

## Genotype characterization of *Haematobia irritans* from different Brazilian geographic regions based on randomly amplified polymorphic DNA (RAPD) analysis<sup>1</sup>

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**ABSTRACT.**- Brito L.G., Regitano L.C.A., Huacca M.E.F., Carrilho E. & Moya Borja G.E. 2007. **Genotype characterization of the horn fly *Haematobia irritans* from different Brazilian geographic regions based on randomly amplified polymorphic DNA (RAPD) analysis.** *Pesquisa Veterinária Brasileira* 27(1):1-5. Laboratório de Sanidade Animal, Embrapa Rondônia, BR 364 Km 5,5, Porto Velho, RO 78900-970, Brazil. E-mail: [luciana@cpafro.embrapa.br](mailto:luciana@cpafro.embrapa.br)

Blood-sucking diptera are important parasites in bovine production systems, especially regarding confinement conditions. *Haematobia irritans*, the horn fly, is one of the most troublesome species within bovine production systems, due to the intense stress imposed to the animals. An important aspect while studying the variability within a species is the study of the geographic structure of its populations and, attempting to find out the genetic flow of Brazilian populations of horn fly, the RAPD technique, which is suited for this purpose, has been used. The use of molecular markers generated from RAPD made it possible to identify the geographic origin of samples from different Brazilian geographic regions, as well as to estimate the genotypic flow among the different Brazilian populations of the horn fly.

INDEX TERMS: *Haematobia irritans*, genetic diversity, RAPD-PCR, molecular markers.

**RESUMO.**- [Caracterização genotípica de *Haematobia irritans* procedentes de diferentes regiões geográficas brasileiras baseada na análise do DNA polimórfico amplificado ao acaso (RAPD-PCR).] Dípteros hematófagos são importantes parasitas dentro de sistemas de produção de bovinos, especialmente em confinamento. *Haematobia irritans*, a mosca-dos-chifres, é uma das espécies que maiores problemas causa em sistemas de produção de bovinos, dado ao intenso estresse que impõe aos animais. Um importante aspecto quando se estuda a variabilidade genética dentro das espécies é o estudo da estrutura geográfica destas populações. Buscando-se estimar a similaridade genotípica das diferentes populações brasileiras da mos-

ca do chifre utilizou-se a técnica do DNA polimórfico amplificado ao acaso (RAPD-PCR), que mostrou-se eficiente para tal propósito. A utilização dos marcadores moleculares gerados através da técnica de RAPD-PCR tornou possível a identificação da origem geográfica das amostras das diferentes regiões geográficas brasileiras, assim como, estimar o fluxo genotípico entre as diferentes populações brasileiras da mosca-dos-chifres.

TERMOS DE INDEXAÇÃO: *Haematobia irritans*, diversidade genética, RAPD-PCR, marcadores moleculares.

### INTRODUCTION

The presence of *Haematobia irritans* in Brazil, popularly known as "horn fly", has been detected since 1970's (Valério & Guimarães 1983). Its spread from the country's north region was rapid and reached important cattle farming regions in main product's beef cattle (Araújo 1991). Nowadays the fly can be found all over the Brazilian territory.

The presence of horn fly has a negative impact on cattle performance and production, expressed by appetite and weight loss and both milk production and alimentary conversion decrease. Every year in the US losses in animal production caused by ectoparasites exceed US\$ 2.26 billion, US\$ 730 million due exclusively to horn fly (Byford et al. 1992).

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In 1990 there was an advance in the field of molecular markers based on PCR (Polymerase Chain Reaction) with the idea of using shorter and randomly sequenced primers to direct the amplification reaction, thus eliminating the need of previous knowledge of the sequence to be amplified. Such technique was named RAPD (Random Amplified Polymorphic DNA) (Williams et al. 1990).

An important aspect to be considered while studying the variability within a species is the study of the geographic structure of its populations, i.e., the study of the richness of genotypes within and among populations, which involves two different but related components: demographic and genetic structure. The demographic structure refers to processes influencing the number and distribution of phenotypical classes of individuals, such as age groups, sex ratio and variant in life history (Roderick 1996).

The second component of population structure is genetic structure, which can be described as the distribution of genetic variation resulting from the action of factors such as migration, selection, mutations and genetic drift. Demographic aspects are included in genetic processes, what makes it possible to use the genetic structure to deduce the history and demographic processes of the species (Roderick 1996).

To analyze genetic variability of horn fly in Brazil, Castiglioni-Ruiz et al. (1997) studied the pattern of esterase bands in four Brazilian populations of this fly. There were eight esterase bands, named EST1 to EST8, from the anode extremity of the gel, verifying that the four populations of horn fly studied were very similar regarding their esterase bands, differing only in bands EST2 and EST7, both found exclusively in one population.

In another study conducted by Castiglioni-Ruiz (2001), different populations of horn fly from four Brazilian localities (two in the state of São Paulo, one in Mato Grosso do Sul and one in Acre) were compared, along with one American sample from Texas, US, using the RAPD technique. The author found similar results regarding the similarity indexes of the populations studied, the sample from Acre being the one which differed most among all of them.

In this study, the RAPD technique was used to analyze genetic variability of populations of horn fly from all five geographic regions of Brazil, attempting to evaluate the genotypical similarity of the different populations studied and to obtain a RAPD marker able to identify the geographic origin of each population studied.

## MATERIALS AND METHODS

### Origin of the samples, DNA extraction and quantification

The analyzed DNA was obtained through samples of adult populations of horn fly from five different Brazilian localities: Boa Vista (RR), Mossoró (RN), Seropédica (RJ), Campo Grande (MS) e Rosário do Sul (RS), each one in a different geographical region of the country.

In the present study, genomic DNA from 250 flies of all different regions was used. DNA extraction was done in groups of five flies each, with 10 repetitions for each locality. In order to avoid the blood meal contamination of host, the flies were decapitated and only the heads, sails and chests of the flies were used in that analysis.

The protocol for DNA extraction for groups of five individuals follows the technique described by Dellaporta et al. (1983), with some modifications.

The concentration and purity of DNA was determined by spectrophotometer at optical densities of 260 and 280nm (Gene Quant, Pharmacia Biotech, Uppsala, Sweden).

Using this data, samples were diluted in such a way that for each amplification reaction by PCR there were 5ng of DNA for ml.

### DNA amplification

PCR amplifications was done in a Mastercycler Gradient Thermocycler (Eppendorf AG, Hamburg, GE) according to the protocol described by Williams et al. (1990), using RAPD 10 mer primers (Operon Technologies, Alameda, CA). Working solutions containing 20mM of each primer diluted in sterile deionized water were prepared and stored at -20°C.

In the samples to be amplified, 3.0 mL primer and 25 ng DNA were used, in 15 mL reaction volume. RAPD amplified fragments were then separated through electrophoresis in 1.5% agarose gel at 0,5 V cm<sup>-1</sup> for approximately 2.5 h. The gel was prepared with tris-borate EDTA buffer (1X), and visualized by ethidium bromide staining. Molecular weight markers with 50 bp DNA Ladder (Amersham Pharmacia) and 250 bp DNA ladder (Amersham Pharmacia) were used, always mixing 3mg of the base pair marker to 1mL of loading buffer and, in order to get homogenous samples, they were centrifuged with 13,000 rpm for 15 seconds.

### Selection of RAPD primers

Samples of different Brazilian populations of horn fly were amplified with 60 RAPD primers. Amplicons products were assessed for the number and quality of polymorphic loci. Sixteen primers that amplified reproducible polymorphic bands were selected for analysis. All select samples were amplified twice to verify patterns of reproducibility. If ambiguous results were obtained from amplicons, the RAPD reaction was repeated. RAPD loci were scored as 0 (band absent) or 1 (band present).

### Data analysis

The gel images were analyzed using Kodak EDAS ("Electrophoresis Documentation and Analysis System") 290 (Kodak Inc.) for estimation of the molecular weights of bands. Statistical analysis was carried out using the computer program NTSYS (*Numerical Taxonomy and Multivariate Analysis System*) (Exeter Softwares, Seauket, NY) (Rohlf 1993). A pair wise similarity matrix between genotypes was generated using the JACCARD coefficient (Jaccard 1901). The similarity coefficient,  $J = a/a + b + c$ , were:  $a$  is the number of positives bands shared by both individuals  $x$  and  $y$ ,  $b$  and  $c$  are the numbers of fragments present in individuals  $x$  and  $y$ . The SAHN clustering program was then used to group the entries based on similarity coefficients using the unweighted pair-group method using arithmetic average (UPGMA).

Objectifying the detection of the polymorphisms generated for RAPD, were used the bionalyzer able to process approximately up to 12 samples of 1mL in 30 minutes. The commercial kit DNA 1000 LabChips were prepared and loaded with the samples as recommended by the manufacturer. The contents of each well on the chip were mixed *in situ* by gentle pipetting and then vortexed for 1 minute. After being vortexed, chips were immediately inserted into the bioanalyzer and processed. All experiments with the amplicons were performed using Agilent Biosizing software (version A.01.10).

From similarity indexes matrix obtained from data of the polymorphics bands generated by RAPD, the analysis was performed

using EINSIGHT 3.0 software (Infometrix Inc., Seattle, WA), which uses hierarchical groups analysis methods as well as the principalities components and through the construction of dendograms makes it possible to observed the interrelations among the several analyzed genotypes and also to estimate the Euclidean distance among the samples.

To estimate the genotype distance among the Brazilian populations of horn fly, made used Penrose method, which tests multivaried distances of two or more populations, considering data of mean, variance and covariance, and also Mahalanobis method, which considers the correlation among variables, besides the numeric values used in Penrose test (Ayres et al. 2000).

## RESULTS AND DISCUSSION

The genomic DNA amplification of horn fly populations using the 16 RAPD selected primers produced 321 fragments. These fragments varied from 1,714 to 229 bp. The total number of bands produced by each primer varied from 10 (primer H20) to 28 (primer G4 and G16). From the 16 selected primers, 12 generated 15 bands or more. Regarding the total number of bands produced by population, the most polymorphic was RN (70 bands), followed by RJ (68 bands), MS and RS (62 bands) and RR (59 bands) (Table 1).

A 0/1 matrix for all 16 primers selected was generated by compiling only those frames which showed a 1 within the individual primer frameworks. The resulting cluster analysis revealed four major clusters that not correlated completely with the origin of samples. The program sharpened the samples which were not aligning in the analysis, which were considered as outliers, this analysis was based on similarity among samples (Fig.1). The exclusion of these samples promoted the improvement of the results and the samples presenting the least similarity inside their group were chosen, in order to enhance the accuracy of the analysis, by raising similarity and diminishing the Euclidean distance among samples

The hierarchical group analysis shows that Roraima (RR) was the farthest population, presenting zero similarity to the others, while the closest populations were Rio Grande do Sul (RS) and Mato Grosso do Sul (MS), with 0.063 similarity and

Rio de Janeiro (RJ) and Rio Grande do Norte (RN) showed 0.036 similarity among these populations (Fig.2).

The similarity between RJ/RN groups and RS/MS groups was 0.025, demonstrating that Brazilian populations, with the exception of Roraima, share approximately 2.5% of nucleotide sequences complementary to the selected primers, which promotes the genotype distinction among Brazilian populations from different geographical origins.

The Penrose and Mahalanobis tests also demonstrated that the genotypically most similar populations were Rio Grande do Sul (RS) and Mato Grosso do Sul (MS) (Table 2).

The mating among the populations of horn fly from the states of Rio Grande do Sul (RS) and Mato Grosso do Sul (MS) generate individuals that share nucleotide sequences, a fact that does not occur among the populations of Roraima (RR) and Rio Grande do Sul (RS), which do not share or share just a few nucleotide sequences.

This result suggests a low contact among the horn fly populations of these regions, a consequence of the non existing

**Table 1. Random amplified polymorphic DNA primers used and number of fragments generated in five different Brazilian populations of horn fly**

Primer	Sequence	Number of RAPDs
OPE1	ccc aag gtc c	21
OPE9	ctt cac ccg a	14
OPE11	gag tct cag g	15
OPE10	cac cag gtg a	13
OPE13	ccc gat tcg g	17
OPE14	tgg cgc tga c	14
OPE15	acg cac aac c	24
OPE18	gga ctg cag a	27
OPG4	agc gtg tct g	28
OPG6	gtg act aac c	27
OPG16	agc gtc ctc c	20
OPG19	gtc agg gca a	15
OPH8	gaa aca ccc c	29
OPH12	acg cgc atg t	26
OPH16	tct cag ctg g	21
OPH20	ggg aga cat c	10
Total		321

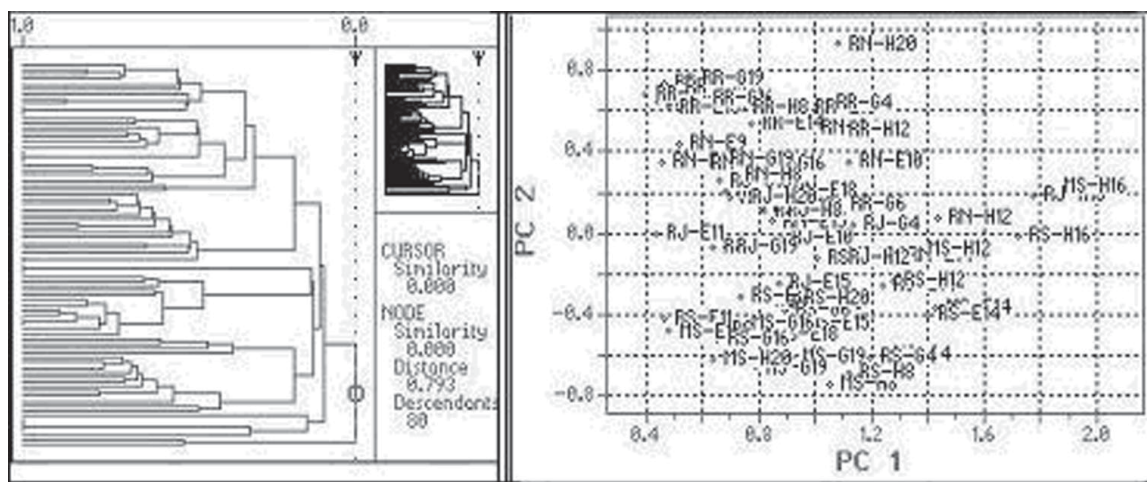


Fig.1. Relationships among Brazilian populations of horn fly generated by principalities components using the selected primers.



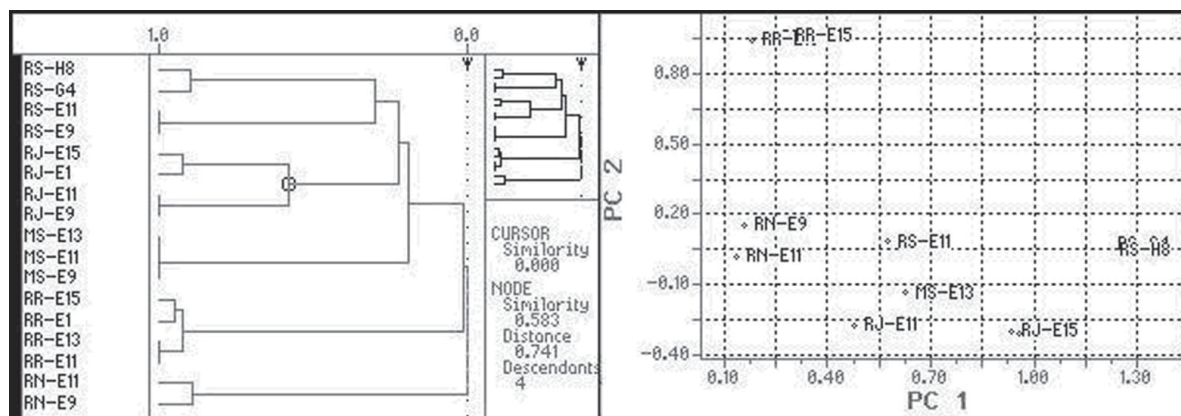


Fig.2. Relationships among Brazilian populations of *Haematobia irritans* based on principal components after the exclusion of the outlier primers.

**Table 2. Comparison among the Brazilian populations of horn fly through the Penrose and Mahalanobis tests**

	RR	RN	RJ	MS	RS
Penrose test					
RR	0	-	-	-	-
RN	1.1469	0	-	-	-
RJ	1.4336	0.0194	0	-	-
MS	1.2892	0.0042	0.0056	0	-
RS	1.4647	0.016	0.0038	0.0002	0
Greatest distance (RR e RS)	1.4647	-	-	-	-
Least distance (MS e RS)	0.0002	-	-	-	-
Mahalanobis test					
RR	0	-	-	-	-
RN	1.1469	0	-	-	-
RJ	1.4336	0.0194	0	-	-
MS	1.2892	0.0042	0.0056	0	-
RS	1.4647	0.016	0.0038	0.0002	0
Greatest distance (RR e RS)	1.4647	-	-	-	-
Least distance (MS e RS)	0.0002	-	-	-	-

trade of animals between these two states. Roraima is not an important state in cattle farming activities, with only one slaughter facility and without any animal commercial business with the rest of the country. These facts promotes an isolation of this horn fly population when compared to the other Brazilian populations of horn fly, blocking new gene flows to it, making it quite distinct from the ones located to the south.

Through the main component analysis it was possible to observe that the Brazilian populations of horn fly showed polymorphic loci able to characterize them genotypically through the OpE9, OpE11, OpE13, OpE15, OpG4 and OpH8 primers.

The primer were best characterized the different Brazilian populations of horn fly was OpE11 (Fig.3), once it generated a single genotype pattern for each one of populations studied, being the best molecular marker that excelled the geographic origin of the samples.

In the present study analysis of the RAPD-PCR products used as molecular markers for identify the geographical origin of the samples of horn fly confirmed the high-resolution capacity and sensitivity found in previous investigations (Valentini et al. 1996).

A RAPD locus as described here consists of a set of comigrating RAPD fragments amplified by the same RAPD primer (Skroch & Neinhuis 1995) Here, by repeated analysis of single RAPD-PCR samples during the evaluation of the procedure, a length-dependent variation was observed in

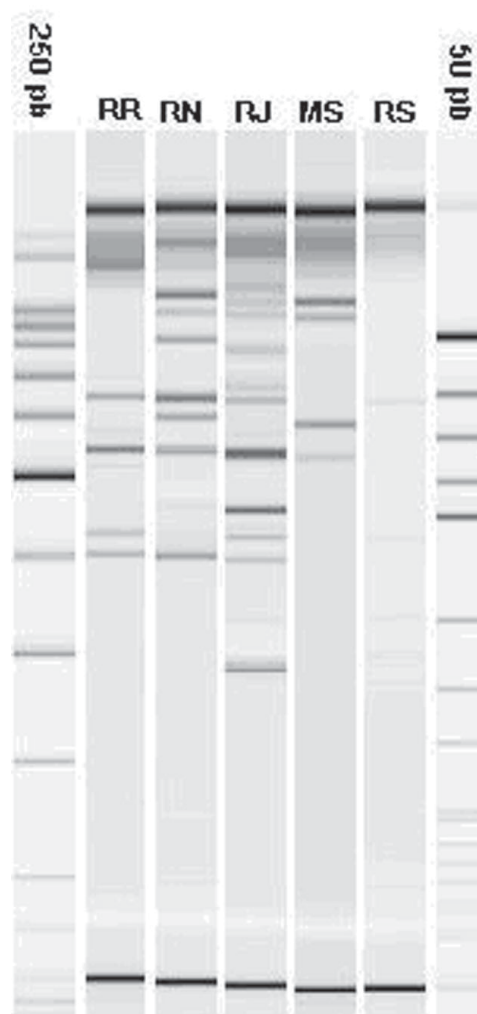


Fig.3. RAPD analyzes obtained from OpE11 primer for Brazilian populations of horn fly.

calculated sizes of individual bands in a range as described earlier (Panaro et al. 2000). In contrast, the pattern of the graphical image generated by the RAPD-PCR fragments during repeated runs of same samples was consistent. Previous populations analysis of parasites by RAPD-PCR demonstrated that this method was suitable for characterize different populations (Pavlicek et al. 1999, Costa et al. 2001, Hampl et al. 2001, Posedi et al. 2004).

In the present investigation, Brazilian populations of horn fly could be assigned by the analysis of RAPD-PCR products from samples of flies were shown to be capable of distinguishing the geographical origin of the horn fly. In other study conducted by Castiglioni-Ruiz (2001) using the products of RAPD-PCR, which compared different horn fly populations from the states of São Paulo, Mato Grosso do Sul and Acre, accompanied by an American sample from Texas, the author could not observed polymorphic loci able to characterizing genotypically the geographic origin of samples studied, where they presented high indexes of similarity between the samples. The fact that the genetic comparison based on the RAPD-PCR product showed that the Brazilian horn fly populations which there were closely relation were Mato Grosso do Sul (MS) and Rio Grande do Sul (RS), and this fact can be explained by the genetic flows between these populations maybe for the animal trade, once the most relevant national herds are in these states (Araújo 1991).

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