Acta Scientiarum
http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v37i2. 25143

Enzyme Polymorphism in *Sitophilus oryzae* (Linnaeus, 1763) and *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera, Curculionidae) in southern Brazil

Danielle das Neves Bespalhok[1*], Erasmo Renesto[2] and Tiago Signorini[1]

*[1]Programa de Pós-graduação em Genética e Melhoramento, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. [2]Departamento de Biologia Celular, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: dani_bespalhok@hotmail.com*

ABSTRACT. Beetles of the species *Sitophilus oryzae* and *S. zeamais* are pests of great economic importance since they attack not only rice and maize but also several other cereals. In fact, these beetles are one of the most visible threats to sustainable food production. Current study estimated the genetic variability of *S. oryzae* in two samples, one from the State of Paraná (PR), Brazil, and another from the state of Rio Grande do Sul (RS), Brazil, and a sample of *S. zeamais* from the State of Santa Catarina (SC), Brazil. Isozyme electrophoresis in starch gel technique was employed to analyze eight enzyme systems (AAT, ACP, GDH, GPI, IDH, MDH, PGM and ME). Average heterozygosity rates were 0.0091, 0.0100 and 0.0000 and expected heterozygosity rates were 0.0419, 0.0452 and 0.0000 respectively for the samples of PR, SC and RS samples. The percentage of polymorphic loci was 30% in the PR sample, 0% in the RS sample and 30% in the SC sample. Genetic identity rates were I=0.9983 between samples from PR and RS; I = 0.6892 between PR and SC, and I = 0.6925 between SC and RS. Nei´s (1978) genetic distance rates were 0.0017, 0.3722 and 0.3675. Samples presented low genetic variability.

Keywords: pest insect, stored grains, starch gel, electrophoresis, heterozygosity.

Polimorfismo Enzimático em *Sitophilus oryzae* (Linnaeus, 1763) e *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera, Curculionidae) no sul do Brasil

RESUMO. Os besouros *Sitophilus oryzae* e *S. zeamais* são considerados pragas de grande importância econômica. Além do arroz e do milho, eles atacam outros diversos cereais. São uma das ameaças mais visíveis para a produção sustentável de alimentos. Este trabalho teve como objetivo estimar a variabilidade genética de *S. oryzae* em duas amostras, uma do Estado do Paraná (PR), e outra do Rio Grande do Sul (RS) e uma amostra de *S. zeamais* de Santa Catarina (SC). Utilizou-se a técnica de eletroforese de isozimas em gel de amido para a análise de oito sistemas enzimáticos (AAT, ACP, GDH, GPI, IDH, MDH, ME e PGM). A heterozigosidade média observada foi de 0,0091, 0,0100 e 0,0000 e a esperada foi de 0,0419, 0,0452 e 0,0000 para as amostras do PR, SC e RS, respectivamente. A porcentagem de locos polimórficos foi de 30, 0 e 30% nas amostras do PR, RS e SC, respectivamente. Os valores para identidade genética foram de I = 0,9983 entre as amostras do PR e RS; I = 0,6892 entre PR e SC e I = 0,6925 entre SC e RS, e os valores da distância genética de Nei (1978) foram 0,0017, 0,3722 e 0,3675, respectivamente. As amostras apresentaram pouca variabilidade genética.

Palavras-chave: inseto-praga, grãos armazenados, gel de amido, eletroforese e heterozigosidade.

Introduction

Sitophilus zeamais (Motschulsky, 1855) and *Sitophilus oryzae* (Linnaeus, 1763) are pests belonging to the Coleoptera order and Curculionidae family. Surveys in São Paulo and in other Brazilian states, demonstrated that *S. zeamais* is the main pest species that attacks rice. These species are morphologically very similar in morphology, even though they are securely distinguished by their genitals (GALLO et al., 1988). These authors also report that, due to similarities between the two species, the biological data obtained for *S. zeamais* could be extended to *S. oryzae* (VIEIRA, 1999).

It has been estimated that around 20% of the total volume of annually harvested grains in Brazil are wasted during harvest, transportation and storage processes, and half of this loss is due to pest attack during storage. Losses in mass and seeds germinating power, product devaluation and an environment favorable to the dissemination of fungi and other microorganisms are among the liabilities (PUZZI, 1986).

Pests are the most relevant cause of physical losses in grains and byproducts, coupled to quality loss. Beetles and moths are two important pest groups that attack stored grains, although the former group causes the greatest damage. *S. oryzae* is the most important pest to rice crops (PACHECO; PAULA, 1995) and *S. zeamais* in the case of corn.

Pests, pathogens and weeds are the most evident threats against sustainable food production. Since the Second World War, the most common reaction towards these agricultural problems has been the application of agrochemicals. Apart from the risks that these products present to human health, their continuous use may lead to the development of resistance in the target organism besides the elimination of natural enemies. Based on these observations, it is currently well-known that synthetic pesticides cause high environmental impact (AZEVEDO, 2003).

Although there are many studies on the control of *S. oryzae* and *S. zeamais*, especially by Martins and Oliveira (2008), Botton et al. (2005), Nukenine et al. (2013), Chu et al. (2011), Wang et al. (2011) and Lira et al. (2015), little is known about the genetic variability of these species. Beiras and Petitpierre (1981), Pintureau et al. (1991) and Grenier et al. (1994) carried out some surveys in which they analyzed the esterase of three European species of *Sitophilus*. Some microsatellites analyses were also made on other species of Curculionidae (LIEWLAKSANEEYANAWIN et al., 2002; KIM et al., 2006).

The characterization of genetic variability in crop pest populations and the factors that maintain this variability are very important in the development of more effective control programs. Over the last 20 years, the use of molecular markers has been a good tool for population genetics studies, taxonomy and conservation biology and provided the analysis of diversity and genetic differentiation of natural populations (VAN OOSTERHOUT et al., 2004).

Current study estimated the genetic variability of two samples of *S. oryzae* - one from the state of Parana, and another one from the State of Rio Grande do Sul - and one sample of *S. zeamais* from the State of Santa Catarina, both based on isozyme electrophoresis to provide subsidies for biological and evolution studies.

Material and methods

Samples of *Sitophilus oryzae* were collected from two different sites. The first sample was collected from a packet of rice in a supermarket in Floresta, a town close to Maringá, Paraná, Brazil, in the northwestern region of the State of Paraná. The sample was taken to the laboratory at the State University of Maringá (UEM) and kept for approximately one year until the analysis conducted on this research. The second sample was collected in the town of Passo Fundo, Rio Grande do Sul State, Brazil, from wheat grains stored at the Embrapa Trigo. This sample was kept for three years at 'Embrapa', during which period its population was replicated in bottles filled with corn; it was later taken to a laboratory at UEM for analysis. The sample of *Sitophilus zeamais* was collected in a corn storage warehouse in Xavantina, Santa Catarina State, Brazil, transported to State University of Maringá and an electrophoresis was carried out soon after. All samples were conditioned *in vivo* in a temperature-controlled room.

The population under analysis comprised 76 specimens of *S. oryzae* from Paraná and 56 from Rio Grande do Sul States, and 56 specimens of *S. zeamais* from the state of Santa Catarina.

Whole adults were homogenized in 30 µL of 0.1 M Tris HCl^{-1} buffer, pH 7.5 and five drops of carbon tetrachloride to precipitate the fat in a 1.5 mL (Eppendorf) plastic tube. The tubes were centrifuged in a refrigerated centrifuge (Mickro 220Rd Hettich), at 14.000 rpm, at 4°C, for 15 minutes.

Starch gels were prepared with 17% corn starch (Penetrose 50®) in a buffer solution approximately four hours before the application of the sample to the electrophoresis run. Current assay comprised five different buffers: Tris-Citrate 7.0 (TC 7.0); Tris-Citrate 7.6 (TC 7.6); Tris-Edta-Maleate 7.4 (TEM 7.4) (SHAW; PRASAD, 1970); Tris-Maleate (TM) (MURPHY et al., 1996) without EDTA; Tris-Citrate 8.0 (TC 8.0) (PASTEUR et al., 1988). However, buffers TC 7.6 and TEM 7.4 yielded the best results for enzymes AAT, GDH, IDH and PGM, and enzymes ACP, GPI, MDH and ME, respectively.

Samples were applied to corn starch gel immediately after centrifugation with small strips of Whatman 3MM paper soaked in the centrifuged supernatant. Electrophoresis was kept under refrigeration for 17 hours at an electric current of approximately 250 V. After electrophoresis, the gel was cut horizontally into slices which were incubated with histo-chemical specific solutions for each isozyme system, following protocol by Murphy et al. (1996).

The eight enzymatic systems analyzed were Aspartate Amino Transferase (AAT), Acid Phosphatase (ACP), Glucose Dehydrogenase (GDH), Glucose 6-Phosphate Isomerase (GPI), Isocitrate Dehydrogenase (IDH), Malate Dehydrogenase (MDH), Malic Enzyme (ME) and Phosphoglucomutase (PGM)

Genetic interpretation was based on the quaternary structure of the enzymes, following Ward et al. (1992) (Table 1). Statistical estimates analyzed in current assay included genetic variability estimated by the heterozygosity indexes (He and Ho), according to Nei (1978); the F statistics (F_{IS}, F_{IT} and F_{ST}) from Wright (1978); the values of the allelic/allele frequencies, the identity (I) and the genetic distance (D) from Nei (1972). All these estimates were calculated with the program Pop gene 1.31 (YEH et al., 1997).

Table 1. Name, Enzyme Commission number (EC) and quaternary structure (QS) of enzymes analyzed in starch gel.

Enzyme (+ Abbreviation)	EC	QS
Aspartate Amino Transferase (AAT)	2.6.1.1	Dimeric
Acid Phosphatase (ACP)	3.1.3.2	Monomeric
Glucose Dehydrogenase (GDH)	1.1.1.47	Monomeric
Glucose 6 - Phosphate Isomerase (GPI)	5.3.1.9	Dimeric
Isocitrate Dehydrogenase (IDH)	1.1.1.14	Dimeric
Malate Dehydrogenase (MDH)	1.1.1.37	Dimeric
Malic Enzyme (ME)	1.1.1.40	Tetrameric
Phosphoglucomutase (PGM)	5.4.2.2	Monomeric

Results and discussion

Electrophoresis analysis of eight enzymatic systems from three *Sitophilus* samples detected 10 loci and 18 alleles. Detected loci were: *Aat, Acp, Gdh, Gpi, Idh, Mdh-1, Mdh-2, Mdh-3, Me* and *Pgm* (Figure 1).

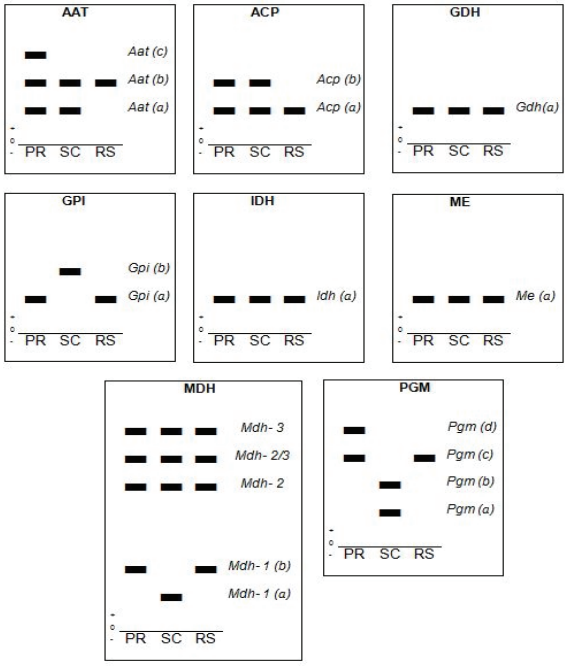

Figure 1. Schematic representation of enzymatic phenotypes from the analysis of eight enzyme systems analyzed in starch gel, with their respective alleles, present in the samples (PR: *S. oryzae* from Floresta in the state of Paraná; SC: *S. zeamais* from Xavantina in the State of Santa Catarina; RS: *S. oryzae* from Passo Fundo in the State of Rio Grande do Sul.

Figure 1 demonstrates the enzymatic expression of the detected alleles. In fact, alleles Acp^a, Gdh^a, Idh^a, Me^a and Aat^b were present in all three samples. All the alleles of loci *Mdh-2* and *Mdh-3* also occurred in all samples. Six enzyme systems (AAT, ACP, GDH, IDH, ME, MDH) revealed all the alleles from all loci of analyzed enzyme systems (Figure 1). Thus, the sample from the state of Paraná represented the greatest frequency of alleles when compared to the other two samples. Among the visualized loci, the enzymes MDH and PGM showed a greater number of alleles per locus (four), while the enzymes GDH, IDH and ME had the lowest number of alleles per locus (one allele).

Allele frequencies observations also identified four exclusive alleles in the *S. zeamais* sample and two exclusive alleles in the *S. oryzae* sample from Paraná. Exclusive alleles found in the *S. zeamais* sample were Gpi^b, $Mdh\text{-}1^a$, Pgm^a and Pgm^b at frequencies 1.0000, 1.0000, 0.0375 and 0.9625, respectively, while in the *S. oryzae* sample from the Paraná, exclusive alleles were Aat^c and Pgm^d at frequencies 0.0568 and 0.0132. Among the six exclusive alleles, Pgm^d at frequency 0.0132 in the sample from Paraná is a rare allele, owing to its low frequency. The remaining exclusive alleles presented high frequencies and they may be employed as genetic markers for such samples.

Table 2 shows allele frequencies in each locus for each sample. In the sample of *S. oryzae* from Paraná, only *Aat, Acp* and *Pgm* loci presented allelic variation, with three, two and two alleles, respectively, while none of the loci presented allelic variation in the sample from Passo Fundo. In the sample of *S. zeamais* from Santa Catarina, only *Aat, Acp* and *Pgm* loci presented any variation, with two alleles each. From the polymorphic loci, only the allele *Pgm* from the *S. zeamais* sample is in Hardy-Weinberg equilibrium.

Allele frequencies estimates, shown in Table 2, were used in a chi-square homogeneity test which revealed differences between samples from PR and SC. In current assay, only *Aat* and *Acp* among 10 loci analyzed in the two samples presented significantly different allele frequencies in all samples ($p < 0.05$).

The percentage of polymorphic loci was 30% in the *S. oryzae* sample from PR and 30% in the *S. zeamais* one. The RS sample did not present polymorphism. In studies performed by Beiras and Petitpierre (1981), the proportion of polymorphic loci was 14% for *S. oryzae*, 16% for *S. zeamais* and 10% for *S. granarius*. According to Grenier et al. (1994), the percentage of polymorphic loci was 62% in *S. oryzae*, 89% in *S. zeamais* and 67% in *S. granarius*.

Table 2. Allele frequency in samples of *Sitophilus oryzae* and *S. zeamais*. PR: *S. oryzae* sample from Floresta in the State of Paraná; RS: *S. oryzae* sample from Passo Fundo in the State of Rio Grande do Sul. SC: *S. zeamais* sample from the town of Xavantina in the State of Santa Catarina.

Loci	allele	PR	SC	RS
Aat	*a*	0.0568	0.2125	-
	b	0.8864	0.7875	1.0000
	c	0.0568	-	-
Acp	*a*	0.1000	0.0200	-
	b	0.9000	0.9800	1.0000
Gdh	*a*	1.0000	1.0000	1.0000
Gpi	*a*	1.0000	-	1.0000
	b	-	1.0000	-
Idh	*a*	1.0000	1.0000	1.0000
Mdh-1	*a*	-	1.0000	-
	b	1.0000	-	1.0000
Mdh-2	*a*	1.0000	1.0000	1.0000
Mdh-3	*a*	1.0000	1.0000	1.0000
Me	*a*	1.0000	1.0000	1.0000
Pgm	*a*	-	0.0375	-
	b	-	0.9625	-
	c	0.9868	-	1.0000
	d	0.0132	-	-

This difference in polymorphic loci percentage was due to the fact that Beiras and Petitpierre (1981) analyzed six loci from several enzymes, while Grenier et al. (1994) examined only three loci of the enzyme Esterase. Further, in research by Laing et al. (1976), the percentage of polymorphic loci ranged between 7.7 and 23.1% in the analysis of 13 isozyme loci in *Ptomaphagus hirtus*.

The average number of alleles per locus, however, ranged from 1.4 to 1.0, and the largest number of alleles was found in the Paraná sample, while the smallest number occurred in the sample from RS, with only one allele for each locus. Therefore, all of them are monomorphic. In studies carried out by Grenier et al. (1994), the authors demonstrated that the average number of allele per locus is 1.90 in *S. oryzae*, 2.48 in *S. zeamais* and 1.67 in *S. granarius*. According to Laing et al. (1976), an average of 1.21 alleles per locus occurred in *Ptomaphagus hirtus* beetles.

According to Laing et al. (1976), low genetic variability for isoenzymatic loci from the cave beetle *Ptomaphagus hirtus* (Leiodidae) was one third smaller than the genetic variability of most invertebrates, which may be attributed to environmental constancy and habitat uniformity. The low genetic variability found in *Sitophilus* has no parallel in other species of weevils, once two bi-sexual strains of *Strophosomus capitatus* and *Otiorrhynchus scaber* have a heterozygosity of 0.170 and 0.309, respectively (SUOMALAINEN; SAURA, 1973), which is clearly higher than that for *Sitophilus*: 0.061, 0.067 and 0.029 S. *oryzae, S. zeamais* and *S. granarius*, respectively (BEIRAS; PETITPIERRE, 1981).

In this survey, the average heterozygosity expected for *S. oryzae* was 0.0419 for the sample from Paraná and 0.0000 for the sample from Rio Grande do Sul. Since the rate was 0.0452 (according to data from Table 3) in the *S. zeamais* sample, it is evident that these pests showed a low genetic variability for allozymes when compared to rate in the study by Ward et al. (1992), featuring average heterozygosity value of 0.137 for insects. It seems that the largest average heterozygosity was observed in the *S. zeamais* sample, followed by the *S. oryzae* sample from Paraná.

Table 3. Measures of genetic variability of *Sitophilus* samples: He: Expected Heterozygosity; Ho: Observed Heterozygosity; P%: proportion of polymorphic loci; K: number of alleles per locus; SD: Standard Deviation; F: fixation index of Wright (Nei,1978).

Sample	He ± SD	Ho ± SD	P%	K ± SD	F
PR	0.0419 ± 0.0821	0.0091 ± 0.0287	30%	1.4000 ± 0.6992	0.7828
SC	0.0452 ± 0.1061	0.0100 ± 0.0242	30%	1.3000 ± 0.4830	0.7787
RS	0.0000 ± 0.0000	0.0000 ± 0.0000	0%	1.0000 ± 0.0000	0.0000

It has been observed that, in comparative terms, the heterozygosity rates found by current survey were lower than the average for other species of invertebrates and for three species of *Sitophilus*, mentioned by Beiras and Petitpierre (1981), and also lower than the average in insects, according to Ward et al. (1992).

According to data in Table 4, *S. oryzae* samples from Paraná and *S. zeamais* samples differed in allele frequencies of the loci *Aat, Acp* and *Pgm*. Expected heterozygosity for samples from Paraná and Santa Catarina was higher than the expected heterozygosity in insects (WARD et al., 1992) only for the *Aat* locus. On the other hand, in the case of *Acp* and *Pgm* loci from Paraná samples and *S. zeamais*, the expected heterozygosity was lower than the expected heterozygosity for the same loci in insects (WARD et al., 1992). However, the other loci of *S. oryzae* from Paraná, in all loci in the sample from Rio Grande do Sul, and in other loci of *S. zeamais*. Or rather, all specimens were homozygotes.

Table 4. Comparison of values of Expected Heterozygosity (He) per locus, for samples of *Sitophilus* from PR, SC, RS and for 170 species of insects (WARD et al., 1992).

Loco	He - PR	He - SC	He - RS	He - Insects
Aat	0.2103	0.3389	0.0000	0.134
Acp	0.1823	0.0396	0.0000	0.199
Gdh	0.0000	0.0000	0.0000	0.126
Gpi	0.0000	0.0000	0.0000	0.229
Idh	0.0000	0.0000	0.0000	0.124
Pgm	0.0261	0.0731	0.0000	0.274
Mdh-1	0.0000	0.0000	0.0000	0.063★
Mdh-2	0.0000	0.0000	0.0000	-
Mdh-3	0.0000	0.0000	0.0000	-
Me	0.0000	0.0000	0.0000	0.095
Average	0.0419	0.0452	0.0000	0.137

★rate referring to MDH enzyme.

When compared to the average for insects in general (WARD et al., 1992) and to other species of *Sitophilus* (GRENIER et al., 1994), the low heterozygosity estimated for *S. oryzae* and *S. zeamais* samples is probably due to the fact that these populations were initiated by few founders with few alleles (Founder´s Principle). Once the population expanded numerically, it maintained low genetic diversity.

Table 5 shows F statistics rates from Wright (1978). According to Wright (1978), when average rates of F_{IS} and F_{IT} are positive, there is an excess of homozygote specimens in the subpopulations and in the total population, respectively, while the resulting rates of F_{ST} measure the differentiation degree among populations. F_{ST} rates up to 0.05 reflect low population differentiation, while those between 0.05 and 0.15 indicate moderate differentiation between populations. On the other hand, rates between 0.15 and 0.25 demonstrate high population differentiation; values above 0.25 reflect a very high distinction.

Table 5. Summary of F statistics for two samples of *S. oryzae* and one of *S. zeamais*. N is sample size, F_{IS} is the average deviation from Hardy-Weinberg proportions in subpopulations, F_{IT} is the deviation from Hardy-Weinberg proportions in the total population and F_{ST} is Wright's standardized variance.

Locus	N	F_{IS}	F_{IT}	F_{ST}
Aat	124	0.7864	0.8041	0.0828
Acp	122	1.0000	1.0000	0.0486
Gdh	120	-	-	0.0000
Gpi	120	-	1.0000	1.0000
Idh	120	-	-	0.0000
Pgm	168	0.2359	0.9454	0.9286
Mdh-1	180	-	1.0000	1.0000
Mdh-2	180	-	-	0.0000
Mdh-3	180	-	-	0.0000
Me	120	-	-	0.0000
Average	143	0.7906	0.9612	0.8145

The average positive rate of the fixation index F_{IS} (0.7906) and the F_{IT} rate (0.9612) for the two *S. oryzae* samples and for the *S. zeamais* sample also confirm the excess of homozygote specimens. The mean value obtained for F_{ST} was 0.8145 and indicated that the three analyzed *Sitophilus* samples were genetically quite different.

Exclusive alleles observed in *S. oryzae* samples from Paraná and in *S. zeamais* from Santa Catarina had high frequencies which might indicate that the alleles were in a process of being rapidly established in these populations. The exception was the Pgm^d allele with a lower frequency, found in the sample from Paraná. According to Thorpe and Solé-Cava (1994), new alleles will appear in the population, initially at low frequencies, and the evolutionary outcome of these new alleles will depend on their relative physiologic performance (adaptive or selective rate) and on random changes in frequency, when transmitted to the following generations. Therefore, the most probable reason for the presence of these exclusive alleles could be the isolation of the populations or to mutations in DNA regions that encode a particular enzyme (which may differ in electrophoretic conditions) that, due to a recent mutation, has a very low frequency. The rapid increase in frequency is due either to natural selection or to genetic drift. Exclusive alleles may be employed as molecular markers for the species, which will be an aid for their identification.

Genetic identity (I) is one of the estimates used to determine whether two populations belong to the same species. Nei (1972, 1978) has provided statistics to calculate the identity of and the genetic distance between two populations. Thorpe and Solé-Cava (1994) used the genetic identity rates from Nei (1972) in their analysis of allopatric populations, including populations composed by specimens of the same species, by specimens from the same genus but different species, and by specimens from different genera. According to the above authors, the genetic similarity index (NEI, 1972) between two populations of the same species varies between 0.85 and 1.00, and between two species of the same genus, it varies between 0.35 and 0.85. Thus, if the genetic similarity between two taxonomic units is less than 0.85, they are certainly two distinct species; however, if it is below 0.35, the two groups are species belonging to different genera.

The genetic identity rates in Table 6 show that I = 0.6892 between samples from SC and PR; I = 0.9983 between PR and RS; I = 0.6925 between SC and RS, or rather, samples from Paraná and Rio Grande do Sul are from the same species, which is not evidenced neither among the SC and PR samples nor between the samples from SC and RS. Beiras and Petitpierre's (1981) survey showed I = 0.42 between *S. oryzae* and *S. zeamais* samples, and I = 0.11 between *S. granarius* and the samples from the two species.

Table 6. Genetic identity rates (above the diagonal line) and genetic distance (below the diagonal line) from Nei (1978) for two samples of *S. oryzae* (PR, RS) and one sample of *S. zeamais* (SC). Population (Pop.)

Pop.	PR	SC	RS
PR	-	0.6892	0.9983
SC	0.3722	-	0.6925
RS	0.0017	0.3675	-

According to method by Nei (1978), the genetic distance rates between PR and SC (0.3722) and between RS and SC (0.3675) indicate that

approximately 37% of the codons that encode for amino acids were replaced during the divergence process between samples of the two species, and that only 0.17% of the codons between samples of *S. oryzae* was replaced.

According to Thorpe and Solé-Cava (1994), in sympatric populations, significant variation in any locus represents a barrier to gene flow and, at least, partial reproductive isolation. In organisms with sexual reproduction by cross-fertilization, this variation indicates that the two populations should be considered different species. Thus, it may be said that there were no significant differences between the two populations and there was an absence of a diagnostic locus. It may be concluded that there are no genetic mechanisms of reproductive isolation. Or rather, the two populations correspond to the same species.

A possible explanation for the low polymorphism frequency of *S. oryzae* in relation to other species (e.g. *S. zeamais* and other Curculionidae weevils) is the fact that the analyzed samples came from lineages that were probably derived from a very small number of specimens or from the same progeny.

Natural selection and genetic drift may have played a main role in the random fixation of alleles in RS population. The environmental constancy of *Sitophilus* in grain warehouses or in laboratory conditions may also enhance similar genetic traits.

Conclusion

The samples studied in current assay have low genetic variability and low He (expected heterozygosity) rates.

Data confirm that samples from the States of Paraná and Rio Grande do Sul belong to the same species, *Sitophilus oryzae,* while the sample from the States of Santa Catarina belongs to a distinct species, *S. zeamais*.

The low genetic variability detected may be due to the fact that analyzed samples proceeded from a lineage that probably derived from a very small number of specimens or from the same progeny.

Isozyme markers are valuable tools for identifying insect-infesting stored grains.

Acknowledgements

We would like to thank the State University of Maringá (UEM) for the opportunity and support in apprenticeship; the Araucaria Foundation for financial assistance, advisor Dr. Erasmo Renesto for his excellent help and advice; Tiago Signorini for his collaboration and assistance and Dr. Paulo Roberto Valle da Silva Pereira for given samples and helped me to identify the species.

References

AZEVEDO, F. A. **As bases toxicológicas da ecotoxicologia**. São Carlos: Rima; Intertox, 2003.

BEIRAS, M. J.; PETITPIERRE, E. Allozymic variability and genetic differentiation in three species of *Sitophilus* L. (Coleoptera, Curculionidae). **Egyptian Journal of Genetics and Cytology**, v. 10, p. 95-104, 1981.

BOTTON, M.; LORINI, I.; AFONSO, A. P. S. Ocorrência de *Sitophilus zeamais* mots. (Coleoptera: Curculionidae) danificando a cultura da videira no Rio Grande do Sul. **Neotropical Entomology**, v. 34, n. 2, p. 355-356, 2005.

CHU, S. S.; LIU, Q. Z.; ZHOU, L.; DU, S. S.; LIU, Z. L. Chemical composition and toxic activity of essential oil of *Caryopteris incana* against *Sitophilus zeamais*. **African Journal of Biotechnology**, v. 10, n. 42, p. 8476-8480, 2011.

GALLO, D.; NAKANO, O.; SILVEIRA NETO, S.; CARVALHO, R. P. L.; BATISTA, G. C.; BERTI FILHO, E.; PARRA, J. R. P.; ZUCCHI, R. A.; ALVES, S. B.; VENDRAMIN, J. D. **Manual de Entomologia Agrícola**. São Paulo: Agronômica Ceres, 1988.

GRENIER, A. M.; PINTUREAU, B.; NARDON, P. Enzymatic variability in 3 species of *Sitophilus* (Coleoptera, Curculionidae). **Journal of Stored Products Research**, v. 30, n. 3, p. 201-213, 1994.

KIM, K. S.; CANO-RIOS, P.; SAPPINGTON, T. W. Using genetic markers and population assignment techniques to infer origin of boll weevils (Coleoptera: Curculionidae) unexpectedly captured near on eradication zone in Mexico. **Environmental Entomology**, v. 35, n. 4, p. 813-826, 2006.

LAING, C.; CARMODY, G. R.; PECK, S. B. Population genetics and evolutionary biology of the cave bee the *Ptomaphagus hirtus*. **Evolution**, v. 30, n. 3, p. 484-498, 1976.

LIEWLAKSANEEYANAWIN, C.; RITLAND, C. E.; EL-KASSABY, Y. A. Inheritance of null alleles for microsatellites in the white pine weevil (*Pissodes strobe* [peck] Coleoptera: Curculionidae). **Journal of Heredity**, v. 93, n. 1, p. 67-70, 2002.

LIRA, C. S.; PONTUAL, E. V.; ALBUQUERQUE, L. P.; PAIVA, L. M.; PAIVA, P. M. G.; OLIVEIRA, J. V.; NAPOLEÃO, T. H.; NAVARRO, D. M. A. F. Evaluation of the toxicity of essential oil from *Alpinia purpurata* inflorescences to *Sitophilus zeamais* (maize weevil). **Crop Protection**, n. 71, p. 95-100, 2015.

MARTINS, T. Z.; OLIVEIRA, N. C. Controle de *Sitophilus zeamais* (Coleoptera: Curculionidae) no milho pipoca (*Zea mays* l.) tratado com terra de diatomácea. **Campo Digital**, v. 1, n. 2, p. 79-85, 2008.

MURPHY, R. W.; SITES JR.; J. W.; BUTH, D. G.; HAUFLER, C. H. Proteins: isozyme electrophoresis. In: HILLIS, D. M.; MORITZ, C.; MABLE, B. K. (Ed.). **Molecular systematics**. Massachusetts: Sinauer Associates, 1996. p. 51-120.

NEI, M. Estimation of average of heterozygosity and genetic distance from small number of individuals. **Genetics**, v. 89, p. 583-590, 1978.

NEI, M. Genetic distance between populations. **American Naturalist**, v. 106, n. 949, p. 283-291, 1972.

NUKENINE, E. N.; CHOUKA, F. P.; VABI, M. B.; REICHMUTH, C.; ADLER, C. Comparative toxicity of four local botanical powders to *Sitophilus zeamais* and influence of drying regime and particle size on insecticidal efficacy. **International Journal of Biological Chemical Sciences**, v. 7, n. 3, p. 1313-1325, 2013.

PACHECO, I. A.; DE PAULA, D. C. **Insetos de grãos armazenados**: identificação e biologia. Campinas: Fundação Cargill, 1995.

PASTEUR, N.; PASTEUR, G.; BONHOMME, F.; CATALAN, J.; BRITTONDAVIDIAN, J. **Pratical isozyme genetics**. Chichester: Ellis Horwood, 1988.

PINTUREAU, B.; GRENIER A. M.; NARDON, P. Polymorphism of esterases in 3 species of *Sitophilus* (Coleoptera, Curculionidae). **Journal of Stored Products Research**, n. 27, v. 3, p. 141-151, 1991.

PUZZI, D. **Abastecimento e armazenagem de grãos**. São Paulo: Instituto Campineiro de Ensino Agrícola, 1986.

SHAW, C. R.; PRASAD, R. Starch gel eletrophoresis of enzymes – A compilation of Recifes. **Biochemical Genetics**, v. 4, p. 297-320, 1970.

SUOMALAINEN, E.; SAURA, A. Genetic polymorphism and evolution in parthenogenic animals. I. Polyploid Curculionidae. **Genetics**, v. 74, p. 489-508, 1973.

THORPE, J. P.; SOLÉ-CAVA, A. M. The use of allozyme electrophoresis in invertebrate systematics. **Zoologica Scripta**, v. 23, n. 1, p. 3-18, 1994.

VAN OOSTERHOUT C.; VAN HEUVEN, M. K.; BRAKEFIELD, P. M. On the neutrality of molecular genetic markers: pedigree analysis of genetic variation in fragmented populations. **Molecular Ecology**, v. 13, p. 1025-1034, 2004.

VIEIRA, N. R. A. **A cultura do arroz no Brasil**. 21. ed. Santo Antonio de Goiás: Embrapa Arroz e Feijão, 1999.

WANG, C. F.; LIU, P.; YANG, K.; ZENG, Y.; LIU, Z. L.; DU, S. S.; DENG, Z. W. Chemical composition and toxicities of essential oil of *Illicium fragesii* fruits against *Sitophilus zeamais*. **African Journal of Biotechnology**, n.10, v. 79, p. 18179-18184, 2011.

WARD, D. R.; SKIBINSKI, D. O. F.; WOODWARK, M. Protein heterozygosity, protein structure, and taxonomic differentiation. In: HECHT, M. K.; WALLACE, Bruce.; MACINTYRE, R. J. (Ed.). **Evolutionary Biology**, New York: Springer Science+Business Media. 1992. p. 73-159.

WRIGHT, S. **Evolution and genetics of populations**. Chicago: The University of Chicago Press, 1978.

YEH, F. C.; YANG, R. C.; BOYLE, T. B. J.; YE, Z. H.; MAO, J. X. **PopGene, the user-friendly shareware for population genetic analysis**. Alberta: Molecular Biology and Biotechnology Centre, University of Alberta, 1997.

Received on September 30, 2014.

Accepted on April 15, 2015.