Haplotype Profile Comparisons Between *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Populations From Mexico With Those From Puerto Rico, South America, and the United States and Their Implications to Migratory Behavior

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ABSTRACT Fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] is a major economic pest throughout the Western Hemisphere of maize, cotton, sorghum, and a variety of agricultural grasses and vegetable crops. Previous studies demonstrated extensive annual migrations occurring as far north as Canada from overwintering locations in southern Florida and Texas. In contrast, migratory behavior in the rest of the hemisphere is largely uncharacterized. Understanding the migration patterns of fall armyworm will facilitate efforts to predict the spread of pesticide resistance traits that repeatedly arise in this species and assess the consequences of changing climatic trends on the infestation range. Four independent fall armyworm colonies derived from widely separated populations in Mexico and two field collections were examined for their mitochondrial *cytochrome oxidase I* (*COI*) gene haplotypes and compared with other locations. The Mexico populations were most similar in their haplotype profile to those from Texas and South America, but also displayed some distinctive features. The data extend the haplotype distribution map in the Western Hemisphere and confirm that the previously observed regional differences in haplotype frequencies are stable over time. The Mexico collections were associated with haplotypes rarely found elsewhere, suggesting limited migratory interactions with foreign populations, including those in neighboring Texas.

KEY WORDS fall armyworm, insect migration, cytochrome oxidase I, haplotypes

Introduction

Spodoptera frugiperda (J. E. Smith), commonly known as fall armyworm, is a major pest of several important agricultural crops in the Western Hemisphere. The wide host range is due in part to the presence of two subpopulations designated rice-strain (RS) and corn-strain (CS) that differ in their host plant preferences, hence their designation as "host strains" (Pashley 1988). While morphologically indistinguishable they differ in their genetic markers, with polymorphisms in the mitochondrial *COI* gene among the best characterized (Levy et al. 2002, Nagoshi et al. 2006, 2008).

Like other *Spodoptera* species fall armyworm exhibits extensive migratory capability (Mitchell 1979, Rose et al. 1987). Trap-capture and radar studies indicate that infestations in areas of North America with prolonged freezing winters result from long-distance migration of populations overwintering in more southern locations (Pair and Sparks 1986, Mitchell et al. 1991, Westbrook 2008). Evidence of fall armyworm migration outside of North America is less clear and indirect, based largely on the absence of detectable genetic differentiation between geographically distant populations using amplified fragment length polymorphism (AFLP) methods (Clark et al. 2007, Belay et al. 2012). This apparent homogenization of fall armyworm on a hemispheric scale is consistent with the widespread mixing of populations expected from migration.

However, a different methodology using mitochondrial haplotypes revealed the existence of two geographically segregated CS populations. Agricultural areas in the southern portions of Florida and Texas serve as the primary sources for the annual northward fall armyworm migration that infests the rest of the United States and Canada (Sparks 1979). These two overwintering locations are separated by the Gulf of Mexico, and their CS populations exhibit differences in

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the frequencies of haplotype profiles (Nagoshi and Meagher 2008, Nagoshi et al. 2008, Nagoshi et al. 2009). The CS population can be divided into four categories, CS-h1–4, based on selected polymorphisms in the mitochondrial *COI* gene. The CS-h4 haplotype predominates in Florida while CS-h2 is the majority form for the Texas population. A simple metric for comparison is the h4/h2 ratio, with values equal to or greater than 1.5 indicative of the Florida profile and 0.5 or less designating the Texas profile (Nagoshi et al. 2008, 2009). The h4/h2 ratios found in Texas and the more northern states lying west of the Appalachian Mountain range were significantly different from those displayed by populations in Florida and along the Atlantic coast as far north as Virginia (Nagoshi et al. 2009). These data define the migratory ranges of the Texas and Florida CS populations and indicate that mixing between the two groups is relatively limited. On a more hemispheric scale, CS fall armyworm from Puerto Rico displayed the Florida h4/h2 profile, while CS populations from Brazil and Argentina resembled those from Texas (Nagoshi et al. 2007a, 2010, 2012b). It appears that the Florida and Puerto Rico CS fall armyworms represent a subpopulation that is relatively isolated from the main group that includes South America and the central regions of North America. Much less is known about the migratory behavior and population structure of the RS fall armyworm population. Appropriate haplotypes have yet to be found for analogous studies, so it is not known whether this group shows a similar pattern of geographical segregation as the CS population.

Understanding how the fall armyworm CS haplotype distribution pattern became established and is being maintained should provide insight on the influence of geography and weather patterns on the large-scale movements of flying insects. However, a major gap in our haplotype distribution map is the region between Brazil and Texas that includes Central America and Mexico. As a first step in filling this deficiency, we analyzed specimens from laboratory colonies derived from widely separated regions in Mexico as well as two field collections from the source locations of two of the colonies. The objective was to use our haplotype frequency methodology to compare CS fall armyworm populations in Mexico with that of the United States. In addition, recent collections from previously surveyed locations were examined and compared with past studies to assess the temporal stability of the haplotype distribution map. The implications of these findings on fall armyworm behavior are discussed.

Materials and Methods

Insect Collections. Four independently isolated laboratory colonies of fall armyworm were derived from widely separated populations in Mexico. Larvae collected from maize (*Zea mays* L.) fields in the proximity of Ciudad Mante Tamaulipas on the northern Gulf Coast, Ciudad Sinaloa along the northern Pacific coast, and Ciudad Durango in the north-central region were used to produce the TAM, SIN, and DUR colonies, respectively (Fig. 1). The CHI colony was generated using larvae from an existing laboratory rearing population maintained at El Colegio de la Frontera (ECOSUR) that originated from a collection near the southern border with Guatemala in Tapachula, Chiapas, Mexico (Table 1). Specimens used for the haplotype comparisons were randomly selected from each colony within seven generations of the colony origin.

Adult field specimens were obtained from cornfields by sweep netting in the proximity of Ciudad Mante, Tamaulipas (Tam-F), and Ciudad Durango, Durango (Dur-F), the same general locations as the source sites of the TAM and DUR colonies, respectively. Collections from Argentina (Arg12) and Brazil (Brz05) were obtained as larvae collected directly from corn plants and stored in ethanol before processing. Field collections from the United States were obtained as adult males in pheromone traps located in corn or sorghum habitats (Table 1).

The colonies were generated as previously described (Rosas-García 2002). Larvae collected from the field or the ECOSUR colony were individually reared on artificial diet contained in 30-ml plastic cups capped with cardboard lids. Pupae were recovered and disinfected with 2% hypochlorite for 2 min, washed in running water, and placed in emergence chambers (4-liter plastic jars lined with paper towel and covered with a 30- by 30-cm cheese cloth held in place with a rubber band) to allow adult eclosion and laying of eggs on the paper towel. Adults were fed with a small cotton ball saturated with 15% sucrose solution. Eggs were collected daily and placed on artificial diet. Rearing was conducted at 26 ± 1°C, with a photoperiod of 14:10 (L:D) h, and 65 ± 5% relative humidity. The number of females used to initiate each generation varied per generation and per colony, and was not recorded.

Pheromone trapping was performed using standard plastic Universal moth traps (Unitraps) baited with a commercially available fall armyworm pheromone and contained insecticide strips (Hercon Environmental Co., Emigsville, PA). Pheromone lures used were either a three-component blend specific for fall armyworm (Suterra LLC, Bend, OR) or a two-component mix designated "Fall Armyworm-PSU" lure (Scentry Biologicals, Inc., Billings, MT). After collection, specimens were stored at −20°C.

Mitochondrial DNA Isolation and PCR Amplification of the *COI* Region. Mitochondrial DNA for use in PCR amplifications was isolated from individual specimens using Zymo-Spin III columns (Zymo Research, Orange, CA) as described previously (Nagoshi et al. 2007a). Two segments of the *COI* gene were amplified. The primers *COI*-101F and *COI*-911R produced an 810-bp PCR amplified product that included the region previously used for barcode comparisons of *Spodoptera* species (Fig. 2A, Nagoshi et al. 2011). Primer pair *COI*-891F and *COI*-1472R generated a 581-bp PCR product that contained the sites that define the CS-h1–4 haplotypes (Fig. 2B).

PCR amplification was performed in a 30-µl reaction mix containing 3 µl 10× manufacturer's reaction buffer, 0.5 µl 10mM dNTP, 0.5 µl 20-µM primer mix, 1–2 µl

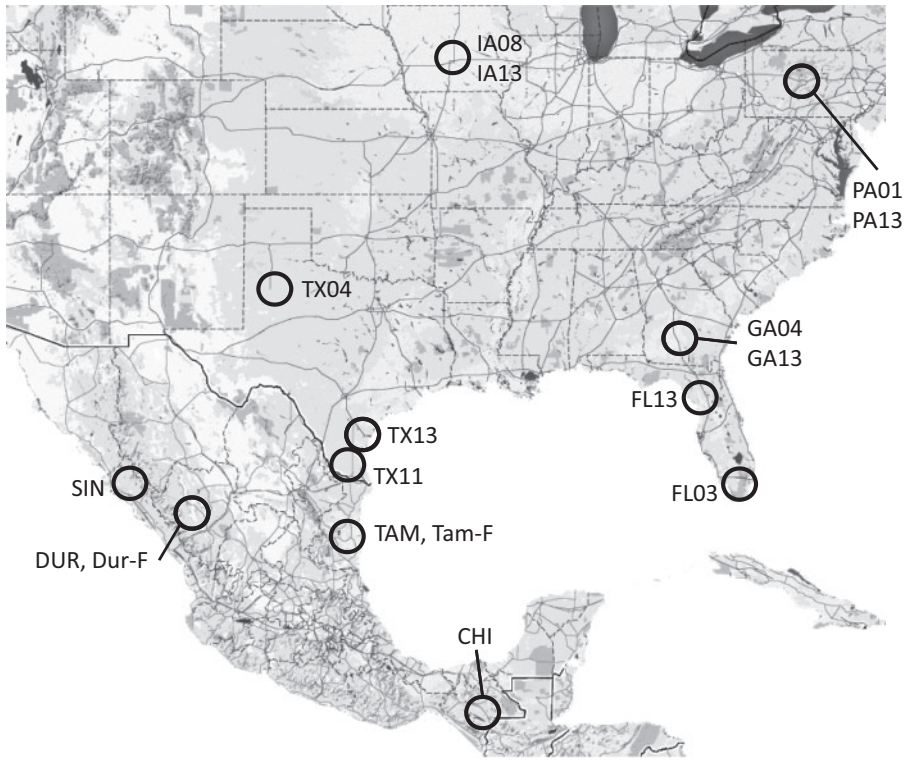

Fig. 1. Sites of origin for the fall armyworm collections used in this study from Mexico and the United States. Map courtesy of Google Maps.

Table 1. Geographical origins of fall armyworm colonies and field collections

Name	U.S. state or country	Location of origin (county or province)	Collection year	Source	Reference
DUR	Mexico	Durango	2013	Colony	This paper
SIN	Mexico	Sinaloa	2013	Colony	This paper
TAM	Mexico	Tamaulipas	2013	Colony	This paper
CHI	Mexico	Chiapas	2013	Colony	This paper
Dur-F	Mexico	Durango	2014	Field	This paper
Tam-F	Mexico	Tamaulipas	2014	Field	This paper
Arg12	Argentina	Tucumán	2012	Field	This paper
FL13	Florida	Hendry Co.	2013	Field	This paper
GA13	Georgia	Tift Co.	2013	Field	This paper
IA13	Iowa	Story Co.	2013	Field	This paper
PA13	Pennsylvania	Centre Co.	2013	Field	This paper
TX11	Texas	Hidalgo Co.	2011	Field	This paper
TX13	Texas	Nueces Co.	2012-13	Field	This paper
Brz05	Brazil	Mato Grosso	2005	Field	Nagoshi et al. 2007b
FL03	Florida	Miami-Dade Co.	2002-3	Field	Nagoshi et al. 2007b
GA04	Georgia	Tift Co.	2004	Field	Nagoshi et al. 2008
IA08	Iowa	Story Co.	2008	Field	Nagoshi et al. 2012a
PA01	Pennsylvania	Centre Co.	2001	Field	Nagoshi et al. 2009
TX04	Texas	Brazos Co.	2004	Field	Nagoshi et al. 2008

DNA template (between 0.05–0.5 μg), and 0.5 unit 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 using either 0.2-ml tube strips or 96-well microtiter plates. Primers *COI*-101F (5′-TTCGAGCTGAATGRACTC-3′), *COI*-911R (5′-GATGTAAAATATGCTC GTG-3′), *COI*-891F (5′-TACACGAGCATATTTTACA

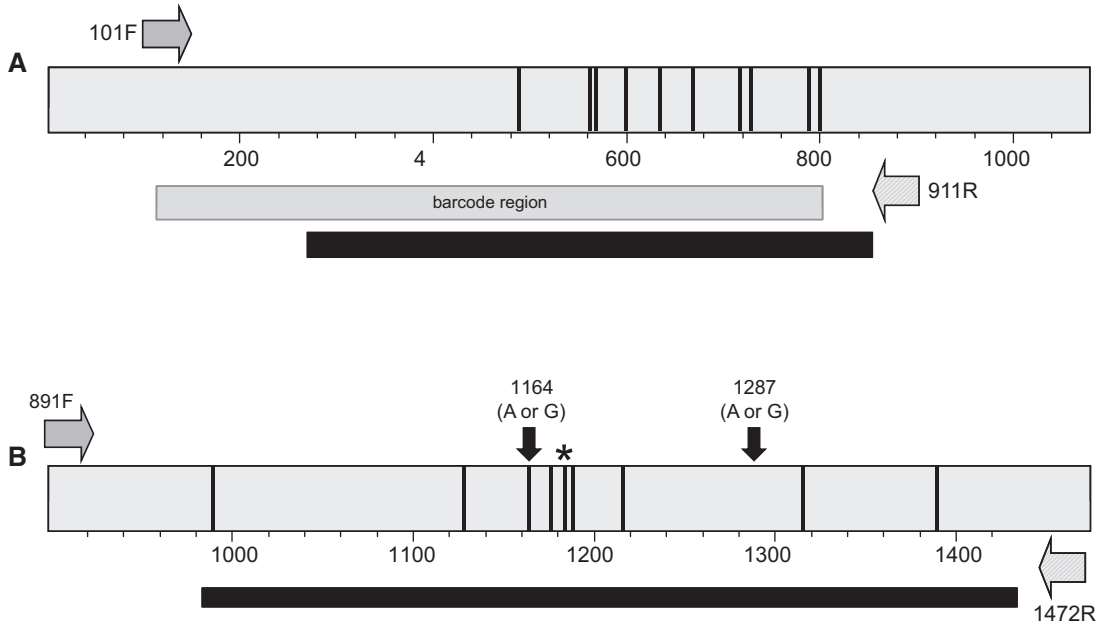

Fig. 2. Maps of two regions of the *COI* gene used for strain and haplotype analysis. (A) Region of the *COI* gene that includes sequences used for DNA barcoding of *Spodoptera* species (Nagoshi et al. 2011). (B) Region of the *COI* gene that includes sites (1164, 1287) used to define the CS-h haplotypes. Asterisk identifies polymorphic EcoRV site present in the RS but not the CS. Block arrows designate locations and directions of relevant primers used for PCR amplification. Vertical lines specify locations of strain-specific polymorphisms used to discriminate CS and RS specimens. Site 1287 is polymorphic but not strain-specific. Solid black bar indicates region used for DNA sequence analysis.

TC-3′), and *COI*-1472R (5′-GCTGGTGGTAAATTTT-GATATC-3′) were synthesized by Integrated DNA Technologies (Coralville, IA).

Confirmation of Species Identity and Determination of Host Strain. The initial larval source populations for the colonies and the larval and adult specimens collected from the field were identified as *S. frugiperda* by morphological criteria. Specimens used in this study were examined for host strain identity by EcoRV digestion of the *COI*-891F and *COI*-1472R PCR amplification product. Only the RS-associated *COI* allele has an EcoRV site in the amplified region (asterisk in Fig. 2), and these were not analyzed further. Uncut fragments were preliminarily identified as CS and were isolated from the gel and prepared for DNA sequencing. The isolated fragments were sequenced using primer *COI*-891F and the *S. frugiperda* and CS identification confirmed by comparison with the CS consensus. All the Mexico specimens were CS by the above criteria, but because we had never characterized fall armyworm from this country, additional analysis was done on a segment of the *COI* gene commonly used for DNA barcoding analysis and previously used to discriminate between *Spodoptera* species (Nagoshi et al. 2011). PCR amplification used the primer combination *COI*-101F and *COI*-911R and the PCR protocol described above, with DNA sequence analysis performed using the *COI*-101F primer (Fig. 2A). Phylogenetic comparisons of the Mexico haplotypes with a subset of *Spodoptera* species barcodes were calculated using the Kimura-2-parameter distance model (Kimura 1980) and graphically displayed as a phenogram (Saitou and Nei 1987). *Spodoptera* haplotypes compared were *S. frugiperda* corn-strain (HM136586–HM136590), *S. frugiperda* rice-strain (HM136593–HM136601), *S. littoralis* (HM756074), *S. pulchella* (HM756075–HM756076), *S. exigua* (HM756077–HM756080), *S. eridania* (HM756081–HM756085), *S. dolichos* (HM756086–HM756089), and *S. litura* (HM756090–HM756093).

For the restriction digest analysis, 5 units of the restriction enzyme EcoRV (New England Biolabs, Beverly, MA) were added to each 20-μl PCR reaction mix along with 1 μl of the manufacturer recommended 10× restriction enzyme buffer (final volume taken to 30 μl with water). Restriction digests were incubated at 37°C 1–3 h. For each reaction, 6 μl of 6× gel loading buffer was added and the entire sample run on a 1.8% agarose horizontal gel containing GelRed (per manufacturer's instructions, Biotium, Hayward, CA) in 0.5× Tris-borate buffer (TBE, 45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). Fragments were visualized on a long-wave UV light box. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research, Orange, CA) according to manufacturer's instructions. The isolated fragments were sequenced using primer *COI*-891F, and the *S. frugiperda* and CS identification was confirmed by comparison of the sequence to the CS consensus.

Table 2. List of CS-h haplotype ratio frequencies from locations sampled at different times

Location	Old collection	*n*	h4/h2	New collection	*n*	h4/h2	*P*-value
Florida	FL03	40	2.2	FL13	36	3.7	0.42
Georgia	GA04	55	1.8	GA13	21	1.5	0.65
Texas	TX04	96	0.2	TX12	100	0.3	0.22
Pennsylvania	PA01	39	0.3	PA13	79	0.2	0.35
Iowa	IA08	60	0.2	IA13	169	0.2	0.55
South America	Brz05	86	0.0	Arg12	56	0.0	0.22

Statistical comparisons between old and new collections were made using two-tailed Fisher's exact test analysis of the numbers of CS-h2 and CS-h4 haplotypes observed.

Sanger DNA sequencing was performed by the University of Florida Interdisciplinary Center for Biotechnology Research (UF-ICBR). DNA comparisons, alignments, restriction site mapping, and phylogeny were performed using Geneious version 5.6.2 created by Biomatters (available from http://www.geneious.com/). The contents in Table 3 of descriptive DNA sequence statistics and calculations of nucleotide variation based on the Jukes-Cantor (JC) model were performed using DNAsp Ver. 5.1 (Librado and Rozas 2009).

Analysis of the h4/h2 Haplotype Categories. The CS-h methodology used to determine the h4/h2 ratio is limited to the CS population. The *COI* region from 990–1430 contains the polymorphic N_{1164} and N_{1287} sites that are both variable for the bases adenine (A) and guanine (G) in CS populations, producing four haplotype categories, CS-h1 (A_{1164} A_{1287}), CS-h2 (A_{1164} G_{1287}), CS-h3 (G_{1164} A_{1287}), and CS-h4 (G_{1164} G_{1287}) that are defined only by these two loci. The CS-h2 and CS-h4 are the majority haplotypes that vary substantially between geographical regions in an inverse relationship; thus, the ratio of the two haplotypes (h4/h2) provides a metric that can discriminate between populations (Nagoshi et al. 2007a, 2008). Chi-square and Fisher's exact test analyses were performed using GraphPad Prism version 6.0d for MacOS X, GraphPad Software, La Jolla, CA, www.graphpad.com.

Novel haplotypes obtained in this study have been deposited in GenBank and include Dur1 (KF872168), Dur2 (KF872169), Sin1 (KF872171), Tam1 (KF872172), and Chi1 (KF872173).

Results

Stability of the h4/h2 Profiles. To test the consistency of the geographical distribution of h4/h2 ratios over time, we analyzed and compared the results of more recent collections from 2012–2013 with our earlier surveys (2001–2008) from the same or nearby locations (Table 2). Collections categorized as displaying the Florida (h4/h2 $\geq$ 1.5) or the Texas (h4/h2 $\leq$ 0.5) profiles in 2001–2008 showed the same association for the later time period. In South America, our earliest analysis was done with Brazilian fall armyworm collected in 2005 (Nagoshi et al. 2007b). Only three specimens out 86 examined had the CS-h4 haplotype (h4/h2 = 0.03 rounded to 0.0, Table 2). A similar result was observed with 2012 collections from the neighboring country of Argentina, where the CS-h4 haplotype was not found in 56 specimens. Statistical comparisons of the CS-h2 and CS-h4 numbers found in the old and new collections showed no significant differences, confirming that the h4/h2 values were consistent over time for all locations (Table 2).

DNA Analysis of the Mexico Colonies and Collections. DNA analysis was performed on two segments of the mitochondrial *COI* gene. These were a 582-bp segment from 267 to 848 that overlaps sequences previously used to discriminate *Spodoptera* species common to the southeastern United States (Nagoshi et al. 2011), and an adjacent but nonoverlapping 441-bp region from 990 to 1430 that contains the sites used for the h4/h2 ratio determination (Fig. 2). Analysis of the combined 1023-bp set of sequences showed low haplotype variability (Table 3). The TAM and CHI colonies were each associated with a single haplotype, Tam1 and Chi1, respectively. Two haplotypes were found for the DUR colony and were present in near equal proportions (Dur1 = 44%, Dur2 = 56%). The SIN colony also displayed two haplotypes, with one (Sin2) represented by a single specimen. All haplotypes displayed the expected open reading frame and none of the polymorphisms altered the predicted amino acid sequence. Phylogenetic comparison of the Mexico barcode haplotypes (267–848 segment) showed unambiguous grouping with the *S. frugiperda* CS clade (Fig. 3), confirming the initial species identification made during the creation of the colonies and the preliminary identification using the *COI* 990–1430 sequence.

The two field collections gave results nearly identical to that of the associated colonies. Tam-F was associated with the same single haplotype as the TAM colony, while the Dur-F field collection contained the same two haplotypes (Dur1 = 67%, Dur2 = 33%) as the DUR colony (Table 3). Fisher's exact test analysis revealed that the haplotype proportions of the DUR and Dur-F1 collections were not significantly different ($P = 0.28$).

Minority Haplotypes Predominate in the Mexico Collections. Analysis of the Mexico *COI* sequences revealed seven sites where single base substitutions generated variants that define the different haplotypes (Table 4). Each site was associated with a choice of two nucleotides, $[C/T]_{450}$, $[A/G]_{666}$, $[C/T]_{684}$, $[C/T]_{711}$, $[C/T]_{759}$, $[C/T]_{1038}$, and $[A/G]_{1044}$. The N_{1164} and N_{1287} sites that define the CS-h haplotypes and are highly polymorphic in U.S. populations were generally invariant within each Mexico collection (Table 4). The only

Table 3. Descriptive statistics of polymorphisms found for regions 267–848 and 990–1430 of the *COI* gene in the Mexico colonies and field collections

	Sample	*n*	Haplo	Ps	SS/NS	Hd (s.d.)	π (s.d.)
Colonies	DUR	18	2	4	4/0	0.52 (0.05)	0.002 (0.0002)
	SIN	20	2	2	2/0	0.10 (0.09)	0.0002 (0.0002)
	TAM	17	1	0	0	0.00 (0.00)	0.00 (0.00)
	CHI	17	1	0	0	0.00 (0.00)	0.00 (0.00)
Field collections	Dur-F	12	2	4	4/0	0.49 (0.05)	0.002 (0.0002)
	Tam-F	15	1	0	0	0.00 (0.00)	0.00 (0.00)

n, number of sequences; Haplo, number of haplotypes; Ps, number of polymorphic sites; SS, synonymous substitution; NS, nonsynonymous substitution; Hd, haplotype diversity; π, pi nucleotide diversity.

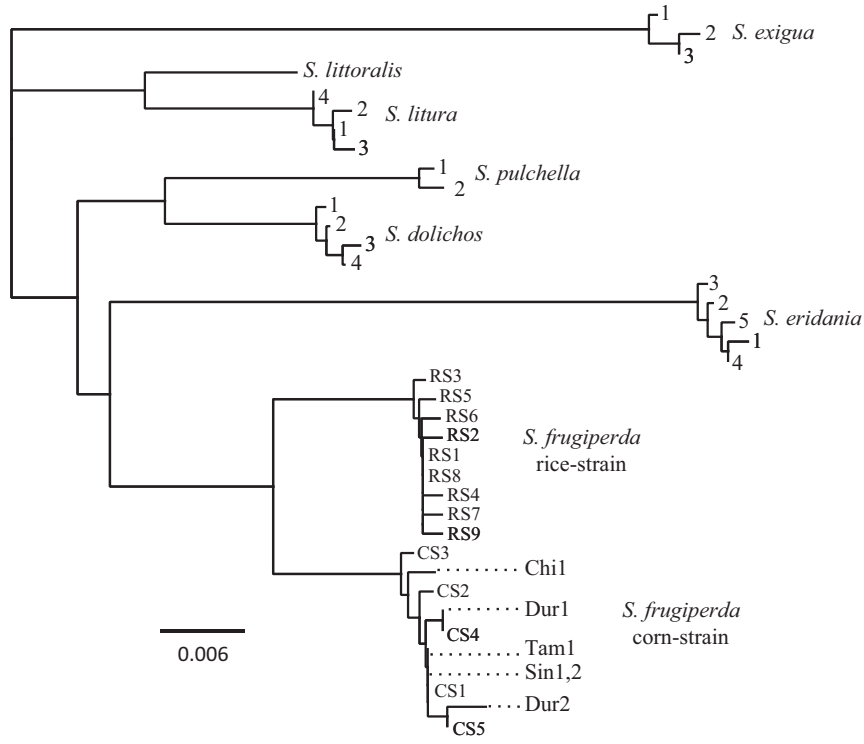

Fig. 3. Phylogenetic tree comparing the Mexico colony haplotypes with a portion of the barcode region from several *Spodoptera* species. The 582-bp portion of the *COI* region (267–848) spanned by the primers *COI*-101F and *COI*-911R from the Mexico colony haplotypes were compared with the corresponding barcode segment from several species of *Spodoptera* common to the southeastern United States. The unrooted phylogenetic tree was derived using the Tamura-Nei genetic distance model (Tamura and Nei 1993) and Neighbor-joining analysis (Sneath and Sokal 1973). Consensus sequences for the *Spodoptera* species, including representative fall armyworm corn-strain (CS1–CS5) and rice-strain (RS1–RS9) sequences, are from Nagoshi et al. (2011). Scale bar measures substitutions per site.

within-population polymorphism at these sites was found in the SIN colony, where one individual carried A_{1287} out of 20 tested. The frequencies of the Mexico haplotypes were examined in wild populations from Hidalgo Co, Texas (TX11), which lies along the Texas–Mexico border (Fig. 1). The single haplotypes found in the CHI colony and in both the TAM and Tam-F collections were not observed in the TX11 field collections (Table 4). Of the two haplotypes in the DUR colony and Dur-F field collection, one was not found in TX11 and the other was present in 6% of specimens.

The Mexico h4/h2 Profiles. The CS-h profile only requires DNA sequence information for the short *COI* segment that spans sites N_{1164} and N_{1287}. This allowed additional specimens to be added to that described in Table 3 for the determination of h4/h2 ratios. Only CS-h1 and CS-h2 were found in the Mexico collections, and their distribution showed substantial segregation (Table 5). All DUR, Dur-F, and all but one SIN specimens were CS-h2, generating an h4/h2 ratio of 0.00 for these collections, identical to that observed in Argentina and generally diagnostic of the Texas profile (h4/

Table 4. A description of the polymorphic nucleotides associated with each of the haplotypes analyzed in Table 3

	Nucleotide location in the *COI* gene									
Haplotype [n]	N_{450}	N_{666}	N_{684}	N_{711}	N_{759}	N_{1038}	N_{1044}	N_{1287}	Freq	TX11 Freq[a]
Dur1 [16][b]	C	A	T	C	T	T	A	G	0.53	0
Dur2 [14][b]	T	G	T	T	T	T	A	G	0.47	0.06
Sin1 [19]	T	A	T	T	T	T	A	G	0.95	0.48
Sin2 [1]	T	A	T	T	T	T	A	A	0.05	0.03
Tam1 [32][c]	T	A	T	T	T	C	A	A	1.00	0
Chi1 [17]	T	A	C	T	C	T	G	A	1.00	0

The haplotype frequency in each Mexico collection is compared with that found in a population from Texas.
[a] Frequency of each haplotype in the TX11 collection ($n = 31$).
[b] Includes the DUR and Dur-F collections.
[c] Includes the TAM and Tam-F collections.

Table 5. Comparison of the nucleotide polymorphism frequencies at sites N_{1164} N_{1287} in the COI gene for different fall armyworm collections

		Proportions with given N_{1164} N_{1287} pattern					
Collection	n	A A CS-h1	A G CS-h2	G A CS-h3	G G CS-h4	h4/h2	$[G/A]_{1164}$
DUR	35	0.00	1.00	0.00	0.00	0.00	0.00
SIN	22	0.05	0.95	0.00	0.00	0.00	0.00
TAM	23	1.00	0.00	0.00	0.00	nd	0.00
CHI	20	1.00	0.00	0.00	0.00	nd	0.00
Dur-F	12	0.00	1.00	0.00	0.00	0.00	0.00
Tam-F	15	1.00	0.00	0.00	0.00	nd	0.00
Arg12	23	017	0.83	0.00	0.00	0.00	0.00
Brz05	84	0.30	0.65	0.01	0.04	0.05	0.05
FL-pool[a]	152	0.11	0.28	0.01	0.61	2.14	1.58
TX-pool[b]	543	0.14	0.70	0.00	0.16	0.22	0.19
S. America[c]	107	0.26	0.73	0.00	0.01	0.02	0.01

nd, not done due to 0 in the denominator.
[a] FL03, FL13, GA04, and GA13.
[b] TX04, TX11, TX13, IA08, IA13, PA01, and PA13.
[c] Arg12 and Brz05.

$h2 < 0.5$, Table 4). The TAM, Tam-F, and CHI collections consisted entirely of CS-h1, a profile that has not been observed in surveys of other locations. CS-h1 was a minority haplotype in both the FL-pool (collections from Florida and Georgia) and TX-pool (Texas, Iowa, and Pennsylvania), representing <15% of U.S. fall armyworm (Table 5). It was somewhat more prevalent in South America, ranging as high as 30% of the collections tested from Brazil, but only 17% in Argentina. The absence of CS-h2 did not allow the calculation of the h4/h2 ratio for the Mexico samples. Instead, we used a second metric based on the ratio of G to A at site N_{1164} ($[G/A]_{1164}$) that we previously showed correlated with the h4/h2 ratio results (Table 5; Nagoshi et al. 2012a). The combined Mexico $[G/A]_{1164}$ of 0.00 was identical to Arg12 and more similar to the values diagnostic of the TX-pool than the FL-pool (Table 5).

Discussion

Comparison of AFLP and Haplotype Ratio Methods. Comparisons of fall armyworm collected from Mexico, the continental United States, Brazil, and Argentina using AFLP, a whole genome measure of genetic variation, did not reveal evidence of populations clustered by geography or by plant host (Clark et al. 2007). There was no correlation between geographic distance and genetic dissimilarity, and principal component analysis generally showed no significant clustering for most populations. Observed clusters encompassed geographically distant groups, reflecting a lack of genetic isolation. A similar study found no evidence of genetic structuring among regions in the United States, Panama, or Puerto Rico, but did reveal a significant correlation between genetic dissimilarity and geographical distance within Argentina locations (Belay et al. 2012). Overall, the implication of the AFLP studies is that fall armyworm essentially constitutes a single interbreeding population in the Western Hemisphere, with little genetic differentiation between regions and no indication of host strains (Clark et al. 2007).

However, studies over a similar geographical range that compared mitochondrial haplotypes indicated a more complex region-specific and host plant-biased distribution of fall armyworm subpopulations. These methods differentiated the RS and CS groups that differ in their host plant preferences (Pashley 1989, Lu and Adang 1996, Nagoshi et al. 2006). The host strains exist in the United States, Argentina, and Brazil,

generally exhibiting the same biased host plant distributions (Prowell et al. 2004, Nagoshi et al. 2007b, Machado et al. 2008, Nagoshi et al. 2010, Nagoshi et al. 2012b; but also see Juarez et al. 2012, Juarez et al. 2014). In addition, the CS-h haplotype method demonstrated that fall armyworm CS populations in Florida and Puerto Rico could be distinguished from those in Texas and South America (Nagoshi et al. 2007a, 2008, 2010). This indication of geographically distinct subpopulations is consistent with a recent observation that the response to pheromone lures by fall armyworm also displays some geographical differentiation (Unbehend et al. 2014).

These studies illustrate that assessments of genetic differentiation between geographical regions can be heavily influenced by the methodological approach. Apparently there is sufficient gene flow among fall armyworm populations to obscure detection of existing subgroups by AFLP, and presumably other strategies based on comprehensive genomic comparisons of polymorphisms. In contrast, our method based on *COI* haplotype frequency (the CS-h comparisons) does not directly measure gene flow. Instead, because mitochondria are maternally inherited, it is an assessment of female exchanges between populations, specifically whether the number of progeny produced by foreign females is sufficient to alter the haplotype profile of a given population. In principle, the h4/h2 approach has the potential to detect populations at intermediate stages of reproductive segregation, when introgression (particularly with males) is still high enough to make genetic differentiation difficult to detect by AFLP, but the exchange of females is sufficiently low to allow the differentiation of mitochondrial haplotype frequencies.

Stability of Haplotype Ratio Differences Between Regions. A central assumption of the h4/h2 ratio approach is that the observed regional differences are sufficiently stable over time to justify extrapolations of past and future population behavior. This requires periodic reassessments of haplotype frequencies. To this end, we compared the CS-h ratios of more recent collections from Florida (FL13), Texas (TX13), and Argentina (Arg12) with those from past studies. We also tested recent migrant collections that should display the same haplotype proportions as their source populations. These include Georgia populations that were previously shown to arise from Florida immigrants, and specimens from Pennsylvania and Iowa that reflect the Texas haplotype profile (Nagoshi et al. 2012a). The consistency observed for each comparison indicates that the haplotype profiles have been stable from at least 2002–2013 in Florida, 2004–2012 in Texas, and 2005–2013 in South America (Table 2). Similarly, the consistency of haplotype frequencies in the Georgia, Pennsylvania, and Iowa samples suggest that at least the broad outlines of the previously described migratory pathways in the United States are reproducible over time (Nagoshi et al. 2008, 2009, 2012a).

Mexico Fall Armyworms Resemble the TX-group CS-h Profile. The DUR, SIN, TAM, and CHI specimens we examined were from laboratory colonies and are therefore a number of generations removed from the original field populations. Prolonged artificial rearing will almost certainly be associated with bottlenecks that will substantially reduce genetic diversity, a possible explanation for the low genetic variability observed for these colonies (Table 3). Therefore, we cannot assume that the colonies currently reflect the full haplotype distribution or genetic variation of the wild populations from which they were derived. However, the likelihood that a particular allele or haplotype will become fixed in a colony due to genetic drift is dependent upon its frequency in the original founder population. While the fixation of rare haplotypes is possible, it is increasingly less likely to occur in multiple independently derived and maintained colonies. Therefore, we believe it reasonable to assume that the CS-h haplotype categories found in the Mexico colonies were significant, if not majority, components of the founder populations. Support for this assumption came from the analysis of wild fall armyworm collected from the source locations of two colonies, DUR and TAM. Both Dur-F and Tam-F were composed of the same haplotypes as the corresponding colonies and displayed similar genetic variability (Table 3).

Three of the six Mexico samplings (DUR, Dur-F, and SIN) were predominated by CS-h2 with no CS-h4 detected, producing a h4/h2 ratio of 0 (Table 5). Only CS-h1 was observed in the TAM, CHI, and Tam-F collections. The combined Mexico results strongly suggest a fall armyworm population that is primarily composed of the CS-h1 and CS-h2 categories, with CS-h4, the variant diagnostic of the Florida profile, rare or absent. These observations are most compatible with the Texas profile, which is consistent with the proximity of the colony origins (particularly SIN and TAM) to Texas and the land connection of Mexico with South America.

Are Mexico Fall Armyworm Populations Isolated?. Substantial exchanges between the Mexico populations with the rest of the hemisphere should lead to the homogenization of genetic variation such that the Mexico haplotype profiles will resemble the hemispheric average. Conversely, isolation of the Mexico populations would provide opportunities for the generation of novel haplotypes and distributions. Suggestive of the latter was that five of the six collections (CHI, DUR, Dur-F, TAM, Tam-F) were dominated by *COI* haplotypes that are rare in the TX11 collections from Hidalgo Co, TX (Table 4). This was most surprising for the TAM and Tam-F collections, as the TX11 collections are only about 500 km from the source location of the TAM colony near Ciudad Mante, Tamaulipas. This distance is within the seasonal migratory range of fall armyworm, which was estimated to move as much as 480 km per generation (Sparks 1979). Despite this relative proximity, the C_{1038} polymorphism observed in all TAM and Tam-F specimens was not found in the TX11 field collections. If the haplotypes of the CHI, DUR, Dur-F, TAM, and Tam-F collections are majority components of the local populations, then their scarcity elsewhere is an indication of limited interactions between Mexico fall armyworm and those from Texas. Only the SIN haplotype profile is compatible with significant exchanges. A more systematic and

extensive survey of wild populations from Mexico is indicated to map the degree of geographical segregation.

In summary, comparisons of haplotype profiles continues to be a useful method for examining the long-distance movements of fall armyworm, identifying complexities in the distribution of populations that have not been detected by other means. In this article we showed that haplotype distribution asymmetries observed in earlier studies have remained broadly stable for at least 6–12 yr. The haplotype distribution map has been expanded to include Mexico and suggest a closer historical connection of Mexico fall armyworms to those from South America and Texas than to those that overwinter in Florida. There are preliminary indications that current interactions between the Mexico populations with the rest of the hemisphere may be limited, but confirmation will require systematic sampling of wild populations from selected regions in Mexico and more detailed comparisons of their haplotype profiles.

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