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ELECTROPHORETIC ANALYSIS OF GENETIC VARIATION IN BROWN PLANTHOPPER *NILAPARVATA LUGENS* (STAL) (HOMOPTERA: DELPHACIDAE) AND GREEN LEAFHOPPER *NEPHOTETTIX VIRESCENS* (DISTANT) (HOMOPTERA: CICADELLIDAE) IN THE PHILIPPINES

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ABSTRACT

Allozyme polymorphisms at 26 gene loci of *N. lugens* and *N. virescens* revealed polymorphism in 11 and 14 loci, respectively. *N. lugens* from 10 localities in the Philippines significantly differ in allelic frequency at 4 polymorphic gene loci. For *N. virescens*, heterogeneity of gene frequencies among 7 local populations was detected at three loci. Partitioning the total variation within and between local populations using the Shannon information index, H and Nei's modification of Wright's F-statistics demonstrated that most of the genetic diversity among population of different localities existed as within-subdivision diversity.

Genetic differentiation of the population susceptible and resistant rice varieties was also observed, emphasizing the importance of host plants as biotic factors associated with the genetic structure of the two species. The potential role of host plants in the process of speciation through biotype or host race formation in *N. lugens* and *N. virescens* is discussed.

Introduction

Host-plant resistance has been used successfully for a number of species of agricultural importance. But sometimes it has been rendered unstable due to the evolution of a pest's nullifying effects on the resistance genes in host plants. Good examples are the two most serious insect pests of rice in tropical Asia, the brown planthopper, *Nilaparvata lugens* (Stal), and the green leafhopper, *Nephotettix virescens* (Distant). *N. lugens* causes "hopperburn" or complete wilting and drying of rice plants (Dyck and Thomas 1979) and transmits the grassy stunt (Ling 1972) and ragged stunt virus diseases (Ling 1967, Ling 1977, Ling *et. al.*, 1981, IRRI 1978). On the other hand, *N. virescens* damages rice plants by excessive feeding and transmission of tungro and other virus diseases (Pathak, 1968).

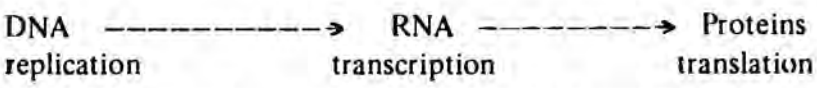
Evolution of insect pest populations which are capable of surviving on and damaging the rice plant with known genes for resistance has been observed in *N. lugens* and are referred to as biotypes. Based on varietal reactions, three rice-infesting biotypes have been recognized at IRRI (Seshu and Kaufmann 1980, IRRI 1976, 1982). Biotype 1 population survives on and damages varieties without genes for resistance; biotype 2 thrives on varieties carrying *Bph* 1 resistance gene, in addition to those susceptible to biotype 1; and biotype 3 can multiply on varieties having *bph* 2 resistance gene, in addition to those susceptible to biotype 1. These three *N. lugens* populations do not damage rice varieties with *Bph* 3 or *bph* 4 resistance gene, and the variety Ptb 33 carrying two unidentified genes. Recently, another biotype infesting a weed grass, *Leersia hexandra* Swartz, was identified on the IRRI farm.

There are *N. virescens* that can survive on and damage varieties of known resistance. Some populations can damage the variety TN1 (lacking resistance gene); others can attack Pankhari 203 (*Glh* 1 resistance gene), IR8 (*Glh* 3 resistance gene), and TAPL (*Glh* 6 resistance gene) (Heinrichs and Rapusas 1984, 1985).

Colonization and subsequent differentiation of insect pests to resistant crops pose problems for plant breeders, chemists, systematists and crop managers. The applied and theoretical aspects of the problem need urgent attention. Failure to recognize the evolution and differentiation of insect pests in nature can have far-reaching and frustrating consequences in pest management (Diehl and Bush 1984).

Studies on genetic differentiation in insect populations infesting rice host plants with known genes for resistance are fundamental in crop and pest management. They provide necessary tools for analyzing pest-cultivar relationships that in turn serve as in programs of breeding pest resistance varieties. Knowledge of the interrelationships between the rice host and pests is helpful to entomologists and plant breeders in combatting pests and at the same time maintaining genetic diversity in rice crop.

In the past, problems were encountered in determining and quantifying the existence of genetic variability of a given population. Traditional methods in genetics led to more questions than answers because traditional genetics only accounted for observable variant traits but not those traits which did not show any variability. However, with the advent of electrophoretic techniques and advances in molecular genetics, most of these problems have been avoided. With electrophoresis, the nature of the genes can be examined by studying the nature of gene-products. The principle follows the concept of the central dogma of molecular biology wherein;



Using electrophoresis, the genotypes of individuals and frequencies of homozygotes and heterozygotes can be determined. The assessment of genetic variation and variability makes use of allozymes (multiple molecular forms of the enzyme coded by alternative forms of a gene) (Lewontin 1974).

The technique has found increasing uses in studies of genetic variation of natural populations (Johnson *et al.*, 1966, Ayala and Powell 1972. Ayala *et al.*, 1972, Selander and Johnson 1973). Among phytophagous insects, genetic variation associated with host plants has been demonstrated within populations of several phytophagous insect species (Edmunds and Alstad 1978, Mitter and Futuyma 1979, Guttman *et al.*, 1981). Genetic differences in host utilization also have been documented in conspecific populations of a few species (Heslop-Harrison 1927, Painter 1951, Dethier 1954, Singer 1971, Knerer and Atwood 1973, Philipps and Barnes 1975, Hsiao 1978, Hsiao and Fraenkel 1968), although with a few exceptions (Hatchett and Gallun 1970, Gould 1979, Mitter *et al.*, 1979).

Using electrophoresis, we studied the genetic variations in *N. lugens* and *N. virescens* populations in the Philippines.

MATERIALS AND METHODS

A. Starch Gel Electrophoresis

Adults of *N. lugens* and *N. virescens* were collected from host plants and stored frozen at - 70°C. Homogenates were prepared by grinding individual insects in wellson a spot-plate with 15ul of homogenizing solution (0.0086M Tris-0.0046M Histidine buffer, pH 8) using a glass rod. Distilled water or 0.1% mercaptoethanol could also be used as a homogenizing solution. Whatman filter paper (No.#3) pieces (eby 9mm) were used to adsorb the homogenates and were inserted directly into the gel slot. Horizontal starch gel electrophoresis was conducted at 4°C and 40mA/ gel slab. The starch gel was prepared using 14% starch (SIGMA) and 0.0086M tris - 0.0046M histidine buffer, pH 8. After electrophoresis, the gels were sliced horizontally and stained following the procedures of Shaw and Prassad (1970) and Brewer (1970).

The genetic basis of biochemical variants was tested by single pair crosses from a sample of the two pest populations. However, in cases where this was not done, the genetic interpretation of the electrophoretic pattern was based on the principles described by Harris (1980), Harris and Hopkinson (1976), and also the published enzyme structure by Klotz (1967), Darnall and Klotz (1975) and Ward (1977).

Each gene locus was analyzed based on the number of genes sampled, the number of alleles, the number of bands in the heterozygotes, and whether the locus was polymorphic or monomorphic.

B. Statistical Analyses

Heterozygosity at each locus was estimated by direct count of heterozygotes (HO) and also by calculating the frequency of heterozygotes expected (He) at

Hardy-Weinberg equilibrium ($He = 1-\Sigma pi^2$), where pi was the estimated frequency of the ith allele in the population).

The goodness-of-fit of observed genotypic proportions to expected proportions was tested by Chi-square (X^2) analysis performed for each of the polymorphic loci tested. When more than two alleles were detected at a locus, the genotypes were pooled into three classes (homozygotes for the commonest allele, heterozygotes for the commonest and another allele, and all other genotypes) to circumvent problems in the Chi-square test when expected frequencies of some classes of genotypes were low. Average deviations of genetic proportions from expected values were estimated using fixation index (Fis) (Wright, 1969). Heterogeneity of gene frequencies among host plants was tested using the method of Workman and Niswander (1970). The formula for computing the Chi-square statistics was as follows:

$$X^2 = \frac{\Sigma(2Ni)\ pi^2 - p\Sigma(2Ni)pi}{\bar{p}\bar{q}}$$

where p and q denote the weighted means of the alleles pi and qi (i.e.,$p = \Sigma((Ni/N)$ pi).

In general, if there are k alleles at a locus, the X^2 value for the corresponding r x k contigency table is given $X^2 = 2N$ (oj/pj), where pj and oj denote the mean and variance of the frequencies of the jth allele. Thus the genic contingency X^2 is a function of the total sample size and the mean and variance of the gene frequencies.

Two methods were employed in partitioning the amount of genetic variation among populations of the two species. These were the Shannon information index, H (Lewontin 1972) and Nei's (1977) modification of Wright's F-statistics. Genetic similarity among insects collected from their different hosts was measured by the coefficient of genetic identity (Nei 1972), where I = Jxy/jxjy and the genetic distance $D = -\log_e$. The key to these two formulae is given as: xi = frequency of the ith allele in population X; yi = frequency of the ith allele in population Y; $jx = \Sigma xi^2$; $jy = \Sigma yi^2$; $jxy = \Sigma xiyi$; Jx, Jy, Jxy are arithmetic means of jx,jy and jxy; ly = normalized identity of genes with respect to one locus; I = normalized identity of genes with respect to all loci; D = genetic distance between X and Y.

RESULTS

I. Genetic Variation in *N. lugens*

Starch gel electrophoresis of 43 enzyme synthesizing *loci* of *N. lugens* revealed 26 active loci in which 11 were polymorphic, while the rest were monomorphic.

The genetic structures of *N. lugens* populations sampled from 10 localities in the Philippines, namely: Albay, Camarines Sur, Isabela, Laguna, Mindoro, Negros

Occidental, North Cotabato, Palawan, South Cotabato and Zamboanga del Sur were investigated. The determinations of allelic frequencies of 6 populations were concentrated on four polymorphic loci such as adenyl kinase (AK), alkaline phosphatase (ALKP), isocitric dehydrogenase (IDH) and malate dehydrogenase (MDH). The allele frequencies in the AK locus of *N. lugens* from the 3 localities of Isabela, Laguna and Zamboanga were not determined yet.

Comparison of the genetic structure of the 10 local populations of *N. lugens* in the Philippines revealed significant deviations from expected proportions at one or more sites in 2 of the 4 loci investigated (Tables 1 to 4). Significant deficiencies in heterozygotes were seen in ALKP for one population (Table 1) and in IDH for 3 populations (Table 2). Significant heterogeneity of gene frequencies among localities was found in all four loci (Tables 1 to 4). Partitioning the total variation within and between populations using Shannon information index, H and Nei's modification of Wright's F-statistics revealed that most of the variability occurred within populations (= 91%) (Table 5).

N. lugens from different rice hosts TN1, Mudgo, ASD7. amd *L. hexandra* were not well-differentiated; coefficient of genetic identity exceeded 0.99. However, when loci were analyzed individually, *N. lugens* from TN1, Mudgo, ASD7, and *L. hexandra* Swarta were significantly differentiated in five loci (Table 6). TN1 and Mudgo populations were differentiated in all five loci (Table 6). TN1 and Mudgo populations were differentiated in all five loci, TN1 and ASD7 populations in three loci, TN1 and *L. hexandra* populations in four loci, Mudgo and ASD7 populations in two loci, Mudgo and *L. hexandra* populations in four loci, ASD7 and *L. hexandra* populations in five loci (Table 7).

II. Genetic Variation in *N. virescens* Populations

Allozyme polymorphisms at 26 enzyme loci of *N. virescens* revealed that 14 out of the 26 possessed more than one allelic form. Four loci (ALKP, EST-A, IDH, and MDH) were investigated in 7 local populations of *N. virescens* sampled from Camarines Sur, Isabela, Laguna (IRRI and Pila), North Cotabato, Quezon and Zamboanga del Sur. Significant deviations from Hardy-Weinberg equilibrium were observed in 3 loci (MDH, IDH, and EST-A). Significant deficiencies in heterozygotes were observed in MDH for 2 populations (IRRI and Quezon), in ALKP for 1 population (IRRI), and in esterase for 6 out of the 7 populations (Tables 8 to 11). Significant spatial heterogeneity of gene frequencies among 7 local populations of *N. virescens* was detected at three loci (Table 8 to 11). Partitioning the total variability observed within and between populations revealed that most of the variability was found within populations of *N. virescens* (Table 12).

Analysis of the gene pool of *N. virescens* from different host plants, such as TN1, P203, IR8, and TAPL were analyzed. Genetic differentiation was observed in 3 of the 5 loci (Table 13). Between TN1 vs. IR8 and P203 vs IR8, significant differentiation existed at three loci. TN1 vs P203, TN1 vs TAPL, and P203 vs TAPL were significantly differentiated at two loci, while IR8 vs TAPL differed in just one locus (Table 14).

Table 1. Allele frequencies for alkaline phosphatase (ALKP) locus in ten *N. lugens* populations

Locality	*Alleles*			*Genes (No.)*	X^{2a}	*Significance*
	97	*100*	*103*			
Albay	0.024	0.976	–	42	0.020	0.886
Isabela	–	0.956	0.043	48	0.100	0.7521
Laguna	–	1.0	–	91	0	
Mindoro	0.007	0.964	0.029	70	0.094	0.759
Negros Occ.	0.050	0.928	0.022	88	2.856	0.091
N. Cotabato	0.033	0.967	–	30	0.030	0.861
Palawan	0.0085	0.983	0.0085	59	0.020	0.886
S. Cotabato	0.004	0.988	0.008	133	0.378	0.539
Zamb. Sur	–	1.0	–	92	0	

$X^{2b}_{p} = 47.841, < 0.001, df = 16$

[a]Test the goodness-of-fit of observed genotypic proportions to the proportion expected according to Hardy-Weinberg Law

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

Table 2. Allele frequencies in the adenyl kinase (AK) locus in seven *N. lugens* populations

Locality	*Alleles*					*Genes (No.)*	X^2	*Significance*
	94	*97*	*100*	*103*	*106*			
Albay	0	0.361	.633	0	.006	93	1.99	0.158
Cam. Sur	0	.033	.889	.078	0	45	0.71	0.399
Mindoro	.020	.259	.721	0	0	120	0.509	0.024
Negros Occ.	.020	0	.976	0	.004	128	0.081	0.776
N. Cotabato	.008	0	.988	.004	0	129	0.021	0.884
Palawan	0	0	1.00	0	0	100	0	
S. Cotabato	0	0	1.00	0	0	34	0	
	$X^{2b} = 391.36, P < .001, df = 24$							

[a]Tests the goodness-of-fit of observed genotypic proportions expected according to Hardy-Weinberg Law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 3. Allele frequencies for isocitric dehydrogenase (IDH) in ten *N. lugens* populations

Locality	*Alleles*						*Genes*	X^{2a}	*Significance*
	97	*100*	*103*	*106*	*109*	*112*			
Albay	0.050	0.851			0.099		111	51.170	7.6×10^{-11}
Cam. Sur		1.000					45	0	
Isabela	0.021	0.958	0.021				24	0.02	0.887
Laguna	0.005	0.979	0.016				92	0.024	0.878
Mindoro	0.021	0.913	0.008		0.058		120	0.117	0.733
Neg. Occ.	0.006	0.994					158	0.081	0.7753
N. Cot.	0.023	0.845	0.019	0.019	0.085	0.013	132	61.040	7.6×10^{-11}
S. Cot.	0.128	0.625	0.037	0.024	0.186	–	148	21.040	3.7×10^{-6}
Palawan	0.037	0.963					109	0.157	0.692
Zamb. Sur		1.000					92	0	
		$X^{2b} = 386.655, P < 0.001, df = 45$							

[a]Tests the goodness-of-fit of observed genotypic proportions to the proportion expected according to Hardy-Weinberg Law
[b]Tests the homogeneity of gene frequencies among *N. lugens* populations using the method of Workman and Niswander (1970)

Table 4. Allele frequencies of malate dehydrogenase (MDH) in 10 local populations of *N. lugens*

Locality	*Alleles*			*Genes*	X^{2a}	*Significance*
	97	*100*	*103*	*(No.)*		
Albay	0.0335	0.895	0.0725	89	1.21	0.27
Cam. Sur	0	1.0	0	45	0	
Negros Occ.	0	1.0	0	158	0	
Isabela	–	1.0	–	96	0	
Laguna		0.989	0.011	91	0.01	0.919
Mindoro	0.042	0.920	0.038	120	0.89	0.345
N. Cotabato	0.034	0.965	–	129		
Palawan	–	1.0	–	109	0	
S. Cotabato	0.002	0.998	–	233	0.005	0.942
Zamb. Sur	0.006	0.961	0.033	92	0.14	0.707
	$X^{2b} = 110.67$b, $P < 0.001$, $df = 18$					

[a]Tests the goodness-of-fit of observed genotypic proportions expected according to the Hardy-Weinberg Law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 5. Partitioning of genetic variability in a population of *N. lugens* using the Shannon information index, H (Lewontin, 1972), and Nei, (1977) modification of F statistics

Locus	*Shannon Index*			*Nei Method*			
	Hepop	*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
HDH	0.143	0.114	97.10	0.053	0.051	0.002	0.039
Mean	0.276	0.195	91.86	0.1185	0.103	0.015	0.089
							% = 91.02

Table 6. Allele frequencies in five protein loci of *N. lugens* populations infesting different host plants

Locus	*Population*	*Genes (No.)*	*Allele Frequencies*			X^{2*}	*df*
			1	*2*	*3*		
AK	TNI	96	0.224	0.776	–	24.60b	3
	Mudgo	77	0.072	0.928	–		
	ASD7	235	0.228	0.772	–		
	*L. hexandra*188		0.147	0.853			
ALKP	TNI	258	0.160	0.838	0.002	75.04b	3
	Mudgo	109	0.023	0.977	0		
	ASD7	249	0.024	0.002			
	L. hexandra	164	0.107	0.888	0.006		
IDH	TNI	322	0.930	.907	0	160.11b	6
	Mudgo	101	0	1.000	0		
	ASD7	228	0.011	0.989	0		
	L. hexandra	254	0.061	0.856	0.083		
MDH	TNI	238	0.954	0.046	–	30.72b	3
	Mudgo	189	0.990	0.010	–		
	ASD7	149	0.991	0.041	–		
	L. hexandra	200	1.000	0	–		
PGI-2	TNi	138	0.018	0.583	0.398	195.04b	6
	Mudgo	378	0.012	0.904	0.084		
	ASD7	102	0.005	0.868	0.128		
	L. hexandra	333	0.047	0.668	0.285		

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

[a]Significant at 5.0% level.

[b]Significant at 0.1% level.

Table 7. Results of homogeneity tests* of gene frequencies between population of *N. lugens* infesting different host plants at five polymorphic loci, indicating level of significant heterogeneity

	ENZYME LOCI				
	AK	*MDH*	*PGI-2*	*ALKP*	*IDH*
TNI vs. Mudgo	14.97b	9.36b	144.67b	27.91b	20.22b
df	(1)	(1)	(2)	(2)	(1)
TNI vs. ASD7	0.01*	5.68a	45.31b	55.47b	32.33b
df	(1)	(1)	(2)	(2)	(1)
TNI vs. *L. hexandra*	5.27a	18.87b	14.29b	5.53a	57.97b
df	(1)	(1)	(2)	(2)	(2)
Mudgo vs. ASD7	18.38b	0.018	4.31a	0.44*	2.24*
df	(1)	(1)	(2)	(2)	(1)
Mudgo vs. *L. hexandra*	5.61a	4.02a	120.43b	15.01b	32.43b
df	(1)	(1)	(2)	(2)	(2)
ASD7 vs. *L. hexandra*	8.83b	3.61*	31.66b	26.36b	58.56b
df	(1)	(1)	(2)	(2)	(2)

a = significant at 5.0% level
b = significant at 0.1% level
* = based on comparison of allelic frequencies according to the method of Workman and Niswander (1970)

Table 8. Observed phenotypes and allelic frequencies in alkaline phosphatase locus in 7 populations of *N. virescens*

Population	*Genes (No.)*	*Allele frequencies*			X^{2a}	*Significance*
		1	*2*	*3*		
Cam. Sur	56	–	.982	.018	.02	.887
Lag. (IRRI)	239	.006	.981	.013	47.5	<.0001
Lag. (Pila)	72	.028	.972	–	.06	.804
Isabela	179	.006	.994	–	.019	.890
N. Cotabato	135	.011	.985	.004	.03	.862
Quezon	67	.009	.991	–	.02	.887
Zambo. Sur	143	.013	.981	.006	.05	.822

$X^{2b} = 18.09, P > 0.1$ df = 12

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law

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

Table 9. Observed phenotypes and allelic frequencies in esterase locus in 7 population of *N. virescens*

Population	*Genes (No.)*	*Allele frequencies*			X^{2a}	*Significance*
		1	*2*	*3*		
Cam. Sur	58	.388	.612	–	5.58	.0182
Isabela	98	.202	.798	–	24.45	< .0001
Lag. (IRRI)	176	.102	.483	.415	61.34	< .0001
Lag. (Pila)	45	.300	.700	–	.46	.5001
N. Cotabato	76	.553	.447	–	13.06	.0003
Quezon	67	.200	.791	.009	32.01	< .0001
Zamb. Sur	12	.151	.817	.032	38.10	< .0001
	$X^{2b} = 490.78, P < .001$ df = 12					

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law

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

Table 10. Observed phenotypes and allelic frequencies in isocitric dehydrogenase locus in 7 populations of *N. virescens*

Population	*Genes (No.)*	*Allele frequencies*			X^{2a}	*Significance*
		1	*2*	*3*		
Cam. Sur	56	–	.982	.018	.02	.887
Isabela	191	–	.987	.013	.032	.858
Lag. (IRRI)	254	.138	.860	.002	2.50	.114
Lag. (Pila)	45	–	.978	.022	.022	.882
N. Cotabato	137	.018	.967	.015	1.04	.308
Quezon	67	–	1.000	–	–	
Zamb. Sur	142	–	.997	.003	.02	.879
		$X^{2b} = 173.47$, $P < 0.001$ df = 12				

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 11. Observed phenotypes and allelic frequencies in malate dehydrogenase locus in 7 populations of *N. virescens*

Population	*Genes*	*Allele frequencies*			X^{2a}	*Significance*
		1	*2*	*3*		
Camarines Sur	58	–	1.00	–	–	–
Isabela	167	–	.953	.047	.126	.723
Laguna (IRRI)	175	–	.974	.026	5.73	.017
Laguna (Pila)	45	–	.978	.022	.022	.882
North Cotabato	137	–	1.00	–	–	–
Quezon	55	.045	.864	.091	32.52	< .0001
Zamboanga Sur	95	–	1.00	–	–	–
	$X^{2b} = 102.82$, $P < .001$ df = 12					

[a]Tests the goodness-of-fit of observed genotypic proportions expected to the Hardy-Weinberg law
[b]Tests the homogeneity of gene frequencies among populations based on the method of Workman and Niswander (1970)

Table 12. Partitioning of genetic variability in a population of *N. virescens* 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	.093	.086	99.3	.0322	.0319	.0003	.0093
AK	.743	.647	90.4	.414	.4810	.0670	.1390
IDH	.162	.124	96.2	.064	.0590	.0050	.0780
MDH	.161	.129	96.8	.064	.0609	.0030	.0470
Mean	.289	.247	95.68	.144	.158	.019	.0683
							% = 93.2

Table 13. Allele frequencies in five protein loci of *N. virescens* populations

Locus	*Population*	*Genes (No.)*	*Allele Frequencies*			X^{2*}	*df*
			1	*2*	*3*		
IDH	TNI	254	0.138	0.860	0.002	100.07b	3
	P203	124	0.016	0.984			
	IR8	132	0	1.0	0		
	TAPL	124	0	1.0	0		
ALKP	TNI	237	0.007	0.989	0.004	57.78b	3
	P203	124	0	1.0	0		
	IR8	198	0	0.940	0.060		
	TAPL	124	0	1.0	0		
MDH	TNI	175	–	0.994	0.006	4.57	3
	P203	124	–	1.0	0		
	IR8	132	–	1.0	0		
	TAPL	124	–	1.0	0		
EST-A	TNI	176	–	0.396	0.604	97.10b	b
	P203	93	–	0.597	0.403		
	IR8	81	–	0.759	0.241		
	TAPL	153	–	0.725	0.275		
AK	TNI	187	–	0.006	0.994	3.61	3
	P203	100	–	–	1.0		
	IR8	100	–	–	1.0		
	TAPL	100	–	–	1.0		

*Tests the homogeneity of gene frequencies among GLH populations based on the method of Workman and Niswander (1970)
a = significant at 5% level
b = significant at 0.1% level

Table 14. Results of homogeneity tests* of gene frequencies between GLH populations at five polymorphic loci, indicating level of significant heterogeneity.

	Enzyme loci				
	IDH	*MDH*	*EST-A*	*ALKP*	*AK*
TNI vs P203	28.66b	1.49	19.76b	2.75	1.21
TNI vs IR8	40.71b	1.59	58.50b	26.25b	1.21
TNI vs TAPL	38.723b	1.49	1.56b	2.75	1.21
P203 vs IR8	4.26a	0	10.32b	15.45b	0
P203 vs TAPL	4.00a	0	8.66b	0	0
IR8 vs TAPL	0	0	0.63	15.45b	0

*Based on comparison of allelic frequencies according to the method of Workman and Niswander (1970)
a = significant at 5% level
b = significant at 0.1% level

Discussion

Population of *N. lugens* and *N. virescens* possess diverse gene pools. Significant heterogeneity of gene frequencies among 10 local populations of *N. lugens* was detected at 2 loci, while in 7 populations of *N. virescens*, it occurred at 3 loci. For example, in *N. lugens*, the frequency of the most common allele in IDH ranged from as high as 1.0 to as low as 0.63 in South Cotabato, while at ALKP, the frequency of the most common allele ranged from 0.96 in Zamboanga del Sur to 0.99 in South Cotabato . On the other hand, in *N. virescens*, the frequency of the most common allele at EST-A ranged from as high as 0.82 in Zamboanga del Sur to as low as 0.45 in North Cotabato, while in ALKP, the most common allele ranged from 0.98 in Zamboanga del Sur to 0.99 in North Cotabato. The magnitudes of these genic differentiation among local populations of *N. lugens* and N. *virescens* were comparable to the host-specific populations of both species.

Despite distinct variations in gene frequencies in some polymorphic loci between populations of either *N. lugens* or *N. virescens*, the overall computed coefficients of genetic identity (Nei, 1972) of 0.99 among *N. lugens* and *N. virescens* from different localities and from their different hosts showed that these populations are closely related to one another. They are simply infraspecies. Early stages of differentiation may not be associated with substantial genetic change (Prakash *et al.*, 1969, Lewontin 1974, Bush 1975). The differences earlier observed in *N. lugens* host plant relationships such as differences in varietal reactions (Oka 1978, Seshu and Kaufmann 1980, IRRI 1982), host-mediated differential responses (Saxena and Pathak 1977), morphological differences (Saxena and Rueda 1983), cytological differences (Saxena and Barrion 1982, 1983a, b) and a certain degree of reproductive isolation (Saxena *et al.*, 1984) all substantiate the existence of genetic differences in the gene pool of the populations (this study). A combination of all these events may contribute to the accumulation of host-adapted gene complexes conducive to genetic events likely to be associated with the evolution of *N. lugens* and *N. virescens* virulent populations or biotypes. The genetic differences detected among *N. lugens* and *N. virescens* may be attributed to selection by the host plants, to population structure, or to a combination of both. Nevertheless, in each case, the subdivision of the populations detected is conducive to genetic events likely to be involved in speciation (Wright 1940, Wilson 1975, Bush *et al.*, 1977, White 1978). Infestations of resistant cultivars and other non-rice hosts such as *L. hexandra* can lead to possible founding populations by genotypes that may not be random samples of the original population. This is a kind of situation that may lead to rapid speciation of groups not specifically adapted to different environments (Templeton 1980a, b). Should these populations be structured along host lines, a host race or biotype may evolve preadapted for life in its new habitat (Sturgeon and Mitton 1982).

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