

## ALLOZYME VARIATION IN LOCAL POPULATIONS OF THE BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (STAL) IN THE PHILIPPINES<sup>1</sup>

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### ABSTRACT

*N. lugens* from 10 localities in the Philippines were assayed, compared, and determined to have significant differences in allelic frequencies at 4 polychromatic gene loci coding for soluble enzymes. Based on Shannon information index (H) and Nei's modification of Wright's F-statistics, most of the genic diversity among populations of *N. lugens* existed within-subdivisions. An average of 9% of the total gene diversity was attributed to between group diversity.

**Key words:** Allozyme variation, electrophoresis, *Nilaparvata lugens*.

### INTRODUCTION

Rice is the world's most important staple food crop being utilized by approximately half of the population on earth (Kush and Coffman, 1977). However, rice yield is often reduced because of the existence of numerous species of insect pests that feed on it. Among insects, the brown planthopper, *Nilaparvata lugens* (Stal), is highly destructive. The insect not only causes complete wilting and drying of rice plants (Dyck and Thomas, 1979) but also transmits the grassy stunt and ragged stunt virus diseases (Ling 1972, 1977). Epidemics of these virus diseases have followed major outbreaks of *N. lugens*. The pest is widely distributed - from India and Pakistan in the West, to Japan and Korea in the North, to the Solomon Islands and Fiji in the East, and to North Australia in the South (Saxena and Barrion, 1985).

Variations among *N. lugens* populations collected from different areas in Southeast Asia have been reported (Claridge *et al.*, 1981). Comparisons of the Australian population with other geographically distant populations of *N. lugens* revealed differences in terms of morphology, biochemistry (electrophoresis), mating behavior, and hybridization. At IRRI in the Philippines, different populations with distinct patterns of virulence on resistant rice varieties have been isolated and termed biotypes (IRRI, 1976; Pathak and Saxena, 1980). Differences in pest behavior and physiology in response to plants of known genotypes, pest morphometrics, and chromosome behavior were observed (Saxena and Rueda 1982, Saxena and Barrion 1985).

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The above studies suggest adoption and possible genetic variation in *N. lugens*. We therefore investigated the gene pools of *N. lugens* populations sampled from ten different localities in the Philippines.

### MATERIALS AND METHODS

*N. lugens* adults were collected from 10 localities in the Philippines: four localities in Luzon -- Albay, Camarines Sur, Isabela and Laguna; three localities in Mindanao -- North and South Cotabato, and Zamboanga del Sur; and islands of Mindoro, Negros Occidental, and Palawan. The insects were brought to the laboratory and assayed by electrophoresis. Polymorphic loci were determined and their inheritance investigated by single pair crosses. Rare alleles were also observed but their inheritance was not studied. Phenotypes and gene frequencies of the four loci investigated were determined from starch gel on single hopper homogenates. Chi-square tests were performed to compare observed numbers of phenotypic classes with those expected under equilibrium conditions. The principal component method (Workman and Niswande, 1970) was used to analyze the gene frequency data. Genetic variability among populations was partitioned using Wright's modified F-statistics (Nei 1977) and Shannon information index (H) (Lewontin, 1972).

### RESULTS AND DISCUSSION

The genetic structures of field populations of *N. lugens* collected from 10 different localities in the Philippines (Table 1) were determined and compared with

Table 1. Localities where *N. lugens* were collected for electrophoretic analysis.

Localities	Number of <i>N. lugens</i> specimens sampled (no.)
Albay	111
Camarines Sur	45
Isabela	96
Laguna	92
Mindoro	120
Negros Occidental	158
North Cotabato	132
South Cotabato	233
Palawan	109
Zamboanga	92

each other based on four polymorphic gene loci, namely: alkaline phosphatase (Alkp), adenyl kinase (Ak), isocitric dehydrogenase (Idh), and malate dehydrogenase (Mdh). The genetic basis of the alleles observed among the four loci was based on single pair crosses (Table 2).

Table 2. Segregation in different crosses made to determine the inheritance of Ak, Alkp, Idh, and Mdh variation in *N. lugens*.

Cross (P <sub>1</sub> X P <sub>2</sub> )	GENOTYPE OF PROGENIES (F <sub>1</sub> )					X <sup>2</sup>
	Ak					
	100/100	91/91				
Ak <sup>100/100</sup> x Ak <sup>100/100</sup>	53(53)	-				ONS
Ak <sup>91/91</sup> x Ak <sup>91/91</sup>	-	57(57)				ONS
	Alkp					
	97/87	97/100	100/100	100/103	103/103	X <sup>2</sup>
Alkp <sup>100/100</sup> x Alkp <sup>100/100</sup>	-	-	53(53)	-	-	ONS
Alkp <sup>100/100</sup> x Alkp <sup>100/103</sup>	-	-	33(35)	37(35)	-	ONS
Alkp <sup>97/97</sup> x Alkp <sup>100/100</sup>	-	48(48)	-	-	-	ONS
	Idh					
	100/100	93/100	93/93			X <sup>2</sup>
Idh <sup>100/100</sup> x Idh <sup>100/100</sup>	97(97)	-	-			ONS
Idh <sup>100/100</sup> x Idh <sup>93/93</sup>	-	53(53)	-			ONS
Idh <sup>100/100</sup> x Idh <sup>93/100</sup>	37(35)	33(35)	-			0.2 NS
	Mdh					
	100/100	100/109	109/109			X <sup>2</sup>
Mdh <sup>100/100</sup> x Mdh <sup>100/100</sup>	177(177)	-	-			ONS
Mdh <sup>100/100</sup> x Mdh <sup>100/109</sup>	39(48)	58(48)	-			3.7NS
Mdh <sup>100/109</sup> x Mdh <sup>100/109</sup>	24(22)	43(43)	20(22)			0.3NS

Significant deviations from expected proportions were observed in allele frequencies for Ak and Idh loci. For instance, the Mindoro population of *N. lugens* had significant deviation in allele frequencies for Ak, while the populations from Albay, North Cotabato, and South Cotabato had allele frequencies for Idh which deviated significantly from expected proportions (Tables 3-6).

In the Alkp locus, five phenotypes with 3 alleles designated as 97, 100, 103 -- were observed. The most common allele was Alkp<sup>100</sup> with frequency ranging from 0.928 to 1.0. The populations from Laguna and Zamboanga del Sur were monomorphic for the most common allele Alkp<sup>100</sup>, while others were polymorphic for 2 to 3 alleles (Table 3). No population was observed to deviate from Hardy-Weinberg equilibrium condition when the frequency of the expected genotypes were compared with the observed. Geographic variation in allelic frequencies for Alkp locus was significant ( $\chi^2 = 47.84$ ,  $P < 0.001$ ,  $df = 16$ ) (Table 3).

Table 3. Allele frequencies for Alkp locus in local populations of *N. lugens*.

LOCALITY	ALLELES			GENES (no.)	X <sup>2a</sup>	SIGNIFICANCE
	97	10	103			
Albay	0.024	0.976	-	42	0.020	0.89
Isabela	-	0.956	0.043	48	0.100	0.75
Laguna	-	1.0	-	91	0	
Mindoro	0.007	0.964	0.029	70	0.094	0.76
Negros	0.050	0.928	0.022	88	2.856	0.09
Occidental North Cotabato	0.033	0.967	-	30	0.030	0.86
Palawan	0.0085	0.983	0.0085	59	0.020	0.89
South Cotabato	0.004	0.988	0.008	133	0.378	0.54
Zamboanga	-	1.0	-	92	0	
$X^{2b} = 47.841$ , $P < 0.001$ , $df = 16$						

<sup>a</sup> Tests the goodness-of-fit of observed genotypic proportions to the proportion expected according to Hardy-Weinberg Law

<sup>b</sup> Tests the homogeneity of gene frequencies among *N. lugens* populations using the method of Workman and Niswander (1970)

The allele frequencies in the Ak locus in seven *N. lugens* populations are presented in Table 4. Five alleles were observed and designated based on their relative distances from their most common allele Ak<sup>100</sup>. Frequencies of the most common allele ranged from 0.633 to 1.0, while rare alleles ranged from as low as 0.004 to as high as 0.361. Only one population, the Mindoro population, showed significant deviation from equilibrium. Geographic variation was observed in allele frequencies in this locus ( $X^2 = 391.36$ ,  $P < .001$ ,  $df = 24$ ).

Table 4. Allele frequencies in the Ak locus in local populations of *N. lugens*.

LOCALITY	ALLELES					GENES (No.)	X <sup>2a</sup>	SIGNIFI- CANCE
	94	97	100	103	106			
Albay	0	0.361	.633	0	0.006	93	1.99	0.16
Camarines Sur	0	0.033	.889	0.078	0	45	0.71	0.40
Mindoro	0.020	0.259	.721	0	0	120	0.509	0.02
Negros Occidental	0.020	0	.976	0	0.004	128	0.081	0.78
North Cotabato	0.008	0	.988	0.004	0	129	0.021	0.88
Palawan	0	0	1.00	0	0	100	0	
South Cotabato	0	0	1.00	0	0	34	0	
$X^{2b} = 391.36$ , $P < 0.001$ , $df = 24$								

<sup>a</sup>Tests the goodness-of-fit of observed genotypic proportions expected according to Hardy-Weinberg Law

<sup>b</sup>Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Idh locus was the most polymorphic locus in *N. lugens* since 6 alleles were observed. The most common allele was idh<sup>100</sup>; its frequency ranged from 0.625 in South Cotabato to 1.0 in Camarines Sur, and Zamboanga del Sur. The frequency of rare alleles varied, ranging from as low as 0.006 in Negros Occidental to 0.186 in South Cotabato. The expected genotype frequencies at Hardy-Wienberg equilibrium obtained from the given gene frequencies differed significantly in these populations (Table 5). Significant variation was observed at this locus among the ten local populations ( $X^2 = 386.65$ ,  $P < 0.001$ ,  $df = 45$ ).

Table 5. Allele frequencies for *Idh* locus in Local *N. lugens* populations.

LOCALITY	ALLELES					GENES (No.)	$X^{2a}$	SIGNIFICANCE
	97	100	103	106	109			
Albay	0.050	0.851			0.099	111	51.170	$7.6 \times 10^{-11}$
Camarines Sur	1.000					45	0	
Isabela	0.021	0.958	0.021			24	0.02	0.89
Laguna	0.005	0.979	0.016			92	0.024	0.88
Mindoro	0.021	0.913	0.008		0.058	120	0.117	0.73
Negros Occidental	0.006	0.994				158	0.081	0.78
North Cotabato	0.023	0.845	0.019	0.019	0.085	132	61.69	$7.6 \times 10^{-11}$
South Cotabato	0.128	0.625	0.037	0.024	0.186	148	21.040	$3.7 \times 10^{-6}$
Palawan	0.037	0.963				109	0.157	0.692
Zamboanga del Sur	1.000					92	0	

$$X^{2b} = 386.655, P < 0.001, df = 45$$

a Tests the goodness-to-fit of observed genotypic proportions to the proportion expected according to Hardy-Weinberg Equilibrium.

b Tests the homogeneity of gene frequencies among *N. lugens* populations using the method of Workman and Niswander (1970).

Table 6. Allele frequencies for Mdh locus in local populations of *N. lugens*.

LOCALITY	ALLELES (No.)		GENES	X <sup>2a</sup>	SIGNIFICANCE
	97	100			
Albay	0.034	0.895	0.073	1.21	0.27
Camarines Sur	0	1.0	0	0	
Negros Occidental	0	1.0	0	0	
Isabela	-	1.0	-	0	
Laguna		0.989	0.011	0.01	0.92
Mindoro	0.042	0.920	0.038	0.89	0.35
North Cotabato	0.034	0.965	-		
Palawan	-	1.0	-	0	
South Cotabato	0.002	0.998	233	0.005	0.94
Zamboanga del Sur	0.006	0.961	92	0.14	0.71

X<sup>2b</sup> = 110.67b, P < 0.001, df = 18

a Tests the goodness-to-fit of observed genotypic proportions expected according to Hardy-Weinberg Equilibrium.

b Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970).

Mdh locus showed as many as 3 alleles. Populations of *N. lugens* from Camarines Sur, Negros Occidental, Isabela, and Palawan were found fixed for the most common allele Mdh<sup>110</sup> (Table 6). The differences between the observed and the expected genotypic values tested using  $X^2$  analysis revealed insignificant variation, suggesting that for this locus all the populations were in equilibrium. However, when frequencies of alleles were compared between populations, significant geographic variations were observed ( $X^2 = 110.67$ ,  $P < 0.001$ ,  $df = 18$ ). Frequency of the most common allele ranged from 0.895 in Albay population to 1.0 in populations from Camarines Sur, Negros Occidental, Isabela, and Palawan.

The total variability observed in *N. lugens* collected from different localities was partitioned into within and between population components. Using the Shannon information index (H) (Lewontin, 1972) and the modification of Wright's F-statistics (Nei, 1977), the variability was found to be within groups. The mean proportions were determined to be 91.86% and 91.02%, respectively (Table 7). Furthermore, the measurement of genetic variation, using the index of genetic similarity (I) (Nei 1972), showed that the I-values ranged from 0.95 to 1.0, indeed very close to unity (Table 8). Strictly these are not "standard genetic distances" (Nei

Table 7. Partitioning of genetic variability in populations of *N. lugens* using the Shannon information index (H) (Lewontin, 1972), and Nei' (1977) modification of F-statistics.

LOCUS	SHANNON INDEX			NEI METHOD			
	Hpop	Hgrp	% Within groups	Ht	Hs	Dst	Gst
ALKP	0.138	0.120	98.20	0.052	0.051	0.001	0.019
AK	0.424	0.266	84.29	0.205	0.165	0.040	0.195
IDH	0.399	0.278	87.86	0.164	0.145	0.017	0.106
MDH	0.143	0.114	97.10	0.053	0.051	0.002	0.039
Mean	0.276	0.195	91.86	0.119	0.103	0.015	0.089
							% = 91.02

where  $n$ :

$H = \sum_{i=1}^n p_i \ln p_i$ , where  $n$  is the number of alleles at a given locus and  $p_i$  is the frequency of the  $i$ th allele

$H_{pop}$  = H value for the whole population

$H_{grp}$  = total gene diversity (subdivided into gene diversity within populations ( $D_{st}$ ))

$G_{st}$  = genetic differentiation among populations

Table 8. Genetic identity/distance<sup>a</sup> among local populations of *N. lugens*.

POPULATION <sup>b</sup>	A	B	C	D	E	F	G	H	I	J
A	..	.957	.997	.997	.978	.999	.984	.998	.999	.983
B	.044	..	.954	.954	.956	.956	.991	.993	.994	.992
C	.003	.047	..	.994	1.0	1.0	1.0	.981	.999	1.0
D	.003	.047	.006	..	.975	.997	.996	.994	.996	.983
E	.022	.045	0	.025	..	.977	.971	.969	.970	.965
F	.001	.045	0	.003	.023	..	.999	.998	.999	.983
G	.016	.009	0	.004	.029	.001	..	.999	1.0	1.0
H	.002	.007	.019	.006	.031	.002	.001	..	.999	.999
I	.001	.006	.006	.004	.031	.001	0	.001	..	.998
J	.017	.008	0	.017	.036	.017	0	.001	.002	..

<sup>a</sup>Genetic identity values above diagonal line; below, genetic distance (Nei, 1972).

<sup>b</sup>Legend:

- A - Albay
- B - Camarines Sur\*
- C - Isabela\*
- D - Laguna\*
- E - Mindoro
- F - Negros Occidental
- G - North Cotabato
- H - South Cotabato
- I - Palawan
- J - Zamboanga del Sur\*

\*Based on three loci.

1977) as they are based on only 4 loci which show genetic variation in one or more natural populations. However, the distances do indicate levels of population differentiation within the species but are not strictly comparable to values determined in other species. It is of interest to point out from the data reported here for the four loci that the common alleles are not necessarily found in higher frequencies and that the frequencies differ between the population in a statistically significant manner. The different *N. lugens* populations seemed to have attained different polymorphic balance equilibrium under natural conditions. This can be explained by a selective process that is acting on this species as has been suggested in *Drosophila* species (Prakash, 1969, Berger, 1970).

Geographically widespread distribution of *N. lugens* conforms with studies on several insect pests which showed genetic differences among different areas of their distribution. The differences observed in the sympatric populations of *N. lugens* as shown by this study presumably must have arisen from degree of isolation by ecological barriers such as resistant hosts or non-host plants, or mere distance with natural selection favoring genotypes in different areas. Isolation of the different *N. lugens* populations is presumed to be a prerequisite for selection to produce statistically demonstrable genetic differentiation, as indicated in this study. Genetic uniformity is always taken to indicate free gene flow among localities and this was not observed in all the populations of *N. lugens* collected from different ricefields in the Philippines. This phenomenon was also observed in studies of *Drosophila pseudoobscura* (Prakash *et al.*, 1969).

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