

Esterase Isozyme Analysis of Asiatic Corn Borer (*Ostrinia Furnacalis* Guenee) Populations from the Philippines and Japan

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Thirteen genotypes of esterase-1 were observed in three populations of Asiatic corn borer, 11 for the Laguna populations with 5 alleles (t, u, v, w, x), 7 for Leyte with 6 alleles (t, u, v, w, x, y), 7 for Cotabato with 5 alleles (t, u, v, w, x). Only the Leyte population had the y allele.

The Asiatic corn borer populations of Laguna and South Cotabato had heterozygosity values of 0.202 and 0.200 higher than that of Leyte with 0.162. The Laguna population was significantly different from that of Leyte and South Cotabato although the latter two were not significantly different from each other.

The Japanese population of corn borers from one site had six genotypes of esterase-1 with 4 alleles (t, u, v, w) and heterozygosity value of 0.286 and which were different from the genotypes of the Philippine populations.

Keywords: Asiatic corn borer, *Ostrinia furnacalis* Guenee, isozymes, esterase, insect biotypes, maize, corn

The development of insect biotypes or new populations of a species which can survive on previously resistant varieties is an important factor to consider in any breeding program for insect pest resistance. For example, the existence of biotypes of the brown planthopper *Nilaparvata lugens* (Stal) based on differences in varietal reactions has been documented in rice-growing countries (Oka, 1978; Seshu and Kaufmann, 1980; IRRI, 1982), thus explaining the instability of rice resistance against this insect pest. Genetic variation in populations of *N. lugens* and *N. virescens* was also shown by isozyme analysis (Demayo et al, 1989).

In New York State, three races of the European corn borer, *Ostrinia nubilalis* (Hubner) (Pyralidae) have been established to exist; these races are morphologically indistinguishable but have distinct differences in voltinism, host plant range and sex pheromone communication (Brindley and Dicke, 1963; Eckenrode et al, 1983; Roelofs et al, 1985).

The presence of ecotypes of the Asiatic corn borer (*O. furnacalis* Guenee) in different corn-growing areas of the country poses a problem in the

usefulness of a resistant cultivar especially if the cultivar was exposed to a different ecotype and bred in a different region. Initial studies by Barrion et al (1981) showed that corn borer (fifth instar larvae) from Musuan, Bukidnon were significantly different in their abdominal chaetotaxy from the Laguna populations (Bay and Los Baños towns). Barrion et al (1980) also observed diffused centromeres of the chromosomes and the extraordinary wide scattering of the frequency and erratic haploid chromosome numbers of the corn borer ranging from 20 to 169 with 27 as predominating number. Their observations indicate that the Asiatic corn borer is chromosomally polymorphic with large genetic variability but with little genetic stability.

A powerful tool in assessing variability among natural populations and in determining evolutionary relationship among organisms is gel electrophoresis of proteins or enzymes. This paper presents results of our initial investigation on variations in and among populations of Philippine and Japanese species of the Asiatic corn borer by isozyme analysis.

MATERIALS AND METHODS

Materials

Larvae of Asiatic corn borer were collected from three sites in the Philippines: University of the Philippines Los Banos, Laguna; Visayas State College of Agriculture (ViSCA), Leyte; and University of Southern Mindanao, South Cotabato. In cooperation with Dr. Yoshio Hirai, larvae of Asiatic corn borer were collected at the National Grassland Research Institute in Japan and frozen immediately.

After removing the midgut, the fifth instar larvae were homogenized in cold distilled water (150 μ l per larva) and polyvinylpyrrolidone (PVPP). The resulting crude extracts were kept on ice until use. All chemicals used were of analytical grade.

Electrophoresis

The samples were electrophoresed on 13% starch gel for 4 h at 250-300 V. A total of 10 enzymes were tested by specific chemical stains (Tanksley and Orton, 1983). Three different buffer systems were used at various ionic concentrations and pH: 0.05 M Tris-0.005 M citric acid at pH 6.5, 8.0 and 8.3 for esterase, peroxidase and malate dehydrogenase, and acid phosphatase, respectively; 0.009 M Tris-0.005 M histidine at pH 6.5, 8.0 and 8.3 for succinic dehydrogenase, leucine aminopeptidase and phosphoglucomutase and glutamine-oxaloacetate transferase; and 0.076 M Tris-0.006 M citric acid at pH 6.5 and 8.0 for isocitrate dehydrogenase and alcohol dehydrogenase, respectively.

RESULTS

Variation in Philippine Populations of Asiatic Corn Borer

Of the ten enzymes analyzed, only esterase showed polymorphism using the Tris/histidine buffer system at pH 8.0 in three populations of Asiatic corn borer from Laguna, Leyte and South Cotabato. Genotypes of each of the individual larvae were designated based on the electrophoretic phenotypes observed (Fig. 1a and b). Thirteen different genotypes of esterase were noted in the three populations. Esterase genotypes ww, vv, uw, xx and tw were present in the three populations. A total of 11 genotypes were observed in the Laguna population and 7 for both Leyte and South Cotabato

populations (Table 1). Asiatic corn borer populations of Laguna and South Cotabato had 5 alleles (t, u, v, w, x) while that of Leyte had 6 (t, u, v, w, x, y) (Table 1). Allele y was observed only in Leyte populations (Table 1). The Laguna and South Cotabato populations were more variable than the Leyte populations as shown by higher heterozygosity values (Table 1). More heterozygotes were observed in these two populations.

Homogeneity among the three populations was compared using the allele frequency data of each of the populations (Workman and Niswander, 1970). Significant differences were observed among the populations (Table 2). In particular, the corn borer population of Laguna was significantly different from those of Leyte and South Cotabato but the latter two were not different from each other (Table 2).

Variation in the Population of Asiatic Corn Borer of Toichigi, Japan

Four esterase genes (t, u, v and w) and six genotypes (tt, uu, tv, tu, uv and uw) were observed in a population of Asiatic corn borer (n=42) obtained from the National Grassland Research Institute in Toichigi, Japan (Table 1).

A higher number of individuals carried the uu genotype (17) and tt genotype (13) in this population compared to the Philippine populations of the Asiatic corn borer. The tt individuals were found only in the Laguna population (3) while uu individuals (4) were observed only in the Leyte population (Table 1). The Asiatic corn borer population of Toichigi, Japan differed significantly from any of the three populations from Laguna, Leyte and South Cotabato (Table 2).

DISCUSSION

The above results suggest local genetic differentiation in and among the populations of the Asiatic corn borer. The Laguna population seemed the most differentiated among the three local populations. The data suggest local adaptation of the corn borer population in Laguna. The corn borer population from one site in Japan also showed variation. The esterase genotypes found in the Japanese population at higher frequencies were very different from any of the local populations indicating local differentiation. These results indicate that screening for resistance in maize varieties against the corn borer need to be done at perhaps regional

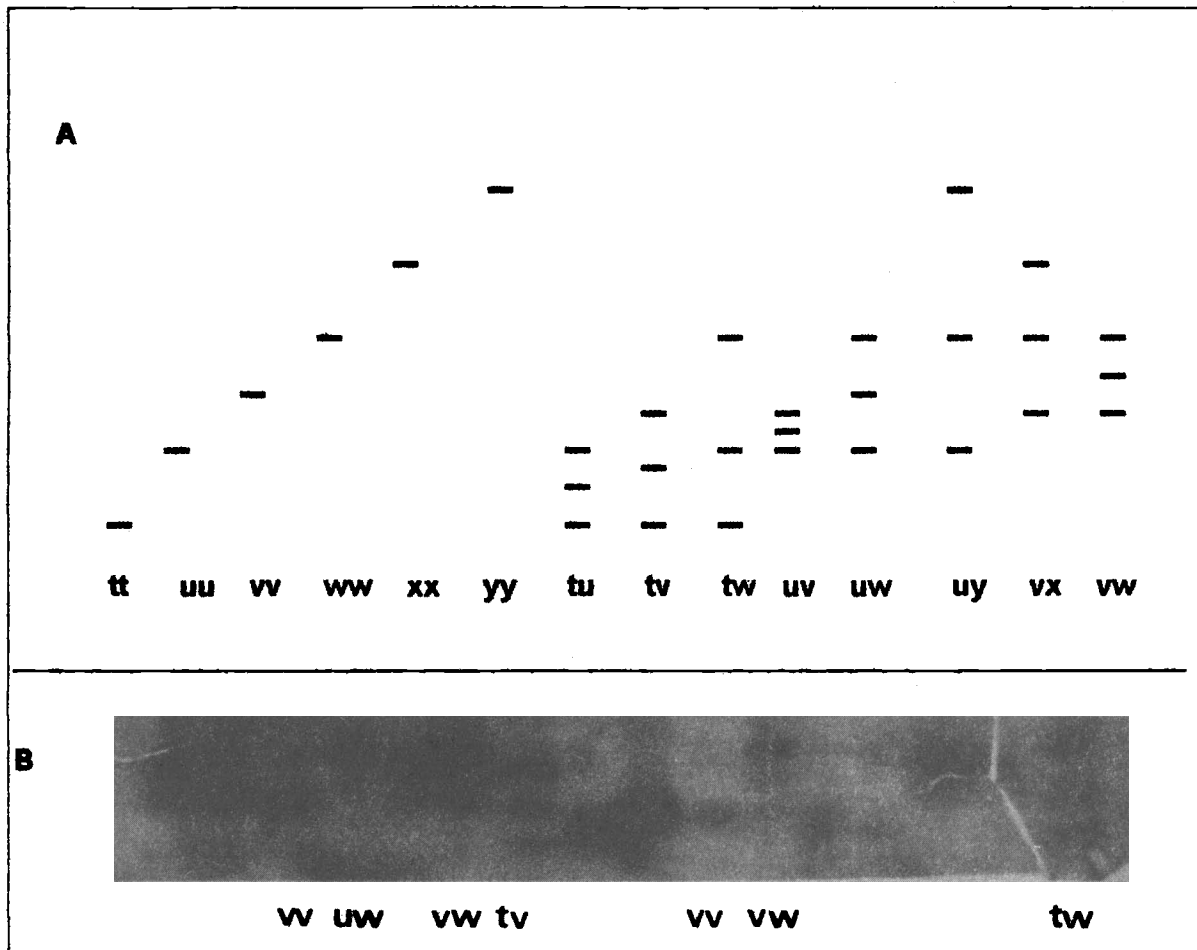


Fig. 1. Schematic diagram (A) and sample electrophoregram (B) of esterase-1 genotypes observed in three local populations of the corn borer, *Ostrinia furnacalis*.

level because of the varying populations of the insect pest. The results also could explain the varying or inconsistent performance of corn varieties against the Asiatic corn borer in different locations in the country.

With the limited sample and sampling strategy, it may not be possible to conclude from the data whether gene flow is occurring. However, the results indicate that the Leyte or South Cotabato population represents the original esterase-genotype composition with Laguna evolving from it. This direction is highly likely due to the presence of relatively higher selection pressures in Laguna, since it has been a center of experimental activities specifically for maize breeding. Using triosephosphate isomerase (TPI) allozyme frequencies of European corn borer moths, Glover et al (1991) obtained evidences supporting the hypothesis that gene flow following hybridization is unidirectional from BE populations into the Z populations. The authors added that the apparent

isolation of the BE race could help explain its more limited geographical range; while Z races predominate over most of the European corn borer range in Europe and North America, the E race is localized in Switzerland, Italy and eastern North America.

To determine if local populations adapt to specific host resources and if gene flow is occurring, it is suggested that the following studies should be further conducted:

1. Genetic variations among local populations of the corn borer (use of wild colony) collected from specific varieties of corn utilizing molecular markers, either enzyme or DNA markers. Assessment can be at both micro and macro-levels. These studies will help assess the extent of genetic variations in natural populations in their utilization of different corn hosts in the field.

2. Assessment of local adaptation in different populations of the corn borer by evaluating the performance of each insect genotype on different

Table 1. Genotypes and gene frequencies in three local populations of the corn borer, *Ostrinia furnacalis*.

Esterase Genotype	Locality			
	Laguna	Leyte	South Cotabato	Japan
ww	56	14	31	0
w	10	5	9	0
uw	2	2	7	0
vx	3	0	4	0
xx	5	8	12	0
vw	5	0	0	1
tv	1	0	0	6
uy	0	3	0	0
tw	6	1	1	0
tu	1	0	0	4
tt	3	0	0	13
uv	1	0	1	1
uu	0	4	0	17
\bar{H}	0.202	0.162	0.200	0.286
Gene				
w	0.665	0.419	0.538	0.012
v	0.160	0.135	0.177	0.095
x	0.069	0.216	0.215	-
u	0.032	0.176	0.062	0.465
t	0.074	0.014	0.008	0.428
y	-	0.041	-	-

$$X^2 = 160.87^{**}$$

corn hosts. This study is important since in most of the studies conducted on insects, substantial geographical variation in host utilization was observed and seemed to be always associated with host availability. While a species of insects may appear to be generalized in feeding habits, it may actually be composed of populations locally specialized on different host species. Parameters such as evaluation of the performance of the different genotypes of a population (morphometric differentiation, life table analysis, survivorship, larval and pupal weight and development time) on different corn hosts should be studied. It is important that populations collected will be reared for one generation in a common host prior to testing on different hosts. Repeated measure analysis of performance tests should be done.

CONCLUDING REMARKS

The above results, indicate wide variations among and within three local populations of the Asiatic corn borer as well as one population obtained from Japan by esterase isozyme analysis. They support earlier although limited studies by Barrion et al (1980, 1981) showing wide genetic variation among and within local populations of the Asiatic corn borer using cytological and morphometric measurements. More comprehensive and detailed studies are needed for a definitive study of the various biotypes or ecotypes of the Asiatic corn borer and their interactions with corn varieties to guide in the proper development of resistant varieties and appropriate pest management strategies.

Table 2. Homogeneity tests among the gene frequencies between the three local populations of the Asiatic corn borer, *Ostrinia furnacalis*.

	Laguna	Leyte	South Cotabato	Tochigi, Japan
Laguna	-	15.75**	7.41**	131.27**
Leyte		-	3.65 ns	58.21**
South Cotabato			-	103.09**
Tochigi, Japan				

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