
OS	Osaka	June 1990	BT, C
MA	Masuda	23 March 1990	O, P
YO	Yokota	6 September 1990	O, P, C
MT	Matsue	23 March 1990	O, P

^aBT, *B. thuringiensis*; O, Organophosphorus; P, Pyrethroid; C, Cartap.

Electrophoresis

Adult moths were homogenized individually in 20-40 μ l mixture of 20% sucrose solution and 0.25% Triton X-100, and 10-20 μ l homogenates were dispensed into pockets in the gel. Electrophoresis was carried out at constant current (30-40 mA) for about 1 hour on 7.5-9% polyacrylamide vertical slab gel, using Tris glycine (pH 8.6) as running buffer. Gels were then stained for 10 min at 36°C for esterase with Fast Blue BB or RR salt in 0.1 M phosphate buffer (pH 7.0) containing 1-naphthyl acetate (dissolved in acetone).

Results and Discussion

Esterase zymograms of DBM were detected in larva, pupa and adult stages. Esterase isozyme patterns of DBM are shown in Fig. 1. There were two distinguishable banding groups (Est-1, Est-2) on the esterase zymogram. These band groups were independent of each other. A slower band group (Est-2) was clear and had many bands. A faster band group (Est-1) was slightly dim and each band was represented by a double band (doublet). In the Osaka population, 9 of 11 adults had the same band in Est-2. However, the Matsue population had different band patterns in Est-2. There was wide variation on Est-2 of the four populations examined in this study. The Osaka population was heavily selected with *B. thuringiensis* application. Therefore, there might be a simple band pattern on Est-2 loci of the Osaka population. A band pattern of Est-1 loci was simple and useful to determine heredity of DBM. It was clear that there could be at least six bands in Est-1 loci.

In crossing tests, theoretical genotype appeared in the F₁ generation. Esterase zymograms of parent and F₁ are shown in Fig. 2. Parents of genotype BB and AC produced AB and BC genotypes in F₁. Therefore, bands of Est-1 must be alleles on Est-1 loci. Genotype frequency is shown in Table 2. There are six alleles and 21 genotypes in Est-1 loci. Eighteen genotypes were detected in four populations. Osaka and Matsue populations had 12 genotypes, and Masuda and Yokota populations had eight genotypes, respectively. Gene frequency of allele is shown in Table 3. In Osaka and Yokota populations, there was high frequency of B allele. Matsue and Masuda populations had a high frequency of C and A alleles, respectively.

These alleles could be available as genetic markers of population characteristics and these markers could be applied to population analysis of DBM with regard to migration and distribution.

Est-2 band of the Osaka population showed homozygote alleles. Hama (1990) reported that *B. thuringiensis* resistance of DBM is incompletely recessive. Therefore, this resistance will appear on homozygote genes. Homozygotes of Est-2 may be coincident with the *B. thuringiensis* resistance gene. However, there is no evidence of coincident homozygote genes. The Osaka population was selected by *B. thuringiensis* (Tanaka 1990), suggesting little genetic variation. If Est-2 loci coincides with *B. thuringiensis* resistance, it will be possible to monitor the change in resistance gene frequently resulting from insecticide selection.

Isozyme analysis was applied to identification of species and biotype, and population genetic studies on many insects such as aphids, egg parasite (*Trichogramma* sp.) and so on (Loxdale and Hollander 1989). This has not been attempted on DBM. Honda and Miyahara (1987) reported that DBM cannot hibernate in Tohoku district, and they suggested that DBM might migrate

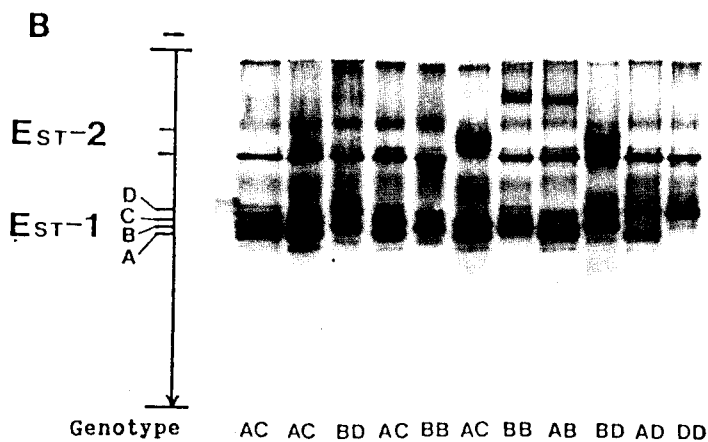
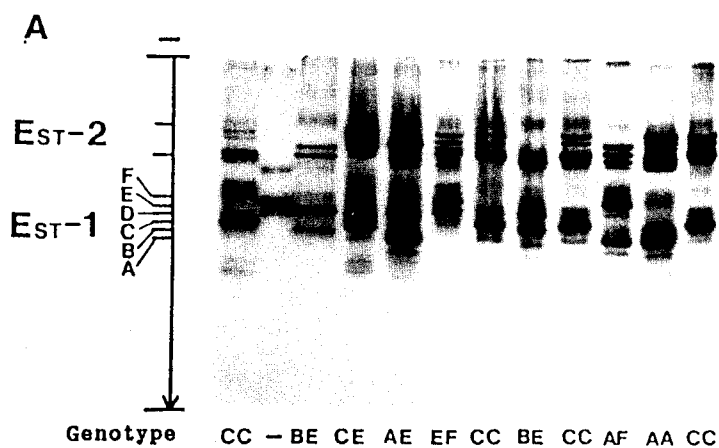


Fig. 1.
Esterase zymograms of two DBM strains: A, Matsue; B, Osaka.

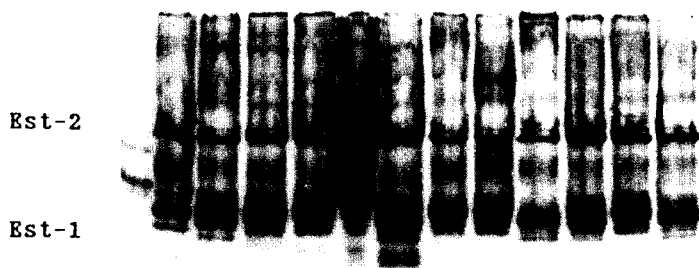


Fig. 2.
Esterase zymogram of parent (P) and F₁ of DBM (Osaka).

Genotype	<u>BB</u>	<u>AC</u>	<u>BC</u>	<u>BC</u>	<u>BC</u>	<u>AB</u>	<u>BC</u>	<u>BC</u>	<u>AB</u>	<u>AB</u>	<u>BC</u>	<u>AB</u>
	P					F ₁						

Table 2. Genotype frequency in Est-1 loci of DBM.

Genotype	Genotype frequencies of each strain			
	OS	MA	MT	YO
AA	1	5	1	0
AB	1	0	0	0
AC	6	4	0	2
AD	4	3	0	1
AE	0	0	1	0
AF	0	0	1	0
BB	15	0	2	4
BC	4	3	0	4
BD	14	1	0	6
BE	3	0	2	1
BF	0	0	1	0
CC	3	1	7	0
CD	2	4	0	0
CE	2	1	3	3
CF	0	0	0	0
DD	1	0	1	0
DE	0	0	1	0
DF	0	0	1	1
EE	0	0	0	0
EF	0	0	1	0
FF	0	0	0	0

OS, Osaka strain; MA, Masuda strain; MT, Matsue strain; YO, Yokota strain.

Table 3. Gene frequencies in Est-1 loci of DBM.

Strain	No. of individuals	Gene frequency of allele					
		A	B	C	D	E	F
OS	56	0.116	0.464	0.178	0.196	0.045	0
YO	22	0.068	0.432	0.205	0.182	0.091	0.023
MT	22	0.091	0.159	0.386	0.091	0.182	0.091
MA	22	0.386	0.091	0.318	0.182	0.023	0

from southern to northern Japan. However, there is no evidence that populations do migrate. In future, isozyme analysis might be used with DBM to estimate the genetic differences between different populations, and to study the levels of genetic variation and the distribution of allele frequencies in natural populations.

Conclusions

Esterase isozymes were studied on larva, pupa and adult DBM. There were two band groups, a faster band (Est-1) and a slower band (Est-2). Est-1 was used to analyze genetic variations. There were at least six alleles on Est-1 loci. These alleles were useful in analyzing the different gene frequencies in four populations. Est-1 bands could be useful as markers to show characteristics of regional populations. The Osaka population, which is resistant to *B. thuringiensis* might coincide with Est-2 loci.

References

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