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ABSTRACT Spatial genetic variability of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), was studied by collecting samples from 31 locations in the United States, Argentina, Panama, and Puerto Rico, and then using amplified fragment length polymorphism to detect genetic variation. Analysis of molecular variance showed significant genetic variation in fall armyworm among all (28%) sample locations and individuals within (71%) sample locations; genetic variation of fall armyworm was minimal between sample locations grouped into regions. The pairwise fixation index (F_{ST}) comparisons showed significant genetic differentiation (0.288) among the 31 locations. However, dendrograms of results from cluster analysis did not provide support of a high level of genetic structuring among regions. The isolation by distance analysis for all sample locations showed the absence of significant correlation between genetic dissimilarity and geographic distance except for fall armyworm samples collected within Argentina. Moreover, the estimate of modest gene flow ($Nm > 1$) may prevent gene fixation within regions. These results indicate that fall armyworm shows little genetic differentiation and high genetic diversity over its range, important information for the development of management strategies and monitoring the development of resistance to management techniques.

KEY WORDS genetic diversity, gene flow, amplified fragment length polymorphism, molecular marker

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a native pest to the subtropical and tropical regions of the Western Hemisphere from the United States, the Caribbean, and Central America to Brazil (Knipling 1980; Pashley et al. 1985; Pashley 1986, 1988). Fall armyworm does not survive conditions of prolonged freezing, and its infestation in the central and northern parts of United States through Canada during spring to fall comes from annual migration of the population that overwinters in southern Florida and Texas (Barfield et al. 1980). This migratory and polyphagous pest is capable of causing substantial production losses in maize, *Zea mays* (L.); sorghum, *Sorghum bicolor* (L.); forage grasses; turfgrasses; rice, *Oryza sativa* (L.); cotton (*Gossypium* spp.); and peanut, *Arachis hypogaea* (L.) (Sparks 1979, Hall 1988).

Studies on genetic diversity and gene flow of fall armyworm populations from the North America and Caribbean regions indicated the existence of two morphologically identical strains that differ in host preference, physiology, behavior, and pesticide susceptibility (Lynch et al. 1983; Pashley 1986, 1988; Pashley et al. 1995, Prowell et al. 2004). One strain was identified as corn (maize) strain that mainly feeds on maize, sorghum, and other large grasses; the other strain was called the rice strain and mainly feeds on rice, Bermuda grass [*Cynodon dactylon* (L.) Pers.], and other small grass species (Pashley 1986). The two strains are morphologically identical and identification is largely dependent on molecular markers (Nagoshi and Meagher 2003). Clark et al. (2007) analyzed the geographic genetic diversity of the corn strain populations from Mexico, the United States, Puerto Rico, Brazil, and Argentina by using amplified frag-

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Table 1. List of FAW collection sites, host plants, life stages and date of collection.

Sample	Country	State/county	Host plant	Life stage	Sample size	Collection date[a]
LA1	USA	Louisiana/St. Joseph	Maize	Larva + pupa	30	7.10.08
LA2	USA	Louisiana/W. Boro	Maize	Larva + pupa	28	7.10.08
TX1	USA	Texas/Hartley	Sorghum	Larva	30	7.31.08
TX2	USA	Texas/Hale county	Sorghum	Larva	25	8.05.08
TX4	USA	Texas/Hale county	Sorghum	Larva	21	8.08.08
NE	USA	Nebraska/Elkhorn	Maize	Larva	34	8.14.08
MS	USA	Mississippi/Washington	Maize	Larva	25	9.25.08
TX3	USA	Texas/Lubbock	Maize	Larva	28	2008
AL	USA	Alabama/Macon	Peanut	Larva	30	9.2.09
IA	USA	Iowa/Johnston	Maize	Larva	30	2009
PAN1	Panama	Panama/Pacora	Maize	Larva	33	6.11.09
PAN2	Panama	Panama/Chepo	Maize	Larva	27	6.24.09
ARG1	Argentina	Alata Gracia	Maize	Larva	32	1.8.09
ARG2	Argentina	Canada de Luque	Maize	Larva	32	1.9.09
ARG3	Argentina	BS,A.S./Tadil.	Maize	Larva	19	1.26.09
ARG4	Argentina	Camilo Aldao	Maize	Larva	32	1.28.09
ARG5	Argentina	BS, AS/La Oria.	Maize	Larva	25	1.22.09
ARG6	Argentina	BS.AS./La Rosa	Maize	Larva	30	1.23.09
ARG7	Argentina	Salto/BS.AS.	Maize	Larva	30	1.23.09
ARG8	Argentina	Chaco/Charata	Maize	Larva	31	1.28.09
ARG9	Argentina	Santa Fe/Fraga	Maize	Larva	30	1.27.09
ARG10	Argentina	Santa Fe/Fraga	Maize	Larva	30	1.27.09
ARG11	Argentina	BS.AS/Guerrico	Maize	Larva	30	1.27.09
ARG12	Argentina	SD Estero/Tanajera	Maize	Larva	30	2.9.09
ARG13	Argentina	Tucumán/Sanda	Maize	Larva	30	2.13.09
ARG14	Argentina	El Azul/ Burrugacu	Maize	Larva	34	2.13.09
ARG15	Argentina	SanLuis/Cuyo	Maize	Larva	22	2.4.09
ARG16	Argentina	SanLuis/Cuyo	Maize	Larva	15	2.4.09
ARG17	Argentina	Cordoba/ Ríos	Maize	Larva	28	2.5.09
PR1	USA	PR/Santa Isabel	Maize	Larva	30	5.17.10
PR2	USA	PR/Isabella	Maize	Larva	28	5.13.10

[a] Collections dates are presented as month.day.year.

ment length polymorphism (AFLP) markers; the results suggested the presence of gene flow between the different geographic areas where fall armyworm was sampled. Martinelli et al. (2007) also found no significant genetic structuring within the *S. frugiperda* sample locations associated with maize and cotton crops in Brazil by using AFLP. However, it is still unclear the extent to which dispersal of fall armyworm in the Western Hemisphere contributes to genetic interaction because other studies have implicated the presence of physiological and behavioral differences between locations of fall armyworm that are consistent with reproductive isolation caused by geographical separation (Young 1979).

Despite the possible benefits that population genetic analysis of the fall armyworm may provide toward understanding their dispersal, monitoring the spread of insecticide resistance, effects of the deployment of transgenic crops, and implementation of area-wide management programs, relatively little information is available in this area. Most research on fall armyworm has concentrated in identifying behavioral and physiological differences between the two host strains; only few studies were done on geographic genetic variability and some limitations in these studies included using limited sample locations, few individuals per sample location or analyzing few molecular markers per individual. The recent discovery of Cry1F resistance in a fall armyworm strain in Puerto Rico (S. Matten, unpublished data; Matten et al. 2008) elevates the need to develop understanding of their genetic diversity to support the monitoring of the potential spread of resistance in transgenic crops. The objective of this study was to evaluate the genetic diversity in samples of the fall armyworm population from the United States, Argentina, Panama, and Puerto Rico by using AFLP.

Materials and Methods

Insect Material Collection. Fall armyworm samples were collected from the United States, Argentina, Panama, and Puerto Rico. We followed a stratified sampling method to get representative fall armyworm samples from the Western Hemisphere, i.e., North America (United States), Caribbean (Puerto Rico), Central America (Panama), and South America (Argentina): sample locations included areas where fall armyworm survive year-round and areas where they only survive the summer growing season (northern and southern extremes). The fall armyworm sample size varied from 15 to 34 individuals; larvae were collected from maize, sorghum, and peanut fields (Table 1). Fall armyworm larvae were received live with diet, preserved in 95% ethyl alcohol, or lyophilized. The samples that were received live were kept in a growth chamber with artificial diet until they reached the fourth larval stadia then fresh frozen in −80°C until DNA extraction. For samples collected in alcohol, the 95% alcohol was changed 2–3 times after collection to

Table 2. List of primers and primer combinations used in AFLP-selective amplification

Primer type	Sequence (5′-3′)	Primer pair
EcoRI IR700-labeled primer	GAC TGC GTA CCA ATT C ACA	E-ACA X M-CAA
EcoRI IR700-labeled primer	GAC TGC GTA CCA ATT C AAC	E-AAC X M-CAA
MseI unlabeled primer	GAT GAG TCC TGA GTA A CAA	E-ACA X M-CAG
MseI unlabeled primer	GAT GAG TCC TGA GTA A CAG	

avoid DNA degradation due to dilution of the alcohol by the water released from the larvae and then stored in a −80°C freezer. Samples that were lyophilized were stored dry on the laboratory bench at room temperature or were place in a −80°C freezer until DNA was extracted (Clark et al. 2009). No difference in quantity or quality of DNA was observed between the three methods, similar to findings by Clark et al. (2009).

DNA Extraction and Quantification. DNA was extracted following the hexadecyltrimethyl-ammonium-bromide (CTAB) method originally developed by Black and Duteau (1997) and modified by Clark (2005). The lyophilized or alcohol preserved larvae were washed and soaked in double-distilled autoclaved water for 10 min before DNA extraction. This enabled the larval tissue to be rehydrated and soften the tissue for removing the gut. Similar washing and dissection procedures were used for samples received at the pupal stage. The larval and pupal tissue was placed in 1.5-ml autoclaved Eppendorf tubes and homogenized manually with a pestle in 250 μl of CTAB.

An additional 250 μl of CTAB buffer and 10 μl of proteinase K (concentration, 200 μg/ml) was added to the homogenate, and then the sample was vortexed at low speed and incubated for 1 h at 65°C. Next, 15 μl of RNase A (concentration, 500 μg/ml) was added to each tube, and the tubes were incubated for 2 h at 37°C to remove the RNA. The homogenate was centrifuged at 14,000 rpm for 5 min at room temperature, and then the supernatant was collected into new tubes and centrifuged at 14,000 rpm for 20 min after adding 500 ml of chloroform:isoamyl alcohol (24: 1). The upper aqueous phase was transferred into a new Eppendorf tube, and the chloroform: isoamyl step was repeated one more time. The DNA was precipitated with 400 μl of chilled (−20°C) isopropanol and incubated overnight at 4°C. The precipitate was centrifuged at 12,000 rpm at 4°C for 30 min, and the isopropanol was decanted off. The DNA pellet was rinsed with 500 μl of 100% chilled ethyl alcohol and centrifuged at 12,000 rpm at 4°C for 5 min. Again, the alcohol was poured off, and the pellet was washed with 500 μl of 70% cold ethanol for 5 min at 12,000 rpm.

The alcohol was poured off, and the pellet was air-dried at room temperature for 30–45 min; 50 μl of 1× TE buffer (10 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA) was added to the pellet and it was stored at −20°C. Quantity and quality of the DNA were estimated using both 1% agarose and a, spectrophotometer (ND-1000 V3.5.1NanoDrop, Wilmington, DE). Once quantified, part of the stock DNA samples was diluted with autoclaved double-distilled water to 20 ng/μl.

A modified polymerase chain reaction (PCR)-AFLP protocol (Vos et al. 1995) was used to assess the genetic variability of fall armyworm samples. The AFLP procedure was completed in three basic steps: 1) DNA template preparation, 2) DNA template preamplification, and 3) selective amplification of the preamplified product.

DNA Template Preparation. The DNA samples were digested with EcoRI and MseI restriction enzymes (New England Biolabs Ltd., Kingston, ON, Canada) following manufacturer's recommendations. Then, oligonucleotide adaptors were ligated to the restriction site of the fragments by adding 5 μl of adapter ligation mixture containing MseI and EcoRI adapters (Integrated DNA Technologies, Inc., Coralville, IA), each 0.5 μl per reaction of 5 pmol/μl T4 DNA ligase (New England Biolabs Ltd.) (0.15 μl per reaction), 10× T4 DNA ligase buffer (New England Biolabs Ltd.) (0.5 μl per reaction), and 3.35 μl of autoclaved double-distilled water. Then, the samples were incubated for 8 h at 25°C. The ligation product was diluted by adding 135 μl of 1× TE buffer to each PCR tube.

DNA Template Preamplification. We mixed 1.25 μl of the ligation product with 12.5 μl of preamplification mix containing preamplification primer mix (1×) (10 μl per reaction) (LI-COR Biosciences, Lincoln, NE), 10× PCR buffer II (1.25 μl per reaction), AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA) (1.25 U per reaction), and 25 mM $MgCl_2$ (0.75 μl per reaction). The mixture was amplified using 20 PCR cycles of 30 s at 94°C, 1 min at 56°C, and 1 min at 72°C. The preamplified product was diluted by mixing 190 μl of double-distilled water with 10 μl of the preamplified product.

Selective Amplification of the Preamplified Product. Two infrared dye (IRD)-labeled EcoRI primers (ACA and AAC) and two unlabeled MseI primers (CAA and CAG) (LI-COR Biosciences) were used in three different combinations (Table 2). A master mix was prepared by mixing 10× PCR buffer II (1.2 μl), AmpliTaq DNA polymerase (0.08 μl; 5 U/μl), 25 mM $MgCl_2$ (0.72 μl), 1.5.0 μl of 1.0 pmol/μl MseI primer, 0.3 μl of 1.0 pmol/μl EcoRI primer, and 4.7 μl of autoclaved double-distilled water. Then, 8.5 μl of the master mix and 2.0 μl of the preamplified product were added into a new PCR tubes. The PCR cycles were set to one cycle for 30 s at 94°C, 30 s at 65°C, 1 min at 72°C; 12 cycles for 30 s at 94°C, 1 min at 72°C; and 23 cycles for 30 s at 94°C, 30 s at 65°C and 1 min at 72°C by using a Gene AMP PCR System 2700 (Applied Biosystems). The reaction was stopped by adding 2.5 μl of blue stop solution (LI-COR Biosciences), denatured at 95°C for 3 min. Samples were stored at −20°C until electrophoresis.

Electrophoresis. Gels were prepared according to manufactures recommendations for a 6.5% polyacryl-

amide gel matrix (LI-COR Biosciences) and polymerized for 2 h. The gel was run for 2.5 h at 45°C and 1,500 V by using a Gene Read IR 4200 DNA sequencer (LI-COR Biosciences) and 1×Tris-borate-EDTA buffer after loading 1 μl of the samples in the middle lanes and 1 μl of IRD-labeled 50–700-bp standard marker (LI-COR Biosciences) on the first and last lanes of the gel as described in Clark (2005). The gel image collected by the camera of the sequencer was opened using the e-Squel SAGA program (LI-COR Biosciences) and saved for latter scoring.

AFLP Gel Scoring (Data Collection). The gel image was imported into the SAGA Generation 2 Software version 3.2 (LI-COR Biosciences) and was calibrated by using the IRD-700 labeled 50–700 bp markers as a reference. Visibility and sharpness of bands were used as criteria for marker selection, and only the most visible and reproducible bands were selected as molecular markers for the genetic analysis. The bands were scored as presence (1) or absence (0). In total, 221 AFLP markers per individual were scored from the three primer combinations and the number of markers scored per primer pair varied from 69 to 80. The size of the fragments scored varied from 58 to 372 bp. Once the gels were scored, the reports were saved in Phylip format.

Data Analysis. The mean coefficient of variation (CV) was calculated after 1,000 bootstrapping replicates using DBOOT version 1.1 (Coelho 2001). This helps to determine the appropriate number of loci required for acceptable precision for genetic studies in determining genetic structure and gene flow. The bootstrapping used the Jaccard similarity coefficient available in the DBOOT program by using the formula $Jac_{ij} = a/(a + b + c)$, where a is the number of cases where a band occurs simultaneously in both individuals, b is number of cases where band occurs only in the *ith* individual, and c is number of cases where band occurs only in the *jth* individual. Once the amount of variation explained by the number of markers used was determined, analysis of molecular variance (AMOVA) was performed to test the genetic variation by using ARLEQUIN version 3.1 (Excoffier et al. 2005). In the AMOVA, the total variation was divided into three nested levels, i.e., among regions, among populations within a region and between individuals within a single population. The fixation index (F_{ST}), an index that measures the genetic distance between populations, was calculated as $F_{ST} = H_T - H_S/H_T$, where H_T and H_S represent the average number of pairwise differences between two individuals sampled from different (between) or the same (within) population, respectively (Nei 1977).

The degree of polymorphism among regions and between sample locations of fall armyworm was assessed using POPGENE version 1.32 (Yeh and Boyle 1997) in unweighted pair-group method with arithmetic average tree by using distance values between samples as an input (Sneath and Sokal 1973). Genetic variation between populations (sample locations) was measured using G_{ST}. G_{ST} is calculated by subtracting heterozygosity of a single location (Hs) from heterozygosity of the total sample locations (H_t) and then by dividing to the heterozygosity of the total sample locations ($G_{ST} = H_t - H_s/H_t$). Gene flow among fall armyworm sample locations (Nm) also was estimated using the corresponding G_{ST} as $Nm = 0.5(1 - G_{ST})/G_{ST}$ (McDermott and McDonald 1993), where N is the number of individuals in a samples, and m is the proportion of those individuals resulting from immigration (Wright 1969). Generally, a value of Nm >1 indicates the presence of significant gene flow between locations (Wolf and Soltis 1992).

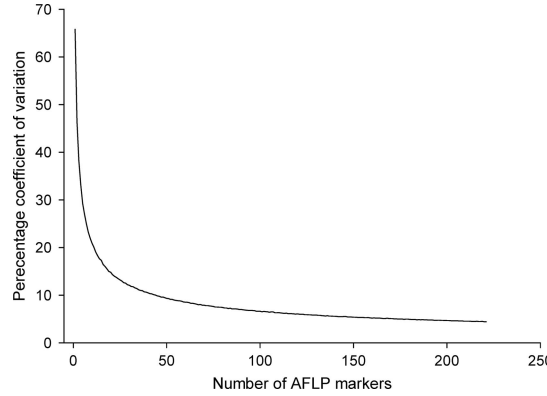

Fig. 1. Coefficient of variation in relation to number of AFLP markers based on analysis of 221 loci in DBOOT with 1,000 iterations for fall armyworm sample locations.

Western bean cutworm, *Striacosta albicosta* (Smith), larvae obtained from Minnesota in the United States were used as an outlier group to test the robustness of the POPGENE analysis. The correlation of geographic distance to genetic distance was estimated using the Mantel test with 1,000 permutations (Mantel 1967) available in ARLEQUIN software. Correlation analysis also was performed using SAS (PROC GLM, SAS Institute 1999); results were compared with those obtained in the Mantel test.

Results

The 221 AFLP markers per individual analyzed in the study explained 95.6% of the variability observed in the fall armyworm populations (Fig. 1), indicating that the number of markers was sufficient to run further analyses. Results from the AMOVA for showed the presence of significant genetic variation among fall armyworm sample locations within a region (28.8%) and between individuals within a location (71.2%; $P = 0.0001$) (Table 3). The F_{ST} comparisons for the 31 fall armyworm locations were also significant ($P < 0.05$). Genetic variability among regions was not significant ($P = 0.1701$). The overall F_{ST} was 0.288, a value that agrees with the AMOVA result in which ≈28% of genetic variability is among locations within a group (region). The average genetic diversity estimate from POPGENE for the entire population also showed that ≈27% of the variation was between fall armyworm locations ($G_{ST} = 0.265$) (Table 3). The Nm value for

Table 3. AMOVA results for fall armyworm samples collected from Argentina, United States, Panama, and Puerto Rico

Source of variation	df	Sum of squares	Variance components	% variation	*P*	G_{ST}	Nm
Among regions	3	1326.957	0.36045 Va	0.79	0.1769		
Among pop/regions	27	10078.499	12.86028 Vb	28.05	0.0001	0.2652	1.3854
Within populations	794	25899.741	32.61932 Vc	71.16	0.0001		
Total	824	37305.198	45.84006	100.00			

Va, variation among regions; Vb, variation among populations within a region; and Vc, variation between individuals within a population.

the entire population as well as pairwise comparisons of regions was >1 (Table 3). The lowest G_{ST} value (0.1992) and higher Nm (2.0100) was recorded among Panama and Puerto Rico fall armyworm sample locations. The number of polymorphic loci using the three primer combinations ranged from 131 (59.3%) to 220 (99.5%) per sample location with average polymorphism of 198.2 (89.7%) loci for the entire population; the lowest and the highest loci polymorphism was recorded from Argentina fall armyworm samples collected from SanLuis/Cuyo (ARG15) and Santa Fe/Fraga (ARG10), respectively (Table 4).

The highest genetic diversity index per (H_S) was recorded from locations TX2 and TX3 (TX for Texas) followed by ARG7 and ARG10 (ARG for Argentina); the lowest H_S value (0.2439) was observed from fall armyworm samples collected from Iowa (Table 4).

Table 4. Genetic diversity estimates from AFLP data in 31 fall armyworm sample locations based on analysis of 221 loci per individual location showing the number of polymorphic loci, percentage of loci polymorphism, and heterozygosity (genetic variation) in a single location (H_S)

Sample location	No. polymorphic loci	% polymorphism	H_S
IA	209	94.57	0.2439
AL	203	91.86	0.3394
TX1	205	92.76	0.3984
TX2	216	97.74	0.4200
TX3	219	99.10	0.4248
TX4	162	73.30	0.2743
MS	191	86.43	0.3434
LA1	143	64.71	0.2641
LA2	199	90.05	0.3352
NE	210	95.02	0.3750
PAN1	212	95.93	0.3292
PAN2	201	90.95	0.3491
ARG1	205	92.76	0.3709
ARG2	217	98.19	0.3325
ARG3	179	81.00	0.3213
ARG4	213	96.38	0.3798
ARG5	202	91.40	0.3627
ARG6	200	90.50	0.3802
ARG7	213	96.38	0.4044
ARG8	191	86.43	0.3175
ARG9	207	93.67	0.3446
ARG10	220	99.55	0.4081
ARG11	182	82.35	0.3360
ARG12	193	87.33	0.3261
ARG13	195	88.24	0.3786
ARG14	196	88.69	0.3460
ARG15	131	59.28	0.2462
ARG16	194	87.78	0.3293
ARG17	216	97.74	0.3637
PR1	203	91.86	0.3751
PR2	217	98.19	0.3995
Entire pop avg	198.2	89.7	0.34901

The dendrogram clustering for the entire population showed no regional clustering of the fall armyworm by sample locations (Fig. 2). Moreover the western bean cutworm samples used as the outlier clearly separated from the fall armyworm population indicating the reliability of the cluster analysis (Fig. 2).

Correlation analysis using the Mantel test with 1,000 iterations for the entire population revealed the absence of significant isolation by distance for the genetic variation in fall armyworm ($r = 0.011014$, $P = 0.06324$) and most of the dissimilarity matrixes lay between 0.2 and 0.3 (Fig. 3). The correlation analysis

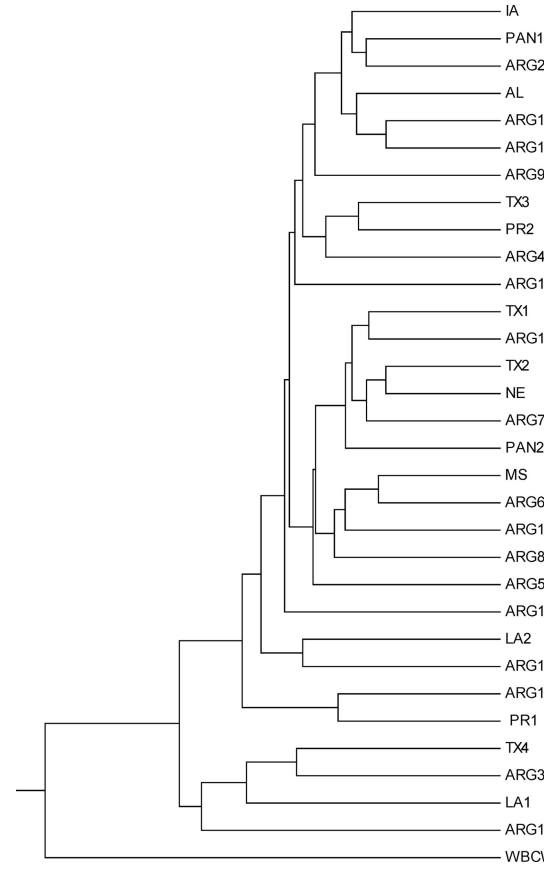

Fig. 2. Dendrogram showing genetic relationships among 31 fall armyworm samples collected from the United States (IA, LA, AL, TX, MS, and NE), Argentina (ARG1–ARG17), Panama (PAN), and Puerto Rico (PR) and an outgroup western bean cutworm (WBCW) population collected from Minnesota.

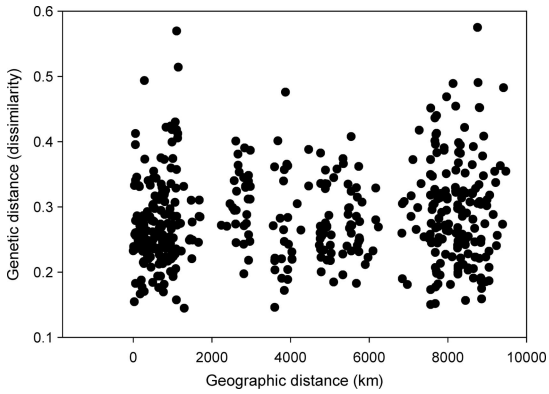

Fig. 3. Correlation between genetic distance (dissimilarity) and geographic distance among fall armyworm samples collected from the United States, Argentina, Panama, and Puerto Rico (USA) (Mantel: $r = 0.011014$, $P = 0.06324$). The black dots represent pairwise genetic distance matrixes between two populations.

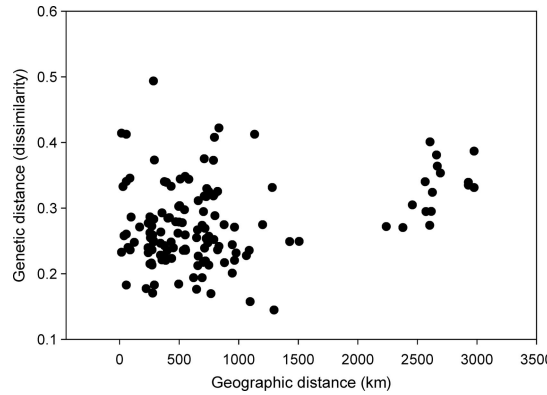

Fig. 4. Correlation between genetic distance (dissimilarity) and geographic distance among fall army worm samples collected from Argentina (Mantel: $r = 0.3322$, $P = 0.0300$). The black dots represent pair-wise genetic distance matrixes between two sample locations.

in SAS also showed a similar trend to that of the Mantel test. According to the Mantel test, the relationship between genetic and geographic distance for the U.S. locations was not significant ($r = 0.098$, $P = 0.2240$). Similarly the SAS results indicated the lack of significant correlation between genetic dissimilarity and geographic distance ($r = 0.23875$, $P = 0.1142$). Moreover, the dissimilarity by geographic distance scatter plot shows random distribution of the matrixes. However, fall armyworm collected from Argentina showed significant isolation by distance ($r = 0.3322$, $P = 0.0300$). The results of the SAS output for data from Argentina also confirmed a significant correlation between genetic dissimilarity and geographic distance ($r = 0.2774$, $P = 0.0011$). The scatter plot graph for data from Argentina also revealed an increasing trend in dissimilarity with increase in isolation distance (Fig. 4).

Discussion

Exploring the geographic genetic structure of a target pest species is necessary before large-scale efforts aimed at control of insect pests (Martinelli et al. 2007) such as the deployment of transgenic crops and the design of resistance management strategies. The increased introduction and adoption of *Bacillus thuringiensis* transgenic maize varieties in the Western Hemisphere that target lepidopteran pests including fall armyworm and the discovery of Cry1F resistant fall armyworm in 2006 in Puerto Rico (S. Matten, unpublished data; Matten et al. 2008) underscores the need to better understand and document the genetic diversity of the species to help in monitoring resistance to transgenic crops (Murúa et al. 2008). Variations in the genetic structure of a pest population in space and time and gene flow among its subpopulations are greatly responsible for the rate of resistance evolution (Fuentes-Contreras et al. 2004).

Although several studies have been conducted on genetic variation in fall armyworm, most were focused on characterizing the corn and rice strains of the pest. Few studies focused on geographic genetic variations and these were based on limited sample size and molecular markers which may not provide enough power to detect genetic variation in fall armyworm throughout its geographic distribution. The first attempt to study geographic genetic variability of fall armyworm (Clark et al. 2007) used seven individuals per sample location; they acknowledged that the sample size was low and further study should be done to confirm their findings. This current study was conducted to further elucidate the geographic genetic variability of the fall armyworm in the Americas by using AFLP, with different primers than in previous studies, with recommended sample size (≈30 individuals) and number of molecular markers (at least 200) (Bonin et al. 2007).

The AFLP principle is based on selective amplification of restriction fragments from a restriction digest of total genomic DNA in which molecular genetic polymorphisms are dominant (Vos et al. 1995). Major advantages of AFLP over other DNA-based molecular markers include its capacity to generate a high number of markers over the entire genome and its reproducibility (Gerber et al. 2000). The nuclear origin of AFLP markers is attractive because markers derived from uniparentally inherited organellar genomes (chloroplast and mitochondrial genomes) might not be sufficiently variable, or even appropriate particularly in organisms where hybridization is important (Meudt and Clarke 2007). Hence, even with being dominant, AFLP fulfills the criteria of a good molecular marker (Gerber et al. 2000).

In a population genetics study, for classical surveys of genetic diversity, population structure, and genetic relatedness, by using low sample size and analyzing few markers may result in a very high sample variance and thus estimates are not reliable. However, sampling above the range does not necessarily increase the power of the analysis (Hollingsworth and Ennos

2004). Although we used 221 AFLP markers per individual (6,630 markers per sample location) that explained 95.6% of the variation, 165 markers were sufficient to detect 95% of the variation. In general, genotyping of 30 individuals and scoring of 200 or more AFLP markers per individual is recommended to get accurate results in population genetics studies (Krauss 2000, Bonin et al. 2007). In this study, using 31 sample locations, 19 samples had ≥30 individuals and 27 had ≥25 individuals; the 221 markers per individual evaluated with these samples were sufficient to obtain reliable genetic diversity estimates of the fall armyworm.

The majority of the total genetic variation in the fall armyworm population was within sample locations followed by variation among sample locations within a region. Variation among regions was not significant, indicating the lack of regional genetic structuring and absence of regional influence on the observed genetic variation among locations. Similar studies in other insect species also showed a higher degree of variation within populations than among populations (Coates and Hellmich 2003, Juan et al. 2004, Timmermans et al. 2005). The pairwise F_{ST} comparisons among all sample locations were significant, suggesting the presence of some genetic structuring among the fall armyworm sample locations used in the study. This indicates that the large sample size used in our study gave us high power to detect genetic variations in fall armyworm population compared with other similar studies with smaller sample size (Clark et al. 2007). Despite the presence of significant genetic structuring among populations, the gene flow (Nm) value for the entire population as well as pair-wise comparison of regions was >1. Generally Nm values of >1 indicate the presence of significant gene flow (Wolf and Soltis 1992) that might prevent distantly located fall armyworm sub-population from large divergence and being genetically fixed, supporting the lack of regional genetic structuring in our analysis. Similar studies in other insect species, as well as that of Clark et al. (2007) for fall armyworm and despite a small sample size, also showed a higher degree of variation within populations than among populations (Coates and Hellmich 2003, Juan et al. 2004, Timmermans et al. 2005). The lack of genetic structuring among regions and higher genetic variations among and within sample locations could be attributed to the highly migratory behavior of the fall armyworm, which can move up to 480 km per generation (Sparks 1986), leading to blending of the genetic variations that may exist among regions and at the same time introducing more genetic variation within subpopulation due to addition of new genes (migrating individuals). Moreover, the high genetic diversity within locations followed by among locations within a region could be due to the fact that although the majority of the samples were collected from maize host plant, there could be strain complexity because both the corn and rice strains of the fall armyworm feed on corn (Nagoshi and Meagher 2004) and the proportion of the two strains in the different locations may vary. Localized selection pressures, such as cropping systems and pest management techniques, might have contributed to the genetic variation among locations within a region of Argentina for this highly mobile species.

The cluster analysis, represented in a dendrogram, also failed to separate sample locations collected from the United States, Panama, Argentina, and Puerto Rico. This further indicates lack of regional genetic structuring and supports AMOVA results in which the majority of the variation was between individuals within a location followed by among locations within a region.

Although the correlation of genetic distance to geographic distance for the entire population was not significant, the Argentina fall armyworm subpopulations showed a statistically significant isolation by distance and genetic structuring. Our findings corroborate a study by Murúa and Baigorí (2004) suggesting that Argentina fall armyworm subpopulations are genetically structured. Similarly, Murúa et al. (2008) observed differences in developmental and reproductive characteristics among Argentinean fall armyworm subpopulations collected from maize. Interestingly, the lowest (59.3%) and the highest (99.6%) loci polymorphism in the present work was recorded from Argentina fall armyworm samples collected from San-Luis/Cuyo (ARG15) and Santa Fe/Fraga (ARG10), respectively; these locations are within the one generation fall armyworm migratory distance (≈500 km). This significant increase in genetic distance for a unit increase in geographic distance in Argentinean fall armyworm subpopulations could be due to the overlapping of the corn and rice cropping systems in most of the sites where the samples were collected. Therefore, samples collected from the maize host plant could be a mixture of both the corn and rice strain individuals, leading to more genetic variation with distance; or perhaps one strain is less genetically diverse than the other using the AFLP primers used here which could give the indication of high genetic differentiation. Another possibility is that there was some interstrain mating in Argentina that confounded our interpretation of the results (Nagoshi et al. 2006). To determine whether the statistical significance of genetic structure in Argentinean subpopulations of fall armyworm is biologically significant will require more fine-scale sampling and analyses.

Our findings are important for the process of developing management strategies of the pest including areawide management programs, deployment of transgenic crops, and resistance monitoring based on genetic structuring and movement of the species. For example, the Cry1F resistant fall armyworm strain which was observed in 2006 in Puerto Rico (S. Matten, unpublished data; Matten et al. 2008) also is reported from samples collected from Florida (Velez et al. 2010). This could be the cumulative effect of gradual northward gene flow (migration) from Puerto Rico to Florida. In addition to geographic variation, determining whether there is temporal genetic variability of a pest species is important to develop appropriate pest management strategies. Hence, future genetic varia-

tion studies in fall armyworm also should consider the temporal genetic variation of the pest within and between regions.

In summary, the current study showed the presence of statistically significant genetic structuring among fall armyworm samples from Argentina that warrants further study. But there was no regional influence on the observed genetic variation among all sample locations from the United States, Puerto Rico, Panama, and Argentina. Furthermore, this study suggests the presence of high genetic diversity and modest gene flow among fall armyworm locations in the United States, Puerto Rico, Panama, and Argentina.

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