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Genetic diversity analysis with RAPD linked to sex identification in the sugar cane borer *Diatraea saccharalis*

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ABSTRACT. *Diatraea saccharalis* is an insect that causes considerable losses in the sugar cane crop. Our aim was to contribute to the knowledge of the biology of *D. saccharalis*, with the report of DNA fragments involved in the differentiation between the male and female of this species using the RAPD sex molecular marker GyakuU-13, which is specific for the W chromosome of *Bombyx mori.* Another point evaluated in this study was the genetic diversity of a *D. saccharalis* population maintained by inbreeding in a laboratory culture. The profile of sex-specific fragments was analyzed, and the genetic variability of this population was estimated. An analysis of the molecular markers showed only one fragment, of approximately 700 bp, that could be considered as a female sex marker in *D. saccharalis.*

Key words: *Diatraea saccharalis*; RAPD; Sex-specific marker; Genetic diversity

INTRODUCTION

In Brazil, the sugar cane borer, *Diatraea saccharalis* (Fabricius, 1794), is a pest that causes considerable damage to the sugar-ethanol agribusiness. The damage caused by this insect results in the formation of galleries in the stem, leaving the plant vulnerable to the entry of fungi, which are responsible for the inversion of sucrose (Botelho, 1992). Biologically, this pest has been controlled since the creation of the massive wasp *Cotesia flavipes* (Hymenoptera, Braconidae) in specialized laboratories, since there is as yet no effective insecticide for its control (Alleyne et al., 2001).

D. saccharalis studies in the literature are about their rearing, population dynamics, parasitoid-host relationships, and some basic aspects of morphology, physiology and molecular biology (Conte, 1994; Dossi and Conte, 2002; Ruvolo-Takasusuki et al., 2002; Beserra and Parra, 2004; Victoriano and Gregorio, 2004; Bravo et al., 2008). Recently, our research group published the first putative antimicrobial peptide from *D. saccharalis* against bacterial infection (Silva et al., 2010). However, whether it is possible to determine molecular markers linked to sex determination in this insect is unknown. The lack of knowledge hampers the implementation of techniques for their biological control, since there is need for basic knowledge of insect biology to conduct evaluations of the interactions between the parasite and its host.

In Lepidoptera, the identification of individual chromosomes based on the size and banding patterns is difficult because they have a large number of chromosomes, which have small and apparent similar sizes (Mandrioli et al., 2003). In this insect order, the females have heterogametic sex chromosomes, represented as WZ, and the male, ZZ. Preliminary cytogenetic studies in *D. saccharalis* indicate the presence of numerous tiny chromosomes with similar morphology (Heideman C, personal communication).

Due to the difficulty in identifying sex in early larval stages and studying the karyotype, several recent studies have tried to correlate DNA markers with sex identification in insects such as *Ostrinia nubilalis* Hübner (Coates and Hellmich, 2003) and *Cydia pomonella* (Fuková et al., 2007). The identification of molecular markers for the sex chromosome of *Bombyx mori* (Abe et al., 1998, 2005) enables the recognition of the genetic sex of an embryo or larva; such marker would be attractive in the case of *D. saccharalis.* In this study, we determined the feasibility of using the molecular marker GyakuU-13, involved in sex identification in the silkworm silk, in sugar cane borer individuals. Another point evaluated in this study was the genetic diversity of a *D. saccharalis* population maintained by inbreeding in a laboratory culture.

MATERIAL AND METHODS

Individuals of *D. saccharalis* were reared under controlled environmental conditions (12-h light/dark cycle, 25 ± 1°C, 70% relative humidity) with artificial diet (Hensley and Hammond, 1968).

The genomic DNA of moths, 14 individuals of each sex, was extracted as described by Monesi et al. (1998), with modifications. Briefly, the moths were macerated in liquid nitrogen, followed by the addition of 350 µL homogenization buffer (10 mM Tris-HCl, pH 7.5, 60 mM NaCl, 10 mM EDTA, pH 8.0, 5% sucrose, 0.15 mM spermidine, 0.15 mM spermine). Proteins

were removed by the addition of an equal volume of proteinase K buffer (200 mM Tris-HCl, pH 9.0, 30 mM EDTA, pH 8.0, 2% SDS) and 100 µg/mL proteinase K, and incubation for 1 h at 60°C. DNA was purified by extraction and precipitation as described elsewhere (Sambrook and Russel, 2001) and stored at -20°C.

The primer GyakuU-13 (5' CCTTGGTCGG 3') was used for amplification reactions from genomic DNA of *D. saccharalis*, as described by Abe et al. (2005). After amplification, the product was subjected to electrophoresis on a 1.5% agarose gel for 1 h at 100 V and a 6% polyacrylamide gel for 8 h at 110 V.

For analysis of the genetic diversity of the population, a matrix was built with the binary data of presence and absence of bands of identical molecular size (same fragment), with identification of each individual. This similarity matrix encoding "1" as the presence of the band in the gel and "0" as its absence was used to determine genetic distances between all possible pairs of individuals. The genetic variability between individuals was estimated using the method of Nei's unbiased genetic distance (1978). The analyses were made in the PopGene 1.32 statistical program (Yeh and Boyle, 1997), considering the parameters for dominant markers in diploids. In order to plot the pattern of genetic divergence, a dendrogram based on Nei's genetic distance (1978) was obtained, using the UPGMA clustering algorithm (unweighted pair-group method using arithmetic average).

RESULTS AND DISCUSSION

Figure 1 shows the molecular marker GyakuU-13, used to detect a sex-specific marker in *D. saccharalis* DNA and the positive control with the amplification of *B. mori* female and male samples. The amplified samples generated several fragments in *D. saccharalis*, and it was evident that the presence of a fragment of approximately 700 bp was unique for the DNA samples from female moths. The size of the amplicon in *D. saccharalis* differs from that obtained by Abe et al. (2005), described as 600 bp in strains of *B. mori* using the same primer. This fact could be explained because this marker sequence is associated with repetitions in the genome, and it is not surprising that the result is different with *D. saccharalis*. These data demonstrate that the primer GyakuU-13 can be used as a sex-specific molecular marker in *D. saccharalis*.

The primer GyakuU-13 produced 39 fragments of molecular size between 220-3054 bp in the experiments. In the total of the analyzed loci, it was observed that all were polymorphic (100%). The high number of bands generated by amplification with primer GyakuU-13 shows the possible variability among the individuals and the ability to estimate its probable genetic distance. The results are shown in the dendrogram depicted in Figure 2. It is evident that the individuals were grouped together according to sex. The male is subdivided into two subgroups and female into four subgroups.

This preliminary study will lead to future studies to develop research strategies and the construction of a molecular genetic marker to identify genes or DNA segments related to sex determination in *D. saccharalis*.

In summary, this study showed that there is binding of a fragment profile with, at least, one fragment related to sexual identity in *Diatraea saccharalis*, and that this population has significant reserves of genetic variability.

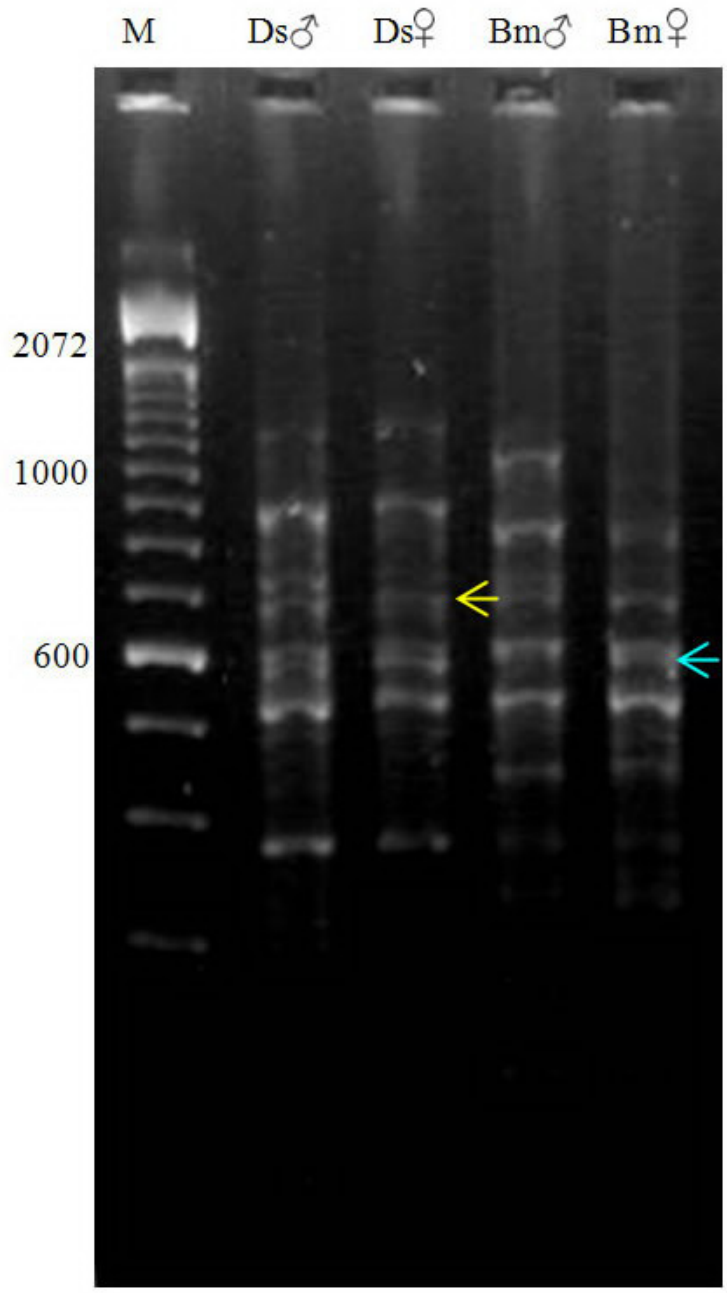

Figure 1. *Diatraea saccharalis* sex molecular marker. The amplicon of ~700 bp (yellow arrow) was detected only in *D. saccharalis* (Ds) females, using the RAPD GyakuU-13 primer. The positive control was carried out with *Bombyx mori* (Bm) DNA, where a fragment of ~600 bp (blue arrow) was detected only in female DNA (6% polyacrylamide gel). M = 100-bp molecular weight marker (Invitrogen).

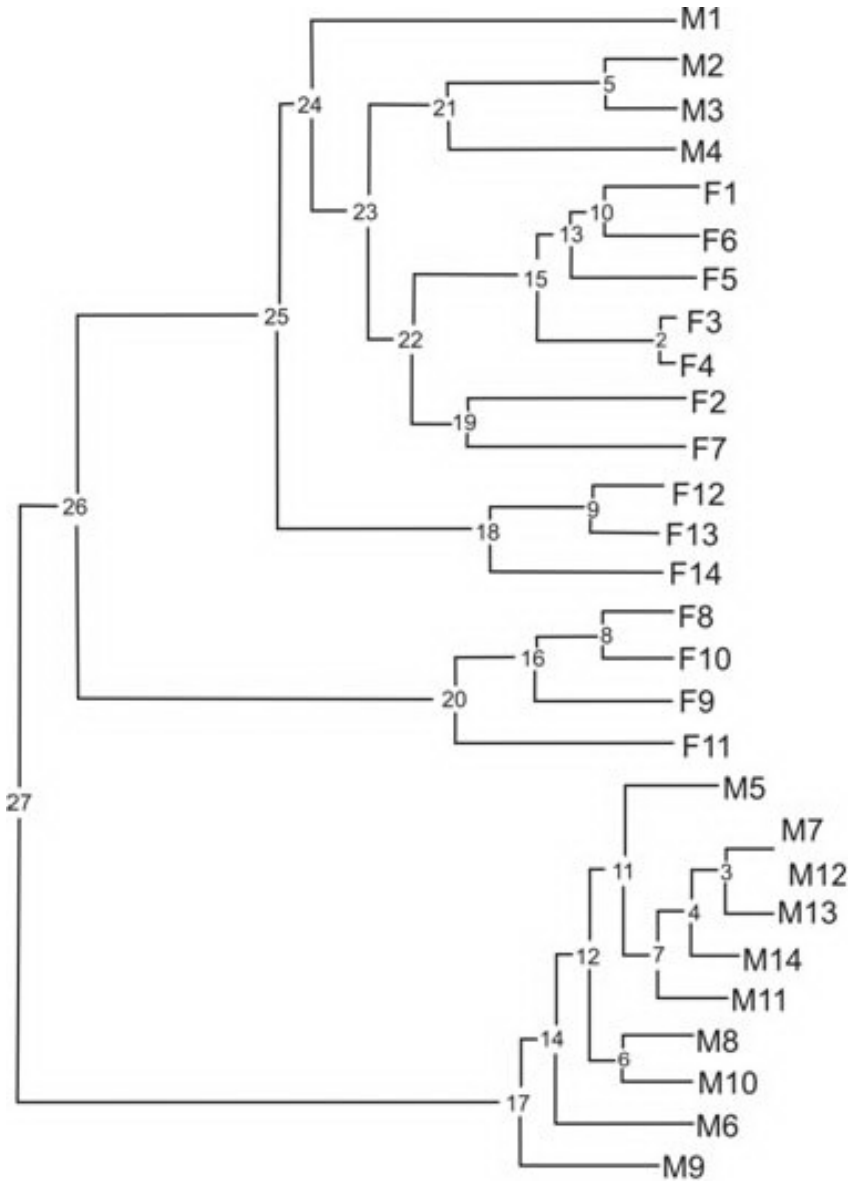

Figure 2. Cluster analysis of the 14 individuals of each sex of *Diatraea saccharalis* using the method of Nei's unbiased genetic distance (1978) and grouping using UPGMA (unweighted pair group method).

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