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First Microsatellites From *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and Their Potential Use for Population Genetics

R. S. Arias
USDA-ARS MSA Genomics and Bioinformatics Research Unit, renee.arias@ars.usda.gov

Carlos A. Blanco
USDA-ARS Southern Insect Management Research Unit, carlos.a.blanco@aphis.usda.gov

Maribel Portilla
USDA-ARS Southern Insect Management Research Unit

Gordon L. Snodgrass
USDA-ARS Southern Insect Management Research Unit

Brian E. Scheffler
USDA-ARS MSA Genomics and Bioinformatics Research Unit

First Microsatellites From *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and Their Potential Use for Population Genetics

RENÉE S. ARIAS,[1,2] CARLOS A. BLANCO,[3,4] MARIBEL PORTILLA,[3] GORDON L. SNODGRASS,[3] AND BRIAN E. SCHEFFLER[1]

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ABSTRACT This is the first report of sequence-specific microsatellite markers (simple sequence repeats [SSRs]) of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), an economically important pest of crops on the Americas. We isolated 192 microsatellite markers by using pyrosequencing and screened 15 individuals from eight isofamilies collected from three geographical areas: Puerto Rico (PR), Texas (TX), and Mississippi (MS). Isofamilies resistant to Cry toxins from *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) also were included. Cluster analysis was performed to determine the potential use of these SSRs in discriminating populations, and colonies were grouped with a reliability of 100% estimated by bootstrap. In this analysis, colonies from TX grouped away from those from PR, but the two MS isofamilies grouped with TX and PR separately. Genetic distance within isofamilies ranged between 0.22 and 0.56, and the minimum distance between isofamilies was 0.83. Unique pattern informative combination (UPIC) scores were calculated, and the 80 SSR markers that had UPIC scores of ≥1 are listed according to their discriminating potential. UPIC scores allow reducing costs by choosing fewer and highly informative markers for future studies. From the best 125 markers, 103 had a maximum of two alleles per sample, making them ideal candidates for population genetic studies. BLAST screening of the sequences points to potential biological meaning of marker polymorphisms. The percentage of alleles shared by the three geographic areas was 14%. The markers reported will significantly enrich the pool of molecular markers available for *S. frugiperda*. In addition, they could be used for monitoring migration of populations, in the development of biocontrol agents and for management practices in general.

RESUMEN Esta es la primera publicación de microsatelites de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), una plaga importante del continente Americano. Hemos aislado 192 marcadores de microsatelites usando un pyrosequenciador, y analizamos 15 individuos de eight isofamilias colectadas de tres áreas geográficas, Puerto Rico (PR), Texas (TX) y Mississippi (MS), incluyendo isofamilias resistentes y susceptibles a *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae). Análisis de cluster SE realizó con el propósito de determinar el potencial discriminatorio de los microsatélites. Este agrupo las isofamilias de TX distantes de las isofamilias de PR, mientras que las de MS SE agruparon con TX y con PR separadamente. La distancia genética dentro de isofamilias fue de 0.22 a 0.56, mientras que la distancia mínima entre isofamilias fue 0.83. Un total de 80 marcadores que tuvieron valores de UPIC ≥1 como potencial discriminante son presentados. Valores de UPIC permiten reducir costos y elegir marcadores que brindan la máxima variabilidad genética en estudios posteriores. Los marcadores listados pueden contribuir significativamente al número de marcadores moleculares disponibles para *S. frugiperda*. De los mejores 125 markers, 103 presentaron un máximo de two alelos por muestra, lo que los hace buenos candidatos para estudios de genética poblacional. Resultados de BLAST indicarían potencial significado biológico del polimorfismo. El porcentaje de alelos compartidos por las tres regiones

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[1] USDA–ARS MSA Genomics and Bioinformatics Research Unit, 141 Experiment Station Rd., Stoneville, MS 38776.

[2] Corresponding author: e-mail, carlos.a.blanco@aphis.usda.gov.

[3] USDA–ARS Southern Insect Management Research Unit, 141 Experiment Station Rd., Stoneville, MS 38776.

[4] USDA–APHIS Biotechnology Regulatory Services, 4700 River Rd., Riverdale, MD 20737.

geográficas fue 14%. Además, estos marcadores podrían ser usados para monitorear migración de poblaciones, en el desarrollo de agentes de control biológico, en programas de mejoramiento, y para prácticas de manejo en general.

KEY WORDS pyrosequencing, fall armyworm, simple sequence repeat, molecular markers, fingerprinting

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is endemic to the American continent (Johnson 1987) where it causes economic losses in important crops such as maize (*Zea mays* L.), cotton (*Gossipium* spp. L.), alfalfa (*Medicago sativa* L.), rice (*Oryza sativa* L.), and grasses (Sparks 1986). In a crop as important as maize, which has a world production of ≈727,000 MT (Index-Mundi 2009), losses caused by *S. frugiperda* can reach up to 20% (Marenco et al. 1992, Polanczyk et al. 2000), and if this insect is not controlled, it can reduce maize yields by up to 73% (Hruska and Gladstone 1988). The control of *S. frugiperda* mostly relies on timely applications of insecticide aimed at susceptible larval developmental stages during incipient plant damage, which gives to this control tactic a narrow window of opportunity for optimal treatment. In maize alone, the amount of insecticide used can reach 30,000 tons of active ingredient per year (Blanco et al. 2010). The alternative method of control is the use of transgenic crops expressing Cry toxins from *Bacillus thuringiensis* (Berliner) (Bt; Bacillales: Bacillaceae) (Jenkins 1999). However, *S. frugiperda* has shown some level of tolerance to Cry1 F toxin (Adamczyk et al. 1997, Chilcutt et al. 2007, de Souza et al. 2009). In particular, Puerto Rico (PR) fall armyworm populations have already shown high tolerance to the δ-endotoxin Cry1 F of Bt (Tabashnik et al. 2009, Nagoshi et al. 2010). Because Puerto Rico is only 1,500 km away from the nearest U.S. coastline, and this island can be affected by numerous yearly weather systems that commonly move northward during high activity of fall armyworm moths, it creates the possibility that this insect, putatively resistant to Bt, can easily move to the continental United States. In fact, Young (1979) and Westbrook and Sparks (1986) have documented the passive movement of this insect via weather atmospheric transport. Thus, numerous questions have arisen regarding the migration of populations, possible interbreeding, and development of resistance in view of the increasing use of transgenic crops expressing Bt toxins.

To control this pest and develop effective management practices it is important to understand the migratory patterns, genetic diversity of the existing populations, and potential development of new populations. So far, two strains have been described of *S. frugiperda* according to their host preference and behavior, but these strains present no morphological differences (Pashley 1986, Pashley et al. 1985, Nagoshi and Meagher 2009) and are only distinguished by molecular methods (Pashley et al. 1985, Nagoshi and Meagher 2003, Martinelli et al. 2006, Machado et al. 2008, Velez-Arango et al. 2008).

Numerous studies have been performed to understand the migrations of *S. frugiperda* in the United States, with sometimes inconsistent results regarding annual migrations, as pointed out by Nagoshi and Meagher (2008). Some molecular tools have been developed to monitor migrations and/or crosses of this insect. For example, two polymorphic loci in the sequence analysis of the mitochondrial cytochrome oxidase subunit I gene (COI) can distinguish four haplotypes in *S. frugiperda* populations across America (Pashley 1989), and the proportions of these haplotypes have been used to identify populations (Nagoshi 2010). Other molecular tools developed to assist in the identification of populations of *S. frugiperda* are a short-repeat element known as Found in Rice (FR) that maps to sex chromosomes (Lu et al. 1994, Nagoshi and Meagher 2003), and polymorphisms in the DNA sequence of the triose phosphate isomerase gene (Tpi) (Nagoshi 2010). However, the need for a multilocus approach to understand the biology of *S. frugiperda* has been emphasized by Prowell et al. (2004), who used combination of molecular tools, including allozymes, amplified fragment length polymorphisms (AFLPs), and mitochondrial DNA to study speciation and introgression in *S. frugiperda.* In fact, the use of large number of loci is more likely to contain information about gene flow during older events even if analyzing small samples (Wang and Hey 2010).

Microsatellites, also known as simple sequence repeats (SSRs), have been the most widely applied class of molecular markers used in genetic studies with applications in many fields of genetics, including genetic conservation, population genetics, molecular breeding, and paternity testing (Ellegren 2004). This range of applications is possible because microsatellite markers are codominant, multiallelic, and highly reproducible; have high resolution; and are based on polymerase chain reaction (PCR) (Oliveira et al. 2006). As a convention, SSRs are regions in the genome where a group of bases (1–8 bp) are repeated in tandem (Richard et al. 2008). These regions can be isolated either by data mining of existing sequences or by generating SSR-enriched libraries (Kijas et al. 1994, Zane et al. 2002).

Genetic resources of *S. frugiperda* are significant, i.e., there are 1,110 nucleotide entries in National Center for Biotechnology Information (NCBI; eight correspond to midgut) and 65,403 expressed sequence tags (ESTs; 11,192 correspond to midgut) reported. However, there have been no publications on microsatellite markers developed for *S. frugiperda.* There have been only eight microsatellite markers developed for a related species, *Spodoptera exempta* (Walker), where it is mentioned that two of the mark-

Table 1. Origin of *S. frugiperda* colonies including their corresponding hosts and locations in the United States

Colony name	Geographic origin[a]	Host	Bt resistance	Generation
456	PR	Maize	Yes	F3
512	PR	Maize	Yes	F3
751	PR	Maize	Yes	F3
778	MS	Grass	No	F2
957	MS	Maize	No	F2
980	TX	Maize	No	F2
985	TX	Maize	No	F2
989	TX	Maize	No	F2

The letters A and B used throughout the manuscript and cluster analysis correspond to DNA samples extracted from two individuals from each population.

[a] PR, Puerto Rico; TX, Texas; MS, Mississippi.

ers also amplified *S. frugiperda* (Ibrahim et al. 2004), and one microsatellite from rice that also amplified a DNA sample of *S. frugiperda* (Zhao and Kochert 1993). Thus, in the current study we used an effective method of microsatellite isolation (Techen et al. 2010), developed sequence-specific microsatellite libraries of *S. frugiperda*, and tested the markers on eight isofamilies from three geographic locations (Table 1). Using cluster analysis and unique pattern informative combination (UPIC) software, we determined the most informative markers and their potential usefulness for multilocus population studies of *S. frugiperda.* In addition, BLASTx and BLASTn screening of the sequences point to potential biological meaning of the marker polymorphisms and could be the basis for future studies.

Materials and Methods

Insect Collection and Rearing. Field-collected *S. frugiperda* larvae (Passoa 1991) were reared in insect artificial diet (Blanco et al. 2009) until adults were obtained. Single pairs from each geographic location formed isofamilies, and their second and third generation (F_2/F_3) were tested on serial dilutions of Bt purified proteins (Blanco et al. 2010; Table 1). All isofamilies were derived from maize sampled in College Station, TX (hereafter TX); Santa Isabel, Puerto Rico (PR), and Stoneville, MS (MS), except isofamily 778 that was collected from grasses in MS.

Isolation of Microsatellites. DNA was extracted from *S. frugiperda* colony 957, from the F_2 generation (Table 1). Only thorax tissue was used for this extraction to avoid contamination with the gut microflora. DNA was extracted with DNeasy Plant Maxi kit (QIAGEN, Valencia, CA), and SSR-enriched libraries were generated following the protocol of Techen et al. (2010), briefly described here. DNA was digested with restriction enzymes AluI, HaeIII, DraI, RsaI, and HpyCH4IV (New England Biolabs, Ipswich, MA) individually and with combinations of RsaI+HaeIII and DraI+AluI. The blunt-end DNA fragments were A-tailed with *Taq*-DNA Polymerase (Promega, Madison, WI) in the presence of dATP for 2 h and then ligated for 3 h at 16°C to a linker made from oligonucleotides (oligos) SSRLIBF3, 5′-CGGGAGAGCAAGGAAGGAGT-3′ and SSRLIBR3, 5′Phos-CTCCTTCCTTGCTCTCTCCCGAAAA-3′ (Techen et al. 2010). The ligated fragments were purified with MinElute (QIAGEN) and amplified by 20 cycles of PCR by using primer SSRLIBF3 and High Fidelity DNA Polymerase (Invitrogen, Carlsbad, CA) at 94°C for 30 s, 60°C for 30 s, and 68°C for 90 s. The amplified products were hybridized to three groups of biotinylated oligo repeats, similar to the groups listed by Glenn and Schable (2005): group 2 [$(AG)_{12}$, $(AAC)_6$, $(AAG)_8$, $(ACT)_{12}$, $(ATC)_8$], group 3 [$(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACAG)_6$, $(ACCT)_6$, $(ACTC)_6$, $(ACTG)_6$], and group 4 [$(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$], primers were purchased from Invitrogen. The final concentration of each oligo in the mix was 1 μM, and 2 μl of each oligo mix was used in 50-μl hybridization reactions. Hybridizations were performed in a gradient thermocycler at 95°C for 10 min, followed by 3 h at the annealing temperature using a gradient block at 50°C for group 2 and 4 and 53°C for group 3, followed by an extension step of 10 min at 68°C in the presence of High Fidelity *Taq* Polymerase (Invitrogen) as indicated in Hayden et al. (2002). Sequences containing repeats were captured using streptavidin-coated magnetic beads M-270 (Invitrogen, Carlsbad, CA) in a Labquake tube shaker/rotator (Barnstead/Thermoline, Dubuque, IA) at 22°C for 1 h (Kijas et al. 1994). After binding, the beads were washed with 0.5× standard saline citrate (SSC) at ambient temperature and 0.5× SSC at 50°C for 5 min each. Elution of the DNA from the biotinylated oligos was done with 60 μl of MilliQ water (Millipore, Billerica, MA) at 96°C for 5 min, twice. The eluate was PCR amplified for 10 cycles as indicated for the ligation step. PCR products were sequenced by pyrosequencing in 1/16th of a plate 454-GS FLX (Roche Diagnostics, Indianapolis, IN) by using a GS Titanium Sequencing kit XLR70 (70 by 75 Titanium Pico-Titer Plates, Roche, Branford, CT; 200 cycles,), using 1/16th of a plate. Sequences were assembled with 454 gsAssembler version 2.0 (Roche). Repeats were searched using SSRFinder (Sharopova et al. 2002), and primers were designed using Primer3 (Rozen and Skaletsky 2000) with stringent parameter conditions: annealing temperature (Tm), 63°C optimum; (60/65°C), min/max; length, 24-bp optimum; (20/28 bp), min/max; 3′GC clamp; and maximum overlap of repeat within the primer, 5 bp. To simplify the recording of the repeated motifs, circular permutations, and reverse complements of the motif sequences were grouped together as one type, i.e., AAC, ACA, CAA, GTT, TGT, TTG were recorded as AAC. Any library/sequence information requirements can be addressed to B.E.S. at brian.scheffler@ars.usda.gov.

Fingerprinting and Cluster Analysis. Using stringent conditions in Primer3 software, we designed 215 primers on the flanking regions of the repeats and tested 192 of them on 15 individuals from eight *S. frugiperda* colonies from three geographical areas as detailed in Table 1. Fourth and fifth instars of *S. frugiperda* were used. From colony 751, the DNA extracted from one individual was sufficient to run 96 markers but not enough for all 192 markers used in this study. These isofamilies are maintained by Dr. M.

Portilla (Southern Insect Management Research Unit, Stoneville, MS). Forward SSR primers were 5′ tailed with the sequence 5′-CAGTTTTCCCAGTCACGAC-3′ to permit product labeling, and reverse primers were tailed at the 5′end with the sequence 5′-GTTT-3′ to promote nontemplate adenylation (Brownstein et al. 1996). Primer 5′-CAGTTTTCCCAGTCACGAC-3′ labeled with 6-carboxy-fluorescein (FAM) (Integrated DNA Technologies, Inc., Coralville, IA) was used for amplification of 10-ng DNA by using Titanium *Taq*DNA Polymerase (Clontech, Mountain View, CA) in 5-μl reactions on an M&J thermal cycler (Bio-Rad Laboratories, Hercules, CA) at 95°C for 1 min, 60°C for 1 min (two cycles), 95°C for 30 s, 60°C for 30 s, 68°C for 30 s (27 cycles), and a final extension at 68°C for 4 min. Fluorescently labeled PCR fragments were analyzed on an ABI 3730XL DNA Analyzer, and data were processed using GeneMapper version 3.7 (both from Applied Biosystems, Foster City, CA). Presence of alleles was converted to a binary matrix. Cluster analysis of SSR marker results for *S. frugiperda* isolates was performed using the unweighted paired group method and arithmetic averages (unweighted pair-group method with arithmetic average) with the Nei's coefficient, algorithm implemented in the SAHN program of NTSYSpc version 2.2 (Exeter Software, Setauket, NY). The confidence levels for the dendrograms were assessed by bootstrap resampling (5,000 replicates) (Felsenstein 1985, Efron et al. 1996) by using WINBOOT (available on line from International Rice Research Institute, Metro Manila, Philippines).

Percentage of Multiallelic Loci, UPICs, BLAST, Number of Effective Alleles, and Expected and Observed Heterozygosity. The percentage of multiallelic loci was calculated for each individual across the 120 SSR markers used for cluster analysis. We also calculated UPIC to identify the most informative markers and marker combinations that could discriminate all the colonies screened. The percentage of multiallelic loci (ML) and UPIC scores were both calculated using UPIC Perl scripts (Arias et al. 2009). The DNA sequences used in this study were screened using BLASTx and BLASTn (Altschul et al. 1990) in NCBI databases, and those with significant hits were indicated on each marker. Effective alleles and heterozygosity were calculated for each locus (Weir 1990).

Results

DNA Isolation, Repeat Symmetry, and Frequency. In total, 4,806 reads were assembled in 1,904 contigs where 474 SSRs were detected. The average length of the contigs containing SSRs was 337 bp with an average of eight reads per contig. The most abundant repeat motif in these libraries was AC, followed by ATC, AG, and AAG. A summary of the abundance of each repeat motif is shown in Table 2. We submitted 185 *S. frugiperda* sequences in total containing microsatellites to GenBank (NCBI), with accession numbers HM752580–HM752766, the numbers indicated in marker ID in Table 3, correspond to the contig numbers of the sequences submitted to the NCBI database. In total, 215 unique primers were designed with stringent conditions of 63°C Tm, 3′-end GC clamp, and optimum length of 24 bp. Out of these 215 primer sets designed, we tested 192 primer sets on 16 DNA samples from eight *S. frugiperda* isofamilies. DNA extracted from individual fifth-instar larvae was used to test the 192 sets of primers. We report here the sequence information and screening results for 174 sequence-specific *S. frugiperda* SSRs that resulted in scorable PCR amplifications of the samples tested. The average length of amplicons for these markers was 134 ± 25 bp. All forward and reverse primers had a Tm of 63 ± 1°C.

Table 2. Motifs and motif frequencies detected on microsatellite-enriched libraries of *S. frugiperda*

Motif	Frequency	Motif	Frequency
AC	93	CG	2
ATC	22	AAAG	1
AG	14	AACG	1
AAG	9	ACGG	1
ACT	5	ACTGCT	1
ACC	4	ACTT	1
ACTG	4	AGA	1
ACAT	3	AGG	1
ATGT	3	AGGT	1
AAC	2	AGT	1
AAT	2	AGTC	1
ACACC	2	ATAA	1
AGC	2	ATAC	1
AGTG	2	ATAG	1
ATT	2	CGT	1
CCG	2		

Marker Description and UPIC Scores. We used the UPIC software to identify the most informative markers obtained by screening *S. frugiperda* samples. Those with UPIC scores >1 from screening 15 DNA samples are listed in Table 4, zero would indicate that no sample is being discriminated by the marker. There were 17 markers with UPIC scores between 10 and 15, what means that those markers can uniquely discriminate between 10 and 15 out of the 15 *S. frugiperda*-DNA samples tested.

Cluster Analysis and Genetic Variation of *S. frugiperda*. Insufficient DNA from one individual of isofamily 751 limited its fingerprinting to only 96 markers, so this sample was excluded from part of the general analysis. In total, 120 markers that amplified at least 93% of the samples were used to calculate genetic distances in a global cluster analysis for 15 *S. frugiperda* DNA samples from eight isofamilies and three geographical origins, PR, TX, and MS. These markers detected 942 alleles with a range of one to 25 alleles per marker across samples, one to seven alleles per marker on individual samples, and an average of 1.4 allele per sample per marker. The average number of alleles per locus was 8 ± 5. In total, 103 of these selected markers had a maximum of two alleles on individual samples. Cluster analysis of the 120 SSR markers was performed. No phylogenetic analysis was intended from this preliminary screening, only the assessment of the usefulness of the markers. Pairs of samples from each

Table 3. Microsatellite markers from *S. frugiperda*

Marker	Forward primer 5′ => 3′	Reverse primer 5′ => 3′
Stv_Spf20	CCATCCTCAATGACAATTCCTATG	ATGTTGTCATCGTCTGTTGGCTAC
Stv_Spf29	TGATTGACGGACAAACATTCAAAC	TGGCAATAGGACTTTTTCCCATTAG
Stv_Spf38	ATTGCGAGATCTGTCAGTTTGTTC	AAATTCACGAATAACACGACAACG
Stv_Spf61	CCAAATTTATGCTACTGATGTACCTG	GGATCTATTGTTGTCGAAATACGG
Stv_Spf69	CCACCTCAACCACTGGTAACCTAC	ATTGACATGGAGTTAGGGTTTTGC
Stv_Spf74	TCGTTCTACGTGGAGGACCAAG	TTGCTTAATTAAACCTTACACGGAAGAC
Stv_Spf85	ACATCTTTCTTTGTTGACGTTTCG	GTATGTGATGGCTGTTTGTTTTGG
Stv_Spf102	ACGTCGGGGTCACCACTGTAG	ATATACGATAGCCAGTCGGCTTCC
Stv_Spf103	CATACAGGACGTGGTGTAATGGC	GAAGCATACAGCCATACGTTACCC
Stv_Spf120	CATTGTACCAGTTGTGGGAGTTTG	AAATCAATCGTACCCTAAGTATTGCAG
Stv_Spf122	AAGGGTAGCCTCCAAAGATAATCC	ACCTAGGTACCTTGACCCCAGAAG
Stv_Spf127	AAACATGACAAATATCCCGTTTCG	CTATGTTCAGCAGTGGACGTCTTG
Stv_Spf143	GCGTTGTGTTCAACTGTTGTTATTAG	CTCTGCAGCACTGTAATAATGTCG
Stv_Spf147	GGGTTGCGACTACTGATCTATGTACC	TAACTAAGCTCACGCAAACACTCG
Stv_Spf148	CCGTCATCTCACGTTCAGTCTAAG	TAAAATTGAGTAGCGTTTTTCGGG
Stv_Spf150	TACTTACGCCATCACAACATCCAC	TCATCATCCTCTGACTCTTCATCG
Stv_Spf151	TGGGGGTCACAGAACAGATAAAAG	GTAGTGGACTGTTAGGGGCAGTTG
Stv_Spf173	TAACTTAGGCTGCAGACTCACCG	ATGTAGAGGAGGCTCGTAGTCCAG
Stv_Spf187	AGAATTTTGTGGAAGACAGGGAAC	GATAACCATAAGGGAGGGGGTG
Stv_Spf188	TGAGACGCATGGTGTTTAGTTTG	ATACACACCACACATCGCACC
Stv_Spf212	TGTGCGATGCAGGATATTTATGAC	GCGAGCATACTAAATACATCAAGGC
Stv_Spf240	GCGGCAAACAATACCTGACATAAC	TTGTATGTTCCTTAGTGGGTTGGG
Stv_Spf255	GTGTAATCGGGACACATACAGCAG	ATGAGGATGGCTTCATCAAAGTTC
Stv_Spf270	TGAACTGACAAACATATTGAATGCG	GTGTGTCCACTTTCCAAAACCTTC
Stv_Spf292	TGATAGACCGTGTGACTACCATGTG	TCATTACATCCAAAGCAACGTCAC
Stv_Spf301	GTATGTAAAGTGGAACAAACCGGC	TTGTATGTTTGTAAACGCACCCAC
Stv_Spf305	TTTGCAGTTGTGTCTGTGTTTGTG	AACTGTATGTGTGCATGCTGTGG
Stv_Spf306	CTCAACATACGCCCCGTCATC	ACCAGAGCCGTTGTCGAAGTTACAC
Stv_Spf343	GTCAAAGTTTTACATGGAAGCGTG	CCCATCTGTTTGTCCACAGTAAAG
Stv_Spf354	GCCAGTCAACAAACACAGTTGC	GTTGTTGCTGTTGCTGGTGTTG
Stv_Spf406	TTAAGTCGTGCATAGATCCCGTAAC	TCGGCACTTGTATACGGAAATTG
Stv_Spf407	CAATAATTACCCGCGCATTATGTC	ACTCCTCCTTGTCCGTGTTGTG
Stv_Spf413	TAAAGATACATACGGAACGGAACCTG	ACAACTTACGTGTATCCCGGTTTG
Stv_Spf417	AACAACAGGGATAGATTGTGGACG	CACCAACTAACAACATCAGACCAATC
Stv_Spf452	ATTGCTTCAGTACTCAGTCGGCTC	ACACGATACATTGTACACGATGGC
Stv_Spf462	CCTAACCAAAGAACATGCACACAC	ACGTTGTTATTGGTGGGATAGCTG
Stv_Spf467	CACTGTTAACGTTTGAAGTGGGTG	TTCAAACAGCGCTCAATACAACTG
Stv_Spf470	CAACTAGCTCTGCGCGCACTATAC	GGGCTACACTCCAACTTTACCGAC
Stv_Spf488	ACATCGAAAGGAGATGAGTTGTCC	GTATGGTCATTCGTAGCTTACGCC
Stv_Spf526	GAACAAAAACAGGCGTGAATATGG	CGAATGTTGTTGACTCCGTATGAC
Stv_Spf538	TGATAAAGGTGATATGTGCTGGGG	ACTCCACAGCAAGTCAACACACAG
Stv_Spf544	TAAAGCAAATCAAACAATTTGGGC	AGTGTACACAGATATGGAAGGACGC
Stv_Spf552	TCACTCCGTACATCATTTCTCAGC	ATCGCCATTTAACTAACGACCATC
Stv_Spf559	CAAGGTGTAGTTCGGAGGTAAACG	CGAACCAGTTGTCATTCTCCATC
Stv_Spf578	TGCAAAAACATTTCCTCAATAACTG	AGTACGGTTGATTTAAGATCGCTATG
Stv_Spf581	GTTTCCTTAAAAAGATCGCGTGC	GCTTCTTTGTTGAGAGTTGAAGCC
Stv_Spf587	CCATAATCGACACCGATTGCTTAC	CGATTATTGATTAGGAATACGAAGATGG
Stv_Spf606_a	CTTAGGTGGGACCATTTTCTTTGC	AGATACGCTGAAAGCCTAAGGGAC
Stv_Spf606_b	CGTGATGTCGTATATAAGGTCGGG	AAGGAGATACGTGGAGTTGTGACG
Stv_Spf615	ATTCCCCCAGAGACGATGAAGTAG	TACAGTGAAATGGGTTGATGTTGG
Stv_Spf636	TATCGATTCCTCCCACACACCTAC	TGGGTGTATGTCCTCTTTTTGTCC
Stv_Spf653	TTGAGTGTTTGTTCAATTCATGGG	CGCTTCAAACATTCACTCACAAAC
Stv_Spf658	TTAAACACCTCACACACCTTGCTG	AACCACGAATCCTTCCAATACACC
Stv_Spf662	TATCATTATCATGAGTTGTCCCGC	ATTGAACAGTTTACTTCGGACGTG
Stv_Spf664	AGCCAAGTAACATGACGAGTAAGC	TGATAATACAATTAAAACCGGTATTGTG
Stv_Spf670	GGGAGAGGTTTCTAGCTTCTACGG	GAGGAGCCTTGGTTCAATAGTGC
Stv_Spf686	CAAGGACATCCTTCCTTGCAGTAG	ACTCACTCACTCACTCACTCGCTC
Stv_Spf688	CTGCCTAATACTCTGCTTCATCCC	TACTTGAACAAGTGGCAGAGCAAC
Stv_Spf692	TGTGACGTCATCTCACAGTCTCG	GATACCAAAACCGAAGCACACAAC
Stv_Spf717	AGGGTCGTTTTCAGGGTTTTAGAG	CCACGAAGTCTACCACATCTACCC
Stv_Spf728	ACTTCGATTGTGGAACTCTTGACC	ACTGTTAATGCTGAGCAAGAAGCC
Stv_Spf738	AATTGAAAGAATTTCCGTGTGTGG	ACGACAGACCGTGAAAATAACTCC
Stv_Spf743	CGAGCAGAGTGAATATGAGAGGTG	TCCAGCTGTGTCTCCATTAATATACG
Stv_Spf746	TATTTCAGACCGATCTGTCCAGTG	GCTTGCTGTGATTAAGCCGATAAC
Stv_Spf747	ACGAGGATGATGATGAAGAAGGTC	ATTATCAGCATCACGATCGCCTAC
Stv_Spf751	GTAAATAAGTCATTGGTCAAAAGCCC	CATGACGAATCTATAAGGGTTCCG
Stv_Spf752	TGTTCTATTGTCTCTGCCTACCCC	TGTGGGCTCAATTTGCATACATAC
Stv_Spf756	TGCCGATACCTAGAGTTCCGAG	GAATAGGTGTAATCAGATGGTGCG

Continued on following page

Table 3. Continued

Marker	Forward primer 5′ => 3′	Reverse primer 5′ => 3′
Stv_Spf764	TAGGTTCCTCTATTGGGTGCAGTC	CGAGCTATCATCTGCCTCATTACC
Stv_Spf783	TGCCATATTATACTGGGCACAATTC	TGCGTTGTTGGTTTAGTGTTCAAG
Stv_Spf789	CGACACGTTGATTGCTCACAG	AATCTTTTATCACAATTCGCAGCC
Stv_Spf807	CAGACGATAGCATGTGTCGATGTAG	TCGATAATTTCTGCCTCACCAATC
Stv_Spf823	GATCAATGTTCAAACCTTCTCGGT	CTAACGCTCTACGCTTGACGAAAT
Stv_Spf824	CGTGCTCATTAGCCTGTTTAATTTTC	GGGTGTGTCGTGGTCCTATTTTATC
Stv_Spf828	CCATGTAGCGTAACATACAGCACTC	AAACCAATTCAACAGAGCGTCTTC
Stv_Spf835	AAATGTGGAACACCTTTGTCTTGG	CTGCGATAATTACAAATCAACAAAACAC
Stv_Spf858	CTGCAAGGAGAGCACCACTGTC	CAAATGGGACGACATTCGCTAC
Stv_Spf868	TTGTTGTAATTGAAAAGATGGCCG	CCTTCAAAACCAAATTTGAATGTATCC
Stv_Spf869	CGTTCTAATTTATCGCACCATTCAC	TGATCGACGTTTACGAAACTTATGG
Stv_Spf904	TATGTCCTGTCGCCTGTCATACAC	TTCTGAAATGAAATGGATTTTCGG
Stv_Spf908	AGTCATTTACAACAAGCTGGAACG	GCATCTAACTTGAACATTTTCCCC
Stv_Spf914	TCCAAATTCAAATTTCATTCCACA	TAGTGTTTGAGCAGCTCTGCTTGT
Stv_Spf918	GCGAAATTGTTTTAATGTGGGTTG	ACGACCTATACGGACCTTGTTACG
Stv_Spf929	TTATTTACGCGGGAATCGTTTATG	ACTACATAAATTCGCACAATCCCG
Stv_Spf941	GAATCTCGGCGAAACAAGGTTAC	ATTATTGTTTGTTTCATCGTCGCC
Stv_Spf950	ATGATATCGTCTGATGCTGACCAC	ATGAGGGTGATTCGAAAAATCTTG
Stv_Spf967	GTTCGATCGGTTTCGAGTAATGTT	CACTCACACCCATAAAACCATTCA
Stv_Spf975	GCAGTGATGAGAGATTAATGTGCG	TTTTTGTCGGATGTGTGTACGTG
Stv_Spf978	GGACATCTGTGGTCAGGATAGCTC	TGTCTCCCAAGGTTTTAAGTCCAC
Stv_Spf988	TGTTGGGTATTGTGTGTAGTTTTGG	CTGACTAAAAACCAACCACGTTCC
Stv_Spf994	CCCTCTTTAATGAAACGGAGTGC	CTTAGTAACAGCGAGGACGTCAGG
Stv_Spf997	TTGATGCATGAATTTTCAAACGAG	ATCACGTTGTGGTCCAATCAATG
Stv_Spf1000	TCGACGAGAGGTTTGTTTAGTTCA	AAAACCACAACGCAAGTAACATGA
Stv_Spf1036	GTGTTAGTAATCTGTGGCTGTGCG	CTAACAAACACTGAGGAGGCAAGTC
Stv_Spf1050	CGGCGAAGTAGGACATAGAGTGAG	CGACATCAAGCAGCATTAGTTCAG
Stv_Spf1057	TGTAATGTTCCATGTAATGGGAGG	TCTCTGCCTACACTCATAGGCTTG
Stv_Spf1058	AAACTTTCACATGATTTGTTGACG	TCGTTCATACAAAACATAACAACAAAC
Stv_Spf1068	CGACACGTAATGCTGCTATACAATG	CGAAGTATTACGGGGCATTTTTC
Stv_Spf1075	TCTCTGGTGGAAAAGCATCTATAAC	CACAAACCACCAAGTCTTGTACTG
Stv_Spf1079	AATAACATGATTCAAGGCTACGGC	CGTATAGGGGAATAATCTCGCTTG
Stv_Spf1098	CATCTAAATCCGAACCGATGAGAC	GCAATGACAGATGCATTATAAATACACG
Stv_Spf1102	CAAACCTTCTCCGTATGAAAAGAG	AAAAGACATAAAACTAACTGTAGCACCC
Stv_Spf1106	CGAAACAAGTGAATCTGTCAGTGC	ATCATCACCATCATCATCACCATC
Stv_Spf1120	AACAACAAGTGAGTATTTTCACACAGTC	CAATAACTTCTTTGAAGGTGCTGC
Stv_Spf1125	GGCTCTTGGGGGTTATGTAAGAAG	GGTTCCAAACTACCACGGATAATG
Stv_Spf1128	ATACATAACCTCACGCCTGTCTCC	TTCTTGTCGTAGTAGTAGCGCAGC
Stv_Spf1134	GTGGTTGGAGACTGTACCGGAG	CTTGTCTCAGTCTTGGGACTCCAC
Stv_Spf1136	ATTACCTCGCACTCAATTAGCCAC	ATAATCACACACCGCCTCAGTACC
Stv_Spf1147	AAACTAATAAACAGAGTTGCTCCATCC	CTCTTGGCCGGATAAAAGGTGTAAC
Stv_Spf1170	AATTGCCGGAATTAGACACTTCTG	AACCGTTCCGGTAAAGTATATGGG
Stv_Spf1171	TTGAACGGGTACGTATTACCAACA	ATAATGGTGACGTGCCATTGAAG
Stv_Spf1176	TTATACCGATATTCCGGTCCAGTC	ACAGCAATATATCTTATCTACGAGCCTG
Stv_Spf1195	GTCGCCATTATTATGGACCTGAAC	GATTTGACTTTGACTTTGAAGGGC
Stv_Spf1221	TTAGTCCAGTGAACAACTGAGGGAG	CGACGATGAACCTGAAATGATTC
Stv_Spf1230	TACAAGGTTCCGGCTTGAAAAATA	CCGTCGCTAGTGATAGAGCAAAGT
Stv_Spf1231	TCAACCGACCAATAGTGATGACAC	AGTTACAAGATTTCACGTCACACACC
Stv_Spf1243	GCTTCATCAAGACGGGAGACAG	GACGTTTATTCGGTTATGGGACAG
Stv_Spf1245	TCTTGGCTTAGATGTGGATTTAGG	TCTGTCATGATACCTTTATCAGTTTTTG
Stv_Spf1260	CACGATCTCCACATGGAACTAGG	ATTGTTGTAAACATCGGCTATCCC
Stv_Spf1264	AAGTTGAGGTCTTTTGGTGACGAG	ATTTTCGGAAGCAATTACTAGGGG
Stv_Spf1268	ATTGACCAGACCTTGCTAAAATGG	GCTGCTCGGTTGTATATTTGTGTG
Stv_Spf1279	AAGCAAATAAACACGTTCACAAGC	CGCTCTCAAAGTTAAGAAATGTATGTG
Stv_Spf1304	TTCAGTAATGGGATGGTTACAGGC	ATTGATGGGCAAATTTGAGAGAAC
Stv_Spf1315	AGATGTGGAGGTGTTAGTGTGTGC	ACCTCACCCATACCAAATCGAAC
Stv_Spf1334	TTCTGCTTGGAATTCATACAGTAACAC	TTTTACATCCCTCAACGTCTCACTC
Stv_Spf1349	TTAATCAAGATTTCCTGCCACACC	GGCTTTTGCTTAACAAACAGAGAGG
Stv_Spf1363	TACCCGGTAGTCGCTTACCCTAAC	ATCGTATTGTGCAGATGGTTTGTG
Stv_Spf1382	TACAGGAAGGTTCATAACTGTGGC	GGTCCGACCTACGTACACTCAAC
Stv_Spf1387	CAGTGGACTGCTATAGGCTGATGA	CAAGTTTACCGATAACCAAAGCCA
Stv_Spf1396	CATGCATGTCTCTTTGCTGTATG	GCAATCTTCTCGTTCAATCAATG
Stv_Spf1401	GTTGCAAGATTCCACTTCACACAC	CGAAAATGTCACTTAATGCACCAG
Stv_Spf1406	TTCATGCAGATTGACGAAAATGAC	TTACGGCCTAAGCCAAGTAAAG
Stv_Spf1409	TAATGACGCGTAAATTATTCGCAC	TAATCCCATTCAACGTCACTCATC
Stv_Spf1419	CCTCAATATAACTCCCGAAGACCAC	TTTCCGATATTTCGGCACTGTTAG
Stv_Spf1432	CCAAATAATTGCACAATACCTGCAC	TCGATAGTTGCTCACATTTGAAGG
Stv_Spf1435	GGGTCTTCACTTCACAAGCACATAC	TGTCAGTTTAACTTTAATTCGTCGGC

Continued on following page

Table 3. Continued

Marker	Forward primer 5′ => 3′	Reverse primer 5′ => 3′
Stv_Spf1447	TGTAAAGAGGTCAATTGGCATGAG	ACTGCACTGAACACGGTGATTAAC
Stv_Spf1460	ACAGTGACTGGGATGAAGAGCTG	AGGCTTATTATACTACGTGCTCACCAG
Stv_Spf1461	CCGGGTCTGTCAAAGTATTACTGG	TTGCCATATACTGGACACAATTCC
Stv_Spf1464	AGCTATAAACACAGGCTCCTCGTC	TAAGAATCAAGCATACAATTGGGG
Stv_Spf1466	GTTTCACGTGCCCCAACTACATAC	AGTACGTTTCTGTACCAACGGCTC
Stv_Spf1471	TCAGTAGTGGTCACTGTTGAAGTGG	TCCCGGGATTAGAAATATTTACCG
Stv_Spf1473	GTTTCATTCACTCGCCACACTG	GGCTGCGTAGTTTAAACAATTAAC
Stv_Spf1486	GCGGTAGTACCAAGGTGAGGTTAC	ATAAATGAAACGTTCACAACACCG
Stv_Spf1502	TTTGCAATTTTAGTTACAAACGTCCTC	TATTGATAGCCTCGTGTTTGACCC
Stv_Spf1539	TTGTAGGGGCATGATTATTGAAGG	TTTTTGAGGAGGGAAAACTCGC
Stv_Spf1552_a	CAAGGGGTGGGGAATAATTAAG	CCACACCTTGCTTACTTTCACTTAC
Stv_Spf1552_b	AAGTGAAAGTAAGCAAGGTGTGGG	TTTGCTCACTCACTCATTCATTCTG
Stv_Spf1561	ATATCAAGATGGGCTACAAAACCG	CTTTCCAGTTGGAACGAACTTACG
Stv_Spf1573	CAATAGGAGAAAGGCGTGAATTTG	TCGTTAGAACTAGCCATTTGGAGC
Stv_Spf1576	CACTAATTACCAGTCACCCTATGCG	TAGCTTAGCTGAGTCCGTTTCCAC
Stv_Spf1582	AAATATTTGCTCGGAGGTATATGGC	AAAACCTTGTCTCCCTTCCACTTC
Stv_Spf1587	TCGTACAAAACCTGCTTAAACTTGG	TCGGACGAACTTTGTGTACGTG
Stv_Spf1592	GGTTCCTGTTATCACCTGCAGTA	CTATGTAGTTTATGTTAATTCGCACGAT
Stv_Spf1600	AAAGTTCCGACGGACAACTTGTAG	AATCGATCGTGTTTGTGTTGTCC
Stv_Spf1604	GGAGGTGTGTATTATCATGTGTTTGG	TTTGTTCCGAAAAATTCGTTAATTG
Stv_Spf1634	TGTAACACTTGATGATAATGCGCC	CGATATTCGCTTACTTGGAAGGTG
Stv_Spf1651	ACATGGAATTGTTTGTTTAATGAAAGG	CATTTAAGACAAGAGACGCGCTG
Stv_Spf1673	TGGCTTTTAGTCAGTAAATGGCTG	AGGCACACAATTCCCTTTTTGTAG
Stv_Spf1680	GGTTGGCATTTGAATAATTCTTTTTG	AGTCTGATGACTCTTTGAGCGGAC
Stv_Spf1683	TCATCAGGGAAAGGAGTTGAACAT	AAAGCTAGGTGTTGTGAGACTCGG
Stv_Spf1685	ATAACTGGCTATTTGCCGTATTGC	AAAGACAAGTTCAAAGTTCACCAACAC
Stv_Spf1690	ACTAGGATTTTTGACGGTGTGTCG	CATTCGATAGTTGACTCGCTTTGG
Stv_Spf1698	CGCATGCTGACCCTAAGTCTTTAC	GACTGTAGGCGGTTGATGTGTG
Stv_Spf1706	CCACTGTACTGTGATAAACAGATGGC	ATGATCATACAAAGTGCATCCGTG
Stv_Spf1707	AAAACATCAAACCTATTCAACGCC	CCTTCACGTCATTCAGCAAACTAC
Stv_Spf1712	AACACAAATTACACCAACCAAGCC	TTTTAGCGCCTCGAGATTGTTATC
Stv_Spf1713	TAAAATACTTATTACGCGAAGCCG	AAGTAAGACACGAATGTATCAATTTTCC
Stv_Spf1723	TTTGTTTAGCAATGGACGTCTCTC	ATCGTGTCTGGTGCTGAATAAATG
Stv_Spf1728	TATGTAGGCAAGGTAACCGGACTC	CGTGTACTCGTTTCCCAACCTATC
Stv_Spf1747	TGTAATTTTGTGTTTGTGTTGTGCC	AGGTCTGTCTAGGTAGCGAGCATC
Stv_Spf1758	TCACACCACACCATATCACAACAC	TGTATTTGTAAGTTTGACCGAGTGAG
Stv_Spf1783	CAAGCGTACATCGAGTCAAGGAG	GCAAATTAATCCCGCACTGTTTAC
Stv_Spf1844	CTACCCCTTCGGGGATAAAAAG	TTGAGTCTCTTTGAGTAAAACCGC
Stv_Spf1856	TCATCATGGAGATTACCTGGACTG	AGTCACACAGTCAGCCACAAAAAC
Stv_Spf1860	AATGTTGAGAAGTTCTGCCTACCG	ATCTACCTGCATGCCAAATTTCAG
Stv_Spf1863	AGGGGGTCTTATTAAAGTGGTGGG	ACCATGACACAACGAGGCCTATAC
Stv_Spf1890	CATCTCATTTTTGAAGAGATCCCG	TTCGCGTGGAATAGTGACTTCC

Marker numbers correspond to the contig names of the sequences submitted to GenBank, NCBI. Max alleles/sample, maximal number of alleles observed on individual samples; N_o, total number of alleles observed for that marker; N_e, effective number of alleles; H_E, expected heterozygosity; H_o, observed heterozygosity; Hits, N indicates BLASTn expected values of $1.0 \times E^{-04}$ or lower, and X indicates BLASTx expected values of $1.0 \times E^{-04}$ or lower; asterisk (*), markers used for cluster analysis in Fig. 1.

population were grouped together by the cluster analysis with a maximum genetic distance of 0.56 (dotted line) within colony, and the minimum genetic distance among colonies was 0.83 (Fig. 1, dashed line). Bootstrap coefficients higher than 50% are shown at the nodes in the dendrogram (Fig. 1). High reliability of the clusters was estimated for the clades at the colony level by bootstrap values of 100%.

Shared Alleles Among Geographical Locations. For the 120 microsatellite markers that amplified across samples resulting in 930 alleles detected, we summarized the number of alleles present in at least one individual of each geographic area and represented the results in Fig. 2. Despite the limited number of samples analyzed, ≈100 more alleles were found in the colonies from TX than in PR and MS, and the percentage of alleles of TX that also were present in PR and MS was the lowest (between 34 and 39%). Only 14% (127) of the alleles were present in at least one individual of each geographical area.

BLAST Results. DNA sequences obtained from the microsatellite-enriched libraries of *S. frugiperda* were masked for repeats and screened using BLASTx and BLASTn and the results are shown in Supp. Table S1 [online only]. Markers for which their corresponding sequences had significant hits were indicated in Table 3. Markers for which sequences had expected values of $1.0 \times E^{-04}$ to $1.0 \times E^{-38}$ for BLASTn and $1.0 \times E^{-04}$ to $1.0 \times E^{-144}$ for BLASTx are shown in Table 3, using a letter N for significant BLASTn hits and a letter X for significant BLASTx hits.

Discussion

Microsatellites for *S. frugiperda*. Microsatellites are desirable over many other molecular markers for being highly polymorphic, highly abundant, codominantly inherited, simple to analyze, and readily transferable (Weber 1990). The microsatellites presented here are not only sequence specific for *S. frugiperda*

Table 4. List of markers with UPIC scores different from zero, calculated for *S. frugiperda* microsatellite markers using the UPIC software (Arias et al. 2009)

Marker	UPIC Score	Marker	UPIC Score	Marker	UPIC Score	Marker	UPIC Score
Stv_Spf01128	15	Stv_Spf01747	9	Stv_Spf01231	7	Stv_Spf01079	4
Stv_Spf00670	13	Stv_Spf01409	9	Stv_Spf00147	7	Stv_Spf00728	4
Stv_Spf00997	13	Stv_Spf01401	9	Stv_Spf00301	7	Stv_Spf01268	4
Stv_Spf00789	13	Stv_Spf00692	9	Stv_Spf00858	7	Stv_Spf01279	4
Stv_Spf01592	13	Stv_Spf00751	9	Stv_Spf01382	7	Stv_Spf00187	4
Stv_Spf01706	13	Stv_Spf00994	9	Stv_Spf01195	7	Stv_Spf01576	3
Stv_Spf00918	13	Stv_Spf00103	9	Stv_Spf01683	7	Stv_Spf01106	3
Stv_Spf00343	11	Stv_Spf00578	9	Stv_Spf00452	6	Stv_Spf00747	3
Stv_Spf00658	11	Stv_Spf00240	9	Stv_Spf01102	6	Stv_Spf00462	3
Stv_Spf00544	11	Stv_Spf00606_b	9	Stv_Spf01334	6	Stv_Spf01260	2
Stv_Spf01486	11	Stv_Spf01419	8	Stv_Spf00908	6	Stv_Spf00606_a	2
Stv_Spf01856	11	Stv_Spf00746	8	Stv_Spf00752	5	Stv_Spf00212	2
Stv_Spf01502	11	Stv_Spf01432	8	Stv_Spf00173	5	Stv_Spf00835	2
Stv_Spf00552	11	Stv_Spf01651	8	Stv_Spf01447	5	Stv_Spf00653	1
Stv_Spf01587	10	Stv_Spf01698	8	Stv_Spf00764	5	Stv_Spf00255	1
Stv_Spf01844	10	Stv_Spf01707	7	Stv_Spf00120	5	Stv_Spf00305	1
Stv_Spf00406	10	Stv_Spf00292	7	Stv_Spf01396	5	Stv_Spf01890	1
Stv_Spf01685	9	Stv_Spf01783	7	Stv_Spf01758	5	Stv_Spf00467	1
Stv_Spf00914	9	Stv_Spf01723	7	Stv_Spf00581	5	Stv_Spf00150	1
Stv_Spf01264	9	Stv_Spf00417	7	Stv_Spf00538	4	Stv_Spf01245	1

but also were designed using stringent conditions regarding runs, overlapping with repeats, 3′GC clamp and a uniform high melting temperature (63 ± 1°C) to minimize the need of PCR optimization. Until now, microsatellites have not been reported for *S. frugiperda*, and there are only eight microsatellites reported for a related species, *Spodoptera exempta* (Walker) (Ibrahim et al. 2004). In the present work we have contributed to the NCBI database with 187 nucleotide sequences of *S. frugiperda* microsatellites from where the markers were isolated. In total, 125 of these microsatellite markers amplified across the samples and 103 of those markers presented no >2 alleles per sample, making them ideal for population studies.

UPIC Scores. The discriminating power of the markers was evaluated by calculating the UPIC scores. UPIC score of a marker represents the number of DNA samples that can be individually discriminated from the rest of the samples by that particular marker, i.e., a UPIC value of 2 indicates that two samples have unique allele patterns for that marker and can be

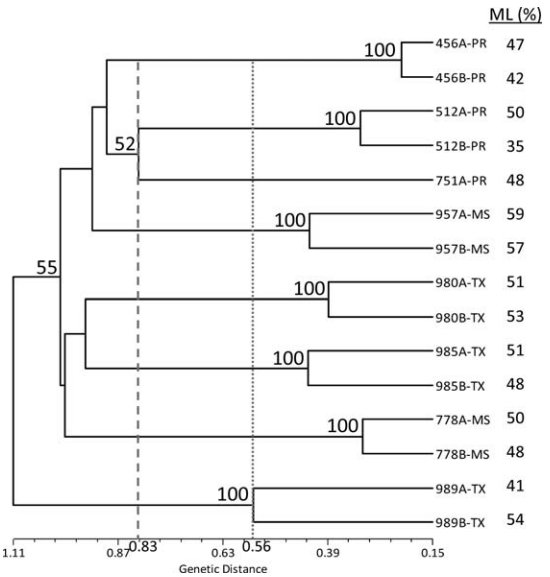

Fig. 1. Cluster analysis of 15 individuals of eight *S. frugiperda* colonies by using 120 microsatellite markers by the unweighted paired group method and arithmetic averages (unweighted pair-group method with arithmetic average). Genetic distances were calculated with Nei's coefficient, and cluster analysis by the algorithm implemented in the SAHN program of NTSYSpc version 2.2. Bootstrap coefficients higher than 50% after 5,000 resampling are shown at the nodes. ML (percentages), multiallelic loci in percentage, represents the percentage of loci that showed multiple amplicons.

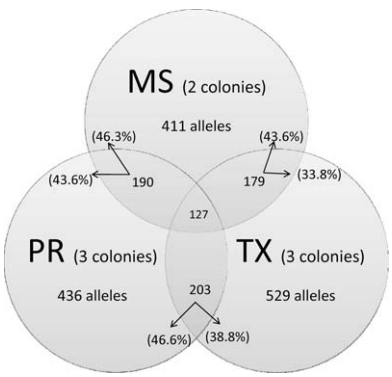

Fig. 2. Alleles shared by *S. frugiperda* individuals from three geographic areas, Puerto Rico (PR), Texas (TX), and Mississippi (MS), in the analysis of 120 *S. frugiperda* specific microsatellite markers. The Ben diagram shows the total number of alleles observed in individuals of each region. Numbers on the overlapping areas of the diagram correspond to the number of alleles observed in at least one individual of each of the geographic regions. Percentages were calculated based on the number of alleles shared and the total number of alleles observed in each group of samples.

distinguished from each other and from the rest of the samples (Arias et al. 2009). UPIC values listed here can be used to plan future experiments, allowing the selection of the most powerful discriminant SSR markers, which are those with the highest UPIC score, and thus reducing the cost of the analyses. For example, the top markers with the highest UPIC scores (Table 4) will provide the maximum discrimination of the genetic variation within and among *S. frugiperda* populations.

Isoline Identification. Two *S. frugiperda* biotypes have been identified so far, the rice and maize biotypes (Pashley et al. 1985, Pashley 1986). Though these biotypes present no morphological differences, they are effectively distinguished by molecular markers such as the Tpi gene (Nagoshi 2010), FR (Lu et al. 1994), the COI gene (Pashley 1989), and AFLPs (Prowell et al. 2004). However, there is evidence of interstrain matings (Nagoshi and Meagher 2003), and in some cases the molecular markers available did not distinguish genetic differences among populations from distant geographical areas, such as Brazil and North America (Machado et al. 2008). Although we do not attempt to make any phylogenetic inferences from the cluster analysis, we do point out that each population was discriminated with bootstrap values of 100%. This indicates that these microsatellite markers will significantly contribute to the pool of molecular markers available for *S. frugiperda* as a large number of loci (174) were analyzed in this work. Such a resource can be used to address issues of biotype, migration, and phylogenetics. The microsatellites reported here can distinguish isofamilies 456 and 512 from each other and from the rest of the colonies; 456 and 512 share geographic origin and present resistance to Bt Cry1F and Cry1Ac proteins (Blanco et al. 2010). Further testing comparing susceptible and Bt-resistant strains from the same geographical origin would be necessary to test the use of these markers in resistance monitoring. Thus, the markers provide a useful tool to differentiate isofamilies (Fig. 1).

Allele Overlapping on Geographic Regions. One advantage of the availability of a large number of molecular markers is, that while studying many individuals at few loci is useful to infer recent population history, a large number of loci are more likely to contain information about older events (Wang and Hey 2010). Using mitochondrial haplotypes, it has been reported that *S. frugiperda* populations of Mississippi and Florida were similar to the haplotype profile found in Texas (Nagoshi et al. 2008). In our cluster analysis, one of the two MS populations analyzed by SSRs did group close to two TX populations, whereas the other MS population grouped with the populations from PR (Fig. 1). The low resolution at the base of the dendrogram indicates that possible crosses may have already taken place between MS and PR populations and between MS and TX populations. In addition, the number of alleles shown in Fig. 2 for individuals of the three geographic regions, PR, TX, and MS, showed that between 39 and 47% of the alleles are shared between geographic regions and only 14% of the alleles were observed in all three regions. The larger number of total alleles in TX and lower percentage of alleles shared with other regions, point to a potentially higher genetic diversity in this region. Having the availability of the markers presented here, it will be feasible to analyze a much larger number of samples from distant areas to better understand the potential migrations and crosses of this insect, and this would influence the management practices.

BLASTn Screening of Marker Sequences. Among the first 125 markers reported in Table 3, several interesting hits were detected using BLASTn. For example, Stv_Spf29 had homology to a gallerimycin, Stv_Spf662 to Serpin-1 alternative splicing isoform, Stv_Spf581 to the ITS 18S ribosomal DNA, Stv_Spf1230 to a chitinase, Stv_Spf301 and Stv_Spf1844 to chitin synthases, Stv_Spf1068 and Stv_Spf1461 to alpha-amylases, Stv_Spf869 to a juvenile hormone, Stv_Spf38 Stv_Spf127 and Stv_Spf1419 to signal transducers, Stv_Spf0747 to a transcription factor, Stv_Spf1230 to a chitinase, Stv_Spf1890 to a nucleopolyhedrovirus mutant, and Stv_Spf417 Stv_Spf746 to cytochromes P450. The potential impact that could result from the genetic polymorphism on the mentioned DNA sequences of *S. frugiperda* is for the most part self-explanatory. We will only add that gallerimycin is an antifungal insect defensin shown to be induced by *Beauveria bassiana* (Bals.-Criv.)Vuill. in *Samia cynthia* (Druri) (Hashimoto et al. 2008), whereas Serpin-1 is a serine-protease inhibitor (Potempa et al. 1994), known to be involved in insect-immune response and to present exon expansion in Lepidoptera (Hegedus et al. 2008). Other sequences with non intuitive function would be those of α-amylases, but these enzymes can be activated up to five times in *S. frugiperda* during larva feeding (Lwalaba et al. 2010).

Marker Stv_Spf746 is very interesting because it had similarity to the antifreeze protein Lu-1 of *Helicoverpa* sp. At this locus (marker Stv_Spf746), whereas all PR samples had the same unique allele, samples from MS and TX had 50 and 100% heterozygosity and a total number of 18 alleles. In the freeze-intolerant moth *Choristoneura fumiferana* (Lederer), the expression of several isoforms of antifreeze proteins serves to this insect as protection for overwintering (Doucet et al. 2002). Considering that in Puerto Rico there are no freezing temperatures and only one allele was detected in samples from that island, the large polymorphism observed at this locus across *S. frugiperda* samples from continental United States suggests a possible biological meaning for the genetic variation observed. *S. frugiperda* is believed to overwinter in the southern U.S. states, becoming source of infestation in northern areas (Nagoshi et al. 2009).

Another interesting marker was Stv_Spf173 that had homology to an EST of antennae tissue of *Spodoptera littoralis* (Boisduval), part of an olfactory and pheromone detection resource, uploaded to NCBI (EZ981883.1) by E. Jacquin (INRA, Versailles, France). The allele distribution of Stv_Spf173 allowed unique identification of each of the eight colonies tested. Because the use of pheromones is essential for pest control (Witzgall et al. 2010), and the

evolution of pheromones in Lepidoptera has an important role in insect speciation (Roelofs and Rooney 2003), the genetic variability at this locus makes us wonder how much more we need to know about the genetics of *S. frugiperda* to develop effective methods of control. Probably, the first target of future work will be to find out whether an association exists between these last two markers Stv_Spf173 and Stv_Spf746, and either cold tolerance or colony recognition in *S. frugiperda.*

BLASTx Screening of Marker Sequences. Also, among the first 125 markers shown in Table 3, significant similarity (E value $\leq 1 \times E^{-04}$) existed between several sequences and protein databases at NCBI. For example, Stv_Spf255 had similarity to a cadherin, Stv_Spf292 to an antennal esterase, Stv_Spf1221 to a calnexin, Stv_Spf686 to the stomato-gastric nerve plexus protein of *Apis mellifera* (L.), Stv_Spf743 to a peroxisomal biogenesis house-keeping protein, Stv_Spf1245 to an x-ray repair protein, and Stv_Spf69 Stv_Spf122 and Stv_Spf1582 to reverse transcriptases. Although the potential effect of genetic variation on these proteins might be self-explanatory, we want to indicate that cadherins are transmembrane proteins known as receptors of the *B. thuringiensis* toxins in Lepidoptera (Vadlamudi et al. 1993, Vadlamudi et al. 1995), and calnexin is a molecular chaperone that participates in potassium channels (Higgins et al. 2003).

Markers With Potentially "Null" Alleles. The second part of Table 3 provides a list of 51 markers that had no amplification in three or more of the samples, or potentially null alleles. In this group, some sequences had significant hits on BLASTx. Among these markers, Stv_Spf688 and Stv_Spf1243 had similarity to a dynactin of *A. mellifera* and to a pyruvate kinase, respectively. Dynactin in *A. mellifera* has shown different degrees of methylation in workers than in queens and is a possible link to environmental response and plasticity (Moczek and Snell-Rood 2008). In marker Stv_Spf688, we observed apparent "null" alleles in all samples from PR. As for pyruvate kinase, this enzyme in *Tenebrio molitor* (L.) varies in expression depending on the developmental stage of the insect and in response to stress, including application of insecticide (Papadopoulos et al. 2005). In addition, markers Stv_Spf74 and Stv_Spf1713 had similarity to reverse transcriptases. Also, within this 51-marker group in Table 3, some had significant hits on BLASTn. The most interesting markers were Stv_Spf1230, with similarity to a larval serum protein in *Bombyx mori* (L.) and Stv_Spf1363, with similarity to a *Spodoptera litura* (F.) nucleopolyhedrovirus (II). Although the markers in this group would not be suitable for direct fingerprinting, it would be interesting to confirm the presence of null alleles and to determine the genetic changes that cause them.

The large number of microsatellite markers for *S. frugiperda* presented in the present work provides a first insight into biological processes that could potentially be affected by genetic variations. Though this work is a preliminary screening of markers, these could become an effective tool for population studies by using multiple loci to help better understand migrations and possible crosses of this insect as well as assist in breeding programs and management practices.

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References Cited

Adamczyk, J. J., Jr., J. W. Holloway, B. R. Leonard, and J. B. Graves. 1997. Susceptibility of fall armyworm collected from different plant hosts to selected insecticides and transgenic Bt cotton. J. Cotton Sci. 1: 21–28.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Arias, R. S., L. E. Ballard, and B. E. Scheffler. 2009. UPIC: Perl scripts to determine the number of SSR markers to run. Bioinformation 3: 353–361.

Blanco, R., M. Portilla, C. A. Abel, H. Winters, R. D. Ford, and D. Streett. 2009. Soybean flower and wheat germ proportions in artificial diet and their effect on the growth rates of the tobacco budworm, *Heliothis virescens.* J. Insect Sci. 9: 59 (insectscience.org/9.59).

Blanco, C. A., M. Portilla, J. L. Jurat-Fuentes, J. F. Sanchez, D. Viteri, P. Vega-Aquino, A. P. Teran-Vargas, A. Azuara-Dominguez, J. D. Lopez, R. S. Arias, et al. 2010. Susceptibility of isofamilies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1Ac and Cry1Fa proteins of *Bacillus thuringiensis.* Southwest. Entomol. 35: 409–415.

Brownstein, M. J., J. D. Carpten, and J. R. Smith. 1996. Modulation of non-templated nucleotide addition by tag DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 20: 1004–1006, 1008–1010.

Chilcutt, C. F., G. N. Odvody, J. C. Correa, and J. Remmers. 2007. Effects of *Bacillus thuringiensis* transgenic corn on corn earworm and fall armyworm (Lepidoptera: Noctuidae) densities. J. Econ. Entomol. 100: 327–334.

de Souza, J., S. Jain, C. de Oliveira, C. Ayres, and W. Lucena. 2009. Toxicity of a *Bacillus thuringiensis israelensis*-like strain against *Spodoptera frugiperda.* Biocontrol 54: 467–473.

Doucet, D., M. G. Tyshenko, P. L. Davies, and V. K. Walker. 2002. A family of expressed antifreeze protein genes from the moth, *Choristoneura fumiferana.* Eur. J. Biochem. 269: 38–46.

Efron, B., E. Halloran, and S. Holmes. 1996. Bootstrap confidence levels for phylogenetic trees. Proc. Natl. Acad. Sci. U.S.A. 93: 13429–13434.

Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. Nat. Rev. Genet. 5: 435–445.

Felsenstein, J. 1985. Confidence-limits on phylogenies—an approach using the bootstrap. Evolution 39: 783–791.

Glenn, T. C., and N. A. Schable. 2005. Isolating microsatellite DNA loci. Methods Enzymol. 395: 202–222.

Hashimoto, K., Y. Yamano, and I. Morishima. 2008. Cloning and expression of a gene encoding gallerimycin, a cysteine-rich antifungal peptide, from eri-silkworm, *Samia cynthia ricini.* Comp. Biochem. Physiol. B Biochem. Mol. Biol. 150: 229–232.

Hayden, M. J., G. Good, and P. J. Sharp. 2002. Sequence tagged microsatellite profiling (STMP): improved isolation of DNA sequence flanking target SSRs. Nucleic Acids Res. 30: e129.

Hegedus, D. D., M. Erlandson, D. Baldwin, X. W. Hou, and M. Chamankhah. 2008. Differential expansion and evolution of the exon family encoding the Serpin-1 reactive centre loop has resulted in divergent serpin repertoires among the Lepidoptera. Gene 418: 15–21.

Higgins, M. K., M. Demir, and C. G. Tate. 2003. Calnexin coexpression and the use of weaker promoters increase the expression of correctly assembled Shaker potassium channel in insect cells. Biochim. Biophys. Acta Biomembr. 1610: 124–132.

Hruska, A. J., and S. M. Gladstone. 1988. Effect of period and level of infestation of the fall armyworm *Spodoptera frugiperda* on irrigated maize yield. Fla. Entomol. 71: 249–254.

Ibrahim, K. M., Y. Yosuf, and E. Amna. 2004. Polymerase chain reaction primers for polymorphic microsatellite loci in the African armyworm, *Spodoptera exempta* (Lepidoptera: Noctuidae). Mol. Ecol. Notes 4: 653–655.

Index-Mundi. 2009. Corn production by country. (http://www.indexmundi.com/agriculture/?commodity=corn&graph=production).

Jenkins, J. N. 1999. Transgenic plants expressing toxins from *Bacillus thuringiensis*, pp. 211–232. *In* F. R. Hall and J. J. Menn (eds.), Methods in biotechnology. Humana Press, Totowa, NJ.

Johnson, S. J. 1987. Migration and life history strategy of the fall armyworm, *Spodoptera frugiperda* in the Western Hemisphere. Insect Sci. Appl. 8: 543–549.

Kijas, J. M., J. C. Fowler, C. A. Garbett, and M. R. Thomas. 1994. Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. Biotechniques 16: 656–660.

Lu, Y. J., G. D. Kochert, D. J. Isenhour, and M. J. Adang. 1994. Molecular characterization of a strain-specific repeated DNA sequence in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insect Sci. Appl. 3: 123–130.

Lwalaba, D., S. Weidlich, K. H. Hoffmann, and J. Woodring. 2010. Exogenous and endogenous protease inhibitors in the gut of the fall armyworm larvae, *Spodoptera frugiperda.* Arch. Insect Biochem. Physiol. 74: 114–126.

Machado, V., M. Wunder, V. D. Baldissera, J. V. Oliveira, L. M. Fiuza, and R. N. Nagoshi. 2008. Molecular characterization of host strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in southern Brazil. Ann. Entomol. Soc. Am. 101: 619–626.

Marenco, R. J., R. E. Foster, and C. A. Sanchez. 1992. Sweet corn response to fall armyworm (Lepidoptera: Noctuidae) damage during vegetative growth. J. Econ. Entomol. 85: 1285–1292.

Martinelli, S., R. M. Barata, M. I. Zucchi, M. C. Silva-Filho, and C. Omoto. 2006. Molecular variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations associated to maize and cotton crops in Brazil. J. Econ. Entomol. 99: 519–526.

Moczek, A. P., and E. C. Snell-Rood. 2008. The basis of bee-ing different: the role of gene silencing in plasticity. Evol. Dev. 10: 511–513.

Nagoshi, R. N. 2010. The fall armyworm triose phosphate isomerase (Tpi) gene as a marker of strain identity and interstrain mating. Ann. Entomol. Soc. Am. 103: 283–292.

Nagoshi, R. N., and R. L. Meagher. 2003. FR tandem-repeat sequence in fall armyworm (Lepidoptera: Noctuidae) host strains. Ann. Entomol. Soc. Am. 96: 329–335.

Nagoshi, R. N., and R. L. Meagher. 2008. Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. Fla. Entomol. 91: 546–554.

Nagoshi, R. N., and R. L. Meagher. 2009. Behavior and distribution of the two fall armyworm host strains in Florida. Fla. Entomol. 87: 440–449.

Nagoshi, R. N., R. L. Meagher, K. Flanders, J. Gore, R. Jackson, J. Lopez, J. S. Armstrong, G. D. Buntin, C. Sansone, and B. R. Leonard. 2008. Using haplotypes to monitor the migration of fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Texas and Florida. J. Econ. Entomol. 101: 742–749.

Nagoshi, R. N., F. Fleischer, and R. L. Meagher. 2009. Texas is the overwintering source of fall armyworm in central Pennsylvania: implications for migrati'on into the northeastern United States. Environ. Entomol. 38: 1546–1554.

Nagoshi, R. N., R. L. Meagher, and D. A. Jenkins. 2010. Puerto Rico fall armyworm has only limited interactions with those from Brazil or Texas but could have substantial exchanges with Florida populations. J. Econ. Entomol. 103: 360–367.

Oliveira, E. J., J. G. Pádua, M. I. Zucchi, R. Vencovsky, and M.L.C. Vieira. 2006. Origin, evolution and genome distribution of microsatellites. Genet. Mol. Biol. 29: 294–307.

Papadopoulos, A. I., A. Anagnostis, and D. Lazarou. 2005. Effect of insecticide injection on pyruvate kinase of the insect *Tenebrio molitor* (Coleopteran). Pestic. Biochem. Physiol. 82: 115–124.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera, Noctuidae)—a sibling species complex. Ann. Entomol. Soc. Am. 79: 898–904.

Pashley, D. P. 1989. Host-associated differentiation in armyworms (Lepidoptera: Noctuidae): an allozymic and mitochondrial DNA perspective, pp. 103–114. *In* H. D. Loxdale and J. D. Hollander (eds.), Electrophoretic studies on agricultural pests. Systematics Association, Oxford, United Kingdom.

Pashley, D. P., S. J. Johnson, and A. N. Sparks. 1985. Genetic population-structure of migratory moths—the fall armyworm (Lepidoptera, Noctuidae). Ann. Entomol. Soc. Am. 78: 756–762.

Passoa, S. 1991. Color identification of economically important *Spodoptera* larvae in Honduras (Lepidoptera: Noctuidae). (Online: July 10, 2010; http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1413&context=insectamundi).

Polanczyk, R. A., R.F.P. da Silva, and L. M. Fiuza. 2000. Effectiveness of *Bacillus thuringiensis* strain against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Braz. J. Microbiol. 31: 165–167.

Potempa, J., E. Korzus, and J. Travis. 1994. The serpin superfamily of proteinase-inhibitors, structure, function, and regulation. J. Biol. Chem. 269: 15957–15960.

Prowell, D. P., M. McMichael, and J. F. Silvain. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 97: 1034–1044.

Richard, G. F., A. Kerrest, and B. Dujon. 2008. Comparative genomics and molecular dynamics of DNA repeats in eukaryotes. Microbiol. Mol. Biol. Rev. 72: 686–727.

Roelofs, W. L., and A. P. Rooney. 2003. Molecular genetics and evolution of pheromone biosynthesis in Lepidoptera. Proc. Natl. Acad. Sci. U.S.A. 100: 9179–9184.

Rozen, S., and H. Skaletsky. 2000. Bioinformatics methods and protocols in the series methods in molecular biology, pp. 365–386. Humana Press, Totowa, NJ.

Sharopova, N., M. D. McMullen, L. Schultz, S. Schroeder, H. Sanchez-Villeda, J. Gardiner, D. Bergstrom, K. Houchins, S. Melia-Hancock, T. Musket, et al. 2002. Development and mapping of SSR markers for maize. Plant Mol. Biol. 48: 463–481.

Sparks, A. N. 1986. Fall armyworm (Lepidoptera, Noctuidae)—potential for areawide management. Fla. Entomol. 69: 603–614.

Tabashnik, B. E., J.B.J. Van Rensburg, and Y. Carrière. 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. J. Econ. Entomol. 102: 2011–2025.

Techen, N., R. S. Arias, N. C. Glynn, Z. Pan, I. Khan, and B. E. Scheffler. 2010. Optimized construction of microsatellite-enriched libraries. Mol. Ecol. Res. 10: 508–515.

Vadlamudi, R. K., T. H. Ji, and L. A. Bulla. 1993. A specific binding-protein from *Manduca sexta* for the insecticidal toxin of *Bacillus thuringiensis* subsp.. Berliner. J. Biol. Chem. 268: 12334–12340.

Vadlamudi, R. K., E. Weber, I. H. Ji, T. H. Ji, and L. A. Bulla. 1995. Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis.* J. Biol. Chem. 270: 5490–5494.

Velez-Arango, A. M., R.E.I. Arango, D. M. Villanueva, E. G. Aguilera, and C.I.B. Saldamando. 2008. Indentificacion de biotipos de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) mediante marcadores mitocondriales y nucleares. Rev. Colomb. Entomol. 34: 145–150.

Wang, Y., and J. Hey. 2010. Estimating divergence parameters with small samples from a large number of loci. Genetics 184: 363–379.

Weber, J. L. 1990. Informativeness of human (Dc-Da)N. (Dg-Dt)N polymorphisms. Genomics 7: 524–530.

Weir, B. S. 1990. Genetic data analysis, pp. 87–125. Sinauer, Sunderland, MA.

Westbrook, J. K., and A. N. Sparks. 1986. The role of atmospheric transport in the economic fall armyworm (Lepidoptera: Noctuidae) infestations in the southeastern United States in 1977. Fla. Entomol. 69: 492–502.

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. J. Chem. Ecol. 36: 80–100.

Young, J. R. 1979. Assessing the movement of fall armyworm (*Spodoptera frugiperda*) using insecticide resistance and wind patterns, pp. 344–351. *In* R. L. Rabb and G. G. Kennedy (eds.), Movement of highly mobile insects: concepts and methodology in research, North Carolina State University, NC.

Zane, L., L. Bargelloni, and T. Patarnello. 2002. Strategies for microsatellite isolation: a review. Mol. Ecol. 11: 1–16.

Zhao, X., and G. Kochert. 1993. Phylogenetic distribution and genetic mapping of a (GGC)n microsatellite from rice (*Oryza sativa* L.). Plant Mol. Biol. 21: 607–614.

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