# Genetic Structure and Gene Flow Among Brazilian Populations of *Heliothis virescens* (Lepidoptera: Noctuidae)

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**ABSTRACT** Population genetic studies are essential to the better application of pest management strategies, including the monitoring of the evolution of resistance to insecticides and genetically modified plants. *Bacillus thuringiensis* Berliner (Bt) crops have been instrumental in controlling tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), a pest that has developed resistance to many common insecticides once used for its management. In our study, microsatellite markers were applied to investigate the genetic structure and patterns of gene flow among Brazilian populations of *H. virescens* from cotton, *Gossypium hirsutum* L., and soybean, *Glycine max* (L.) Merr., fields, aiming to propose means to improve its management in the field. In total, 127 alleles were found across nine microsatellites loci for 205 individuals from 12 localities. Low levels of gene flow and moderate to great genetic structure were found for these populations. Host plant association, crop growing season, and geographic origin were not responsible for the genetic structuring among Brazilian populations of *H. virescens*. Other factors, such as demographic history and seasonal variability of intrapopulation genetic variation, were suggested to be molding the current pattern of genetic variability distribution.

**RESUMO** Estudos de genética de populações são essenciais para uma aplicação eficiente de estratégias de manejo de praga, incluindo o monitoramento da evolução da resistência a inseticidas e plantas geneticamente modificadas. Plantas-*Bacillus thuringiensis* Berliner (Bt) têm sido determinantes no controle de *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), uma vez que essa espécie desenvolveu resistência a muitos dos inseticidas normalmente usados no seu controle. Em nosso estudo, marcadores microssatélites foram usados para investigar a estrutura genética e os padrões de fluxo gênico entre populações brasileiras de *H. virescens* coletadas em campos de algodão e soja, para propor meios de incrementar seu manejo no campo. Foi encontrado um total de 127 alelos em nove lócos microssatélites para 205 espécimes de 12 localidades. Fluxo gênico baixo e estrutura genética moderada a alta foram encontrados para estas populações. Cultura, safra e localidade de origem não foram responsáveis pela estrutura genética entre as populações brasileiras de *H. virescens*. Outros fatores, tais como história demográfica e variabilidade sazonal na variação genética intrapopulacional, podem estar moldando o presente padrão de distribuição da variabilidade genética nessas populações.

**KEY WORDS** cotton, microsatellite, pest management, resistance, soybean

Population genetics studies of agricultural pests are essential to estimate their genetic variability and the amount of gene flow to assess genetic and ecological factors molding their present distribution and invasiveness (Scott et al. 2005, Endersby et al. 2006, Rollins et al. 2006). This information can be exploited for a more effective application of pest control measures and to maximize the use of pest management technologies (Krafsur 2005, Franck et al. 2007). The evolution of resistance to insecticides, including the bacterium *Bacillus thuringiensis* Berliner (Bt) proteins expressed by genetically modified (GM) plants, can be closely monitored when the gene flow among populations of the target species, and consequently the change of genetic content among them, is estimated (Caprio and Tabashnik 1992, Alstad and Andow 1995, Tabashnik and Carrière 2008). In fact, one of the main

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**Table 1. Populations of *H. virescens* collected during 2008–2010**

| Sample location (city, state) | Code | Latitude | Longitude | Mo yr | Host plant | Sample size |
|---|---|---|---|---|---|---|
| Chapadão do Sul, MS | MS08 | 18° 47′ S | 52° 37′ W | April 2008 | Cotton | 18 |
| | MS10Gm | | | Jan. 2010 | Soybean | 19 |
| Luiz Eduardo Magalhães, BA | BA08 | 12° 05′ S | 45° 48′ W | Mar. 2008 | Cotton | 15 |
| | BA09 | | | Feb. 2009 | Cotton | 18 |
| | BA10 | | | Jan. 2010 | Cotton | 21 |
| | BA10Gm | | | Jan. 2010 | Soybean | 15 |
| Palmeiras, GO | GO08 | 16° 48′ S | 49° 55′ W | May 2008 | Cotton | 14 |
| Primavera do Leste, MT | MTP09 | 15° 31′ S | 54° 20′ W | Mar. 2009 | Cotton | 12 |
| Riachão das Neves, BA | BAR09 | 11° 50′ S | 44° 54′ W | Mar. 2009 | Cotton | 26 |
| Rio Verde, GO | GO09 | 17° 47′ S | 50° 55′ W | April 2009 | Cotton | 21 |
| Sapezal, MT | MT09 | 13° 32′ S | 58° 48′ W | June 2009 | Cotton | 18 |
| Sinop, MT | MT08 | 11° 50′ S | 55° 38′ W | May 2008 | Cotton | 8 |
| Total | | | | | | 205 |

approaches used to delay resistance to GM plants expressing Bt proteins, the random mating between Bt-susceptible and Bt-resistant insects (Gould 1998), considers gene flow among populations of the target insects (Tabashnik and Carrière 2008). In this way, pests' gene flow between populations from refuge areas and Bt crops usually focuses on genes directly related to resistance (Morin et al. 2004) as well as neutral markers (Martel et al. 2003, Tabashnik and Carrière 2008); that is, genes of which polymorphisms should be maintained by random allelic extinction by genetic drift (Avise 2004). The former may indicate any shift in the frequency of susceptible individuals within populations and reflect how quickly resistance genes are spread all over the distribution range of the insect (Caprio and Tabashnik 1992, Tabashnik 1994). The latter points to the amount of genetic content shared by populations and how the variability is distributed (Pritchard et al. 2000, Behura 2006). Genetic variability and structure inferred with neutral markers were shown to be indicative of different levels of susceptibility to toxins expressed by GM plants (Monnerat et al. 2006).

Bt-expressing cotton, *Gossypium hirsutum* L., has been efficiently used to control among other Lepidoptera, the noctuid tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), one of the main insect pests attacking cotton in the American continent, especially because the species developed resistance to many insecticides once used for its management (Sparks 1981, Wolfenbarger et al. 1981, McCaffery 1998, Teran-Vargas et al. 2005, Ali and Luttrell 2006, Blanco et al. 2008a, Blanco 2012). However, the tobacco budworm has shown natural variability in the susceptibility to Cry1Ac protein even before the commercial widespread use of Bt cotton (Gould et al. 1992, Stone and Sims 1993, Gould et al. 1995, Luttrell et al. 1999).

*H. virescens* is found in Brazil, Bolivia, and Peru and throughout the United States and southern Canada (Poole et al. 1993). In Brazil, the species is distributed in northeastern and central regions, in all major cotton-producing areas (Fitt 1989, Degrande 1998). *H. virescens* was described feeding on at least 99 species in 14 families of host plants (Waldvogel and Gould 1990, Blanco et al. 2007), and its polyphagy, coupled with its facultative migratory behavior (Schneider 1989), allows the species to exploit several cultivated and wild hosts. As a consequence, *H. virescens* can display quick growth rates and a great potential to damage cultivated hosts. In Brazil, besides attacking cotton, it has recently become an important pest of soybean, *Glycine max* (L.) Merr. (Tomquelski and Maruyama 2009).

There is scarce information on the genetic variability and structure of populations of *H. virescens* in Brazil. A recent study applying mitochondrial (mtDNA) sequences revealed a weak genetic structure among populations of *H. virescens* from Brazil and strongly indicated a recent event of demographic expansion that could be responsible for the low population structure recorded (Albernaz et al. 2012). Other studies in the United States and Mexico using different biochemical and molecular markers also found low genetic structure among populations of the tobacco budworm (Korman et al. 1993, Roehrdanz et al. 1994, Han and Caprio 2004, Groot et al. 2011).

The aim of this study was to apply microsatellite markers to infer intra- and interpopulation genetic variability of populations of *H. virescens* from the major cotton- and soybean-producing areas in Brazil and to estimate the gene flow among them to propose means to improve the integrated management of this pest in the field. We also considered whether different growing seasons, host plant, and geographical localities have molded the current genetic structure among populations of *H. virescens*.

## Materials and Methods

**Sampling and DNA Extraction.** Samples of *H. virescens* were collected from 12 localities in Brazil (Table 1), 11 of them previously characterized using mtDNA (Albernaz et al. 2012). Each sampling location was treated as a population. Larvae were sampled early in the growing season from cotton-growing areas in 2007–2008, 2008–2009, and 2009–2010 and from soybean crops in 2009–2010. In the laboratory, these field-collected larvae were reared on a white bean-based artificial diet until pupation (Greene et al. 1976). Adults were immediately frozen after eclosion at −20°C and kept under this condition until DNA extraction.

**Table 2. Features of nine microsatellite loci isolated from *H. virescens* (Perera et al. 2011)**

| Locus shortname | Locus name (GenBank accession) | Repeat | Alele size range (bp) | Annealing temp (°C) | A | $H_O$ | $H_E$ | PIC |
|---|---|---|---|---|---|---|---|---|
| 1 | HvMS117 (JF310676) | TGA | 104–174 | 59 | 14 | 0.352 | 0.569 | 0.513 |
| 2 | HvMS138 (JF310679) | ATG | 129–168 | 59 | 15 | 0.538 | 0.721 | 0.665 |
| 3 | HvMS222 (JF310679) | GAT | 120–180 | 54 | 15 | 0.148 | 0.371 | 0.326 |
| 4 | HvMS227 (JF310680) | TC | 104–245 | 54 | 18 | 0.210 | 0.359 | 0.306 |
| 5 | HvMS326 (JF310681) | TGAC | 104–174 | 50 | 14 | 0.125 | 0.300 | 0.268 |
| 6 | HvMS566 (JF310683) | GTT | 100–180 | 59 | 16 | 0.404 | 0.442 | 0.384 |
| 7 | HvMS1942 (JF310686) | CAT | 105–172 | 59 | 9 | 0.187 | 0.225 | 0.199 |
| 8 | HvMS1974 (JF310687) | CAA | 108–189 | 50 | 12 | 0.422 | 0.635 | 0.551 |
| 9 | HvMS596944 (JF310690) | GAA | 102–202 | 54 | 14 | 0.346 | 0.319 | 0.282 |
| Mean | | | | | 14.11 | 0.303 | 0.438 | 0.388 |
| SE | | | | | | 0.047 | 0.055 | 0.051 |

Locus names, repeat sequence, size range of observed alleles, annealing temperature for amplification reactions, number of alleles per locus (A), observed and expected heterozygosity ($H_O$ and $H_E$, respectively), and PIC for each locus are provided.

Genomic DNA was isolated from the thorax of each adult by using the Invisorb Spin Tissue Kit (STRATEC Molecular, Berlin, Germany), following the manufacturer's protocol. A 2-$\mu$l aliquot of genomic DNA was quantified by comparing the intensity of the band with the LowMass ladder (Invitrogen, Carlsbad, CA), and DNA concentration was standardized for 3 ng of total DNA/$\mu$l.

**Microsatellites Genotyping.** Each individual was genotyped at nine polymorphic microsatellite loci using primers previously designed and tested (Perera et al. 2011) (Table 2). Each forward primer was end-fluorescent–labeled either with 6-FAM, HEX, or NED (Applied Biosystems, Carlsbad, CA) as described previously (Schuelke 2000). The amplification reaction mixture contained 3 ng of DNA, 0.5 $\mu$M fluorescent dyes, 2.0 $\mu$M reverse and forward primers, 40 $\mu$M dNTPs, 2.0 mM $MgCl_2$, 4.0 $\mu$l of dimethyl sulfoxide 5%, 1× of *Taq* buffer, 1 U of GoTaq DNA Polymerase (Promega, Madison, WI), and 7.55 $\mu$l of Milli-Q (Millipore, Billerica, MA) water in a total volume of 20 $\mu$l. Amplification programs included an initial denaturation step at 95°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at specific temperatures for each locus (Table 2) for 45 s, and elongation at 72°C for 45 s; followed by eight cycles of denaturation at 94°C for 30 s, annealing at 45°C for 45 s, and elongation at 72°C for 45 s; finalizing with an extension step at 72°C for 10 min (Schuelke 2000). Products of polymerase chain reaction (PCR) with each fluorescent dye were electrophoresed together using a multiplex approach (Missiaglia and Grattapaglia 2006) and detected on an ABI 3730 DNA Analyzer (Applied Biosystems) by using GeneScan-500 ROX-labeled size standard (Applied Biosystems). Electropherograms were detected and analyzed by using GeneMarker v. 1.91 (SoftGenetics LLC, State College, PA). We statistically tested for genotyping errors and for the presence of null alleles by using Micro-Checker v. 2.2.3 (Van Oosterhout et al. 2004). Raw data are available from us upon request.

**Data Analysis.** Population genotypic frequencies for each allele at each locus were tested for concordance to Hardy–Weinberg equilibrium (HWE) by applying the Fisher exact test (Weir 1996), with the program Genepop v. 3.4 (Raymond and Rousset 1995). This program follows a Markov Chain procedure that was set for 5,000 dememorizations, 2,000 batches, and 2,000 iterations per batch. A locus was considered under disequilibrium if Hardy–Weinberg deviation was significant for all populations. Each locus also was tested for selection by using the hierarchical island model (Excoffier et al. 2009), as implemented in Arlequin v. 3.5 (Excoffier and Lischer 2010), by using default parameters. The linkage disequilibrium was tested by using the program FSTAT v. 2.9.3.2 (Goudet 2002), with the significance level adjusted by sequential Bonferroni correction.

The genetic diversity of each population was estimated through the number of alleles per locus (A), observed and expected heterozygosity ($H_O$ and $H_E$, respectively), and polymorphic information content (PIC) by using the Excel Microsatellite Toolkit (Park 2001). The genetic structure of populations was estimated by the parameters $\theta$, $F$, and $f$ (Weir and Cockerham 1984), by using the program FSTAT v. 2.9.3.2 (Goudet 2002), and the parameter $R_{ST}$ (Slatkin 1995), by using the program Genalex v. 6.4 (Peakall and Smouse 2006). To test for differences in the genetic structure of *H. virescens* populations through time, these parameters also were estimated within each growing season.

Pairwise $\theta$ values (Weir and Cockerham 1984) were computed between every population as implemented in FSTAT v. 2.9.3.2 (Goudet 2002). Locus HvMS227 was pruned of the data set because it did not amplify for two out of 12 populations.

The effect of geographic distance in the genetic differentiation among sampling sites of *H. virescens* was inferred using a Mantel test (Mantel 1967) as implemented in Genalex v. 6.4 (Peakall and Smouse 2006). Under the null hypothesis of isolation by distance (Wright 1931), a linear relationship between genetic ($\theta = F_{ST}/F_{ST} - 1$) and geographical distances is expected. The significance was established by 9,999 permutations.

Three different clustering methods were applied to determine the pattern of population structure of *H. virescens*, a principal coordinate analysis (PCA), a Bayesian assignment test, and a network analysis of

**Table 3. Features of 12 populations of *H. virescens* genotyped at nine microsatellite loci**

| Sample location | A | $A_p$ | $H_O \pm SE$ | $H_E \pm SE$ | PIC | HWD |
|---|---|---|---|---|---|---|
| MS08 | 3.67 | 1 | 0.270 ± 0.039 | 0.373 ± 0.091 | 0.327 | 3 (3,6,8) |
| MS10Gm[a] | 3.56 | 3 | 0.313 ± 0.039 | 0.452 ± 0.087 | 0.389 | 1 (3) |
| BA08 | 3.33 | 1 | 0.206 ± 0.035 | 0.352 ± 0.093 | 0.312 | 2 (3,6) |
| BA09 | 4.13 | 3 | 0.370 ± 0.041 | 0.410 ± 0.087 | 0.37 | 3 (1,5,9) |
| BA10 | 5.22 | 7 | 0.233 ± 0.033 | 0.490 ± 0.110 | 0.454 | 1 (7) |
| BA10Gm | 4.67 | 7 | 0.345 ± 0.043 | 0.553 ± 0.070 | 0.485 | 4 (1,3,4,6) |
| GO08 | 4.56 | 5 | 0.294 ± 0.041 | 0.439 ± 0.095 | 0.398 | 3 (4,6,9) |
| MTP09 | 3.89 | 5 | 0.316 ± 0.047 | 0.496 ± 0.066 | 0.433 | 4 (3,4,5,7) |
| BAR09 | 4.00 | 4 | 0.292 ± 0.030 | 0.302 ± 0.081 | 0.267 | 2 (5,6) |
| GO09 | 5.78 | 8 | 0.366 ± 0.035 | 0.486 ± 0.074 | 0.447 | 4 (1,2,5,8) |
| MT09 | 4.11 | 4 | 0.331 ± 0.040 | 0.489 ± 0.085 | 0.426 | 2 (1,8) |
| MT08 | 3.11 | 1 | 0.317 ± 0.056 | 0.414 ± 0.094 | 0.353 | 2 (1,8) |
| Mean | 4.17 | | 0.304 | 0.438 | 0.389 | |

Sample location names; A, avgerage number of alleles per locus; $A_p$, number of private alleles; observed and expected heterozygosity ($H_O$ and $H_E$, respectively); PIC; HWD, number of loci with significant deviations from Hardy–Weinberg equilibrium ($P < 0.01$) (in parentheses, loci short name, as presented in Table 2) are shown.

[a] Gm, *G. max.*

genetic covariance, testing for the role of three variables, time, locality, and host of sampling. The multivariate PCA was performed by using the pairwise $\theta$ matrix (Weir and Cockerham 1984), by using the program Genalex v. 6.4 (Peakall and Smouse 2006) with default parameters.

The Bayesian assignment test was performed by using the program STRUCTURE v. 2.3.3 (Pritchard et al. 2000). The number of populations (K) was estimated under the "admixture ancestry model," with allele frequencies "correlated" and K ranging from 1 to 20. For each value of K, 20 independent iterations with 500,000 repetitions of Markov Chain Monte Carlo and burn in of 50,000 iterations were performed. To estimate the value of K that best fitted the data, the ΔK of Evanno et al. (2005) was calculated by using the application Structure Harvester v. 0.6.1 (Earl 2011). The graph of the genetic structure was produced by using the programs CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) and Distruct v. 1.1 (Rosenberg 2004).

The program Population Graphs v. 2 (Dyer and Nason 2004) was applied to develop the network analysis, using a web-based interface (http://dyerlab.bio.vcu.edu/software/). The graph topology was visualized using the program Graph, a component of GeneticStudio (Dyer 2009).

Recent migration rates among populations collected within each growing season (2007–2008, 2008–2009, or 2009–2010) were estimated to infer the percentage of migrants among locations in different years by using the program BayesAss v. 1.3 (Bayesian Inference of Recent Migration Using Multilocus Genotype) (Wilson and Rannala 2003). Twenty million iterations were executed, discarding the first four million as burn in, with data sampled from the chain every 2,000 iterations. The confidence interval of 95% was calculated for each estimate.

## Results

No genotyping errors or null alleles were detected over the nine microsatellite loci. Fisher's exact test indicated deviation from the HWE at all loci in at least one population (Table 3). However, all loci were kept for further analyses because no locus had significant departures for HWE ($P < 0.01$) for all populations. There was no evidence of selection at any locus under the significance level of $P = 0.05$. Results of tests for loci under selection and Fisher's exact test indicated the neutrality of all loci investigated here. There was no linkage disequilibrium after applying a Bonferroni correction for multiple testing.

In total, 127 alleles were found across nine microsatellites loci for 205 individuals of *H. virescens* from 12 localities. Allelic variation ranged from nine to eighteen alleles per locus (average = 14.11) (Table 2), indicating high intrapopulation variability. All loci showed at least one common allele at high frequency in all populations; some of them presented only one allele within populations, which is indicative of allelic fixation (HvMS227, HvMS326, HvMS1942, and HvMS596944). The average PIC was 0.388 (range, 0.199–0.665). For all estimates, loci HvMS1942 and HvMS138 were the lesser and the most diverse, respectively (Table 2).

The average number of alleles per locus within populations was 4.17, ranging from 3.11 (MT08) to 5.78 (GO09) (Table 3). Forty-nine from the 127 alleles scored (38%) were private alleles; that is, they were found only in one or two populations usually at low frequency. Ten private alleles were detected only in soybean populations from two localities ≈1,000 km apart (BA10Gm and MS10Gm), and one of them (locus HvMS566) was shared by the two populations at a high frequency (>0.20). The average observed heterozygosity for populations was 0.304, ranging from 0.206 (BA08) to 0.370 (BA09), and average expected heterozygosity was 0.438, varying from 0.302 (BAR09) to 0.553 (BA10Gm). Mean PIC for populations was 0.389 [range, 0.312 (for BA08)–0.485 (for BA10Gm)].

The genetic structure inferred for all studied populations of *H. virescens* was moderate to great (Hartl and Clark 2007), with a significant value of $\theta = 0.132$, and estimated $R_{ST} = 0.252$ (Table 4). Grouping populations by growing season resulted in moderate to very great values of parameters $\theta$ and $R_{ST}$ (Table 4).

**Table 4. Estimates of parameters *f*, F, $\theta$ (Weir and Cockerham 1984) and $R_{ST}$ (Slatkin 1995) for populations of *H. virescens***

| | *f* | F | $\theta$ | $R_{ST}$ |
|---|---|---|---|---|
| All samples | 0.294 (0.178–0.406) | 0.387 (0.273–0.512) | 0.132 (0.072–0.218) | 0.252 |
| Growing season 2007–2008 | 0.324 (0.163–0.517) | 0.388 (0.230–0.584) | 0.093 (0.042–0.163) | 0.318 |
| Growing season 2008–2009 | 0.206 (0.109–0.341) | 0.298 (0.196–0.441) | 0.115 (0.057–0.181) | 0.393 |
| Growing season 2009–2010 | 0.414 (0.218–0.547) | 0.44 (0.260–0.561) | 0.045 (0.012–0.082) | 0.089 |

Numbers in parentheses are lower and upper values of confidence interval at 95%.

Pairwise $\theta$ values were not significant for any pair of populations. The correlation analysis between genetic and geographical distances revealed the lack of a significant association between them (Mantel test, $r^2 = 0.0135$, $P = 0.146$).

PCA of all individuals from 12 sampling localities revealed two groups recovered by components X (explains 35.55% of variation) and Y (explains 16.99% of variation). No clear geographical, temporal, or nutritional (crop) pattern was observed between groups 1 and 2 (Fig. 1). The coordinate X, which explains most of the variation, joined individuals sampled from cotton crops from different localities in group 1, and both soybean and cotton-samples in group 2.

The Bayesian assignment test identified $\Delta K = 2$ (Fig. 2a). This grouping is very similar to the one recovered with PCA, with no indication of structure by sampling collection, growing season or crop (Fig. 2b). The two clusters, however, were suggested to change genetic information between them, especially for populations BA09, MT09, and MS08. The network analysis recovered the same two groups (Fig. 3).

The Bayesian inference of migrants among localities sampled within every growing season indicated low migration rate because most individuals within each locality were residents (>97%) (Table 5). Despite the low migration rate, movements from the states of Goiás (GO) and Mato Grosso (MT) to areas of Bahia (BA) were suggested. Samples collected in the 2009–2010 growing season from different crops showed migration from soybean to cotton fields.

## Discussion

$H_O$ and $H_E$ and the PIC were high for *H. virescens* populations (Table 3). Perera et al. (2011) found similar results characterizing 15 microsatellite loci for samples from Stoneville, MS, with nine of them applied here. These results are congruent with the high genetic variability found within Brazilian populations by using mtDNA because 35 haplotypes were found in the 97 mtDNA sequences obtained (Albernaz et al. 2012).

Populations of pest species undergo periodic size reduction when management tactics are applied for their control and may lose genetic variability every crop growing season, so new alleles are presumed to be foreign. Albernaz et al. (2012) raised the hypothesis that besides the rise of new haplotypes by mutation, wild host plants nearby crop fields may keep individuals with variable combinations of alleles and may be the place of origin of rare mtDNA haplotypes found in populations of *H. virescens* sampled from cultivated hosts. This hypothesis agrees with the polyphagy (Blanco et al. 2007) and facultative migratory habits (Schneider 1989) of this species, and with the vege-

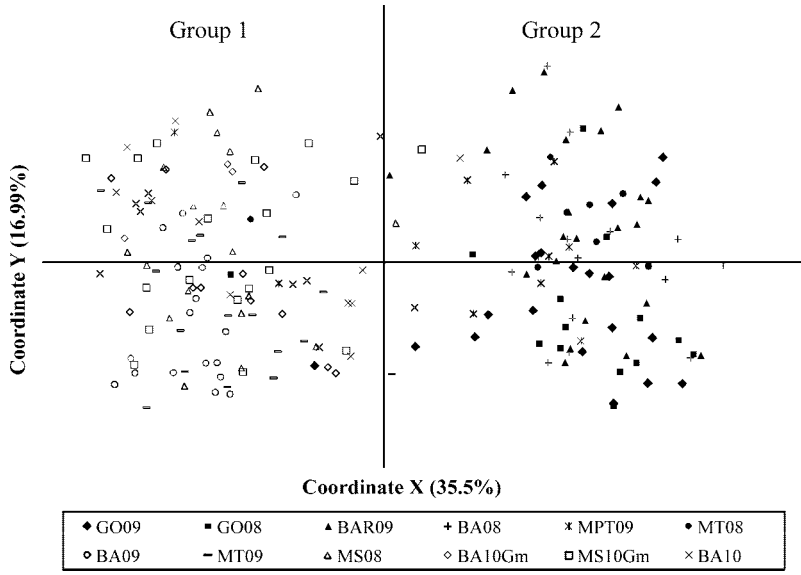


**Fig. 1.** PCA plot of individuals of *H. virescens*. Names of populations are described in Table 1.

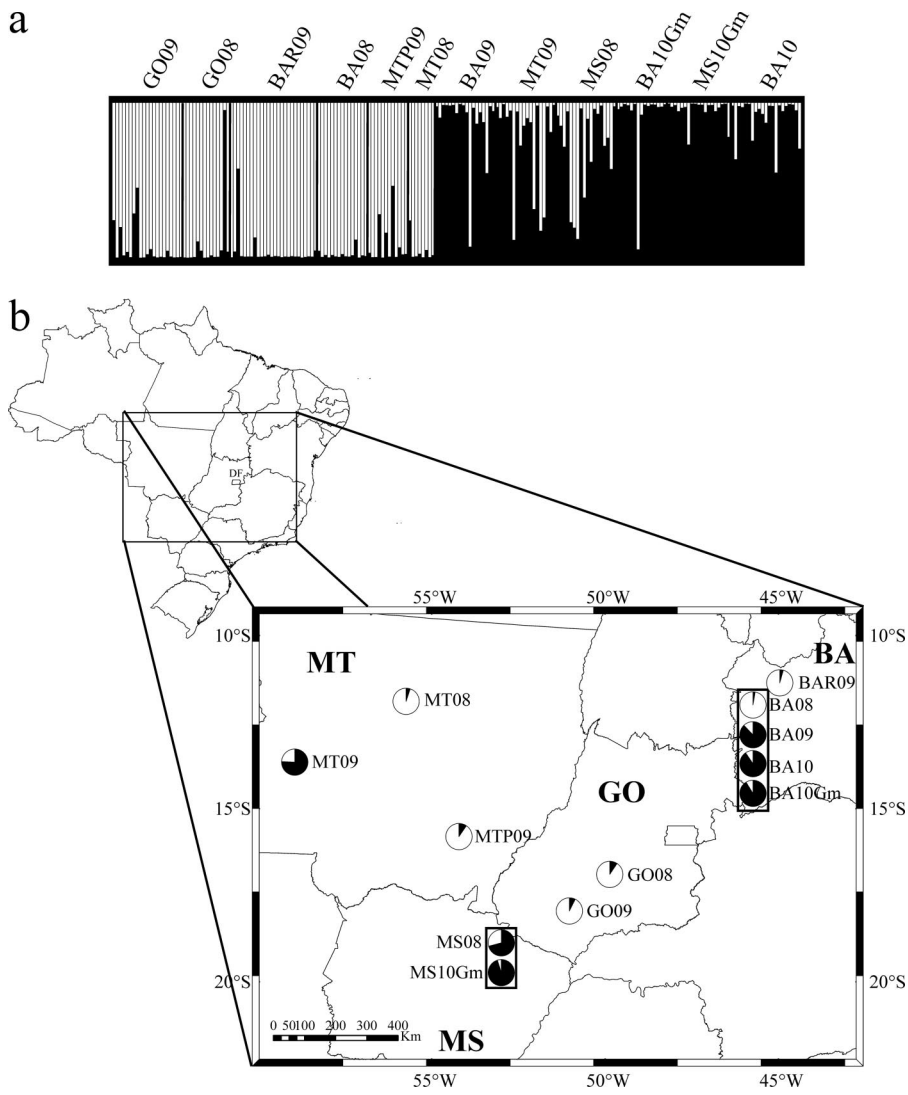


**Fig. 2.** (a) Bar plot for K = 2 groups for *H. virescens* populations assigned by the program STRUCTURE. Every bar corresponds to one individual. (b) Map of distribution of sampled localities of *H. virescens* populations. Each pie chart indicates the fraction of the genome of each population belonging to each of the two groups recovered by the program STRUCTURE. Pie charts are arranged on the geographical position of each sampled locality. Charts from the same sampled locality, but from different growing seasons or crops, were joined by a frame.

tation diversity found around cotton and soybean fields in Brazil (Werneck 2011).

The analysis of *H. virescens* populations based on nine microsatellite loci indicated a high number of private alleles within sampled localities (49 of 127 alleles in total), although insufficient sampling should always be considered in population genetic studies. The high level of private alleles may be indicative of restricted gene flow among populations that can consequently increase the genetic divergence among them. Estimates of genetic structure among populations of *H. virescens* indicated moderate to great population structure, with $\theta = 0.132$ and $R_{ST} = 0.252$. This is the first time that significant genetic structure is suggested for this species, because low genetic structure has been reported for populations of *H. virescens* from different regions of the United States and Mexico in studies using different biochemical and molecular markers (Table 6).

Microsatellites are preferred for population genetic studies because they are highly polymorphic in nature (Behura 2006) and allow for population differentiation where other markers fail. In fact, a weak structure in the same set of populations evaluated here was demonstrated when mtDNA sequences were investigated instead (Albernaz et al. 2012). However, microsatellites and mtDNA can be complementary because each one highlights different aspects of genetic variability. For Brazilian tobacco budworm populations, mtDNA evidenced a recent demographic ex-

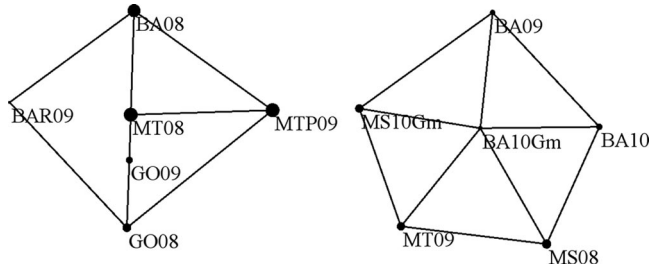


**Fig. 3.** Population graph of all sampled populations of *H. virescens* (node size indicates within population variance, and thin lines are retained edges indicating genetic covariance).

pansion that might be responsible for the current low genetic structure among populations recovered with that marker, that is in fact much more prone to demographic effects (Ballard and Whitlock 2004). Here, microsatellites support an incipient differentiation among them that was only slightly suggested by mtDNA sequences.

All clustering analysis, PCA, Bayesian assignment test, and network analysis of genetic covariance, supported the genetic structure suggested by the fixation indexes. Although these analyses are quite different in the way they cluster individuals, all of them recovered the same pattern of structure in two major groups which were not correlated with the sample geographical origin, crop growing season, or host plant from where they were collected. In addition, the Bayesian assignment test indicated the occurrence of current or past gene flow among populations of *H. virescens* in Brazil, despite the moderate genetic structure.

Similar allelic frequencies among populations of *H. virescens*, coupled with reduced gene flow, indicate a historically homogeneous population. This pattern might be because of demographic events operating on this species (Albernaz et al. 2012), suggesting that the observed gene flow could be historical. In this way, two hypotheses could explain the current pattern of genetic variability and gene flow among these populations. First, a recent event of demographic expansion could have built the present pattern of high levels of genetic variability and moderate to great differences among populations (Albernaz et al. 2012). According to this hypothesis, *H. virescens* populations might have followed the distribution of cotton crops in Brazil from the Meridian region toward the Midwest (mainly to Goiás, Mato Grosso, and Mato Grosso do Sul states) and west of Bahia (CONAB 2009/2010). After this event of demographic expansion, populations were free to diversify in relative isolation. If this hypothesis is correct, older populations of *H. virescens* might have behaved as metapopulations and could explain the nonsignificant isolation by distance test and the lack of correlation of the two recovered clusters with spatial, nutritional, and temporal factors. Only a very sensitive marker such as a microsatellite could have shown an initial process of structuring among these populations.

The second hypothesis suggests that the seasonality of intrapopulation variability could be responsible for the pattern of genetic variability and gene flow among populations of the tobacco budworm. Short-lived organisms such as *H. virescens* present several generations (three in Brazil) (Degrande 1998) each crop growing season, perhaps leading to variation in the population size and in the metapopulation structure (Shpak et al. 2010). According to this proposition, at the beginning of each crop growing season, the earliest generations are characterized by small and relatively isolated populations, whereas later generations experience bigger and connected populations. The increase of the population size favors gene flow when individuals from larger populations migrate to new areas after reaching the ecological support capacity of their habitats (Shpak et al. 2010). For insect pests, the size of the populations is usually dependent upon

**Table 5. Percentage of migrants between populations of *H. virescens* sampled within different growing seasons**

| Growing season | *P* value residence, 83.3% | | *P* value migration, 5.53% | |
|---|---|---|---|---|
| | BA08 | GO08 | MT08 | MS08 |
| 2007–2008 | | | | |
| BA08 | 97.99 | 0.66 | 0.66 | 0.69 |
| GO08 | 28.13 | 68.79 | 1.41 | 1.67 |
| MT08 | 25.63 | 2.10 | 69.95 | 2.32 |
| MS08 | 1.24 | 0.64 | 0.64 | 97.49 |

| | *P* value residence, 83.3% | | *P* value migration, 4.17% | | |
|---|---|---|---|---|---|
| | BA09 | BAR09 | GO09 | MTP09 | MT09 |
| 2008–2009 | | | | | |
| BA09 | 98.08 | 0.57 | 0.44 | 0.46 | 0.44 |
| BAR09 | 0.41 | 0.36 | 0.64 | 0.34 | |
| GO09 | 0.83 | 9.88 | 68.12 | 20.42 | 0.79 |
| MTP09 | 1.46 | 17.43 | 1.26 | 78.55 | 1.30 |
| MT09 | 23.42 | 6.15 | 1.01 | 1.06 | 68.35 |

| | *P* value residence: 83.3% | | *P* value migration: 8.37% |
|---|---|---|---|
| | BA10 | BA10Gm | MS10Gm |
| 2009–2010 | | | |
| BA10 | 97.28 | 0.92 | 1.81 |
| BA10Gm | 16.60 | 69.03 | 14.37 |
| MS10Gm | 13.39 | 1.28 | 85.33 |

The direction of migration is toward row-column. Values of cells of a population with itself report resident individuals.

**Table 6. Estimates of genetic structure in populations of *H. virescens***

| Locality (no. of sampled sets) | Maximum geographical distance among populations (km) | Marker | Genetic structure* | Reference |
|---|---|---|---|---|
| Brazil (12) | ≈1,500 | Microsatellite | $\Theta_{ST} = 0.132$<br>$R_{ST} = 0.252$ | This study |
| USA (11) | ≈3,200 | Allozyme | $F_{ST} = 0.002$ | Korman et al. (1993) |
| USA and Mexico (14) | ≈2,300 | PCR-RFLP | No genetic structure | Roehrdanz et al. (1994) |
| USA (5) | ≈3,000 | Allozyme<br>Sodium channel *Hpy*<br>Juvenil hormone esterase *Hejs* | $F_{ST} = 0.002$<br>$F_{ST} = 0.041$<br>$F_{ST} = 0.001$ | Taylor et al. (1995, 1996) |
| USA (6) | Within 10 km-radius | Allozyme<br>RAPD | $\theta = 0.0036–0.0239$<br>$\theta = 0.001–0.077$ | Han and Caprio (2004) |
| USA and Mexico (8) | ≈2,500 | PCR-AFLP | $F_{ST} = 0.0067$ | Groot et al. (2011) |
| Brazil (11) | ≈1,500 | Mitochondrial DNA | Nonsignificant $F_{ST}$ | Albernaz et al. (2012) |

*, $P < 0.05$.

control measures such as insecticides or Bt plants. Therefore, populations never reach the support capacity of their food resources and, as a consequence, gene flow is reduced. However, individuals could migrate to regions with larger food availability, even if the biological responses of the species is suboptimal on the new hosts (Abney et al. 2007, Blanco et al. 2008b). Sampling at different times during several crop growing seasons could highlight seasonal patterns of gene flow and genetic structure of the tobacco budworm. The two hypotheses are not mutually exclusive and both demographic history and seasonal variation in gene flow might be responsible for the relatively high population structure found among Brazilian populations of *H. virescens.*

The low number of migrants suggested by the Bayesian inference agrees with a low level of gene flow among sampled localities. Tobacco budworm can be considered a facultative migrant (Schneider 1989) because migration events are usually related with the local unavailability of oviposition sites and food sources (Farrow and Daly 1987, Fitt 1989). Therefore, considering the extensive areas in which cotton and soybean crops are cultivated in Brazil (CONAB 2009/2010), resident populations of *H. virescens* are expected. Remnant host plants after harvesting can function as reservoir of tobacco budworm individuals during overwintering times, and, in addition, its polyphagy would allow for population maintenance year-round in Brazil on any available host plant (Blanco et al. 2008b). Migratory events over long distances are unknown under Brazilian conditions.

The inferred migration of *H. virescens* from soybean to cotton suggests a local movement between these host plants. In Brazil, soybean is cultivated from October to February, whereas cotton is cultivated from December to May, with an overlap in some areas (CONAB 2009/2010). When soybean is harvested, *H. virescens* adults could move locally to cotton and to other alternative hosts. There is currently no available information on the direction or migration rate of *H. virescens* in Brazilian field conditions. A more detailed investigation on the interchange of individuals between the main crops used by this species in Brazil could help to clarify the invasiveness of this pest and help to avoid new invasions.

Pest management measures to control *H. virescens* in Brazil, including procedures to delay the evolution of resistance to insecticides and Bt toxins used to control this lepidopteran species in the field, should consider that the moderate to great population structure among its populations does not seem to be related to the host plant where populations were sampled from. The high dose–refuge strategy could take advantage of the fact that individuals of the tobacco budworm occupying both cotton and soybean crops are not genetically differentiated, and other plant species could, in theory, function as refuge areas (Abney et al. 2007, Blanco et al. 2008b). Therefore, soybean crops could be used as a refuge to maintain susceptible individuals of *H. virescens*, in addition to non-Bt cotton, in areas where they are cocultivated. However, the use of soybean areas as refuge could not be considered if this crop also expresses genes coding for Bt proteins and, in fact, Bt soybean expressing Cry1Ac protein was recently approved for commercialization in Brazil (CTNBio 2011).

Furthermore, the moderate to great genetic structure observed for Brazilian populations of *H. virescens* also indicates that care must be taken to avoid the spread of resistance alleles throughout the species geographical distribution. Gene flow between populations from refuge areas and Bt crops can be reflected at small and large scales. At a small or local scale, gene flow can dilute resistance because of the possible free mating between susceptible and resistant individuals, although other factors can influence this oversimplified model (Blanco et al. 2010). At a large scale, if populations can freely exchange genetic information, resistance alleles could quickly increase their frequency within the total population of the target pest. However, these events are prevented by the high dose strategy that lowers the frequency of heterozygote individuals carrying the resistance alleles (Tabashnik and Carrière 2008).

Despite the significant genetic structure found among Brazilian populations of *H. virescens*, it was not yet possible to relate it to ecological features measured

here, such as host plant use, habitat, and crop growing season. Because it is the first time that genetic structuring is reported for these populations, other biological and ecological attributes would deserve further investigation to apply this information for tobacco budworm management in Brazil.

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## References Cited

**Abney, M. R., C. E. Sorenson, and J. R. Bradley, Jr. 2007.** Alternate crop host as resistance management refuges for tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae) in North Carolina. J. Cotton Sci.11: 35–39.

**Albernaz, K. C., K. L. Silva-Brandão, P. Fresia, F. L. Cônsoli, and C. Omoto. 2012.** Genetic variability and demographic history of *Heliothis virescens* (Lepidoptera: Noctuidae) populations from Brazil inferred by mtDNA sequences. Bull. Entomol. Res. 102: 333–343.

**Ali, M. I., and R. G. Luttrell. 2006.** Susceptibilities of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) populations to Cry1Ac insecticidal protein. J. Econ. Entomol. 99: 164–175.

**Alstad, D. N., and D. A. Andow. 1995.** Managing the evolution of insect resistance to transgenic plants. Science 268: 1894–1896.

**Avise, J. C. 2004.** Molecular markers, natural history, and evolution. Sinauer, Inc., Sunderland, MA.

**Ballard, J.W.O., and M. C. Whitlock. 2004.** The incomplete natural history of mitochondria. Mol. Ecol. 13: 729–744.

**Behura, S. K. 2006.** Molecular marker systems in insects: current trends and future avenues. Mol. Ecol. 15: 3087–3113.

**Blanco, C. A. 2012.** *Heliothis virescens* and Bt cotton in the United States. GM Crops Food 3: 1–12.

**Blanco, C. A., A. P. Teran-Vargas, J. D. Lopez, J. V. Kauffman, and X. K. Wei. 2007.** Densities of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in three plant hosts. Fla. Entomol. 90: 742–750.

**Blanco, C. A., N. P. Storer, C. A. Abel, R. Jackson, R. Leonard, J. D. Lopez, G. Payne, B. D. Siegfried, T. Spencer, and A. P. Teran-Vargas. 2008a.** Baseline susceptibility of tobacco budworm (Lepidoptera: Noctuidae) to Cry1F toxin from *Bacillus thuringiensis.* J. Econ. Entomol. 101: 168–173.

**Blanco, C. A., A. P. Teran-Vargas, C. A. Abel, M. Portilla, M. G. Rojas, J. A. Morales-Ramos, and G. L. Snodgrass. 2008b.** Plant host effect on the development of *Heliothis virescens* F. (Lepidoptera: Noctuidae). Environ. Entomol. 37: 1538–1547.

**Blanco, C. A., F. Gould, A. T. Groot, C. A. Abel, G. Hernandez, O. P. Perera, and A. P. Teran-Vargas. 2010.** Offspring from sequential matings between *Bacillus thuringiensis*-resistant and *Bacillus thuringiensis*-susceptible *Heliothis virescens* moths (Lepidoptera: Noctuidae). J. Econ. Entomol. 103: 861–868.

**Caprio, M. A., and B. E. Tabashnik. 1992.** Gene flow accelerates local adaptation among finite populations: simulating the evolution of insecticide resistance. J. Econ. Entomol. 85: 611–620.

**Comissão Técnica Nacional De Biossegurança [CTNBio]. 2011.** Comissão Técnica Nacional De Biossegurança-Liberação comercial da soja geneticamente modificada resistente a inseto e tolerante a herbicida soja MON 87701 ×89788: Parecer Técnico Prévio Conclusivo N° 2542/2010. (http://www.ctnbio.gov.br/index.php/content/view/15348.html).

**(CONAB) Companhia Nacional de Abastecimento. 2009/2010.** Companhia Nacional de Desenvolvimento-Séries históricas relativas às safras 1976/77 a 2009/2010 de área plantada, produtividade e produção. (http://www.conab.gov.br/).

**Degrande, P. E. 1998.** Guia prático de controle das pragas do algodoeiro. Federal University of Mato Grosso do Sul, Dourados, Brazil.

**Dyer, R. J. 2009.** GeneticStudio: a suite of programs for the analysis of genetic-marker data. Mol Ecol Resour. 9: 110–113.

**Dyer, R. J., and J. D. Nason. 2004.** Population graphs: the graph theoretic shape of genetic structure. Mol. Ecol. 13: 1713–1727.

**Earl, D. A. 2011.** Structure harvester v0.6.1 computer program. (http://taylor0.biology.ucla.edu/structureHarvester/).

**Endersby, N. M., S. W. McKechnie, P. M. Ridland, and A. R. Weeks. 2006.** Microsatellites reveal a lack of structure in Australian populations of the diamondback moth, *Plutella xylostella* (L.). Mol. Ecol. 15: 107–118.

**Evanno, G., S. Regnaut, and J. Goudet. 2005.** Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. Resour. 14: 2611–2620.

**Excoffier, L., and H.E.L. Lischer. 2010.** Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10: 564–567.

**Excoffier, L., T. Hofer, and M. Foll. 2009.** Detecting loci under selection in a hierarchically structured population. Heredity 103: 285–298.

**Farrow, R. A., and J. C. Daly. 1987.** Long range movements as an adaptive strategy in the genus *Heliothis* (Lepidoptera, Noctuidae) - a review of its occurrence and detection in four pest species. Aust. J. Zool. 35: 1–24.

**Fitt, G. P. 1989.** The ecology of *Heliothis* species in relation to agroecosystems. Annu. Rev. Entomol. 34: 17–52.

**Franck, P., M. Reyes, J. Olivares, and B. Sauphanor. 2007.** Genetic architecture in codling moth populations: comparison between microsatellite and insecticide resistance markers. Mol. Ecol. 16: 3554–3564.

**Goudet, J. 2002.** FSTAT, a program to estimate and test gene diversities and fixation indices version 2.9.3.2. (http://www.unil.ch/izea/softwares.fstat.html).

**Gould, F. 1998.** Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

**Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferre, F. J. Silva, and W. J. Moar. 1992.** Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens.* Proc. Natl. Acad. Sci. U.S.A. 89: 7986–7990.

**Gould, F., A. Anderson, A. Reynolds, L. Bumgarner, and W. Moar. 1995.** Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera, Noctuidae) strain with high lev-

els of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545–1559.

**Greene, G. L., N. C. Leppla, and W. A. Dickerson. 1976.** Velvetbean caterpillar (Lepidoptera, Noctuidae) rearing procedure and artificial medium. J. Econ. Entomol. 69: 487–488.

**Groot, A. T., A. Classen, O. Inglis, C. A. Blanco, J. López, Jr., A. T. Vargas, C. Schal, G. Heckel, and G. Schöfl. 2011.** Genetic differentiation across North America in the generalist moth *Heliothis virescens* and the specialist *H. subflexa.* Mol. Ecol. 20: 2676–2692.

**Han, Q., and M. A. Caprio. 2004.** Evidence from genetic markers suggests seasonal variation in dispersal in *Heliothis virescens* (Lepidoptera: Noctuidae). Environ. Entomol. 33: 1223–1231.

**Hartl, D. L., and A. G. Clark. 2007.** Principles of population genetics. Sinauer, Inc., Sunderland, MA.

**Jakobsson, M., and N. A. Rosenberg. 2007.** CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23: 1801–1806.

**Korman, A. K., J. Mallet, J. L. Goodenough, J. B. Graves, J. L. Hayes, D. E. Hendricks, R. Luttrell, S. D. Pair, and M. Wall. 1993.** Population structure in *Heliothis virescens* (Lepidoptera, Noctuidae)-an estimate of gene flow. Ann. Entomol. Soc. Am. 86: 182–188.

**Krafsur, E. S. 2005.** Role of population genetics in the sterile insect technique, pp. 389–406. *In* V. A. Dyck, J. Hendrichs, and A. S. Robinson (eds.), Sterile insect technique: principles and practice in area-wide integrated pest management. Springer, Amsterdam, The Netherlands.

**Luttrell, R. G., L. Wan, and K. Knighten. 1999.** Variation in susceptibility of noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis.* J. Econ. Entomol. 92: 21–32.

**Mantel, N. 1967.** The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209–220.

**Martel, C., A. Réjasse, F. Rousset, M.-T. Bethenod, and D. Bourguet. 2003.** Host-plant-associated genetic differentiation in northern French populations of the European corn borer. Heredity 90: 141–149.

**McCaffery, A. R. 1998.** Resistance to insecticides in heliothine Lepidoptera: a global view. Philos. Trans. R. Soc. Lond. B Biol. Sci. 353: 1735–1750.

**Missiaglia, A., and D. Grattapaglia. 2006.** Plant microsatellite genotyping with 4-color fluorescent detection using multiple-tailed primers. Genet. Mol. Res. 5: 72–78.

**Monnerat, R., E. Martins, P. Queiroz, S. Ordúz, J. Jaramillo, G. Benintende, J. Cozzi, M. D. Real, A. Martinez-Ramirez, C. Rausell et al. 2006.** Genetic variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) populations from Latin America is associated with variations in susceptibility to *Bacillus thuringiensis* Cry toxins. Appl. Environ. Micro. 72: 7029–7035.

**Morin, S., S. Henderson, J. A. Fabrick, Y. Carriere, T. J. Dennehy, J. K. Brown, and B. E. Tabashnik. 2004.** DNA-based detection of Bt resistance alleles in pink bollworm. Insect Biochem. Mol. Biol. 34: 1225–1233.

**Park, S.D.E. 2001.** Trypanotolerance in West African cattle and the population genetic effects of selection. University of Dublin, Dublin, Ireland.

**Peakall, R., and P. E. Smouse. 2006.** GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6: 288–295.

**Perera, O. P., C. A. Blanco, L. Ballard, K. L. Silva-Brandão, F. A. Domingues, and C. A. Abel. 2011.** Evaluation of anonymous and expressed sequence tag-derived polymorphic microsatellite markers in the tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae). Southwest. Entomol. 36: 287–294.

**Poole, R. W., C. Mitter, and M. Huettel. 1993.** A revision and cladistic analysis of the *Heliothis virescens* species-group (Lepidoptera: Noctuidae) with a preliminary morphometric analysis of *Heliothis virescens.* Mississippi Agricultural and Forestry Experiment Station Technical Bulletin, Mississippi State University, Mississippi State, MS.

**Pritchard, J. K., M. Stephens, and P. Donnelly. 2000.** Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

**Raymond, M., and F. Rousset. 1995.** Genepop (version 1.2), population genetics software for exact tests and ecumenicism. J. Hered. 86: 248–249.

**Roehrdanz, R. L., J. D. Lopez, J. Loera, and D. E. Hendricks. 1994.** Limited mitochondrial-DNA polymorphism in North-American populations of *Heliothis virescens* (Lepidoptera, Noctuidae). Ann. Entomol. Soc. Am. 87: 856–866.

**Rollins, L. A., A. P. Woolnough, and W. B. Sherwin. 2006.** Population genetic tools for pest management: a review. Wildl. Res. 33: 251–261.

**Rosenberg, N. A. 2004.** DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4: 137–138.

**Schneider, J. C. 1989.** Role of movement in evaluation of area-wide insect pest management tactics. Environ. Entomol. 18: 868–874.

**Schuelke, M. 2000.** An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18: 233–234.

**Scott, K. D., N. Lawrence, C. L. Lange, L. J. Scott, K. S. Wilkinson, M. A. Merritt, M. Miles, D. Murray, and G. C. Graham. 2005.** Assessing moth migration and population structuring in *Helicoverpa armigera* (Lepidoptera: Noctuidae) at the regional scale: example from the Darling Downs, Australia. J. Econ. Entomol. 98: 2210–2219.

**Shpak, M., J. Wakeley, D. Garrigan, and R. C. Lewontin. 2010.** A structured coalescent process for seasonally fluctuating populations. Evolution 64: 1395–1409.

**Slatkin, M. 1995.** A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457–462.

**Sparks, T. C. 1981.** Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. Bull. Entomol. Soc. Am. 27: 186–192.

**Stone, T. B., and S. R. Sims. 1993.** Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera, Noctuidae) to *Bacillus thuringiensis.* J. Econ. Entomol. 86: 989–994.

**Tabashnik, B. E. 1994.** Evolution of resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 39: 47–79.

**Tabashnik, B. E., and Y. Carrière. 2008.** Evolution of insect resistance to transgenic plants, pp. 267–279. *In* K. J. Tilmon (ed.), Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. University of California Press, Berkeley, CA.

**Taylor, M.F.J., Y. Shen, and M. E. Kreitman. 1995.** A population genetic test of selection at the molecular level. Science 270: 1497–1499.

**Taylor, M.F.J., P. Park, and Y. Shen. 1996.** Molecular population genetics of sodium channel and juvenile hormone esterase markers in relation to pyrethroid resistance in *Heliothis virescens* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 89: 728–738.

**Teran-Vargas, A. P., J. C. Rodriguez, C. A. Blanco, J. L. Martinez-Carrillo, J. Cibrian-Tovar, H. Sanchez-Arroyo, L. A. Rodriguez-Del-Bosque, and D. Stanley. 2005.** Bollgard cotton and resistance of tobacco budworm (Lepidoptera: Noctuidae) to conventional insecticides in southern Tamaulipas, Mexico. J. Econ. Entomol. 98: 2203–2209.

**Tomquelski, G. V., and L.C.T. Maruyama. 2009.** Lagarta-da-maçã em soja. Rev. Cultivar. 117: 20–22.

**Van Oosterhout, C., W. F. Hutchinson, D.P.M. Wills, and P. Shipley. 2004.** MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4: 535–538.

**Waldvogel, M., and F. Gould. 1990.** Variation in oviposition preference of *Heliothis virescens* in relation to macroevolutionary patterns of heliothine host range. Evolution 44: 1326–1337.

**Weir, B. S. 1996.** Genetic data analysis II. Sinauer, Inc., Sunderland, MA.

**Weir, B. S., and S. S. Cockerham. 1984.** Estimating F-statistics for the analyses of population structure. Evolution 38: 1358–1370.

**Werneck, F. P. 2011.** The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. Quat. Sci. Rev. 30: 1630–1648.

**Wilson, G. A., and B. Rannala. 2003.** Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163: 1177–1191.

**Wolfenbarger, D. A., P. R. Bodegas, and R. Flores. 1981.** Development of resistance in *Heliothis* spp. in the Americas, Australia, and Asia. Bull. Entomol. Soc. Am. 27: 181–185.

**Wright, S. 1931.** Evolution in mendelian populations. Genetics 16: 97–159.

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