NOTAS CIENTÍFICAS

Polymorphism of the grasshopper *Rhammatocerus schistocercoides* populations revealed by RAPD[1]

João Batista Tavares da Silva[2], Myrian Silvana Tigano[2], Bonifácio Peixoto Magalhães[2] and Célia Maria Torres Cordeiro[2]

Abstract – The objective of this work was to study the genetic variability of the grasshopper *Rhammatocerus schistocercoides* (Orthoptera: Acrididae) using RAPD analysis among individuals from three populations, one from Colombia and two from Brazil (Goiás and Mato Grosso States). Ninety scorable binary markers were obtained by fingerprinting with 11 oligonucleotide primers. Most of the polymorphism was attributed to 42 markers with variable frequency among the different populations. Although the existence of significant difference among populations ($P<0.0001$), most of the genetic variability was found within populations (87.7% of total variation). Pairwise distances between Colombian and Brazilian populations were 0.12 ($P<0.0001$) and 0.18 ($P<0.0001$) for Goiás and Mato Grosso, respectively. The pairwise distance between Goiás and Mato Grosso populations was 0.06 ($P<0.0001$). These data indicated that the phenotypic differences among populations are associated mainly with the geographical distances between the Brazilian and Colombian populations.

Index terms: Insecta, genetic marker, genetic variation, genetic distance.

Polimorfismo em populações do gafanhoto *Rhammatocerus schistocercoides* revelado por marcadores RAPD

Resumo – O objetivo deste trabalho foi estudar a variabilidade genética do gafanhoto *Rhammatocerus schistocercoides* (Orthoptera: Acrididae) por meio da análise de RAPD entre indivíduos de três populações, uma da Colômbia e duas do Brasil (Goiás e Mato Grosso). Noventa marcadores binários foram selecionados através de análise de polimorfismo com o uso de 11 oligonucleotídeos. A maior parte do polimorfismo observado foi atribuída a 42 marcadores com freqüência variável entre as diferentes populações. Apesar da existência de diferença significativa interpopulacional ($P<0,0001$), grande proporção da variabilidade genética foi detectada dentro das populações (87,7% da variação total). As distâncias entre as populações colombianas e brasileiras foram 0,12 ($P<0,0001$) e 0,18 ($P<0,0001$) para Goiás e Mato Grosso, respectivamente. A distância obtida entre Goiás e Mato Grosso foi 0,06 ($P<0,0001$). Estes dados indicam que as diferenças fenotípicas entre populações estão associadas principalmente às distâncias geográficas entre as populações do Brasil e a da Colômbia.

Termos para indexação: Insecta, marcador genético, variação genética, distância genética.

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[2] Embrapa-Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia, Caixa Postal 02372, CEP 70849-970 Brasília, DF. E-mail: jtavares@cenargen.embrapa.br, myrian@cenargen.embrapa.br, boni@cenargen.embrapa.br, celia@cenargen.embrapa.br

The grasshopper *Rhammatocerus schistocercoides* (Rehn, 1906) is found in the savanna area of Central Brazil causing severe damages to rice, soybean, maize and sugarcane crops, and native pastures. The hoppers concentrate mainly between the parallels 12° and 15° S and meridians 52° and 61° W. This area occupies partially the states of Rondônia, Mato Grosso, and Goiás, where, since 1983 severe outbreaks have been registered (Miranda et al., 1996). The insect is also present in Colombia, where it is reported as one of the main problems to the agriculture (Lecoq & Assis-Pujol, 1998).

Polymerase Chain Reaction (PCR) based DNA markers, such as those generated by random amplified polymorphic DNA-RAPD (Williams et al., 1990), also known as arbitrarily primed PCR markers (Welsh & McClelland, 1990), have been extensively used to detect polymorphism in several organisms, including grasshoppers within the genus *Melanoplus* (Chapco et al., 1992) and the species *Schistocerca pallens* (Silveira et al., 1998).

By comparing several individuals, the presence or absence of a specific amplified DNA fragment may identify each individual by its DNA fingerprint or polymorphic fragments as a character to study population dynamics.

The RAPD technique has become very popular because it allows to easily generate polymorphic markers using very small amounts of DNA, independently of any previous knowledge about the target DNA sequence (Mori et al., 1999). Knowledge of the variability between populations from different regions may be useful in the establishment of new strategies to control *R. schistocercoides*. The objective of this study was to obtain preliminary data on the intra and interpopulational genetic variability of *R. schistocercoides*, through RAPD markers.

Thirty female adults from Colombia (Puerto Gaitan, Department of Meta) and 55 from Brazil (29 from Silvania, Goiás, and 26 from Campos de Júlio, Mato Grosso) were used in this study. The insects were stored in ethanol at -20°C until use.

Femoral muscles of the grasshopper individuals were used for genomic DNA extraction, based on the method developed by Aljanabi & Martinez (1997). PCR amplifications were performed in a final volume of 30 μL, using 12 ng of template DNA, as described by Silveira et al. (1998). Eleven 10-mer primers (Operon Technologies Inc, Alameda, California) were used: OPD-02, OPE-01, OPE-06, OPE-08, OPAB-01, OPAB-05, OPAB-08, OPAB-12, OPAB-16, OPAB-17, and OPAB-18. These primers were selected based on the reproducible and informative amplification products, that ranged from 170 to 3,000 bp and were scored as presence or absence for all individuals.

Results were analyzed using Arlequin ver. 2000 software (Schneider et al., 2000). The relationship among individuals using the RAPD markers was evaluated according to hierarchical structure, considering populations from the different regions and individuals within regions. The estimate of the variance components associated with the different hierarchical levels was obtained with the aid of AMOVA (Analysis of Molecular Variance). The variance components among populations from different regions and individual variance within populations represent the sum of the variance component estimates of each RAPD marker. The pairwise variability between populations

was based on distances measured as proposed by Reynolds et al. (1983). The levels of significance for the estimates of variance component and the distances among population pairs were computed by non-parametric permutational procedures (Hull et al., 1993).

Ninety scorable binary characters were obtained by the amplification of 11 primers. Out of the 90 scorable bands, 63 were polymorphic (Figure 1). From these 63 polymorphic bands, 42 were present in the sample of each population with variable frequency (3% to 96%), 11 were fixed in one or two populations with frequency varying from 80% to 100%, and 10 were absent in one or two populations with frequency varying from 0% to 30%. This frequency distribution of bands shows that the differentiation among these populations was mostly attributable to the band frequency shared by the three populations.

Although the existence of significant difference among populations (Vp = 1.05, P<0.0001), most of the variability was found within populations (87.7% of total variation) (Table 1). The pairwise distances between Colombian and Brazilian populations were 0.12 (P<0.0001) for Goiás, and 0.18 (P<0.0001) for Mato Grosso, respectively. The distance obtained between Goiás and Mato Grosso populations was 0.06 (P<0.0001). These results indicate that the

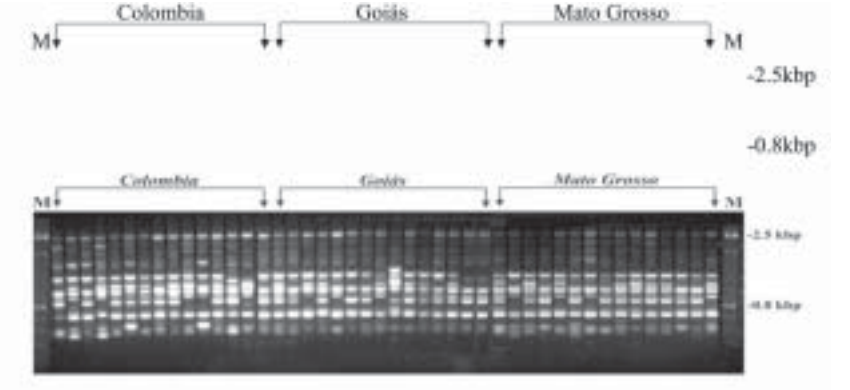

Figure 1. Agarose gel showing RAPD amplification pattern of *Rhammatocerus schistocercoides* populations from Colombia and Brazil (Goiás and Mato Grosso). Data are related to 15 individuals from each population. The primer used was OPAB-05. M: molecular marker (100 bp ladder).

Table 1. Variance components among and within populations of *Rhammatocerus schistocercoides* based on RAPD markers using AMOVA (molecular analysis of variance).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-values[(1)]
Among populations	2	74.85	1.05 (Vp)[(2)]	12.35	<0.0001
Within populations	82	615.62	7.50	87.65	
Total	84	690.47	8.55		

[(1)]P-value is the probability of obtaining a larger variance component by chance alone under the null hypothesis that the variance is zero; P-value is estimated from 16,002 sampling permutations. [(2)]Estimate of variance component for populations.

differences among populations are associated mainly with the distances between the Brazilian and Colombian populations. The geographic isolation between Colombia and Central Brazil may be responsible for these differences. This hypothesis is supported by the findings of Montealegre et al. (1998) and Lecoq & Assis-Pujol (1998). They reported that in early 1994, the Colombian Orinoquia suffered an unexpected *R. schistocercoide*s outbreak, which affected many natural grasses and some crops in a large part of the region. After comparing climatic conditions of the two countries, they concluded that *R. schistocercoides* did not spread to Colombia from Brazil.

The RAPD technique was successfully used to show polymorphism among and within *R. schistocercoides* populations. However, further analysis, using a larger number of insects and markers, as well as different populations, will be necessary to confirm these preliminary findings.

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