

## QUANTIFICATION OF PROTEIN VARIATION IN TWO NATURAL POPULATIONS OF *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE)

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### ABSTRACT

We analyzed 22 loci of *Spodoptera frugiperda* from a previous electrophoretic study of 12 proteins (Brazil. J. Genetics. 13: 711-729, 1990) to estimate the degree of genetic variation within and between two populations separated by a distance of approximately 500 km. Eleven polymorphic loci were detected with a mean  $\bar{P}$  value of 41.7%. The genotypic frequencies of the different loci were homogeneously distributed both between sexes (within each sample) and between samples. We estimated mean  $\pm$  standard deviations (SD) of observed heterozygosity ( $\bar{H}_o$ ) to be  $0.0816 \pm 0.0002$  and mean  $\pm$  SD of expected heterozygosity ( $\bar{H}_e$ ) to be  $0.0935 \pm 0.0030$ . A comparative analysis of  $\bar{P}$  and  $\bar{H}_e$  showed that our data are quite similar to those obtained for other *S. frugiperda* populations. Joint analysis of the present data and the literature permitted us to estimate the  $\bar{P}$  and  $\bar{H}_e$  of *S. frugiperda* to be 47.2% and  $0.0778 \pm 0.1503$ , respectively, based on 36 loci.

### INTRODUCTION

The fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797), family Noctuidae, is a crop pest, originally from the tropical and subtropical regions of the Western Hemisphere (Luginbill, 1928), and distributed from Southern Canada to Chile and Argentina (Todd and Poole, 1980). In the Americas, it is considered to be one of the

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major pests of corn, rice, sorghum and forage grasses (Carvalho, 1970; Pantoja *et al.*, 1987; Sparks, 1979; Wiseman *et al.*, 1966).

The limited amount of data on protein variation in South American populations and the interest in quantifying such variation for the largest possible number of loci, led us to study Brazilian populations of this insect. The electrophoretic profiles of 12 protein systems analyzed in two natural Brazilian populations of *S. frugiperda*, with the number of activity regions, number of loci involved and possible allelic variants, have been reported by Lima and Contel (1990).

The objectives of the present paper were to present populational data on allelic and genotypic frequencies, to compare the distribution of genotypic frequencies between sexes, to report intralocus and mean heterozygosity and the proportion of polymorphic loci, and to compare these data for these two disjunct populations.

## MATERIAL AND METHODS

Description of the samples and of the electrophoretic conditions are given in detail in Lima and Contel (1990).

Allele and genotype frequencies were estimated by gene counts. Data on genotype frequency distribution between sexes and on allele and genotype distribution at each locus between the two populations were analyzed statistically by the homogeneity test (chi-square). A locus was considered to be polymorphic when the frequencies of two or more alleles were higher than 0.01. The proportion of polymorphic loci ( $\bar{P}$ ) for each sample was calculated as the ratio of the number of polymorphic loci to the total number of sampled loci (cf. Ayala and Valentine (1979)). The corresponding intralocus and mean heterozygosities ( $\bar{H}_o$ ) were obtained by direct counting, and the expected intralocus and mean heterozygosities ( $\bar{H}_e$ ) were calculated according to Nei and Roychoudhury (1974) and Nei (1978, 1987). For loci presenting a high level of polymorphism (*PEP1*, *EST3*, *GDH1*, and *PGM1*) we used the chi-square test to determine the probability of association among them.

For polyallelic loci, such as *EST3*, rare alleles were grouped on the basis of close electrophoretic mobility to avoid both probable reading errors and errors of very small expected genotypic classes.

## RESULTS

Twenty two loci were studied for the 12 protein systems analyzed: two for peptidases (PEP), three for carboxylesterases (EST), one for malic enzyme (ME), one for glucose dehydrogenase (GHD), three for alcohol dehydrogenase (ADH), two for isocitrate dehydrogenase (IDH), one for glycerol-3-phosphate dehydrogenase

(G-3-PDH), one for leucineaminopetidase (LAP), five for nonspecific proteins (PT), one for phosphoglucumutase (PMG), one for superoxide dismutase (SOD) and one for malate dehydrogenase (MDH). The *PT2* locus was not considered in either population because of problems of precision in phenotype definition, and the *PT1* locus was not considered in population 1 for the same reason.

Allele frequencies for the 13 variable loci are presented in Table I. No significant differences at the 5% level from expected values, on the basis of genetic equilibrium, were detected at any locus, except for *PEP2* and *EST3* in population 1, and for *PEP1*, *PEP2* and *SOD* in population 2. Comparative analysis showed that the allele frequency distribution for the different loci was the same for the two populations, except for loci *LAP* and *SOD* (Table I).

Table I - Gene frequencies for 13 variable loci os *Spodoptera frugiperda* in samples from Sertãozinho and Londrina, BR.

Locus	Allele	Sertãozinho population			Londrina population			X <sup>2</sup> Test of homogeneity between samples
		Sample size	Frequency	Hardy-Weinberg <sup>A</sup> X <sup>2</sup>	Sample size	Frequency	Hardy-Weinberg <sup>A</sup> X <sup>2</sup>	
PEP1	PEP1*1	315	0.1540	0.6247	216	0.1250	8.9064*	0.8906
	PEP1*2		0.8317			0.8611		
	PEP1*3		0.0143			0.0139		
PEP2	PEP2*1	315	0.0984	315.0731***	216	0.0694	158.7292***	1.3623
	PEP2*2		0.9016			0.9306		
EST1	EST1*1	299	0.9699	0.2907	216	0.9630	0.3202	0.1872
	EST1*2		0.0301			0.0370		
EST3	EST3*1	257	0.0934	38.2095***	186	0.0323	24.4457	9.8636
	EST3*2		0.1245			0.1855		
	EST3*3		0.5370			0.5484		
	EST3*4		0.1829			0.1532		
	EST3*5		0.0564			0.0699		
	EST3*6		0.0058			0.0108		
EST3 <sup>B</sup>	EST3*3'	257	0.7549	0.1743	186	0.7661	1.9423	1.1044
	EST3*4		0.1829			0.1532		

Continued

Table I - Continued

Locus	Allele	Sertãozinho population			Londrina population			X <sup>2</sup> Test of homogeneity between samples
		Sample size	Frequency	Hardy-Weinberg <sup>A</sup> X <sup>2</sup>	Sample size	Frequency	Hardy-Weinberg <sup>A</sup> X <sup>2</sup>	
	EST3*5'		0.0622			0.0807		
GDH1	GDH1*1	293	0.3123	0.1531	214	0.2547	0.5829	2.0010
	GDH1*2		0.6877			0.7453		
ADH1	ADH1*1	296	0.0068	0.0146	216	0.0023	0.0012	0.5184
	ADH1*2		0.9932			0.9977		
IDH1	IDH1*1	234	0.0086	0.0174	198	0.0051	0.0251	1.3816
	IDH1*2		0.9914			0.9898		
	IDH1*3		0.0000			0.0051		
IDH2	IDH2*1	234	0.0021	0.0101	197	0.0051	0.0118	0.3772
	IDH2*2		0.9936			0.9924		
	IDH2*3		0.0043			0.0025		
LAP	LAP*1	300	1.0000		215	0.9814	0.0774	5.6235*
	LAP*2		0.0000			0.0186		
PT1	PT1*1				216	0.9653	0.2799	
	PT1*2					0.0347		
PT3	PT3*1	278	0.9730	3.3154	171	0.9737	0.1251	0.0019
	PT3*2		0.0270			0.0263		
PGM1	PGM1*1	316	0.0348	2.4390	214	0.0350	4.2690	0.2505
	PGM1*2		0.8212			0.8131		
	PGM1*3		0.1313			0.1425		
	PGM1*4		0.0127			0.0094		
SOD	SOD*1	300	0.9983	0.0012	216	0.9792	170.3852***	4.7716*
	SOD*2		0.0017			0.0208		

<sup>A</sup> Chi-square calculated to test Hardy-Weinberg equilibrium (within each sample) and the chi-square of the homogeneity of allelic frequencies between samples.

<sup>B</sup> The alleles were grouped according to electrophoretic mobility: EST3\*3' - EST3\*1 + EST3\*2 + EST3\*3; EST3\*5' - EST3\*5 + EST3\*6.

In population 1, seven of 21 loci were polymorphic, with an estimated P value of 33.34%. Among the 22 loci studied in population 2, 11 were polymorphic, with a P value of 50%.

The data obtained by the chi-square test on the basis of genetic equilibrium showed that loci *PEP1*, *EST3*, *GDH1* and *PMG1* are not associated.

In the analysis of genotypic frequency distribution between sexes, which was carried out on loci presenting variation but with a frequency higher than 1% for at least one of the rarer alleles, we obtained nonsignificant chi-square homogeneity values.

The genotypic frequencies of variable loci in genetic equilibrium were homogeneously distributed among the two populations. An exception was the *EST3* locus when analyzed without grouping the alleles of lower frequency and of close electrophoretic mobility. However, when this grouping was done, the chi-square homogeneity test was nonsignificant.

Table II shows the observed and expected intralocus heterozygosity values for populations 1 and 2, and the estimated  $\bar{H}_o$  and  $\bar{H}_e$  values for both populations. The mean  $\pm$  standard deviation (SD)  $\bar{H}_o$  value estimated for the two populations was  $0.0816 \pm 0.0002$  and the mean  $\pm$  SD  $\bar{H}_e$  estimated value was  $0.0985 \pm 0.0030$ .

## DISCUSSION

The allelic and genotypic frequencies for the different loci analyzed were found to be homogeneously distributed among the two populations of *S. frugiperda*, suggesting the absence of genetic heterogeneity between them.

According to Johnson (1987), there is considerable circumstantial evidence which indicates that migration is the major strategic component in the life cycle of *S. frugiperda*. Since this is doubtlessly a species of tropical origin (Luginbill, 1928), it cannot survive the winters of colder regions of North America because it does not possess the diapause mechanism (Sparks, 1979; Johnson, 1987). The species survives in warmer areas in which food is constantly available and migrates to colder regions during the spring and summer (Andrews, 1980). The migration rate may reach 450 km/generation; a possible hypothesis to explain this rate is that *S. frugiperda* adults may be transported over large distances by wind currents (Sparks, 1979; Johnson, 1987). In Brazil, a country with a climate ranging from temperate to warm and where corn and other gramineae are grown throughout most of the year, *S. frugiperda* finds all the conditions necessary for constant reproduction and propagation.

According to Nei (1987), migration, even in low amounts, is sufficient to prevent appreciable genetic differentiation between populations, except in the presence of strong differential selection. This suggests that the homogeneity we observed in allelic and

Table II - Intralocus and mean observed and expected heterozygosities for the *Spodoptera frugiperda* populations analyzed.

Locus	Sertãozinho population				Londrina population			
	No. of Heterozygous individuals		Intralocus heterozygosity		No. of heterozygous individuals		Intralocus heterozygosity	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
PEP1	87	89.6361	0.2762	0.2846	44	52.4207	0.2037	0.2427
PEP2	0	55.8920	0.0000	0.1774	4	27.9001	0.0185	0.1292
EST1	18	17.4580	0.0602	0.0584	16	15.3926	0.0741	0.0713
EST3	142	167.2401	0.5525	0.6507	108	118.2086	0.5807	0.6353
GDH1	123	125.8545	0.4198	0.4295	77	81.2464	0.3598	0.3797
ADH1	4	3.9982	0.0135	0.0135	1	0.9913	0.0046	0.0046
IDH1	4	3.9902	0.0171	0.0171	4	4.0083	0.0202	0.0202
IDH2	3	2.9802	0.0128	0.0127	3	2.9766	0.0152	0.0151
LAP	-	-	-	-	8	7.8492	0.0372	0.0365
PT1	-	-	-	-	15	14.4702	0.0694	0.0670
PT3	13	14.6067	0.0468	0.0525	9	8.7580	0.0526	0.0512
PGM1	99	97.0178	0.3133	0.3070	75	67.8912	0.3505	0.3173
SOD	1	1.0183	0.0033	0.0034	1	8.7987	0.0046	0.0407

$$\bar{H}_{O1} = 0.0817 \pm 0.1616; \bar{H}_{E1} = 0.0956 \pm 0.1774$$

$$\bar{H}_{O2} = 0.0814 \pm 0.1544; \bar{H}_{E2} = 0.0914 \pm 0.1628.$$

genotypic frequencies in the two populations may be due to gene flow, despite the distance of approximately 500 km between the two sites.

In their study of several *S. frugiperda* populations from Mexico, the United states and Puerto Rico, Pashley *et al.* (1985) concluded that, except for the Puerto Rican population, no genetic heterogeneity was present and that all populations belonged to a large intercrossed population. The same fact was observed in *S. exempta* (Den Boer, 1978). Other migratory pests such as *Alabama argillacea*, *Pieris rapae* and *Anticarsia gemmatalis* also present genetic homogeneity among populations (Pashley *et al.*, 1985). McDonald *et al.* (1987) obtained the same result for populations of the dipteran *Haematobia irritans* and concluded that the absence of significant genetic differentiation seems to be due to the lack of barriers against gene flow between populations, probably because of the recent introduction of this species into the United States. Genetic

heterogeneity has been detected in sedentary populations (Berger, 1973; McKechnie, 1975).

The proportion of polymorphic loci reported for lepidopterans varies considerably, with values ranging from 2.5% to 62.5% (Lokki *et al.*, 1975; Menken, 1982; Harrison *et al.*, 1983; Willhite and Stocks, 1983). Estimates ranging from 67.8% to 84.2% have been reported for the family Noctuidae (Sluss *et al.*, 1978; Pashley and Johnson, 1986). The  $\bar{P}$  value estimated for the two populations of *S. frugiperda* studied here, 41.7%, is below the estimates reported for the family Noctuidae, but within the range for other lepidopteran species.

The mean expected heterozygosity ( $\bar{H}_e$ ) of 9.35% for the populations reported here is below the value estimated for 46 lepidopteran species (13.2%) but is similar to the value estimated for 263 insect species (10.7%) (Lima, 1989). Singh and Rhomberg (1987) reported two factors affecting heterozygosity estimates that present a problem for interspecific comparisons. First, enzymes and other proteins with different functions have variable levels of heterozygosity and, second, representation of such enzymes in the sampled loci in different organisms is equally variable.

When comparing our  $\bar{H}_o$  and  $\bar{P}$  data with those presented in Table I of the paper by Pashley *et al.* (1985), we see that the  $\bar{H}_o$  estimated for each of their 10 different populations (excluding the Puerto Rican one) ranges from 0.107 to 0.195 and  $\bar{P}$  ranges from 50% to 91.7%. On the basis of this Table, we estimated a mean  $\bar{H}_o$  value of  $0.1507 \pm 0.0246$  and a mean  $\bar{P}$  of 70%, values that are much higher than those estimated for our populations. This discrepancy may be explained by the fact that Pashley *et al.* (1985), performed an electrophoretic investigation of 22 loci in a sample of 100 individuals but excluded 10 monomorphic loci from these initial 22 in later analyses of the ten populations, and from the calculations of  $\bar{H}$  and  $\bar{P}$ . The main objective of the study by Pashley *et al.* (1985) was to determine the migratory routes of *S. frugiperda* in relation to Mexico, Puerto Rico and the United States; on this basis, it was very important for them to use loci known to be polymorphic and which may be used as biochemical markers in tests of homogeneity between populations. In addition, the estimate of mean heterozygosity is greatly affected by the choice and number of sample loci (Nei, 1987). We recalculated the  $\bar{H}_e$  and  $\bar{P}$  values for the populations of Pashley *et al.* (1985) while including the 10 monomorphic loci and obtained a range of variation of 0.0751 to 0.1111 for  $\bar{H}_e$  and of 27.27% to 50% for  $\bar{P}$ . The mean  $\pm$  SD estimated  $\bar{H}_e$  value was  $0.0977 \pm 0.0166$  and the mean estimated  $\bar{P}$  value was 38.18% values very similar to those estimated for our populations.

To obtain an estimate of  $\bar{H}_e$  for *S. frugiperda* based on a larger number of loci, we combined the present data with those obtained by Pashley *et al.* (1985) for wild populations collected on corn. Data for the Puerto Rican (collected on rice) and Ecuadorian (collected on cotton) populations and for a Brazilian laboratory population

were excluded, since previous data suggest the possibility of host-associated races or sibling species, (Pashley *et al.*, 1985; Pashley, 1986, 1988a, 1988b, 1989; Pashley and Martin, 1987; Pantoja *et al.*, 1987). Eight loci (both variable and monomorphic) were common to both studies, 14 were unique to Pashley *et al.* (1985) and an additional 14 were studied only in the present investigation. For a total of 36 loci, 18 were monomorphic and 18 variable.

Table III shows the variable loci with their respective expected intralocus heterozygosities and the estimated  $\pm$  SD  $\bar{H}_e$  value for *S. frugiperda*,  $0.0778 \pm 0.1503$ . Since among the variable loci *ADH1* was not polymorphic (according our criteria, see Material and Methods), the  $\bar{P}$  for the species is 47.2%.

Table III - Expected intralocus and mean heterozygosity for *Spodoptera frugiperda* obtained by joint analysis of the present data with those reported by Pashley *et al.* (1985).

Locus	Intralocus heterozygosity <sup>A</sup> of the population of Pashley <i>et al.</i>	Intralocus heterozygosity of populations 1 and 2	General intralocus heterozygosity
PEP1	0.2687 $\pm$ 0.0659	0.2637 $\pm$ 0.0296	0.2679 $\pm$ 0.0603
PEP2	0.0170 $\pm$ 0.0370	0.1533 $\pm$ 0.0341	0.0400 $\pm$ 0.0632
EST3	0.5130 $\pm$ 0.1490	0.6431 $\pm$ 0.0107	0.5354 $\pm$ 0.1438
IDH1	0.0020 $\pm$ 0.0063	0.0187 $\pm$ 0.0022	0.0048 $\pm$ 0.0086
IDH2	0.0040 $\pm$ 0.0084	0.0139 $\pm$ 0.0017	0.0056 $\pm$ 0.0085
LAP	0.0590 $\pm$ 0.0870	0.0183 $\pm$ 0.0258	0.0522 $\pm$ 0.0803
PGM1	0.3769 $\pm$ 0.0744	0.3122 $\pm$ 0.0073	0.3661 $\pm$ 0.0719
AATF	0.0156 $\pm$ 0.0239		0.0156 $\pm$ 0.0239
AK	0.0432 $\pm$ 0.0559		0.0432 $\pm$ 0.0559
HBDH	0.1465 $\pm$ 0.0405		0.1465 $\pm$ 0.0405
MPI	0.5325 $\pm$ 0.0369		0.5325 $\pm$ 0.0369
PGI	0.1705 $\pm$ 0.0410		0.1705 $\pm$ 0.0410
EST1		0.0649 $\pm$ 0.0091	0.0649 $\pm$ 0.0091
GDH1		0.4046 $\pm$ 0.0352	0.4046 $\pm$ 0.0352
ADH1		0.0091 $\pm$ 0.0063	0.0091 $\pm$ 0.0063
PT1		0.0670 $\pm$ 0.0000	0.0670 $\pm$ 0.0000
PT3		0.0519 $\pm$ 0.0009	0.0519 $\pm$ 0.0009
SOD		0.0221 $\pm$ 0.0264	0.0221 $\pm$ 0.0264

<sup>A</sup>The data presented for the populations analyzed by Pashley *et al.* (1985) were calculated by us.

$\bar{H}_e$  - 0.0778  $\pm$  0.1503.

Singh and Rhomberg (1987) detected a highly significant negative correlation between the number of loci examined and mean heterozygosity in several species of *Drosophila*. This correlation seems to exist also for *S. frugiperda* since, with an increasing number loci analyzed,  $\bar{H}_e$  decreased from 0.0977 (Pashley *et al.*, 1985) and 0.0935 (present data) (22 loci analyzed in both cases) to  $0.0778 \pm 0.1503$ , when 36 loci are included.

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## RESUMO

Em um estudo eletroforético de 12 sistemas protéicos (*Brazil. J. Genetics 13*: 711-729, 1990), analisamos 22 locos de *Spodoptera frugiperda* com a finalidade de estimar o grau de variabilidade genética entre populações naturais dessa espécie. Foram detectados onze locos polimórficos com um valor médio de  $\bar{P}$  de 41,7%. As frequências genotípicas dos diferentes locos são distribuídas homoganeamente entre os sexos (dentro de cada população) e entre as populações. O valor estimado para a heterozigidade média ( $\bar{H}_o$ )  $\pm$  desvio padrão (DP) foi de  $0,0816 \pm 0,0002$  e a esperada ( $\bar{H}_e$ )  $\pm$  DP de  $0,0935 \pm 0,0030$ . A análise comparativa de  $\bar{P}$  e  $\bar{H}_e$  mostrou que esses dados são muito semelhantes a aqueles obtidos para outras populações de *S. frugiperda*. Análise conjunta dos dados do presente trabalho com os já existentes na literatura permitiu estimar, com base no estudo de 36 locos, a  $\bar{P}$  e  $\bar{H}_e$  de *S. frugiperda* como sendo 47,2% e  $0,0778 \pm 0,1503$ , respectivamente.

## REFERENCES

- Andrews, K.L. (1980). The whorlworm *Spodoptera frugiperda*, in Central America and neighboring areas. *Fla. Entomol.* 63: 456-467.
- Ayala, F.J. and Valentine, J.W. (1979). *Evolving. The theory and process of organic evolution*. Benjamin Cummings Publishing Company, Menlo Park, California, pp. 452.
- Berger, E.M. (1973). Gene-enzyme variation in three sympatric species of *Littorina*. *Biol. Bull. Mar. Biol. Lab. Woods Hole* 145: 83-90.
- Carvalho, R.P.L. (1970). Danos, flutuação da população, controle e comportamento de *Spodoptera frugiperda* (J. E. Smith, 1977) e susceptibilidade de diferentes genótipos de milho em condições de campo. Doctoral Thesis, Escola Superior de Agricultura Luiz de Queiróz, USP, Piracicaba, SP.
- Den Boer, M.H. (1978). Isoenzymes and migration in the African armyworm *Spodoptera exempta* (Lepidoptera, Noctuidae). *J. Zool.* 185: 539-553.

- Harrison, R.G., Wintermeyer, S.F. and Odell, T.M. (1983). Patterns of genetic variation within and among gypsy moths, *Lymantria dispar* (Lepidoptera: Limantriidae) populations. *Ann. Entomol. Soc. Amer.* 76: 625-656.
- Johnson, S.J. (1987). Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the Western Hemisphere. *Insect. Sci. Applic.* 8: 543-549.
- Lima, L.M.K.S. (1989). Variabilidade proteica em populações naturais de *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Doctoral Thesis, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto, São Paulo.
- Lima, L.M.K.S. and Contel, E.P.B. (1990). Electrophoretic analysis of 12 proteins in natural populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Brazil J. Genetics* 13: 711-729.
- Lokki, J., Soumalainen, E., Saura, A. and Lankinen, P. (1975). Genetic polymorphism and evolution in parthenogenetic animals. II. Diploid and polyploid *Solenobia triquetrella* (Lepidoptera: Psychidae). *Genetics* 79: 513-525.
- Luginbill, P. (1928). The fall armyworm. *Tech. Bull.* 34: 1-91.
- McDonald, P.T., Hilburn, L.R. and Kunz, S.E. (1987). Genetic similarities among natural populations of the horn fly (Diptera: Muscidae). *Ann. Entomol. Soc. Am.* 80: 188-192.
- McKoechnie, S.W., Ehrlich, P.P. and White, R.R. (1975). Population genetics of *Euphydryas* butterflies. I. Genetic variation and the neutral hypothesis. *Genetics* 81: 571-594.
- Menken, S.B.J. (1982). Biochemical genetics and systematics of small cwmrmine moths (Lepidoptera, Yponomeutidae). *Z. Zool. Syst. Evolt-forsch* 20: 131-143.
- Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
- Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press, New York, pp. 512.
- Nei, M. and Roychoudhury, A.K. (1974). Sampling variance of heterozygosity and genetic distance. *Genetics* 76: 379-390.
- Pantoja, A., Smith, C.M. and Robinson, J.F. (1987). Development of fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), strains from Louisiana and Puerto Rico. *Environ. Entomol.* 16: 116-119.
- Pashley, D.P. (1986). Host-association genetic differentiation in fall armyworm (Lepidoptera: Noctuidae) a sibling species complex? *Ann. Entomol. Soc. Am.* 79: 898-914.
- Pashley, D.P. (1988a). Quantitative genetics, development, and physiological adaptation in host strains of fall armyworm. *Evolution* 42: 93-102.
- Pashley, D.P. (1988b). Current status of fall armyworm host strains. *Fla. Entomol.* 71: 227-233.
- Pashley, D.P. (1989). Host-associated differentiation in armyworm (Lepidoptera: Noctuidae); an allozymic and mitochondrial DNA perspective. In: *Electrophoretic Studies on Agricultural Pests* (Loxdale, H.D. and den Hollander, J., eds.). Systematics Association Special Volume no. 39, Clarendon Press, Oxford, pp. 103-114.
- Pashley, D.P. and Johnson, S.J. (1986). Genetic population structure of migratory moths: the velvetbean caterpillar (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 79: 26-30.
- Pashley, D.P. and Martin, J.A. (1987). Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 80: 731-733.

- Pashley, D.P., Johnson, S.J. and Sparks, A.N. (1985). Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 78: 756-762.
- Singh, R.S. and Rhomberg, L.R. (1987). A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. II. Estimates of heterozygosity and patterns of geographic differentiation. *Genetics* 117: 255-271.
- Sluss, T.P., Sluss, E.S., Graham, H.M. and DuBois, M. (1978). Allozyme differences between *Heliothis virescens* and *H. zea*. *Ann. Entomol. Soc. Am.* 71: 191-195.
- Sparks, A.N. (1979). A review of the biology of the fall armyworm. *Fla. Entomol.* 62: 82-87.
- Todd, E.L. and Poole, R.W. (1980). Keys and illustrations for the armyworm moths of the Noctuid genus *Spodoptera* Guenee from the Western Hemisphere. *Ann. Entomol. Soc. Am.* 73: 722-738.
- Willhite, E.A. and Stocks, M.W. (1983). Genetic variation among spruce budworm (*Choristoneura occidentalis*) (Lepidoptera: Tortricidae) outbreak in Idaho and Montana. *Can. Entomol.* 155: 41-54.
- Wiseman, B.R., Painter, R.H. and Wasson, C.E. (1966). Detecting corn seedling differences in the greenhouse by visual classification of damage by the fall armyworm. *J. Econ. Entomol.* 59: 1211-1214.

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