Genetic Diversity and Structure of Brazilian Populations of *Diatraea saccharalis* (Lepidoptera: Crambidae): Implications for Pest Management

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ABSTRACT The sugarcane borer, *Diatraea saccharalis* (F.), is the main pest of sugarcane in Brazil. Genetic variability and gene flow among 13 Brazilian populations of the species were evaluated based on mitochondrial DNA sequences to estimate the exchange of genetic information within and among populations. We found high genetic structure among sampled localities ($\Phi_{ST} = 0.50923$), and pairwise genetic distances were significantly correlated to geographic distances. Demographic analysis and genealogical network of mitochondrial sequences indicate population growth and admixture of *D. saccharalis* populations, events likely related to the sequential expansion of the corn and sugarcane crops in Brazil. The implications of these findings for pest management are discussed.

KEY WORDS corn, genetic structure, geographic variation, mitochondrial DNA, sugarcane field

Introduction

For insect populations in their natural habitats, genetic diversity and population structure at neutral markers are shaped by intrinsic and extrinsic traits of a species. Intrinsic traits include migration behavior and dispersal ability, while extrinsic features include host plant use, and distribution range of populations (Kirk et al. 2013). Population genetics tools have been successfully applied to insect pest management studies characterizing gene flow among populations (Groot et al. 2011, Medina et al. 2012), identification of invasive species (Silva-Brandão et al. 2012), and characterization of host races (Busato et al. 2004), among others (Rollins et al. 2006, Kirk et al. 2013).

The sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), is a serious pest in Brazil, responsible for significant damages and economic losses to several cultivated grasses (Hughes and Dorn 2002), yet comprehensive population genetics studies for Brazilian populations are lacking. *D. saccharalis* can attack sugarcane (*Saccharum* spp.), corn (*Zea mays* L.), rice (*Oryza sativa* L.), and sorghum (*Sorghum bicolor* (L.) Moench) (Dyar and Heinrich 1928, Box 1931, Myers 1935, Long and Hensley 1972, Pashley et al. 1990). The insect larvae feed in the interior of plants, as a borer, and all life stages, from egg to adult, are usually completed within one plant (Guagliumi 1972). The sugarcane borer is currently widely distributed from the southwestern United States through Central America (including the Caribbean islands) to South America (Bleszynski 1969, Pastrana 2004). In Brazil, the species is found in sugarcane fields from the Amazon basin across the northeastern, southeastern, and southern regions of the country (Guagliumi 1972). It is believed that the sugarcane borer was originally a riparian species probably restricted to tropical South America, where it fed on aquatic and semi-aquatic native hosts (Myers 1935). The species may have expanded its range after corn domestication ~7,500 years ago, and later after sugarcane introduction in the South and Central Americas in the 15th century (Pashley et al. 1990). In Brazil, the initial expansion of populations of *D. saccharalis* may have been facilitated by the presence of endemic species of *Saccharum* in the region [such as *Saccharum angustifolium* (Nees), *Saccharum asperum* (Nees) Steudel, and *Saccharum villosum* Steudel], and of wild rice [such as *Oryza alta* Swallen, *Oryza glumipatula* Steudel, and *Oryza grandiglumis* (Döll) Prodoehl], including species found on flooded areas in the northern region (Vianna et al. 2006).

Morphological variation in size and coloration has been reported for *D. saccharalis* along its distribution, and genital traits are believed to be the only reliable characters for species diagnosis (Box 1931). Besides morphological variances, populations of *D. saccharalis* also present behavioral and pheromone differences (Katiyar and Long 1961, Fuchs et al. 1979, Cortés et al. 2010). Phylogenetic analyses point to lineages divergence: two cryptic species classified as *D. saccharalis*, one occurring in the United States and Mexico and the other in Brazil, were inferred based on molecular data (Pashley et al. 1990, Lange et al. 2004). More than one

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strain of the sugarcane borer has also been proposed within the United States (Laca et al. 2009). Noteworthy, none of those phylogenetic studies tested for the genetic diversification due to host plant use, although one would expect adaptive genetic structure to be more common in generalist phytophagous species that feed in the interior of plants (Mopper 1996).

Because of the recent expansion of Brazilian agriculture to the Cerrado region (The Economist 2010), and increase in the use of genetically modified corn (Bt corn), reaching up to 80% of the corn-cultivated area during the 2012–2013 cropping season in Brazil (Céleres 2012), wide-ranging estimates of gene flow and population structure are necessary to establish the occurrence of random mating among Brazilian populations of *D. saccharalis*. With this kind of a study, it is possible to evaluate whether sugarcane fields can contribute with susceptible individuals for managing resistance to Bt corn in a specific agroecosystem. Thus, the main objective of this study was to assess the genetic variability and structure of Brazilian populations of the sugarcane borer based on sequences of mitochondrial markers. The second aim of this study was to investigate the demographic history for populations of *D. saccharalis* in Brazil to propose hypothesis on the expansion of this pest under Brazilian conditions.

Material and Methods

Sample Collection. In total, 125 specimens of *D. saccharalis* representing 13 corn and sugarcane-producing areas were sampled in the growing seasons 2009–2010 and 2010–2011 (see Table 1 and Figure 1 for sampling details). For corn fields, larvae were sampled and reared on a white bean-based artificial diet until pupation (Greene et al. 1976). Newly emerged adults were immediately frozen at –20°C. This procedure permitted the record of larvae performance in laboratory conditions (data not shown). For specimens from sugarcane fields, larvae were preserved in ethanol 96% until laboratory manipulation. Each sampling locality was treated as a separate subpopulation for analytical purposes.

DNA Extraction, Amplification and Sequencing. Total genomic DNA was extracted from thoracic tissues of adults or from whole larvae with Invisorb Spin Tissue kit, following the manufacturer's protocol (STRATEC Molecular, Berlin, Germany). All larvae were evaluated for the presence of parasitoids before DNA extraction. Two mitochondrial genes were evaluated, the subunit I of *cytochrome* c *oxidase* (COI, ~1,500 bp) and the subunit 6 of NADH (*nad6*, ~500 bp). Reactions for amplifying these regions via polymerase chain reactions (PCR) were done by using 1 µl of total DNA, 2.0 mM of $MgCl_2$, 40 µM of dNTPs, 0.2 µM of each primer (LCO + HCO; Folmer et al. 1994), and Jerry + PatII (Caterino and Sperling 1999) for COI; and tPro-J10090 + ND6-N10624 for *nad6* (Silva-Brandão et al. 2011)), 1 U of GoTaq DNA Polymerase (Promega, Madison, WI), and 10% of 10 × *Taq* buffer. PCRs were completed in 25 µl reactions under the following conditions: for COI, the PCR program was composed by initial denaturation at 95°C (3 min), followed by 35 cycles of denaturation at 94°C (30 s), annealing at 45–47°C (30 s), and polymerization at 72°C (1.5 min), with final extension at 72°C (10 min). For *nad6*, the program included an initial denaturation at 94°C (5 min), 35 cycles of denaturation at 94°C (45 s), annealing at 45°C (45 s), and elongation at 60°C (1.5 min), followed by extension at 60°C (5 min). Amplicons were purified with ExoSAP-IT (GE Healthcare, Bucks, United Kingdom) or with Invisorb Fragment CleanUp (STRATEC Molecular, Berlin, Germany). Samples were sequenced on an ABI 3700 automated sequencer with both forward and reverse primers. Electropherograms were checked to infer sequences quality and proceed alignment with the software Geneious (Drummond et al. 2011).

Genetic Diversity and Population Structure Analyses. Unless otherwise indicated, all data analyses were conducted by using concatenated datasets of COI and *nad6* sequences.

Mean genetic distance among all combined sequences and within and among sampled localities were calculated with the uncorrected *p*-distance model of nucleotide substitution (Nei and Kumar 2000) in

Table 1. Samples of *D. saccharalis* with sampling locality in Brazil, code (Zm = *Z. mays*; So = *Saccharum* spp), geographical coordinates, host plant, and number of collected specimens

Number	Locality		Code	Date	Latitude; longitude	Host plant	*N*
	City	State					
1	Catalão	Goiás (GO)	CAGO_Zm	Feb. 2009	18° 9′ S; 47° 56′W	Corn	7
2	Passo Fundo	Rio Grande do Sul (RS)	PFRS_Zm	Feb. 2009	28° 16′ S; 52° 24′ W	Corn	12
3	Ponta Grossa	Paraná (PR)	PGPR_Zm	Feb. 2009	25° 5′ S; 50° 12′ W	Corn	10
4	Primavera do Leste	Mato Grosso (MT)	PLMT_Zm	Feb. 2009	15° 34′ S; 54° 18′ W	Corn	8
5	Coruripe	Alagoas (AL)	COAL_So	Jan. 2010	10° 7′ S; 36° 10′ W	Sugarcane	9
6	Goianésia	Goiás (GO)	GOGO_So	April 2010	15° 19′ S; 49° 7′ W	Sugarcane	10
7	Jaboticabal	São Paulo (SP)	JASP_So	Jan. 2010	21° 15′ S; 48° 19′ W	Sugarcane	9
8	Monte Alegre	Minas Gerais (MG)	MAMG_So	Jan. 2010	21° 24′ S; 46° 15′ W	Sugarcane	10
9	Maringá	Paraná (PR)	MAPR_So	Feb. 2010	23° 26′ S; 51° 56′ W	Sugarcane	9
10	Paranaiguara	Goiás (GO)	PAGO_So	April 2010	18° 54′ S; 50° 39′ W	Sugarcane	13
11	Pederneiras	São Paulo (SP)	PDSP_So	Jan. 2010	22° 21′ S; 48° 19′ W	Sugarcane	9
12	Pradópolis	São Paulo (SP)	PRSP_So	Jan. 2010	21° 21′S; 48° 4′ W	Sugarcane	10
13	Tangará da Serra	Mato Grosso (MT)	TSMT_So	Jan. 2010	14° 38′ S; 57° 32′ W	Sugarcane	9
Total							125

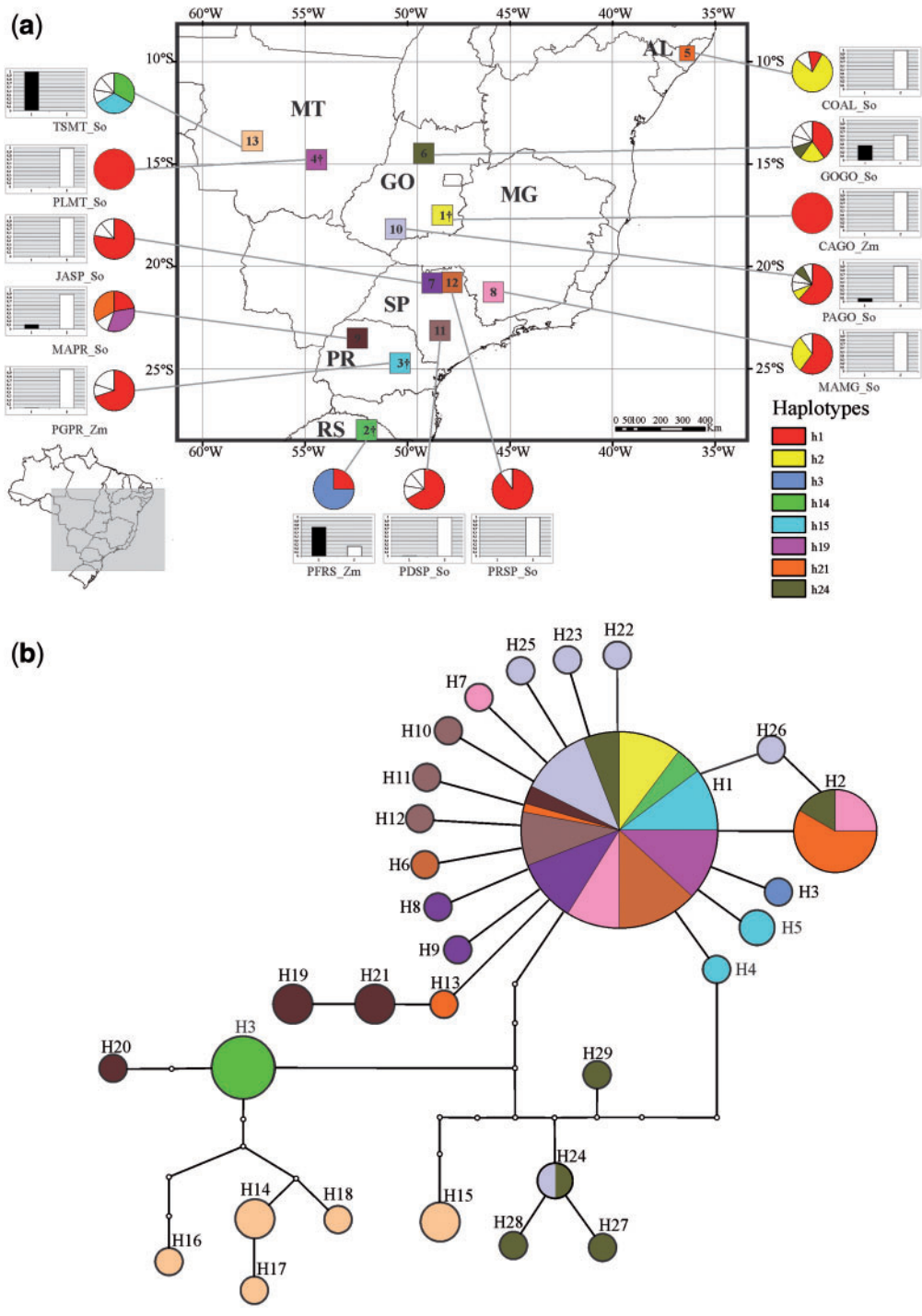

Fig. 1. (a) Sampling sites of *D. saccharalis* specimens (squares), where each locality is shown by a different color and number; † designates corn fields (for locality details, see Table 1). Pie charts indicate the frequency of more common haplotypes found at each locality. Rare haplotypes (found in only one specimen) are indicated in white in the pie charts. Bars indicate proportion of genetic clusters recovery with Structure in each locality (black = 1, white = 2); (b) minimum spanning network of mtDNA haplotypes of combined genes COI and *nad6* reported in 13 populations of *D. saccharalis*. Colors correspond to sampling sites where haplotypes were recorded. Area of the circles is directly proportional to the number of specimens showing such haplotype; smallest circles correspond to rare haplotypes. Small white circles designate nonsampled haplotypes. Each branch is equivalent to one base pair change.

MEGA v. 5.0 (Tamura et al. 2011). The average number of nucleotide substitutions (k) for each locality, and current (θ_π) and historical (θ_W) genetic diversities for all sampled sites, was estimated in DnaSP v. 5.0 (Librado and Rozas 2009). Nucleotide diversity (π) and haplotype diversity ($\hat{H}$; Nei 1987) were estimated in Arlequin v. 3.5 (Excoffier and Lischer 2010).

A minimum spanning network to estimate genealogies of haplotypes (Templeton et al. 1992) was estimated in TCS v. 1.21 (Clement et al. 2000). Number of haplotypes for each population was calculated in the same program. The relatedness of two haplotypes was limited by a probability of parsimony for DNA pairwise differences <0.95.

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was applied to assess molecular variation among and within populations of *D. saccharalis* with the software Arlequin v. 3.5 (Excoffier and Lischer 2010). The analysis was conducted to calculate the distribution of the molecular variation attributed to the presence of genetic structure among individuals from all sampling localities (nonhierarchic). A hierarchic AMOVA was applied between corn and sugarcane samples to test host plant effect on genetic structure of populations. The degree of structure was interpreted by the statistics Φ associated with the different hierarchical levels in which the variation is distributed (Excoffier et al. 1992). Significance of the Φ_{ST} values was evaluated using 10,000 permutations, computed distance matrix using pairwise difference, and gamma a value equal 0.

Genetic structure was also estimated using a Bayesian assignment test as implemented in the program Structure v. 2.3.3 (Pritchard et al. 2000). The number of clusters (K) in all sequenced sampling localities was estimated after the conversion of the complete sequence data set to a single-nucleotide polymorphism (SNP) formatted data set using MEGA v. 5.0 (Tamura et al. 2011). Each variable nucleotide was numerically coded as follows: A = 1, T = 2, C = 3, G = 4, all other characters = 0, and missing data = −9. Run parameters included 20 runs with 500,000 iterations following a burn-in period of 50,000 iterations for K = 1–15, under the "admixture ancestry model" and allele frequencies "correlated." The ΔK of Evanno (Evanno et al. 2005) was calculated by using the application Structure Harvester v. 0.6.93 (Earl and Vonholdt 2012) to estimate the number of clusters (K). Genetic structure of clusters was visualized by using the programs CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) and Distruct v. 1.1 (Rosenberg 2004).

In addition, spatial analysis of molecular variance (SAMOVA) (Dupanloup et al. 2002) was applied to identify major genetic differentiation among samples, using SAMOVA v. 1.0 (available at: http://cmpg.unibe.ch/software/samova/). This analysis tests all possibilities to set up groups of populations that maximize the "among-groups" component of the total genetic variance and reduce the "among populations within groups" component, and was applied to define groups of populations of *D. saccharalis* that can be considered geographically homogeneous but maximally differentiated from each other. Significance of F_{ST} (overall allele frequency divergence among populations), F_{SC} (divergence among populations within groups), and F_{CT} (divergence among groups) were computed by a nonparametric permutation procedure with 10,000 iterations.

A matrix of Slatkin's pairwise linearized distances ($t/M = F_{ST}/(1 - F_{ST})$; M = N for haploid data; Slatkin 1995) was estimated with Arlequin v. 3.5 (Excoffier and Lischer 2010). To assess whether gene flow correlates with geographic distance, pairwise linearized distances were correlated with a matrix of linear geographic distances to test the hypothesis of genetic isolation by geographical distance (isolation by distance [IBD]), using a Mantel test (Mantel 1967), with 10,000 permutations.

Principal coordinate analysis (PCoA) was applied to infer the dissimilarity among sampled localities. The complete sequence data set was converted to a SNP formatted data set as before. PCoA based on a pairwise matrix of Φ_{PT} genetic distances for populations was calculated with the software GENALEX (Peakall and Smouse 2006), with default parameters.

Demographic Analysis. The distribution of paired differences among sequences (mismatch distribution analysis; Slatkin and Hudson 1991, Rogers and Harpending 1992) was applied to infer the demographic history of all sampled localities of *D. saccharalis*, and specifically for specimens from corn and from sugarcane fields, as implemented in Arlequin v. 3.5 (Excoffier and Lischer 2010). In this approach, the distribution of pairwise differences is supposed to be multimodal when sampled populations are at demographic equilibrium, while populations that have undergone a sudden demographic expansion should show a unimodal pattern (Slatkin and Hudson 1991, Rogers and Harpending 1992). The adjustment to a model of population expansion was estimated from the sum of the squared deviations (SSD) and the raggedness index (r), with significance evaluated by 10,000 permutations under the sudden expansion model. Parameters of demographic expansion, such as moment estimators of time to the expansion Tau (τ), the mutation parameter before ($\theta_0 = 2\mu N_0$) and following ($\theta_1 = 2\mu N_1$) demographic expansion were estimated for all populations using $\alpha = 0.05$ and 10,000 permutations. Deviations from neutrality of the mtDNA sequences (which would be otherwise under population expansion) were tested by means of Fu's parameter F_S (Fu 1997) and of Tajima's parameter D (Tajima 1989), using Arlequin. Significance of both parameters was assessed by 10,000 randomizations.

Results

The sequences of COI and *nad6* of samples of *D. saccharalis* were 1,429 and 497 bp in length, respectively (GenBank accession numbers for COI: JN108957–86 and JX442547–648; for *nad6*: JN108987–016 and JX442649–750). The mean genetic distance among all concatenated sequences was 0.002 (range from 0 to 0.007). Combined

Table 2. Percentage of pairwise genetic distances (*p*-distance; Nei and Kumar 2000) among populations of *D. saccharalis* (upper right) and pairwise F_{ST} (Slatkin 1995; lower left)

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. CAGO_Zm		0.2	0	0	0	0.1	0	0	0.1	0	0	0	0.5
2. PFRS_Zm	**1.74834**		0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3
3. PGPR_Zm	0.02857	**1.80272**		0	0.1	0.1	0	0	0.1	0.1	0	0	0.5
4. PLMT_Zm	0	**1.86676**	0.04750		0	0.1	0	0	0.1	0	0	0	0.5
5. COAL_So	**1.64069**	**2.02962**	**1.04157**	**1.77588**		0.2	0.1	0	0.2	0.1	0.1	0.1	0.5
6. GOGO_So	0.23804	**0.53765**	**0.28969**	0.27093	**0.38489**		0.1	0.1	0.2	0.2	0.1	0.1	0.5
7. JASP_So	0	**1.76820**	0.04286	0	**1.10526**	0.27430		0	0.1	0	0	0	0.5
8. MAMG_So	0.12857	**1.77692**	0.14545	0.15417	**0.28571**	0.25729	0.11628		0.1	0.1	0	0	0.5
9. MAPR_So	**1.10774**	**1.43434**	**1.10195**	**1.20486**	**1.30937**	**0.57566**	**1.08333**	**1.08692**		0.2	0.2	0.1	0.6
10. PAGO_So	0	**1.28256**	0.02129	0	**0.39953**	0.11840	0	0.02678	**0.72307**		0.1	0	0.5
11. PDSP_So	0	**1.67756**	0.03763	0	**0.91304**	0.26241	0	0.10000	**1.00000**	0		0	0.5
12. PRSP_So	0	**1.97544**	0.05882	0	**1.48870**	**0.31461**	0.00456	0.15385	**1.26459**	0	0.00654		0.5
13. TSMT_So	**1.72024**	**0.43728**	**1.94828**	**1.86111**	**1.99187**	**0.83917**	**1.86667**	**1.93008**	**1.54094**	**1.68442**	**1.80645**	**2.06252**	

Significant values of paired F_{ST} at $\alpha = 0.05$ are in bold.

Table 3. Distribution of haplotypes and estimates of genetic diversity for sampled localities of *D. saccharalis* based on combined datasets of COI and *nad6*

Population code	N_{hap}	Haplotype (*n* of specimens)	Mean genetic *p*-distance	*k*	$\hat{H} \pm SD$	$\pi \pm SD$
CAGO_Zm	1	H1(7)	0.0000	0	0	0
PFRS_Zm	2	H1(3); H3(9)	0.0012	1.227	0.7273 ± 0.1133	0.001354 ± 0.000876
PGPR_Zm	3	H1(7); **H4**(1); H5(2)	0.0003	0.556	0.5111 ± 0.1643	0.000288 ± 0.000290
PLMT_Zm	1	H1(8)	0.0000	0	0	0
COAL_So	3	H1(1); H2(7); **H13**(1)	0.0003	0.611	0.7222 ± 0.1592	0.000317 ± 0.000312
GOGO_So	6	H1(4); H2(2); H24(1); **H27**(1); **H28**(1); **H29**(1)	0.0020	3.756	0.8444 ± 0.1029	0.001950 ± 0.001214
JASP_So	3	H1(7); **H8**(1); **H9**(1)	0.0002	0.444	0.4167 ± 0.1907	0.000231 ± 0.000255
MAMG_So	3	H1(6); H2(3); **H7**(1)	0.0003	0.667	0.7556 ± 0.1295	0.000350 ± 0.000330
MAPR_So	4	H1(2); H19(3); **H20**(1); H21(3)	0.0011	1.833	0.8333 ± 0.0980	0.001053 ± 0.000739
PAGO_So	6	H1(8); **H22**(1); **H23**(1); H24(1); **H25**(1); **H26**(1)	0.0008	1.385	0.7949 ± 0.1091	0.000785 ± 0.000567
PDSP_So	4	H1(6); **H10**(1); **H11**(1); **H12**(1)	0.0003	0.667	0.5833 ± 0.1833	0.000346 ± 0.000331
PRSP_So	2	H1(9); **H6**(1)	0.0001	0.200	0.2000 ± 0.1541	0.000104 ± 0.000158
TSMT_So	5	H14(3); H15(3); **H16**(1); **H17**(1); **H18**(1)	0.0032	6.222	0.8889 ± 0.0910	0.003231 ± 0.001922

N_{hap}, number of different haplotypes found at each sampled locality (rare haplotypes, found only in one specimen, are indicate in bold); *k*, average number of nucleotide differences; $\hat{H}$, haplotype diversity; π, nucleotide diversity (Nei, 1987).

sequences of all sampled sites resulted in 37 variable nucleotides. From those, 8 were nonsynonymous; 3 of the 30 variable sites of COI and 5 of the 7 variable sites of *nad6*.

Pairwise genetic distances between sampled localities (Table 2) ranged from 0 (between CAGO_Zm and PLMT_Zm) to 0.0056 (between TSMT_So and MAPR_So; see Table 1 for the meaning of codes). The mean genetic distances within localities ranged from 0 in CAGO_Zm and PLMT_Zm (each locality with one reported haplotype) to 0.0032 in TSMT_So, with five reported haplotypes (Table 3). The average number of nucleotide substitutions (*k*) ranged from 0 to 6.222 for those same localities. Low values of mean nucleotide diversity (π; average = 0.00077; range: 0–0.003231) and high values of haplotype diversity ($\hat{H}$; average = 0.5598; range 0–0.8889) were observed for all sampled localities (Table 3). Current genetic diversity (θ_π) per site for all localities was 0.00098, while historical genetic diversity (θ_W) was 0.003. The lower value of θ_π relative to θ_W is indicative of population growth (Hamilton 2009).

The 125 mtDNA sequences were linked in a single parsimony network (Fig. 1). The minimum spanning network method reported 29 haplotypes; 25 were exclusive occurrences from sugarcane samples, 3 from corn samples, and 1 was shared by corn and sugarcane samples. A main star-shaped structure directly linked 15 haplotypes to the most frequent haplotype (H1), which was present in 68 of the 125 sampled specimens (54.4%). This haplotype was usually the most frequent in all localities but TSMT_So (Table 3). Two other haplotypes were also frequently reported: H2 (found in 9.6% of samples), and H3, found only in the population of Passo Fundo, RS (PFRS_Zm; approximately 7% of samples; Fig. 1). The remaining haplotypes were represented by one to three specimens, and 20 of them were considered rare haplotypes (represented by only one specimen; Table 3, haplotypes in bold). The two samples set from the state of Goiás (GOGO_So and PAGO_So) showed the highest number of haplotypes, six each, three of them shared by both localities (Table 3). Haplotype 24 was only found within those two localities (Fig. 1).

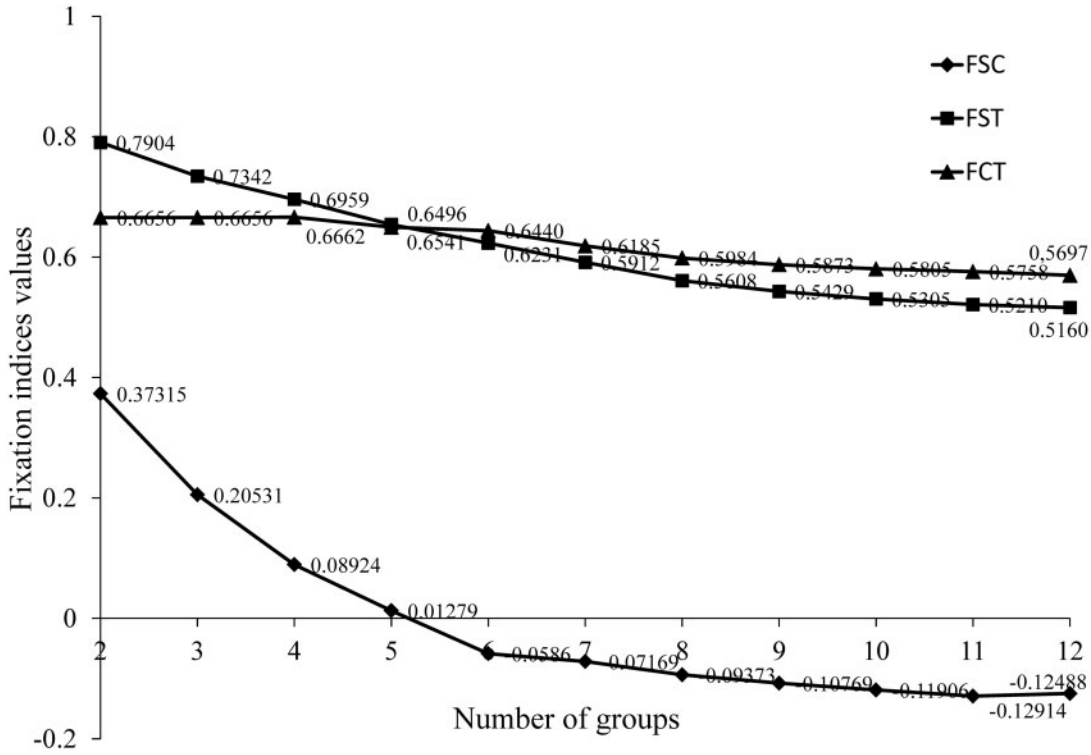

Fig. 2. SAMOVA of the 13 sampled populations of *D. saccharalis*. Values of fixation indices, F_{CT}, F_{ST}, and F_{CT}, for $k = 2$–12 groups.

Nonhierarchical AMOVA among all populations of *D. saccharalis* indicated a highly structured genetic variability, with a significant value of $\Phi_{ST} = 0.50923$ ($P < 0.001$), which corresponds to high genetic differentiation (*sensu* Hartl and Clark 2007). The exclusion of three marginal localities (TSMT_So, PFRS_Zm and COAL_So, see map in Fig. 1) resulted in a lower value of $\Phi_{ST} = 0.26530$ ($P < 0.001$), still corresponding to great genetic differentiation. Hierarchic AMOVA between samples from different host plants (corn vs. sugarcane) resulted in a $\Phi_{ST} = 0.4915$ ($P < 0.001$). Genetic variation is equally distributed among populations within groups and within populations (55.86 and 50.85% of variation, respectively). No variation was attributed to host plant origin of samples.

Bayesian assignment test conducted in Structure recovered $\Delta K = 2$ with the highest likelihood, which indicated two genetic clusters (Fig. 1). The clusters are not associated to geographical or host plant similarity.

SAMOVA indicated higher values of F_{CT} for $k = 2$–4 groups (Fig. 2). The most divergent populations were MAPR_So, PFRS_Zm, and TSMT_So. All remaining populations were composed of one group with lower genetic structure.

Slatkin's (Slatkin, 1995) linearized pairwise F_{ST} values ranged from 0 to 2.062 among all samples. The highest significant value was reported between samples collected in Tangará da Serra, MT (TSMT_So), and in Pradópolis, SP (PRSP_So), ~1,300 km apart (Table 2). In agreement with the occurrence of genetic structure due to the geographic distance among sampling sites, the Mantel test for IBD indicated significant correlation between geographic distances and Slatkin's pairwise linearized distances ($r^2 = 0.468$; $P = 0.015$).

The PCoA indicated dissimilarities among sampled sites of *D. saccharalis* analogous to the ones inferred by SAMOVA with the higher values of F_{CT}, with the population TSMT_So as the most isolated by the coordinate 1, which explains 72.37% of the variation (Fig. 3). Population PFRS_Zm also appears isolated by this same coordinate 1, while the population MAPR_So was isolated by coordinate 2, which explains 13.69% of the variation. The remaining localities were all clustered together. Individuals belonging to genetic cluster 1 partially or totally compose populations distinct by coordinate 1 (Fig. 1).

The overall mismatch distribution for the combined datasets of COI and *nad6* for all sampled localities showed a bimodal profile, one mode corresponding to the number of differences among sequences of samples from sugarcane fields (mode at one pairwise difference) and the other to differences among sequences from corn fields (two modes, at 1 and at 6 pairwise differences; Fig. 4). A bimodal pattern might result from constant population size or an admixture population (Rogers and Harpending 1992). However, mismatch distribution is believed to be very insensitive in

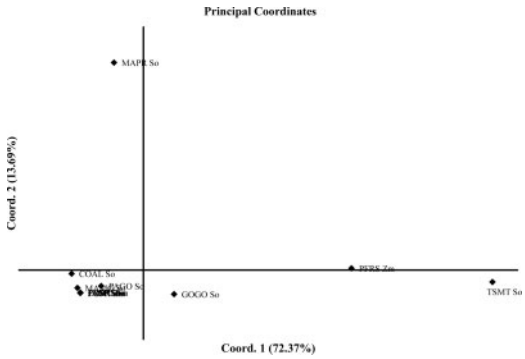

Fig. 3. PCoA of SNPs of combined sequences of COI and *nad6* from populations of *D. saccharalis.* Codes represent sampling localities as detailed in Table 1.

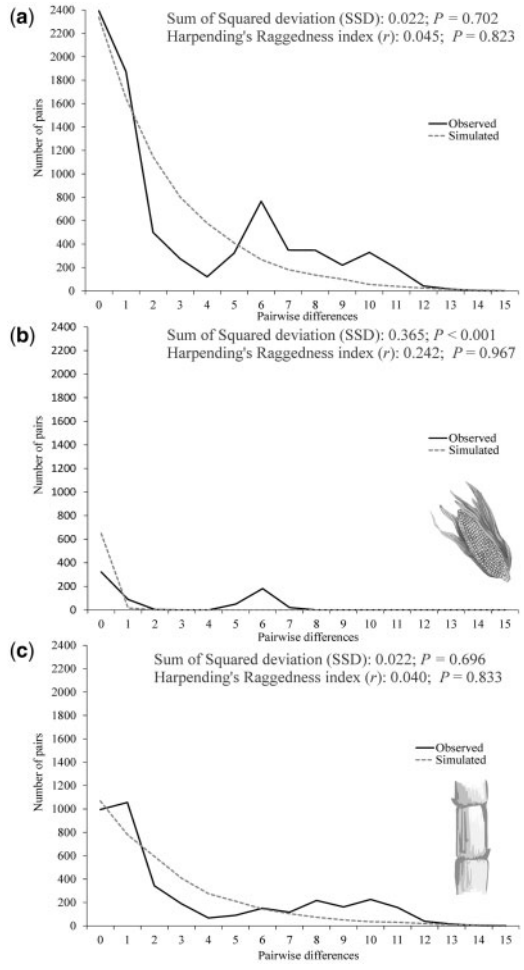

Fig. 4. Mismatch distributions for all pairwise combinations of the 125 analyzed sequences of mitochondrial regions of *D. saccharalis.* (a) for all sampled populations; (b) for samples from corn fields; (c) for samples from sugarcane fields.

detecting population growth (Ramos-Onsins and Rozas 2002), and all other tests for constant population size indicated population growth indeed. The values of SSD and r were both low and nonsignificant for all populations analyzed together (Fig. 4), as expected under the population growth model (Ramos-Onsins and Rozas 2002). The values of population size before ($\theta_0 = 0.002$) and after ($\theta_1 = 2.254$) the demographic expansion of the populations, and the value of $\tau = 9.104$, indicate a departure of constant effective population size, as well as the negative and significant values of Tajima's D ($D = -1.6324$, $P = 0.008$) and Fu's F_S ($F_S = -14.7864$, $P < 0.001$), which point out to a departure of neutrality and stable population size. Altogether, those results indicate that the effective population size of *D. saccharalis* changed through time.

Discussion

Population genetics studies are suitable to aid in pest control approaches by, among other things, indicating whether different populations of an insect are able to exchange genetic information. If samples from the complete geographical distribution of an insect pest behave as a unique genetic population, i.e., if there is enough gene flow to maintain them as a single genetic entity, control measures can be more effectively and uniformly applied in all areas.

In our study in Brazil, the high and significant value of Φ_{ST} (0.50923) suggests high level of genetic structure among samples of *D. saccharalis.* The same mtDNA markers and procedures were used to study Brazilian populations of *Heliothis virescens* (F.) (Lepidoptera, Noctuidae) and the Φ_{ST} estimated then was low and not significant (Albernaz et al. 2012). Despite the low number of samples from some localities, which could result in artificial genetic structure, we are confident that there is enough data to propose at least three possible scenarios to explain the high Φ_{ST} found among *D. saccharalis* populations. In the first scenario, the current gene flow is in fact low among Brazilian populations of *D. saccharalis*, and it is lower among distant populations (although some geographically distant populations still share genetic material, as we show in Fig. 1). Geographic distance is one of the many causes of reduced gene flow among populations of phytophagous insects, and it is usually associated to low dispersal capability (Peterson and Denno 1998). We did find significant correlation between genetic and geographic distances, which is indicative of geographic structure (Roderick 1996). In addition, *D. saccharalis* is indeed a short distance flyer (Guagliumi 1972), and geographic structure due to low dispersal ability would be expected. Moreover, as mitochondria, the molecular marker investigated, is primarily maternally inherited, sexual dimorphism in dispersal would result in nonsignificant IBD if females dispersed more than males at field conditions, as actually found. Regrettably, there are no available data on differential dispersion ability for *D. saccharalis*.

The second possible scenario is genetic structure due to demographic events. Populations of pest insects

undergo periodic size reductions because of control tactics, and may be subject to repetitive events of genetic bottlenecks. Another possible cause of genetic loss, specific to the system we studied, is the manual harvest after burning of sugarcane plants applied in Brazil until the 1990s. That procedure was likely prone to extinguish most of the borers, stochastically wiping out genetic variability each harvest season. In this regard, the main advantage in using mitochondrial sequences in population genetics studies is the possibility of inferring historical demographic patterns of the target species. The low nucleotide diversity ($\pi = 0.000707$) and medium or high haplotype diversity ($\hat{H} = 0.5598$) we reported for *D. saccharalis* (Table 3), and the negative and significant values of Fu's F_S and Tajima's *D*, reflect a recent demographic expansion (Excoffier et al. 2009), as found for other moth in Brazil, *H. virescens* (Albernaz et al. 2012). This scenario was also supported by the "star-like"-shaped haplotype network we obtained with mtDNA data (Fig. 1; an arrangement typical of species that have undergone a recent demographic expansion, with interior haplotypes expected to be ancestral, whereas peripheral ones are derived; Crandall and Templeton 1993, Castelloe and Templeton 1994). However, the mismatch distribution showed two peaks, one for sugarcane samples and two for corn samples (Fig. 4), which might be a consequence of constant population size or an admixtured population (Rogers and Harpending 1992). We believe the later possibility can better explain the demographic history of the sugarcane borer in Brazil, as the history of host plant use by this species could have produced this pattern: *D. saccharalis* is considered a riparian species, and its original host might have been aquatic or semiaquatic plants, such as wild rice (Myers 1935). The species may has invaded corn after the domestication of this crop, at least 5,000 years ago (Brieger et al. 1958). For that reason, the first population growth we deduce in this work of *D. saccharalis* may have occurred in corn. *D. saccharalis* may have diversified in corn and accumulated differences at pairwise mitochondrial comparisons. We also hypothesize that a more recent demographic expansion happened in Brazil after the introduction of sugarcane, as the large availability of sugarcane as a food source to the sugarcane borer allowed the exploitation of this host to increase its population's size and to expand its distribution range into new biomes. As the *D. saccharalis* specimens analyzed here were sampled mainly from sugarcane fields, the overall pattern of demographic expansion of *D. saccharalis* we report might be a reflection of the sampling strategy.

A third scenario is the contribution of adjacent wild and endemic hosts as repository and source of individuals (and, consequently, new alleles) of the sugarcane borer to crop fields, resulting is geographic structure. The impact of wild hosts to maintain pest insects populations was investigated for north American populations of *H. virescens* (Blanco et al. 2008); however, the role of alternative noncultivated hosts in maintaining *D. saccharalis* populations in Brazil is unknown. Indeed, endemic species of *Saccharum* are available in Brazil (Vianna et al. 2006), besides a large diversity of wild plants.

Some landscape traits in the sampled areas give incidental support to that scenario. The main populations accounting for the genetic structure found among populations of *D. saccharalis* were from Tangará da Serra, MT (TSMT_So), and Passo Fundo, RS (PFRS_Zm), as suggested by the SAMOVA analysis (Fig. 2). Both regions present very unique environmental phytophysiognomies (i.e., vegetation features) in relation to the overall distribution of *D. saccharalis* that could result in genetic isolation of the individuals found there. The same pattern of high genetic isolation of samples from Tangará da Serra in relation to other populations within Brazil was found for another sugarcane pest, the giant sugarcane borer *Telchin licus* (Drury) (Castniidae) (Silva-Brandão et al. 2012).

Elements from all three putative scenarios may have contributed to the genetic structure we inferred for Brazilian populations of *D. saccharalis*. Overall, our data on mtDNA variability of *D. saccharalis* in the main corn- and sugarcane-producing areas in Brazil indicate that there is substantial genetic variation on this species, which in this case could be of utmost importance for its control. Some authors went further, as to raise the possibility of cryptic variation within *D. saccharalis* from other localities. In one of them, allozyme variation recorded for sugarcane borer populations from United States, Mexico, and Brazil led to the suggestion that *D. saccharalis* could actually be two species (Pashley et al. 1990). In the other, two phylogenetic groups were observed, one composed of populations from South and Central America, while the other contained Caribbean and North American populations (Lange et al. 2004).

From a pest control perspective, our data on the genetic variability within and among sampled localities of *D. saccharalis* from corn and sugarcane crops raise at least one important conclusion: samples from different localities may not show significant gene flow among them. Furthermore, irrespective of low sampling, there is indication of genetic variation within the species *D. saccharalis* that warrants further investigation. On the other hand, samples from closer localities share most of their haplotypes and are genetically more similar at the markers investigated here. These inferences should be considered to improve the control strategies of *D. saccharalis* at field conditions. Therefore, our findings support that Brazilian populations of *D. saccharalis* from sugarcane fields can contribute with susceptible individuals for management resistance to Bt corn in a specific agroecosystem as there is no genetic variation associated to host plant origin.

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