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Allozyme Variation Among Biotypes of the Brown Planthopper *Nilaparvata lugens* in the Philippines

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Allozyme variation was studied in three Nilaparvata lugens *biotypes infesting specific rice varieties and a biotype infesting a weed grass,* Leersia hexandra. *Of the 20 enzymes in* N. lugens *for which activity was noted, 9 were polymorphic. Eleven enzyme loci were monomorphic for the same allele in all biotype populations; the rest were polymorphic for two or more alleles. The mean number of alleles per polymorphic locus was 2.3, while the mean number of alleles per locus was 1.5; heterozygosity ranged from 0.02 to 0.06 (biotype 1 > biotype 3 >* Leersia*-infesting biotype > biotype 2). Allelic frequency differences were observed in five loci among the four biotypes. However, the coefficient of genetic identity (I) of 0.99+ showed that the four biotype populations were genetically close relatives or merely populations of* N. lugens *undergoing genetic differentiation.*

KEY WORDS: *Nilaparvata lugens,* allozyme variation, biotypes, brown planthopper

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (Stål), is a major pest of rice in tropical Asia. It causes "hopperburn" and complete wilting and drying of rice plants (Dyck and Thomas, 1979) and also transmits the grassy stunt and ragged stunt viral diseases (Ling, 1977). Large-scale rice crop damage caused by the pest was reported in the 1970s in several South and Southeast Asian countries (Dyck and Thomas, 1979).

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Host plant resistance has played a key role in the management of *N. lugens.* However, the stability of resistant varieties is threatened by the occurrence and evolution of insect pest populations capable of surviving on and damaging the varieties with known genes for resistance. In the case of *N. lugens,* these populations are known as biotypes. Studies conducted at IRRI in the Philippines have demonstrated the existence of three rice-infesting biotypes based on differential varietal reactions. Biotype 1 damages and survives on varieties without any genes for resistance to *N. lugens,* biotype 2 thrives on varieties carrying the *Bph* 1 resistance gene and on those susceptible to biotype 1, and biotype 3 can multiply on varieties having the *bph* 2 resistance gene and on those susceptible to biotype 1 (Seshu and Kaufman, 1980; Saxena and Barrion, 1985). Biotypes 1, 2, and 3 do not attack rice varieties with resistance genes *Bph* 3 and *bph* 4 and Ptb 33 carrying two resistance genes. Recently, another *N. lugens* population was found to infest a weed grass, *Leersia hexandra* Swartz, that grows abundantly in canals near irrigated rice fields in Southeast Asia (Domingo *et al.,* 1983; Sogawa *et al.,* 1984). The *Leersia*-infesting *N. lugens* population fails to survive on rice plants. Conversely, rice-infesting *N. lugens* biotypes do not thrive on *Leersia.* The differences in host-plant affinity are presumed to be genetically determined. Studies of hybridization between *N. lugens* biotypes showed differences in crossability (Saxena and Barrion, 1985).

Genetic differences and variability can be assessed precisely by analyzing allozyme variations (Lewontin, 1974; Ayala *et al.,* 1972). We therefore studied the genetic differentiation in *N. lugens* biotypes by allozyme analysis.

MATERIALS AND METHODS

Adults of rice-infesting *N. lugens* biotypes 1, 2, and 3 and *Leersia*-feeding *N. lugens* were frozen at −70°C for at least 30 min prior to use. Individual insects were ground and homogenized in wells of a spot plate in 15 µl of a buffer solution (0.0086 M Tris–0.0046 M histidine, *p*H 8), using a glass rod. Small pieces (4 × 9 mm) of Whatman filter paper No. 3 were used to adsorb the homogenates and were inserted directly into starch gel slots. Horizontal starch gel electrophoresis was conducted at 4°C and 40 mA/gel slab. The starch gel was prepared using 14% starch (Sigma, St. Louis, Missouri) and 0.0086 M Tris–0.0046 M histidine buffer (*p*H 4.8). After electrophoresis, the gels were sliced and stained, following the procedure of Shaw and Prassad (1970).

Each gene locus was analyzed based on the number of (a) genes sampled, (b) alleles, and (c) bands in the heterozygotes and on whether the locus was polymorphic or monomorphic. Heterozygosity at each locus was estimated by direct counting of heterozygotes (Ho).

The goodness of fit of observed genotypic proportions to expected proportions was tested by chi-square (χ^2) analysis performed for each of the polymorphic loci tested. Heterogeneity of gene frequencies among biotypes was tested using the method of Workman and Niswander (1970). Genetic similarity among biotype populations was measured by the coefficient of genetic identity (Nei, 1972).

RESULTS AND DISCUSSION

Of the 20 enzyme systems in *N. lugens,* 9 were found to be polymorphic ($P = 0.01$) (Table I). Some of these polymorphic loci are shown in Fig. 1. Except for acid phosphatase (Acp), tetrazolium oxidase-1 (To-1), and malate dehydrogenase-1 (Mdh-1), which migrated cathodally, others migrated anodally.

Eleven enzyme loci were monomorphic for the same allele in all biotype populations. The rest were polymorphic for two or more alleles. The variability of main bands was explained by the polymorphism of one gene coding for dimeric enzymes, such as alkaline phosphatase (Alkp), isocitric dehydrogenase (Idh), malate dehydrogenase (Mdh), and phosphoglucose

Table I. Number of Presumptive Loci in Different Enzyme Systems of *N. lugens*

Enzyme	IUB No.[a]	Migration	Locus	Polymorphism
Alkp	3.1.3.1	Anodal	1	Yes
Ak	2.7.4.3	Anodal	1	Yes
Cat	1.11.1.6	Anodal	1	Yes
Est	3.1.1.1	Anodal	4	Yes
Idh	1.1.1.42	Anodal	1	Yes
Mdh	1.1.1.37	Anodal	1	Yes
		Cathodal	1	No
Me	1.1.1.40	Anodal	1	Yes
Pgm	2.7.5.1	Anodal	1 or 2	Yes
Pgi	5.3.1.9	Anodal	2	Yes
Acp	3.1.3.2	Cathodal	1 or 2	No
Lap	3.4.11.1	Anodal	1 or 2	No
Ald	4.1.2.13	Anodal	1	No
Glydh	1.1.1.8	Anodal	1	No
Xdh	1.1.1.204	Anodal	1	No
Gk	2.7.1.2	Anodal	1	No
Nodh		Anodal	1	No
Hk	2.7.1.1	Anodal	1	No
G-6pd	1.1.1.49	Anodal	1	No
6-Pgd	1.1.1.43	Anodal	1	No
To	1.15.1.1	Anodal	1	No
		Cathodal	1	No

[a]IUB, International Union of Biochemistry classification of the enzyme.

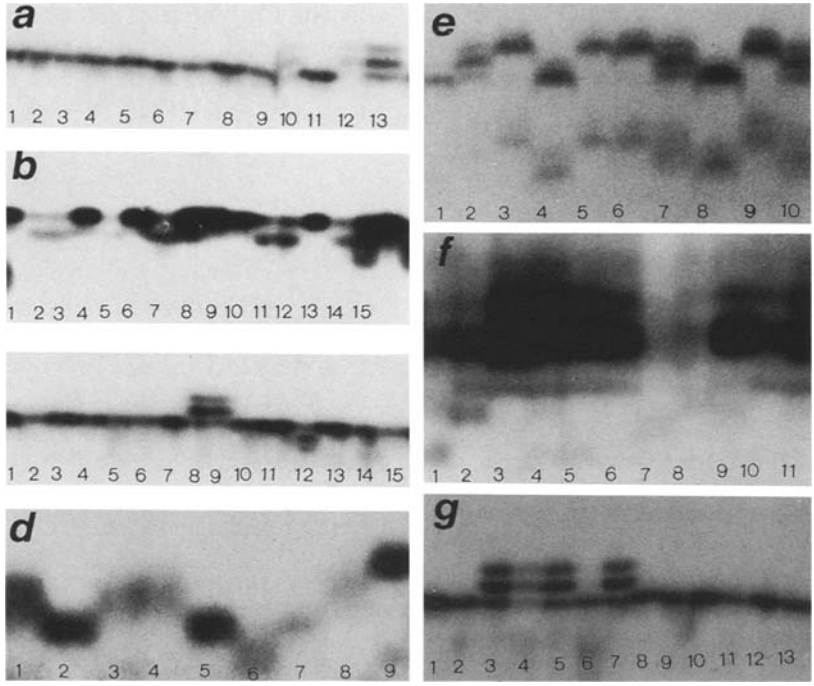

Fig. 1. Electrophoretic phenotypes of (left to right) (a) Alkp—1–9, 11 (100), 10, 12, 13 (100/103); (b) Ak—1, 4, 6, 9, 10, 13 (106/106), 2, 3, 7, 8, 11, 12, 14, 15 (106/106); (c) Idh—1–7, 10–11, 13–15 (100/100), 8, 9 (100/103), 12 (97/103), 12 (97/103); (d) Me—1, 3, 4, 8 (103/103), 2, 5, 7 (100/100), 9 (106/106); (e) Pgi—1, 4, 8 (100/100), 3, 5, 6, 9 (105/105), 2, 7, 10 (100/105); (f) Est—Est-1, 1 (100/100), Est-2, 1, 3–6 (100/100), 2, 8, 9 (97/100), 7 (97/97); (g) Mdh—1, 2, 6, 8–13 (100/100), 3–5, 7 (100/109).

isomerase (Pgi), and for one gene coding for a monomeric enzyme, such as adenyl kinase (Ak). This was also confirmed by two indirect lines of evidence. (1) The inferred heterozygote genotype for a particular locus displayed an intermediate band more intense than both the parental bands for dimeric enzyme and two equally intense bands for the monomeric enzyme Ak. (2) The inferred genotype distributions observed in the populations did not deviate significantly from Hardy–Weinberg equilibrium. Furthermore, the genetic interpretation of the variants was determined by doing single-pair crosses from a sample of the test population and observing the segregation of alleles. The foregoing interpretation of the variants was also based on the principles described by Harris (1980) and on the published enzyme structure (Klotz, 1967; Darnal and Klotz 1975). Rare alleles were observed, but their genetic control was not investigated.

The *Pgi* gene had two loci, one of which had three allelles: Pgi^{95}, Pgi^{100},

and Pgi^{105}, which were correspondingly designated 1, 2, and 3. The *Mdh* gene also had two loci; one was anodal and the other was cathodal. The cathodal form was monomorphic, while the anodal form had two allelles, Mdh^{100} and Mdh^{109}, designated 2 and 3. The *Alkp* gene had three allelles, designated $Alkp^{97}$, $Alkp^{100}$, and $Alkp^{103}$. The *Ak* gene had two allelles, Ak^{100} and Ak^{106}, designated 2 and 3. The *Idh* gene had three allelles, Idh^{100}, Idh^{97}, and Idh^{103}, designated 1, 2, and 3. The *Est* gene had four loci, all of which were polymorphic. However, due to some difficulty in the genetic analysis of the patterns, this was not included in the computation of heterozygosity values. This was also true with *Me* and *Pgm*, which showed the existence of several electrophoretic phenotypes.

The amount of genetic variation in the four biotypes of *N. lugens* is given in Table II. The mean number of alleles per polymorphic locus (K_p) ranged from 2.0 (rice-infesting biotype 2) to 2.4 (biotypes 1 and 3 and *Leersia*-infesting biotype). Also, the mean number of alleles per locus (K) ranged from 1.40 to 1.53, respectively. The observed heterozygosity (H_o) was highest in biotype 1 (0.062), followed by biotype 3 (0.041) and the *Leersia*-infesting biotype (0.036), and lowest in biotype 2 (0.019).

Genetic differentation between biotype populations was determined by chi-square analysis of variations in genic proportion among biotypes (Workman and Niswander, 1970). The chi-squares from independent comparisons, i.e. independent loci, were combined to provide an overall test of heterogeneity. The total chi-squares were significant at 0.01% level (Table III). Specifically, biotypes 1 and 2 significantly differed in all five loci; biotypes 1 and 3 in four loci; biotype 1 and *Leersia*-infesting biotype in five loci; biotypes 2 and 3 in two loci; biotypes 2 and the *Leersia*-infesting biotype in four loci; and biotypes 3 and the *Leersia*-infesting biotype in four loci (Table IV).

Our findings complement earlier studies on *N. lugens*–host plant interactions, such as differential varietal reactions (Seshu and Kaufman, 1980),

Table II. Percentage of Polymorphic Loci (P), Mean Number of Alleles per Polymorphic Locus (K_p), Mean Number of Alleles per Locus (K), and Observed Heterozygosity (H_o)

Biotype[a]	P[b]	K_p	K	H_o
B1	0.52	2.4	1.53	0.062
B2	0.48	2.0	1.40	0.019
B3	0.52	2.4	1.53	0.041
BL	0.48	2.4	1.53	0.036
Mean	0.53	2.3	1.50	0.039

[a]B1, B2, B3, and BL—biotypes 1, 2, and 3 and the *Leersia*-infesting biotype, respectively.
[b]Included are the 11 monomorphic loci studied.

Table III. Allele Frequencies in Five Protein Loci of *N. lugens* Biotype Populations

Locus	Biotype[a]	Genes (No.)	Allele frequency 1	2	3	χ^{2b}	df
Ak	B1	96	0.224	0.776	—	24.6*	3
	B2	77	0.072	0.928	—		
	B3	235	0.228	0.772	—		
	BL	188	0.147	0.853	—		
Alkp	B1	258	0.160	0.838	0.002	72.44*	3
	B2	109	0.023	0.977	0		
	B3	249	0.024	0.974	0.002		
	BL	164	0.107	0.888	0.006		
Idh	B1	322	0.093	0.907	0	79.88*	3
	B2	101	0	1.000	0		
	B3	228	0.011	0.989	0		
	BL	254	0.061	0.856	0.083		
Mdh	B1	238	0.954	0.046	—	30.72*	3
	B2	189	0.990	0.010	—		
	B3	149	0.991	0.041	—		
	BL	200	1.000	0	—		
Pgi-2	B1	138	0.018	0.583	0.398	195.04*	6
	B2	378	0.012	0.904	0.084		
	B3	102	0.005	0.868	0.128		
	BL	333	0.047	0.668	0.285		

[a]B1, B2, and B3—biotypes 1, 2, and 3, respectively; BL—*Leersia*-infesting biotype.
[b]Test for the homogeneity of gene frequencies among biotype populations based on the method of Workman and Niswander (1970).
*Significant at the 0.01 probability level.

Table IV. Results of Homogeneity Tests[a] of Gene Frequencies Between *N. lugens* Biotype Populations at Five Polymorphic Loci Indicating the Level of Significant Heterogeneity

Biotype[b]	Locus *Ak*	*Mdh*	*Pgi-2*	*Alkp*	*Idh*
B1, B2	14.97**	9.36**	144.67**	27.91**	20.22**
B1, B3	0.01 (NS)	5.68*	45.31**	54.41**	32.33**
B1, BL	5.27*	18.87**	14.29**	3.95*	7.23**
B2, B3	18.38**	0.018 (NS)	4.31*	0.06 (NS)	2.24 (NS)
B2, BL	5.61*	4.02*	120.43**	14.89**	32.43**
B3, BL	8.83**	3.61 (NS)	31.66**	26.28**	57.07**

[a]Based on comparison of allelic frequencies according to the method of Workman and Niswander (1970). NS, not significant.
[b]B1, B2, and B3—biotypes 1, 2, and 3, respectively; BL—*Leersia*-infesting biotype, respectively.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.

host-mediated behavioral and physiological responses, morphological differences, cytological behavior, and crossability differences (Saxena and Barrion, 1985). Accumulation of host-adapted gene complexes in the pest may have led to differentiation of populations on rice varieties with genes for resistance and on the nonrice host, *L. hexandra.* This probably is conducive for genetic events likely to be involved in speciation (White, 1978).

The polymorphism among phytophagous insects associated with host plants is considered to represent the first stage of the process of sympatric speciation (Malavasi and Morgante, 1983). Epidemic infestations of rice cultivars with known genes for resistance to *N. lugens* may have led to possible founding populations by genotypes that were not random samples of the original population. This kind of situation may lead to rapid speciation of groups not specifically adapted to specific environments (Templeton, 1980a,b). Should these populations be substructured along host lines, a host race or biotype may become a new species preadapted for life in its new habitat. This is a probable case for *N. lugens,* which has succeeded in establishing itself not only on resistant rices but also on *L. hexandra.* Based on differences in acoustical behavior and host-plant affinity, the *Leersia*-feeding *N. lugens* has been considered to be a separate species (Claridge *et al.,* 1985, 1988; Claridge and Morgan, 1987).

However, while statistical differences in allelic frequencies among the four sympatric *N. lugens* biotypes (Tables III and IV) were observed, the coefficient of genetic identity showed that they are closely related to each other (Table V). The calculated value of $I = 0.99+$ for *N. lugens* was typical for different populations of the same species of organisms investigated to date as in *Drosophila* (Ayala *et al.,* 1972). Similar results were obtained for 11 populations of a fruit fly, *Anastrepha fraterculus* Wied., found thriving on eight different hosts (Malavasi and Morgante, 1983). Genetic distance

Table V. Genetic Identity (*I*) Values of Five Polymorphic Loci Between Biotypes of *N. lugens*

	Biotypes					
Locus	B1, B2	B1, B3	B1, BL	B2, B3	B2, BL	B3, BL
Ak	0.979	1.000	0.994	0.978	0.996	0.993
Alkp	0.986	0.987	0.998	1.000	0.995	0.995
Idh	0.995	0.996	0.995	1.000	0.993	0.994
Mdh	0.999	0.999	0.999	0.995	1.000	0.995
Pgi-2	0.874	0.899	0.980	0.999	0.951	0.966
			$I^{b}_{xy} = 0.99^{+}$			

[a]B1, B2, and B3—biotypes 1, 2, and 3, respectively; BL—*Leersia*-infesting biotype.
[b]Mean genetic identity comparisons between the biotypes all exceeded 0.99+, including the 11 monomorphic loci investigated.

($D = 0.002$) was small and heterozygosity was also low ($H = 0.05$). Likewise, electrophoretic survey of populations of the pea aphid, *Acyrtosiphon pisum* (Harris), disclosed a lack of variability in clones collected over a wide geographical range but which comprised recognized biotypes (Simon *et al.,* 1982). Early stages of species differentiation may not be associated with substantial genetic change (Prakash *et al.,* 1969; Lewontin, 1974; Bush, 1975; Harrison and Vawter, 1977). No distinct electrophoretic differentiation between *Lethe eurydice* L. and *Lethe appalacia* L. was found, although the two species were found to be good species (Harrison and Vawter, 1977; Cardé *et al.,* 1978). Differentiation of the two species may have resulted from single-gene substitutions or chromosomal rearrangements, provided that these changes act to interrupt the gene flow between populations (Bush, 1975). Similar genetic events may have led to the evolution of *N. lugens* biotypes which were observed to have considerable differences in cytological behavior and crossability (Saxena and Barrion, 1985). In another study, we have analyzed the allozyme variation of *N. lugens* populations collected from different geographical locations in the Philippines (Demayo *et al.,* 1990).

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