Using Haplotypes to Monitor the Migration of Fall Armyworm (Lepidoptera: Noctuidae) Corn-Strain Populations from Texas and Florida

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ABSTRACT Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), infestations in most of North America north of Mexico arise from annual migrations of populations that overwinter in southern Texas and Florida. A comparison of the cytochrome oxidase I haplotype profiles within the fall armyworm corn-strain, the subgroup that preferentially infests corn (*Zea mays* L.) and sorghum (*Sorghum vulgare* Pers.), identified significant differences in the proportions of certain haplotypes between the Texas and Florida populations. These proportional differences were preserved as the populations migrated, providing a molecular metric by which the source of a migrant population could be identified. The migratory pattern derived from this method for several southeastern states was shown to be consistent with predictions based on analysis of historical agricultural and fall armyworm infestation data. These results demonstrate the utility of haplotype proportions to monitor fall armyworm migration, and they also introduce a potential method to predict the severity of cotton crop infestations in the short term.

KEY WORDS fall armyworm, migration, haplotype, *Spodoptera frugiperda*

Many lepidopteran species are capable of moving long distances within a single generation (for review, see Gatehouse 1997). Such behavior is a characteristic of several important economic pests, including the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), whose annual migration from sites in Florida and Texas accounts for infestations in central and eastern North America (Luginbill 1928). A detailed description of the pattern of these movements would contribute greatly toward efforts to predict the timing and severity of infestations in areas that are the targets of migration.

Fall armyworm is responsible for substantial economic damage to sweet corn (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), and several perennial grass varieties (Sparks 1979, Pashley 1988a, Foster 1989). It also is considered a sporadic late-season pest of cotton (*Gossypium* L.), and it attacks young sugarcane plants (interspecific hybrids of *Saccharum* L.) (Hall 1988, Pashley 1988a). Contributing to this wide host range is the presence of two strains that differ in host preference (Pashley 1986, Prowell et al. 2004). The corn-strain is associated with corn and sorghum, whereas the rice-strain is preferentially found in rice and turfgrass. The two strains are morphologically identical; thus, they can only be reliably distinguished by molecular methods (Pashley 1986, Lu et al. 1992, Pashley and Ke 1992, Lu and Adang 1996, McMichael and Prowell 1999, Prowell et al. 2004). There are reported differences between strains in their physiology, development, and behavior that are consistent with their representing genetically distinct populations (Pashley 1988b, Whitford et al. 1988, Pashley et al. 1995, Veenstra et al. 1995).

Various attempts have been made to describe the annual migrations of fall armyworm in North America. Methods include comparing chemical or viral susceptibility of fall armyworm from different locations (Young 1979, Fuxa 1987, Pitre 1988), monitoring adult

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Table 1. Source locality and host information

Location	Collection	Plant/habitat	Date
Florida[a]	Pheromone/larva	Mixed	Feb.–Dec. 2002–2005
Brazil[a]	Larva	Mixed	June–Dec. 2005–2006
Weslaco, TX	Pheromone	Corn	April 2004, July–Sept. 2006, Jan. 2007
College Station, TX	Pheromone/larva	Corn	May–Sept. 2004
San Angelo, TX	Pheromone/larva	Corn	Sept. 2006
Milstead, AL	Pheromone	Mixed	May–Aug. 2005
Auburn, AL	Pheromone	Mixed	May–Aug. 2005
Camp Hill, AL	Pheromone	Mixed	Oct. 2005
Tifton, GA	Pheromone	Pasture	Aug.–Sept. 2004
Williamson, GA	Larva	Corn	Sept. 2006
Winnsboro, LA	Pheromone	Mixed	Sept. 2006
Washington Co., MS	Pheromone	Cotton	Oct.–Nov. 2004
Washington Co., MS	Pheromone	Cotton	July–Oct. 2005
Washington Co., MS	Larva	Corn	July–Aug. 2006

[a] Data from Nagoshi et al. (2007a).

moths by pheromone trapping and radar (Rose et al. 1975, Pair et al. 1987), and correlating trap collections with wind and weather patterns (Luginbill 1928; Mitchell 1979, Pair et al. 1986, Westbrook and Sparks 1986, Mitchell et al. 1991). These studies provided estimations of the general direction of the annual migration from the overwintering sites, but at resolutions too low to conclusively identify the relative contributions of the Texas and Florida populations to infestations in the migratory areas. Furthermore, because rapid and efficient molecular methods for distinguishing strains have only recently become available, the possibility of strain-specific migration behavior was not examined.

An alternative approach to following migrating populations was made possible by the identification of haplotype subgroups within the corn-strain population (Nagoshi et al. 2007a). Sequence analysis of the mitochondrial *Cytochrome oxidase I* (*COI*) gene have identified >20 sites that display strain-specific single nucleotide polymorphisms (Nagoshi et al. 2006, 2007b). In the majority of these sites, >95% of each strain will be associated with a particular nucleotide, providing a number of molecular markers that are diagnostic of strain identity. However, two sites exhibited substantial polymorphism within the corn-strain population, with the nucleotide combinations generating four distinct haplotype categories (Nagoshi et al. 2007a). These haplotypes were found to be distributed in a consistent profile within the Florida corn-strain population that was independent of season, location, or plant type. Similar consistency was observed in an examination of corn-strain samples from Brazil, but the pattern was substantially different from the Florida distribution (Nagoshi et al. 2007a). This suggested the possibility that haplotype proportions could be used to monitor the relative movements of the corn-strain populations from Brazil and Florida.

A potential method for determining the migration patterns of the corn-strain into cotton growing regions was suggested by a recent study indicating that the fall armyworm infesting cotton grown in the Mississippi delta likely arose from migrants that developed on corn (Nagoshi et al. 2007c). If it is generally true that corn is the primary source of the fall armyworm that initially infests cotton, then there might be a linkage between the amount of corn acreage in the source location and the severity of the cotton infestation at the migration destination. Higher corn acreage would be expected to lead to increased fall armyworm numbers and, presumably, to a larger migratory population. In this scenario, there should be a positive correlation between the severity of the cotton infestation in a give area and the amount of corn acreage at the migratory source.

This study examined the feasibility of using differences in haplotype proportions to monitor the annual northward migration of fall armyworms in North America. Differences in haplotype distribution were identified for the corn-strain populations endemic to Texas and Florida, including areas where fall armyworm is thought to overwinter. The proportional differences were then tested to see whether they were sufficiently robust to produce a consistent pattern of fall armyworm movements in selected southeastern states. The proposed migratory pattern was compared with estimations of fall armyworm movements based on correlations between corn acreage and the proportion of infested cotton derived from historical agricultural data.

Materials and Methods

Specimen Collections and Sites. Fall armyworm specimens were obtained at several locations in the southern United States (Table 1). Adult males were collected using pheromone traps as described previously (Meagher and Gallo-Meagher 2003). Standard plastic Universal moth traps (Unitraps) were baited with a commercially available fall armyworm pheromone (Suterra LLC, Bend, OR), and contained insecticide strips (Hercon Environmental Co., Emigsville, PA). Collections from traps were made at various intervals, ranging from 1 to 14 d. After collection, specimens were stored at −20°C. Larvae were collected from host plants, and they were identified by morphological criteria. These were then preserved in 95% ethanol until DNA isolation, or they were placed

individually in 22.5-ml (0.75-ounce) plastic cups with artificial diet (Heliothis Premix, Stonefly Industries, Bryan, TX) to complete development. DNA was isolated from either adults or late (post-fourth) instars.

DNA Preparation. Individual specimens were homogenized in 4 ml of phosphate-buffered saline (PBS; 20 mM sodium phosphate and 150 mM NaCl, pH 8.0) in a 15-ml test tube by using a tissue homogenizer (PRO Scientific Inc., Oxford, CT). Cells and tissue were pelleted by centrifugation at 6,000 × *g* for 5 min at room temperature. The pellet was resuspended in 800 μl of cell lysis buffer (0.2 M sucrose, 0.1 M Tris-HCl, pH 8.0, 0.05 M EDTA, and 0.5% sodium dodecyl sulfate), transferred to a 1.5- or 2.0-ml microcentrifuge tube, and incubated at 55°C for 5 min. Proteins were precipitated by the addition of 100 μl of 8 M potassium acetate. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Orange, CA), and it was processed according to the manufacturer's instructions. The DNA preparation was increased to a final volume of 40 μl with distilled water. Each polymerase chain reaction (PCR) reaction required 1 μl of the DNA preparation (≈0.02 μg).

PCR Analysis and Cloning. PCR amplification of the mitochondrial *COI* gene was performed in a 30-μl reaction mix containing 3 μl of 10× manufacturer's reaction buffer, 1 μl of 10 mM dNTP, 0.5 μl of 20 μM primer mix, 1 μl of DNA template (between 0.05 and 0.5 μg), and 0.5 U of *Taq*DNA polymerase (New England Biolabs, Beverly, MA). The thermocycling program was 94°C (1 min), followed by 33 cycles of 92°C (30 s), 56°C (45 s), 72°C (45 s), and a final segment of 72°C for 3 min. Typically, 96 PCR amplifications were performed at the same time by using either 0.2-ml tube strips or 96-well microtiter plates. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Amplification of the *COI* region used the primer pair *COI-893 F* (5′-CACGAGCATATTTTACATC-WGCA-3′) and *COI-1303R* (5′-CAGGATAGTCA-GAATATCGACG-3′) to produce a 410-bp fragment.

For fragment isolations, 6 μl of 6× gel loading buffer was added to each amplification reaction, and the entire sample was run on a 1.8% agarose horizontal gel containing GelRed (Biotium, Hayward, CA) in 0.5× Tris-borate buffer (45 mM Tris base, 45 mM boric acid, and 1 mM EDTA, pH 8.0). Fragments were visualized on a long-wave UV light box, and they were cut out from the gel. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research) according to manufacturer's instructions. The isolated fragments were analyzed by DNA sequencing performed by Northwoods DNA, Inc. (Bemidji, MN). All other DNA sequences were obtained from National Center for Biotechnology Information GenBank. DNA comparisons, alignments, and restriction site mapping were performed using the DS Gene program (Accelrys, San Diego, CA).

Statistical Analysis. Corn acreage data were obtained from the National Agricultural Statistics Service (http://www.nass.usda.gov/), and fall armyworm infestation in cotton statistics were from the Mississippi State University archive of Beltwide Cotton Crop Loss (http://www.msstate.edu/Entomology/resources/CTNLSTAB.html). We estimated correlations between corn acreage of different states and proportion of cotton acreage infested by fall armyworm by calculating the Pearson's correlation coefficient using SPSS 15.0 for Windows (Lead Technologies, Inc., Chicago, IL). Haplotype ratios were analyzed by one-way analysis of variance (ANOVA) with Tukey–Kramer post test by using GraphPad InStat version 5.1 (GraphPad Software Inc., San Diego, CA).

Results

Haplotypes Differentiate Texas and Florida Fall Armyworm. The polymorphisms in the mitochondrial COI gene previously shown to distinguish between Florida from Brazil corn-strain were used to examine populations from Texas (Nagoshi et al. 2007a). The polymorphisms generated four haplotype subgroups (labeled as CS-h1, CS-h2, CS-h3, and CS-h4) that together make up the entire corn-strain population examined. Analysis of haplotype proportions in Texas fall armyworm from both pheromone trap and larval collections consistently displayed the pattern of CS-h2 > CS-h1 = CS-h4, with the CS-h3 haplotype detected sporadically and at low levels (Fig. 1). This haplotype profile is similar to that found in Brazil corn-strain populations and distinct from profiles found in Florida (Fig. 1; Nagoshi et al. 2007a). The primary difference lies in the relative proportions of the CS-h2 and CS-h4 haplotypes, which are present in inverse proportions in Florida populations compared with those in Texas and Brazil. Therefore, a ratio of the two haplotype proportions should provide a simple metric to quickly distinguish between the haplotype profiles. The CS-h4/CS-h2 ratio was calculated for the data in Fig. 1 with the samples now categorized according to sampling areas within the three locations (Fig. 2). Collections in Florida, Brazil, and Texas gave CS-h4/CS-h2 ratios of 2.4, 0.05, and 0.15, respectively. Pairwise comparisons of the data demonstrated a statistically significant difference between the CS-h4/CS-h2 ratios observed in Florida populations with those from Texas or Brazil (Table 2). There was no indication of a significant difference between Texas and Brazil corn-strain.

Corn-strain samples were next obtained from sites in Louisiana, Alabama, Georgia, and Mississippi, and CS-h4/CS-h2 ratios were calculated and compared with those from Texas and Florida. The Georgia samples displayed the Florida profile, whereas those from Alabama, Mississippi, and Louisiana were more similar to the Texas ratio (Fig. 2). The data from these states were combined into two groups, FL-GA and AL-MS-LA, and compared in pairwise combinations with the results from Texas, Brazil, and Florida (Table 2). The ratio of the FL-GA group was statistically significantly different from fall armyworms in Brazil, Texas, and the AL-MS-LA collection. No significant differences were observed between the fall armyworms analyzed from AL-MS-LA and those from Brazil or Texas.

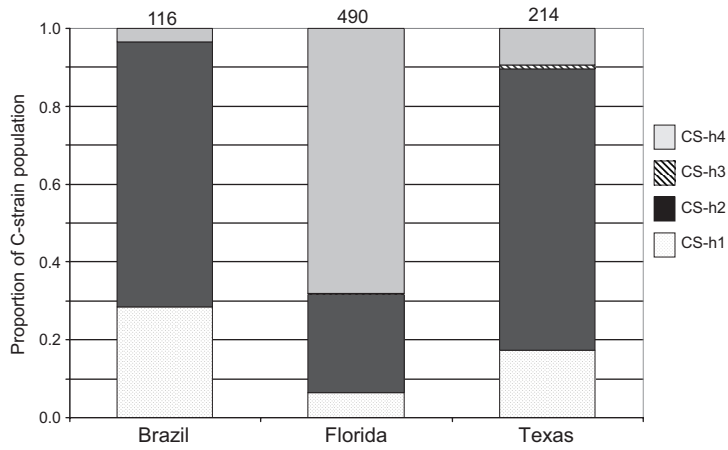

Fig. 1. Proportions of the corn-strain haplotypes present in samples collected from sites in Brazil, Florida, and Texas. A portion of the data for Florida and Brazil were described previously (Nagoshi et al. 2007a). Numbers above columns indicate total number of samples.

Correlations between Corn Acreage and Cotton Infestation. Tabulations were made for total corn acreage planted annually and the proportion of cotton acreage infested annually by fall armyworm (calculated by dividing the number of acres infested by total cotton acres planted) for selected southeastern states that bordered Texas and Florida for the period from 1995 to 2006 (Table 3). Comparisons of annual corn acreage planted showed very strong positive correlations (>99.9% confidence) between Alabama, Florida, and Georgia (Table 4). In addition, the contiguous arrangement of these states suggests that the level of corn plantings is responding to the same economic and environmental factors; therefore, it can be considered as a single agricultural unit designated A-F-G (Table 4). A similar high correlation was observed for corn acreage in the adjacent states of Louisiana and Mississippi, resulting in their consideration as a single unit designated L-M.

Corn acreage planted in Texas is larger than that of A-F-G and L-M combined for the period from 1995 to 2006 (Table 3). The Texas acreage showed a significant positive correlation (>95% confidence) with corn acreage in the A-F-G region, but no evidence of a significant correlation with corn acreage in region L-M (Table 4).

Comparisons of cotton infestation levels and corn acreage were next calculated for the different regions and states (Table 5). The proportion of fall armyworm infested cotton acreage in Alabama and Georgia only displayed a significant correlation with corn acreage planted in the A-F-G region, consistent with local corn

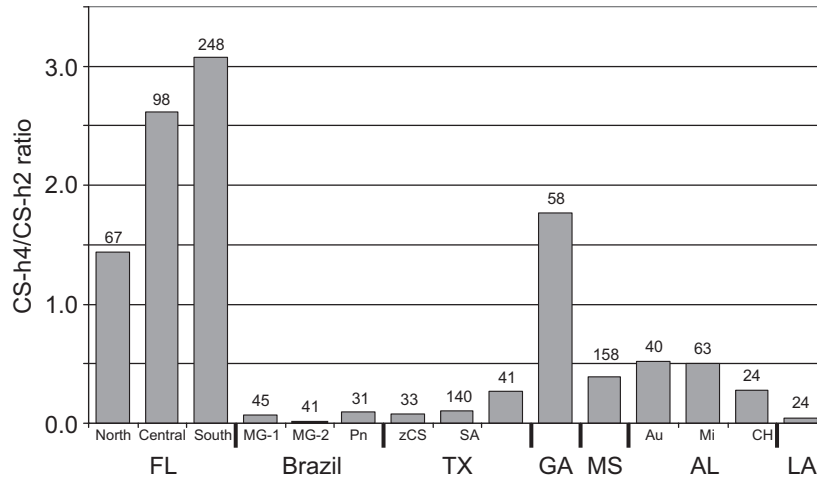

Fig. 2. CS-h4/CS-h2 ratios for corn-strain samples analyzed in Brazil and selected states in the southern United States. A portion of the data for Florida and Brazil were described previously (Nagoshi et al. 2007a). Numbers above columns indicate total number of samples. MG-1, cotton/sorghum sites in Mato Grosso; MG-2, corn site in Mato Grosso; Pn, corn site in Parana; Ws, Weslaco; CS, College Station; SA, San Angelo; Au, Auburn; Mi, Milstead; CH, Camp Hill; FL, Florida; TX, Texas; GA, Georgia; MS, Mississippi; AL, Alabama; LA, Louisiana.

Table 2. Analysis of the CS-h4/CS-h2 haplotype ratios for fall armyworm populations described in Fig. 2 by the Tukey–Kramer multiple comparisons test

Comparison	Mean difference	q	P value
Brazil vs. TX	−0.10	0.33	$P > 0.05$
FL-GA vs. Brazil	2.17	7.97	$P < 0.001$*
FL-GA vs. TX	2.07	7.61	$P < 0.001$*
FL-GA vs. AL-MS-LA	1.88	7.85	$P < 0.001$*
Brazil vs. AL-MS-LA	−0.29	1.12	$P > 0.05$
Texas vs. AL-MS-LA	−0.19	0.75	$P > 0.05$

Statistically significant differences (ANOVA) are indicated by *, $P < 0.05$.

[a] The q value is a measure of statistical distance.

being the source of the population infesting cotton in these states. The Florida fall armyworm infestation pattern also correlated with A-F-G corn, but it differed in that it showed a significant correlation with Texas corn acreage as well. However, it is possible that the latter is a reflection of the high positive correlation observed between the corn acreage in these two states (Table 4).

Fall armyworm cotton infestation in Mississippi showed no evidence of correlation with locally (L-M) grown corn, but it did show a significant positive correlation with Texas corn acreage. In contrast to Florida, this correlation was not associated with similarities in corn planting patterns between Mississippi and Texas (Table 4). Louisiana cotton infestation levels showed no evidence of correlation with corn acreages in the L-M region or in the state of Texas as a whole (Table 5). But a significant correlation was observed with corn grown in two districts in northeastern Texas (designated NE TX) that are adjacent to the eastern border of Louisiana. These results suggest that the fall armyworm infesting cotton in Louisiana and Mississippi directly originate from Texas rather than local cornfields.

Infestation levels in Texas cotton showed no evidence of statistically significant correlation with corn acreage from any state or region. However, the data for this state may be problematic because there were 2 yr (1999, 2001) when no fall armyworm infestation in cotton was reported (Table 3), and numerous instances of no reported fall armyworm infestations in individual districts with large cotton acreages (data not shown). These observations suggest that the reporting of fall armyworm in Texas may have been inconsistent during the 1995–2006 period.

Discussion

Using Haplotypes to Study Fall Armyworm Migration Patterns. The differences in the haplotype distribution pattern between corn-strain from Texas and those from Florida make possible a novel method for distinguishing between these populations that can be used to monitor their seasonal migration. Although the corn-strain haplotypes are present in both locations, we found significant differences in their proportions that could distinguish between the two populations. The haplotype profile associated with Florida fall armyworms was shown to be in equilibrium throughout

Table 3. Historical data for corn acreage planted[a] and cotton acreage infested[b] with fall armyworm for selected states

Region	Category	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
AL	Corn acres (× 1,000)	250	300	280	300	220	230	180	200	220	220	220	200
	Proportion of FAW infested cotton	0.56	0.84	0.93	1.00	0.07	0.01	nd	0.39	0.39	0.12	0.35	0.43
FL	Corn acres (× 1,000)	100	140	120	160	90	85	65	75	75	70	65	60
	Proportion of FAW infested cotton	0.14	0.17	0.64	0.78	0.01	0.07	0.003	0.02	0.003	0.17	0.13	0.11
GA	Corn acres (× 1,000)	400	580	500	500	350	360	265	340	340	335	270	280
	Proportion of FAW infested cotton	0.05	0.30	0.70	0.62	0.01	0.02	0.01	0.14	0.10	0.10	0.14	0.23
LA	Corn acres (× 1,000)	230	535	430	700	340	380	315	580	520	420	340	300
	Proportion of FAW infested cotton	0.14	0.09	0.11	0.27	0.03	0.35	0.10	0.05	0.16	0.31	0.61	0.45
MS	Corn acres (× 1,000)	300	630	460	550	340	390	400	550	550	460	380	340
	Proportion of FAW infested cotton	0.24	0.13	0.31	1.00	0.18	0.60	0.15	0.35	0.25	0.25	0.42	0.39
A-F-G	Corn acres (× 1,000)	750	1020	900	960	660	675	510	615	635	625	555	540
	Proportion of FAW infested cotton	0.19	0.44	0.74	0.72	0.02	0.02	nd	0.20	0.18	0.11	0.21	0.28
L-M	Corn acres (× 1,000)	530	1165	890	1250	680	770	715	1130	1070	880	720	640
	Proportion of FAW infested cotton	0.20	0.12	0.23	0.73	0.13	0.51	0.14	0.26	0.22	0.27	0.49	0.41
TX	Corn acres (× 1,000)	2100	2100	2000	2400	1950	2100	1600	2050	1830	1830	2050	1760
	Proportion of FAW infested cotton	0.26	0.07	0.06	0.10	nd	0.02	nd	0.10	0.02	0.01	0.14	0.18
NE TX[c]	Corn acres (× 1,000)	460	470	438	554	441	529	363	675	640	620	784	624

nd, no data; indicates no fall armyworm infestation was reported.

[a] National Agricultural Statistics Service (http://www.nass.usda.gov/).

[b] Mississippi State University archive of Beltwide Cotton Crop Loss (http://www.msstate.edu/Entomology/resources/CTNLSTAB.html).

[c] Pooled data from Texas agricultural districts 4 and 5 (codes D40 and D51, http://www.nass.usda.gov/Statistics_by_State/Texas/Charts_&_Maps/distmap2.htm).

Table 4. Interstate comparisons as measured by Pearson's correlation analysis for selected regions for annual corn acreage planted from 1995 to 2006

Region	Statistical measure	Corn acreage						
		AL	FL	GA	LA	MS	TX	A-F-G
FL	Pearson correlation	0.95*						
	Significance (two-tailed)	0.000						
	N	12						
GA	Pearson correlation	0.95*	0.93*					
	Significance (two-tailed)	0.000	0.000					
	N	12	12					
LA	Pearson correlation	0.49	0.59**	0.53				
	Significance (two-tailed)	0.108	0.045	0.075				
	N	12	12	12				
MS	Pearson correlation	0.46	0.50	0.58	0.89*			
	Significance (two-tailed)	0.132	0.102	0.050	0.000			
	N	12	12	12	12			
TX	Pearson correlation	0.75**	0.76**	0.64**	0.53	0.30		
	Significance (two-tailed)	0.005	0.005	0.025	0.077	0.346		
	N	12	12	12	12	12		
A-F-G[a]	Pearson correlation				0.54	0.54	0.70**	
	Significance (two-tailed)				0.069	0.068	0.012	
	N				12	12	12	
L-M[b]	Pearson correlation	0.49	0.56	0.57			0.44	0.56
	Significance (two-tailed)	0.106	0.057	0.054			0.151	0.059
	N	12	12	12			12	12

* Correlation is significant at >99.9% confidence level (two-tailed).
** Correlation is significant at >95% confidence level (two-tailed).
[a] Pooled data for Alabama, Florida, and Georgia.
[b] Pooled data for Louisiana and Mississippi.

the state and consistent over a 4-yr period from 2003 to 2006 (Nagoshi et al. 2007a). The Texas profile also seems to be in equilibrium based on captures at three different locations over a similar length of time (Fig. 2; Table 1). As a proof of concept, we measured and compared the haplotype proportions of the corn-strain populations in Georgia, Alabama, Louisiana, and Mississippi, states that lie adjacent to Texas or Florida and along an east–west line from the Atlantic Ocean to the Gulf of Mexico. Our results indicated that the most eastern of the states, Georgia, was infested by corn-strain populations that were indistinguishable in haplotype distribution from those overwintering in southern Florida. Corn-strain populations in Louisiana, Mississippi, and Alabama were statistically indistinguishable to populations sampled in central and southern Texas. This distribution pattern suggests a plausible migratory pattern in which the fall armyworm overwintering in Texas migrate north and eastward through Louisiana, MS, and into Alabama, whereas Florida populations move northward into Georgia (Fig. 3).

These observations conform to descriptions of migratory pathways by Luginbill (1928) that were based on trap captures and seasonal wind patterns. They are also supported by correlations between corn acreage and cotton infestation levels that suggest a linkage between fall armyworm from Texas (or at least portions of the state) and those infesting Louisiana and Mississippi (Table 5). The consistency of the haplo-

Table 5. Comparisons between corn acreage and the proportion of cotton acreage infested by fall armyworm as measured by Pearson's correlation analysis for selected states and regions[a]

Corn acreage	Statistical measure	% cotton acreage infested by fall armyworm					
		AL	FL	GA	LA	MS	TX
A-F-G	Pearson correlation	0.79*	0.70**	0.71**	−0.33	0.27	−0.11
	Significance (two-tailed)	0.004	0.011	0.010	0.296	0.398	0.770
	N	11	12	12	12	12	10
L-M	Pearson correlation	0.49	0.41	0.50	−0.26	0.35	−0.55
	Significance (two-tailed)	0.127	0.191	0.096	0.414	0.267	0.097
	N	11	12	12	12	12	10
TX	Pearson correlation	0.51	0.60**	0.48	0.05	0.67**	0.13
	Significance (two-tailed)	0.109	0.040	0.116	0.874	0.016	0.713
	N	11	12	12	12	12	10
NE TX[b]	Pearson correlation	−0.28	−0.13	−0.08	0.66**	0.27	−0.15
	Significance (two-tailed)	0.410	0.690	0.810	0.019	0.389	0.676
	N	11	12	12	12	12	10

* Correlation is significant at the >99% level (two-tailed).
** Correlation is significant at the >95% level (two-tailed).
[a] Pooled data from two districts in northeastern Texas (see Table 1).

Fig. 3. Map of the southeastern United States showing locations of fall armyworm collections with CS-h4/CS-h2 haplotype ratios characteristic of Texas or Florida overwintering populations. Open circles show similarity to the Texas ratio. Striped circles display the Florida ratio. Map courtesy of the United States Geological Survey (http://nmviewogc.cr.usgs.gov/viewer.htm).

type results with the expected migratory pattern supports the use of haplotype distribution patterns for monitoring the long-range movements of the corn strain, demonstrating that it is now possible to genetically monitor the migratory patterns of this important agricultural pest.

It has been suggested that there is a return migration of fall armyworm in the fall during which the populations in the northern states move southward to repopulate the overwintering sites (Pair et al. 1987, Mitchell et al. 1991). If there is substantial mixing of the overwintering populations among the migrants followed by a southern return, then we should see a gradual homogenization of the Florida and Texas populations with respect to haplotype proportions. Alternatively, the long-term persistence of the asymmetrical haplotype distribution would argue that return migrations either do not occur or that any genetic mixing is limited in scope. The second alternative seems to be the case as the haplotype profiles of Florida from 2003 to 2006 and Texas from 2004 to 2007 has remained relatively constant.

Using Corn Acreage to Predict the Level of Fall Armyworm Infestations in Cotton. Our working model was that fall armyworm initially established themselves in corn, by using secondary plant hosts such as cotton only as necessitated by higher populations. This would explain the sporadic nature of cotton infestations and suggest that there might be a correlation between the severity of such infestations and the amount of local corn acreage. Historical data are available for corn acreage planted and the percentage of cotton acres infested with fall armyworm. However, the latter may be problematic as fall armyworm control efforts in one or both crops are subject to change over time and location (for example, by the introduction of genetically modified plant lines), and there is likely to be substantial variation in the methodology used to assess infestation levels between states or even counties. Despite these potentially confounding variables, highly significant correlations were observed between fall armyworm infestations of cotton in Alabama, Florida, and Georgia with in state corn acreage, consistent with the working model.

Analogous correlations were not found for Mississippi and Louisiana, suggesting a different dynamic may be at work. Instead, statistically significant correlations were observed for infestations in these states with corn acreage in Texas (Table 5). Although inferences based on correlations must be treated with caution, such linkages could indicate a direct colonization of Mississippi and Louisiana cotton by migrating fall armyworm arising from Texas cornfields. This suggestion is supported by the similarities in haplotype proportions between the Texas, Mississippi, and Louisiana corn-strain populations. We note that a similar direct infestation between migrating fall armyworm from Florida and cotton grown in Georgia might also be occurring, but could be "masked" by the strong correlations in corn acreage between these two states.

It should be possible to exploit the observed correlations to predict the severity of fall armyworm infestations. For example, our results indicate that an early estimate of corn acreage to be planted in Texas generally predicts the extent of infestation in the cotton grown in the Mississippi delta. Similarly, regional estimates of corn acreage are predictive of cotton infestation levels in Alabama, Florida, and Georgia. If this correlation represents a causal relationship, as we suspect, then the identification of other factors that influence the quality of corn plants available for fall armyworm colonization should allow additional refinements that will improve predictability. This will require a two-step strategy of first identifying by haplotypes the likely primary source of the infesting population and then correlating the severity of infestations with environmental conditions at the source location.

In summary, we describe mitochondrial haplotypes that make possible a detailed description of the migratory patterns in North America that is only limited by the availability of collected samples. We also identified correlations that could provide a more accurate means of estimating future levels of fall armyworm infestations in cotton. We believe these findings have potentially important ramifications toward monitoring and predicting migratory behavior and infestation levels of this important agricultural pest.

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