Genetic Characterization of Fall Armyworm (Lepidoptera: Noctuidae) Host Strains in Argentina

RODNEY N. NAGOSHI,[1,2] M. GABRIELA MURÚA[3,4] MIRIAN HAY-ROE,[1] M. LAURA JUÁREZ,[3] EDUARDO WILLINK,[3] AND ROBERT L. MEAGHER[1]

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ABSTRACT Fall armyworm is a major economic pest throughout the Western Hemisphere. Previous studies of populations in the southern United States, Brazil, and the Caribbean demonstrated the existence of two morphologically identical but genetically distinct host strains that can only be distinguished using genetic markers, including polymorphisms in the mitochondrial *Cytochrome Oxidase I* (*COI*) gene and in the Z-chromosome linked *Triose phosphate isomerase* (*Tpi*) gene. The strains differ in some physiological and behavioral characteristics, most notably their preference for different plant hosts, but are capable of hybridizing in the laboratory and in the field. These traits suggest that the strains are in the process of divergence, which may or may not be hemispheric in scope. The objective of this study was to determine whether the two strains are present in Argentina. It was found that the strain-diagnostic haplotypes of the *COI* and *Tpi* genes subdivided the Argentina population into two major groups. Each group displayed biases in their distribution among different host plants that were generally consistent with expected strain behavior. The overall results indicated that Argentina fall armyworm exhibit similar genetics and behavior to populations in the rest of the hemisphere. In addition, the Argentina populations had comparable haplotype frequencies to those from Brazil and Texas, consistent with possible interactions with these fall armyworm groups, but appeared to have had minimal exchanges with those from Puerto Rico or Florida.

KEY WORDS *Spodoptera frugiperda*, haplotype, triose phosphate isomerase, cytochrome oxidase I

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), commonly known as the fall armyworm, is one of the major agricultural pests in the Western Hemisphere, infesting corn, sorghum, turf grasses, and a number of other crops (Luginbill 1928). Although a semitropical insect with permanent populations restricted to areas with mild winters, fall armyworm annually infests most of the United States and the southern portion of Canada. This reflects its long-range mobility, with annual migrations extending thousands of kilometers from its overwintering sites in Florida and Texas (Mitchell et al. 1991).

The fall armyworm species is subdivided into two host strains based on the observation that certain molecular markers were asymmetrically distributed in populations feeding on rice and corn (Pashley 1986, 1989). These markers included several allozyme electrophoretic variants and DNA polymorphisms, which still remain the only reliable means of discriminating between the morphologically indistinguishable strains (Lu et al. 1992, Lu and Adang 1996, McMichael and Prowell 1999, Levy et al. 2002, Prowell et al. 2004). Surveys of primarily U.S. populations have correlated these markers with plant hosts and have found that the "rice-strain" is preferentially found on turf grasses (e.g., Bermuda grass) and pasture grasses while the "corn-strain" is most common on corn, sorghum, and cotton (reviewed in Nagoshi and Meagher 2004b). Fall armyworm has a large host range, with 186 host plant species from 42 different families recorded to date (Casmuz et al. 2010), only a small fraction of which has been linked with a host strain.

The association of the strains with specific plants is not absolute. It has been estimated that ≈18% of the larvae collected from corn have one or more markers indicative of the rice-strain, and several instances were found both in and outside the United States when the unexpected strain was the majority on a particular plant host (Prowell et al. 2004). Initial surveys of populations from Argentina, Brazil, and Paraguay also found some inconsistent associations of strain-specific mitochondrial haplotypes with host plants (M. L. Juárez, unpublished data), and the rice-

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[1] Center for Medical, Agricultural and Veterinary Entomology, USDA–ARS, Gainesville, FL 32604.

[2] Corresponding author, e-mail: rodney.nagoshi@ars.usda.gov.

[3] Estación Experimental Agroindustrial Obispo Colombres. Sección Zoologia Agrícola, CC 9, Las Talitas (T4101XAC), Tucumán, Argentina.

[4] CONICET Sección Zoología Agrícola Estación Experimental Agroindustrial Obispo Colombres William Cross 3150 Las Talitas, 4101 Tucumán, Argentina.

strain made up the majority of the collection from corn at some Florida sites during limited, but recurring, periods in the growing season (Nagoshi and Meagher 2004a, b). The reasons for this variability are not known, but could reflect plasticity in strain behavior (Stuhl et al. 2008, Nagoshi 2011), the occurrence of interstrain hybrids (Nagoshi and Meagher 2003, Nagoshi et al. 2006a), or inaccuracies with the strain markers, among others. Attempts to find reproducible strain differences under controlled laboratory conditions have had some notable, but limited success. The two strains appear to differ in the timing of important aspects of mating behavior and in the components of the female sex pheromone (Pashley et al. 1992, Groot et al. 2008, 2010; Lima and McNeil 2009; Schofl et al. 2009; Groot et al. 2010). There are also reports of strain differences in ovipositional substrates, developmental time and viability on various hosts, and mating preferences, though these have often not been in agreement (Pashley and Martin 1987, Whitford et al. 1988, Quisenberry 1991, Pashley et al. 1995, Veenstra et al. 1995, Meagher et al. 2004, Meagher Jr. et al. 2011).

Despite these issues, the evidence for the existence of two behaviorally distinct strains is strong. The great majority of surveys comparing corn and pasture habitats in Florida consistently show the expected correlation between strain markers and host plant (Nagoshi and Meagher 2004a; Nagoshi et al. 2007c, 2008a). Similar results were obtained in studies of Brazilian fall armyworm (Nagoshi et al. 2007c, Machado et al. 2008), indicating that the strains are present in South America and are likely to be ubiquitous in the Western Hemisphere.

Critical to these studies is the availability of efficient methods of strain identification that can be used on single specimens on a large enough scale for population surveys. One such method uses haplotypes of the mitochondrial *Cytochrome oxidase subunit I* gene (*COI*), which has been shown to be at least as accurate as allozymes or whole genome approaches (McMichael and Prowell 1999). One portion of the *COI* gene has been developed as a DNA barcoding region capable of distinguishing fall armyworm from other *Spodoptera* species as well as differentiating the two strains (Nagoshi et al. 2011). A second segment of the *COI* region has been shown to not only encode for the haplotypes that identify the two strains, but also for those that can be used (in combination) to examine the geographical distribution of different populations (Nagoshi et al. 2007a, 2008b, 2010). Another gene of note is *Tpi*, which encodes for the enzyme Triose phosphate isomerase (EC 5.3.1.1). *Tpi* haplotypes can also distinguish the two strains, and because it is on the Z-chromosome it undergoes a different inheritance pattern from *COI.* This makes it useful as a complement to the *COI* method and, in combination with *COI*, provides a means to identify potential instances of interstrain hybridization (Nagoshi 2010).

The application of each of these methods on a population survey can provide substantial information about the distribution of haplotypes in a given area and on strain behavior in different habitats. As part of a long-term project to map haplotype frequencies in the Western Hemisphere we analyzed specimens from 14 fall armyworm colonies representing seven provinces in Argentina. We tested whether the *COI* and *Tpi* haplotypes capable of differentiating strains in the United States and Brazil are present in Argentina and whether they exhibit the expected biases in host plant distribution. We further compared the *COI* haplotype ratios in Argentina populations to those found in Brazil and Puerto Rico. The implications of these results on presence and behavior of the fall armyworm strains in Argentina were discussed.

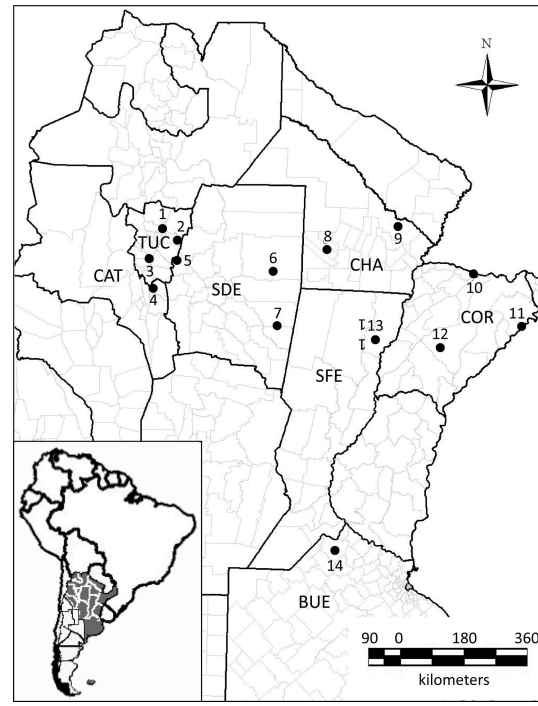

Fig. 1. Map of locations in Argentina where fall armyworm larvae were collected for the generation of colonies. Abbreviations of provinces, BUE: Buenos Aires, CAT: Catamarca, COR: Corrientes, SDE: Santiago del Estero, SFE: Santa Fé, TUC: Tucumán, CHA: Chaco. Numbers identify site locations as described in Table 1.

Materials and Methods

Insect Collections. Fall armyworm larvae were collected from commercial fields in seven Argentine provinces in January or December of 2010 and from January to February of 2011 (Fig. 1). The crops sampled were corn (*Zea mays* L.), rice (*Oriza sativa* L.), and sorghum (*Sorghum bicolor* (L.) Moench subsp *bicolor*) (Table 1). To optimize the genetic diversity present within each colony as much as practical a minimum of 250 larvae (from instars 3–6) were collected from each site and placed individually in glass tubes (12 cm high and 1.5 cm diameter) with leaves of the host plant. The collected larvae were placed in growth chambers under controlled conditions (27 ±

Table 1. Sample localities from Argentine colonies associated with host plants, year of collection, and colony identification log

Site no.	Colony	Year founded	Plant host	Collection site (nearest city)	Longitude	Latitude	Province
1	TUC[cn2]	2010	Corn	Tafí Viejo	WO 55° 13′ 59.5′	S 26° 44′ 41.2′	Tucumán
2	TUC[cn1]	2010	Corn	Los Pereyra	WO 64° 53′ 36.9′	S 26° 55′ 09.0′	Tucumán
3	TUC[cn3]	2010	Corn	Concepción	WO 65° 41′ 29.6′	S 27° 19′ 56.8′	Tucumán
4	CAT[cn]	2011	Corn	Los Altos	WO 65° 29′ 50.5′	S 28° 03′ 02.6′	Catamarca
5	SDE[cn3]	2011	Corn	YutuYacu	WO 64° 54′ 26.6′	S 27° 23′ 48.8′	Santiago del Estero
6	SDE[cn2]	2011	Corn	Quimilí	WO 62° 21.249′	S 27° 38.680′	Santiago del Estero
7	SDE[cn1]	2011	Corn	Bandera	WO 62° 08′ 41.6′	S 28° 51′ 54.4′	Santiago del Estero
8	CHA[so1]	2011	Sorghum	Las Breñas	WO 61° 02′ 37.6′	S 27° 04′ 24.5′	Chaco
9	CHA[so2]	2011	Sorghum	Gral José de San Martín	WO 59° 20′ 36.66′	S 26° 31′ 11.3′	Chaco
10	COR[ri]	2011	Rice	ItaIbate	WO 57° 39′ 50.3′	S 27° 23′ 47.9′	Corrientes
11	COR[cn1]	2011	Corn	Santo tomé	WO 56° 03′ 31.6′	S 28° 33′ 25.4′	Corrientes
12	COR[cn2]	2011	Corn	Mercedes	WO 58° 02′ 18.5′	S 29° 11′ 53.3′′	Corrientes
13	SFE[cn]	2011	Corn	Avellaneda	WO 59° 33′ 22.2′	S 28° 55′ 47.2′	Santa Fe
14	BUE[cn]	2010	Corn	Pergamino	WO 60° 39′ 45.5′	S 33° 42′ 16.7′	Buenos Aires

2°C, 70–75% RH, 14:10 L:D photoperiod) until adult emergence. Late larval instars and adults were examined using taxonomic morphological markers to confirm species. Pure cultures of each sampled crop in each province were maintained as a separate population and used to establish laboratory colonies. Sampled insects from each of these populations were deposited as voucher specimens in the collection of Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina.

Insect Rearing. Colonies were started with 200 adults raised from the collected larvae. Matings were initiated within 24 h after eclosion using four females and four males in cylindrical polyethylene-terephthalate oviposition cages (30 cm high and 10 cm diameter). For aeration, the top was covered with a nylon mesh cloth, and a hole was made on one side. The cages contained paper that allowed the females to lay eggs. Food was provided via a cotton plug saturated with honey and water (1:1) mixture, which was renewed every day. Cages were checked daily for oviposition and adult mortality. Approximately 15 egg masses were collected per cage and introduced in glass tubes (12 cm high and 1.5 cm diameter). Once emerged, 15 neonate larvae from different egg masses were isolated at random and placed individually in glass tubes with artificial larval diet (Osores et al. 1982), which was renewed every two or three days. As larvae pupated, they were placed in cylindrical cages until adult emergence. Two hundred adults were used to initiate a new generation with the same breeding procedure as described above. Individuals from the second generation and from different egg masses were preserved in 70% ethanol until DNA isolation.

***COI* Haplotype Analysis.** Genomic/mitochondrial DNA for use in polymerase chain reaction (PCR) amplifications were isolated from individual specimens using Zymo-Spin III columns (Zymo Research, Orange, CA) as described previously (Nagoshi et al. 2007a). Two methods were used to analyze *COI* haplotypes. The primers *COI*-101 F/*COI*-1058R produce a 958 bp PCR amplified product that contains an *Msp*I site at two locations, one specific to the rice-strain and the other the corn-strain (Fig. 2A). Digestion by *Msp*I produces diagnostic banding patterns of 319 and 639 bp associated with the rice-strain (*COI*-RS) or 456 and 502 bp for the corn-strain (*COI*-CS) haplotypes (Nagoshi et al. 2007b, Meagher and Nagoshi 2010). This method has the advantage of requiring *Msp*I activity to occur to diagnose either strain. Therefore, the failure of the enzyme to digest an existing site, perhaps the most likely potential technical artifact, would result in a single 958-bp band that is distinguishable from the strain diagnostic patterns.

In the second method, primers *COI*-893 F/*COI*-1303R generate a 410-bp PCR product from a more downstream region that contains a single *Eco*RV site present only in the rice-strain (Fig. 2B). The presence of two bands after *Eco*RV digestion is diagnostic of the *COI*-RS haplotype. If only one band was present, it was purified from the gel and analyzed by DNA sequencing to confirm the absence of the *Eco*RV site. In addition, sites 1164 and 1287 in this region are polymorphic within the *COI*-CS haplotype, with either an A or G observed at either site (Nagoshi et al. 2007a). This results in four possible haplotype subcategories: CS-h1 (A:A, 1164:1287), CS-h2 (A:G), CS-h3 (G:A), CS-h4 (G:G). All have been detected in surveys of fall armyworm populations in the United States and Puerto Rico, but populations can differ with respect to the ratio of CS-h4/CS-h2 (Nagoshi et al. 2007a, 2008b, 2010).

***Tpi* Haplotype Analysis.** *Tpi* haplotypes were analyzed by modifications of an earlier method that used 10 single base polymorphisms, distributed over two exons and one intron, as identified by direct DNA sequencing of PCR products (Nagoshi 2010). The original method was often ambiguous for male specimens because these are homogametic (Z/Z) in Lepidoptera and therefore carry two copies of the *Tpi* gene. As such, specimens heterozygous for size polymorphisms in the relevant intron (that were found to occur frequently) generated overlapping and out-of-register sequencing chromatographs that could not be deciphered. To avoid this problem, the method was modified to use only the subset of the strain-diagnostic polymorphisms located at the distal end of exon-4, with one of these (designated C370) associated with a polymorphic *Msp*I site (Fig. 2C). Because these sites

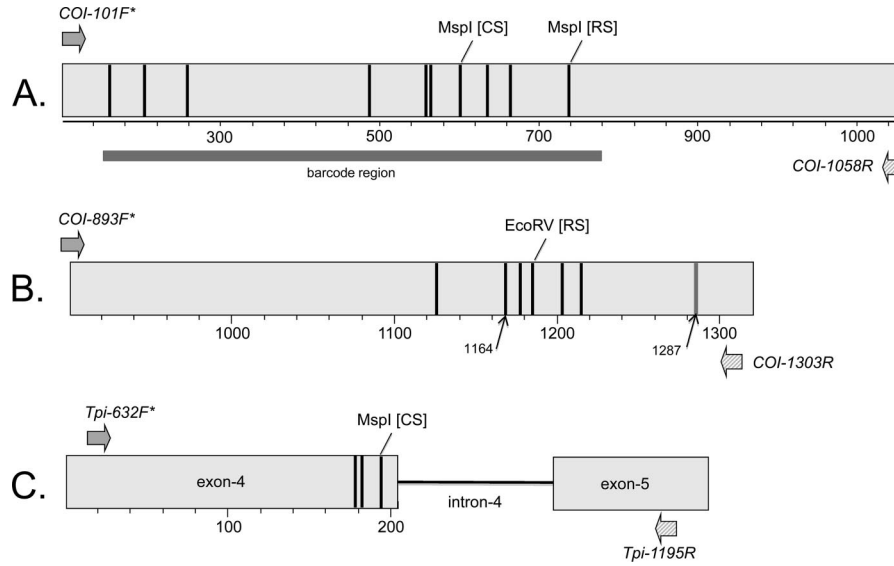

Fig. 2. Maps of the regions from the *COI* and *Tpi* genes used for the strain analysis. A. Region of the *COI* gene that includes sequences used for DNA barcoding of *Spodoptera* species (Nagoshi et al. 2011). Includes two strain-specific *Msp*I sites. B. Region of the *COI* gene that includes sites (1164, 1287) used to determine haplotype ratios for discriminating between geographically dispersed populations. C. Portion of the fall armyworm *Tpi* gene including exon-4, intron-4, and a part of exon-5. Block arrows designate locations and directions of relevant primers used for PCR amplification, with asterisks indicating primers used for DNA sequencing. Dark vertical lines specify locations of relevant strain-biased polymorphisms described in the text. Site 1287 is polymorphic but not strain-specific. CS: Corn-strain specific. RS: Rice-strain specific.

lie upstream of the variable length intron with respect to the start of DNA sequencing, intron-induced changes in the sequencing frame were made irrelevant. In this procedure, primers *Tpi*-632 F/*Tpi*-1195R were used to produce an ≈600-bp fragment from the beginning of exon-4 to the middle of exon-5, which includes the variable length intron (typically ≈250 bp in length). DNA sequencing was initiated using *Tpi*-632 F, which lies at the proximal end of exon-4. *Tpi* haplotypes as identified by this subset of polymorphisms and by the single C370 site were highly correlated with that defined when using all 10 loci (R. N Nagoshi, unpublished data). Therefore, *Tpi* haplotypes were primarily defined by examination of the C370 polymorphism as determined by sequence analysis or *Msp*I restriction mapping.

The *Msp*I-based analysis uses the same PCR-amplified fragment produced by *Tpi*-632 F/*Tpi*-1195R, which carries the C370 *Msp*I site present only in the *Tpi*-C haplotype. There is another *Msp*I site in the region contained in the 632 F primer, but its presence in both strains and proximity to the end of the fragment makes it irrelevant to this analysis. After *Msp*I digestion, the *Tpi*-C haplotype will produce at least two fragments, a diagnostic 162-bp fragment containing the region from the 632 F primer to C370, and a typically larger one that contains the variable length intron and parts of exon-4 (from C370) and exon-5. A more complicated pattern could emerge if the variable intron happens to carry a *Msp*I site, but the diagnostic 162-bp fragment will be unaffected. In comparison, the *Tpi*-R haplotype will typically be associated with a band of ≈600-bp corresponding to the uncut amplified fragment.

In addition to the two strain-diagnostic haplotypes (*Tpi*-C and *Tpi*-R) the *Tpi* analysis also detected a more ambiguous genotype that appeared to be a combination of *Tpi*-C and *Tpi*-R. This was characterized in DNA sequence analysis by overlapping chromatographs at the strain-diagnostic polymorphic loci, with the two curves specifying the alternative strain-specific nucleotides. In the *Msp*I digestions, it appeared as an overlap in the strain-specific restriction patterns with the bands diagnostic of both strains present. It is possible that these represent *Tpi*-C/*Tpi*-R heterozygotes where both haplotypes are present in the same specimen because of interstrain mating, but could also be the result of sample contamination. These ambiguous samples were rare, representing <4% (13/340) of the total specimens tested, and were not included in the data analysis.

PCR Amplification and Restriction Enzymes Digestion. PCR amplification for all reactions used the profile: 94°C (1 min), followed by 32 cycles of 92°C (30 s), 56°C (30 s), 72°C (1 min), and a final segment of 72°C for 3 min. For direct DNA sequencing, the PCR-amplified fragments were isolated from 1.5% agarose gels using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, CA). The isolated fragments were sent to the University of Florida ICBR center for DNA sequence analysis. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Amplification of the *COI* region used the primer pairs *COI-101 F* (5′- TTCGAGCTGAATTAG-

GAACTC-3′) and *COI-1058R* (5′- ACACCTGTTAATCCTCCTACAG-3′) or *COI-893 F* (5′-CACGAGCATATTTTACATCWGCA-3′) and *COI-1303R* (5′-CAGGATAGTCAGAATATCGACG-3′). Amplification of the *Tpi* gene used primers *Tpi*-632 F (5′-GGTTGCCCATGCTCTTGAGTCCGGACTGAAGg-3′) and *Tpi*-1195R (5′-AGTCACTGACCCACCATACTG-3′). Digestions with restriction enzymes (New England Biolabs, Beverly, MA) used manufacturer-provided buffers. Each reaction used 10–20 U of restriction enzyme and was incubated at 37°C for 3 h to overnight. For gel electrophoresis, 6 μl of 6× gel loading buffer was added to each reaction and the entire sample run on a 2% agarose horizontal gel containing GelRed (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 ultra violet light box using the Alpha Imager Mini photodocumentation system (Cell Biosciences Inc., Santa Clara, CA).

DNA Sequence Analysis. DNA comparisons, alignments, and restriction site mapping were performed using Geneious Pro 5.4.3 (Biomatters Ltd., Auckland, New Zealand). Statistical analyses involving correlations and paired *t*-tests were performed with GraphPad InStat version 5.1, GraphPad Software, San Diego, CA (www.graphpad.com). Sequence divergences among individuals were calculated using the Tamura-Nei genetic distance model (Tamura and Nei 1993) and unweighted pair-group method with arithmetic average analysis (Sneath and Sokal 1973). Argentine *S. frugiperda COI* haplotypes identified and used in this study have been deposited in GenBank with voucher specimens deposited at CMAVE (Gainesville, FL). GenBank accession numbers of sequences used in this study are *S. littoralis* (HM756074.1), *S. litura* (HM756090.1), *S. dolichos* (HM756086.1), *S. pulchella* (HM756075.1), *S. exigua* (HM756077.1), *S. eridania* (HM756085.1), FAW (fall armyworm) corn-strain (HM136586.1), FAW rice-strain (HM136593.1), FL COI-RS (JN621261), FL COI-CS (JN573290), ArgR1–2 (JN621268–9), ArgC1–6 (JN621262–7).

Results

DNA Sequence Comparisons. Laboratory colonies derived from 14 different collection sites representing seven Argentine provinces provided the genetic material for this study (Table 1; Fig. 1). Subsets of these colonies were used as needed to estimate the diversity of Argentine fall armyworm populations with respect to the genetic markers commonly used to identify and categorize U.S. populations.

To preliminarily assess the genetic similarity between Argentine and United States fall armyworm, DNA sequence comparisons were made using a portion of the *COI* gene (extending from sites 167–773, Fig. 2A) being developed as a barcoding region to discriminate between *Spodoptera* species (Nagoshi et al. 2011). DNA sequences from nineteen specimens representing six Argentine colonies, including those from the most eastern (TUC), northern (CHA), western (COR), and southern (BUE) collection sites were compared with the barcodes diagnostic of seven *Spodoptera* species (Fig. 3). All 19 Argentina samples clustered with fall armyworm, segregating with the consensus barcodes of one or the other strain. The strain segregation is based on the presence of nine strain-specific, single nucleotide polymorphisms within the 606-bp barcoding segment (Nagoshi et al. 2006b, 2011). Each Argentine specimen carried the complete set of nucleotides consistent with either the *COI*-RS or *COI*-CS haplotypes (Table 2), providing evidence that the genetic markers indicative of fall armyworm host strains are intact in Argentine populations.

More extensive sampling was done in a more downstream region of the *COI* gene as part of the study investigating haplotype ratios. The 200-bp region between 1100 and 1300 contains six strain-specific, single-nucleotide, polymorphisms (Nagoshi et al. 2007a, 2008b; Fig. 2B). Sequence analysis of 167 Argentine specimens representing 11 colonies from seven provinces found that 163 carried the six nucleotide combinations at the strain-specific sites indicative of either *COI*-RS or *COI*-CS, including site 1182 responsible for a rice-strain specific *Eco*RV restriction site (Table 2). The four exceptions matched *COI*-CS at five sites, but carried an A typically associated with *COI*-RS at site 1197 (Table 2). Sequence comparisons identified an additional polymorphic site in the *COI*-RS group creating two haplotypes designated ArgR1 and ArgR2. The *COI*-CS group had five additional polymorphic sites, creating six additional *COI*-CS haplotypes designated as ArgC1–6. Phylogenetic analysis showed that the ArgR and ArgC sequences clustered with the fall armyworm *COI*-RS and *COI*-CS consensus haplotypes, respectively (Fig. 4).

Polymorphisms in the *Tpi* gene provide a Z-chromosome alternative to *COI* for identifying fall armyworm strains (Nagoshi 2010). Sequence comparisons of a portion of exon-4 showed identity between the sequences of 148 Argentine samples and the consensus sequences from fall armyworm (Table 2). All polymorphisms were located at three sites previously associated with strain specificity (Nagoshi 2010; unpublished results), with the Argentine sequences displaying either the *Tpi* haplotype designating the rice-strain (*Tpi*-R) or corn-strain (*Tpi*-C). The association of an *Msp*I restriction site with one of these loci (C370) indicates that restriction mapping using this enzyme can be used as a substitute for DNA sequencing.

Association of Strain Markers With Plant Hosts. Having established that the strain-diagnostic *COI* and *Tpi* haplotypes were present in Argentina, we tested whether they were associated with the expected host plants. This was largely the case (Table 3). The *COI*-CS haplotype was the majority in nine of the 11 colonies derived from corn-feeding larvae, with an average frequency of 87% for those nine populations and an overall average of 74% for the 11 colonies. All the samples tested from the two sorghum-derived colonies were also *COI*-CS, while 97% of the single colony derived from larvae feeding on rice was of the *COI*-RS

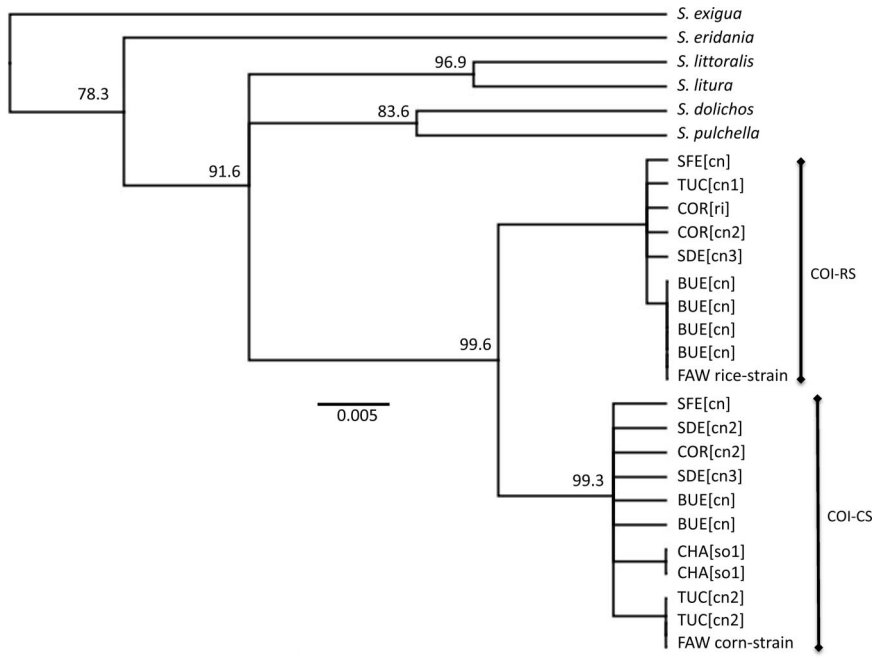

Fig. 3. Consensus phylogenetic tree comparing Argentina specimens with barcode region from several *Spodoptera* species. The 606-bp portion of the *COI* region between primers *COI*-101 F/*COI*-1058R was compared from multiple samples representing nine Argentine colonies. The unrooted phylogenetic tree was derived using the Tamura-Nei genetic distance model (Tamura and Nei 1993) and unweighted pair-group method with arithmetic average analysis (Sneath and Sokal 1973), with confidence assessed by bootstrapping at 1,000× repetition. *Spodoptera* species are represented by consensus sequences derived from Nagoshi et al. (2011). Only confidence values above 75% are indicated and nodes below 70% are collapsed. Scale bar measures substitutions per site.

haplotype (Table 3). Two colonies from corn, SDE[cn2] and BUE[cn], were the exceptions as they displayed large *COI*-RS majorities of 75 and 95%, respectively.

The colonies were reexamined for the *Tpi* haplotypes. In total, 327 samples from the 14 colonies were tested at various times by DNA sequencing or *Msp*I restriction mapping. Both methods were used to an-

Table 2. Nucleotide composition at strain-diagnostic sites in tested regions of the *COI* and *Tpi* genes

Strain analysis	*n*	Nucleotide position									
A. *COI* (58F/914R)		171	207	258	489	564	570	600[a]	634	663	738[b]
Consensus: *COI*-RS[c]		C	A	T	C	C	T	T	C	A	G
COI-CS		T	T	C	T	T	C	C	T	T	A
Argentina: *COI*-RS	9	C	A	T	C	C	T	T	C	A	G
COI-CS	10	T	T	C	T	T	C	C	T	T	A
Other	0	—	—	—	—	—	—	—	—	—	—
B. *COI* (893F/1303R)		1125	1164	1176	1182[d]	1197	1216				
Consensus: *COI*-RS[e]		C	T	C	T	A	A				
COI-CS		T	G/A	T	C	G	T				
Argentina: *COI*-RS	62	C	T	C	T	A	A				
COI-CS	101	T	A	T	C	G	T				
Other	4	T	A	T	C	A	T				
C. *Tpi* (632F/1095R)		C351	T355	C370[a]							
Consensus: *Tpi*-R[f]		T	C	T							
Tpi-C		C	T	C							
Argentina: *COI*-RS	34	T	C	T							
COI-CS	114	C	T	C							
Other	0	—	—	—							

Argentine samples were compared with the consensus strain sequences derived from Florida populations.
[a] Associated with corn-strain specific MspI site.
[b] Rice-strain specific MspI site.
[c] Nagoshi 2007 (7651).
[d] Rice-strain specific EcoRV site.
[e] Nagoshi 2007 (4729).
[f] Nagoshi 2010 (7652).

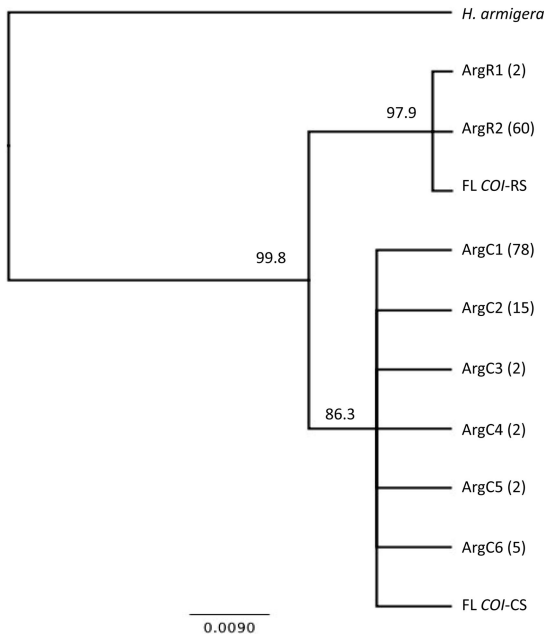

Fig. 4. Consensus phylogenetic tree comparing observed Argentina *COI* haplotype variants with the consensus strain haplotypes derived from United States populations. *COI*-RS (ArgR1–2) and *COI*-CS (ArgC1–6) variants were detected from the sequence analysis of a 240-bp fragment of the *COI* region used to determine haplotype ratios. Number of each haplotype found is in parentheses. Phylogeny was based on the Tamura-Nei genetic distance model (Tamura and Nei 1993) and using unweighted pair-group method with arithmetic average analysis (Sneath and Sokal 1973). Only confidence values above 75% are indicated and nodes below 70% are collapsed. Scale bar equals 0.009 substitutions per site. The *COI* sequence from *H. armigera* was used as an outgroup. Numbers at branches denote bootstrap values (%) based on number per 1,000 replicates.

alyze the same 82 specimens and in each case were in complete agreement on the designated *Tpi* haplotype. Therefore, the data from the two procedures were pooled (Table 3). Of the 11 colonies from corn-feeding fall armyworm, *Tpi*-C averaged 90% of the sampled population compared with 74% for *COI*-CS. In 13 of the 14 colonies the *Tpi* and *COI* methods were in agreement about the majority strain, and a two-tailed paired *t*-test analysis of these 13 colonies showed a significant positive correlation ($r = 0.97$; $P < 0.0001$). The only substantial deviation between *Tpi*-C and *COI*-CS was observed with the BUE[cn] colony, which had a *COI*-CS proportion of 5% compared with 100% for *Tpi*-C. In contrast, the other corn colony that showed a *COI*-RS majority (SDE[cn2]) produced only a modest difference between methods, with a *COI*-CS proportion of 25% compared with 33% for *Tpi*-C.

Association of *COI* and *Tpi* Haplotypes. The *COI* and *Tpi* strain diagnostic haplotypes were both determined in 333 specimens, allowing a direct assessment of their association. In 12 of the 14 colonies (262 sampled specimens) the concordance between *COI* and *Tpi* was >70%, with an average of 92%. The two outlying colonies were those derived from corn-feeding larvae that exhibited a *COI*-RS majority. The SDE[cn2] colony had 67% concordance between the *Tpi* and *COI* haplotypes with a roughly equal split between the *COI*-RS *Tpi*-C and *COI*-CS *Tpi*-R discordant classes (Table 3). In contrast, the BUE[cn] colony displayed only a 5% concordance frequency, with all 41 of the discordant samples of the *COI*-RS *Tpi*-C configuration. Even when excluding the BUE[cn] colony, *COI*-RS *Tpi*-C was the most common discordant class, representing 90% (18/20) of the discordant configurations from the other 13 colonies (Table 3).

Assessing Corn-Strain Haplotype Ratios. Polymorphisms at sites 1164 and 1287 in the *COI* gene generate four *COI*-CS haplotypes, CS-h1–4 (Nagoshi et al. 2007a). The ratio between the CS-h4 and CS-h2 haplotypes is particularly significant as it can differentiate between populations from Florida and Texas (Nagoshi et al. 2008b). A total of 105 *COI*-CS specimens from a sampling of Argentine colonies from seven provinces

Table 3. Comparison of *COI* and *Tpi* strain identifications for the 14 Argentina colonies

Site no.	Colony	Host plant	No. samples			Frequency			No. discordants	
			n (*COI*)	*n* (*Tpi*)	*n* (*COI*+*Tpi*)	*COI*-CS	*Tpi*-C	Concordant[a]	*COI*-CS *Tpi*-R	*COI*-RS *Tpi*-C
14	BUE[cn]	Corn	44	43	43	0.05	1.00	0.05	0	41
4	CAT[cn]	Corn	25	24	20	0.76	1.00	0.71	0	3
11	COR[cn1]	Corn	28	24	24	1.00	1.00	1.00	0	0
12	COR[cn2]	Corn	29	19	18	0.72	0.74	0.95	0	0
7	SDE[cn1]	Corn	30	22	22	0.77	0.82	0.86	0	3
6	SDE[cn2]	Corn	16	15	15	0.25	0.33	0.67	2	3
5	SDE[cn3]	Corn	20	20	20	0.85	1.00	0.85	0	3
13	SFE[cn]	Corn	25	22	22	0.88	1.00	0.86	0	3
2	TUC[cn1]	Corn	28	24	22	0.93	1.00	0.83	0	2
1	TUC[cn2]	Corn	18	18	18	0.94	1.00	0.94	0	1
3	TUC[cn3]	Corn	30	29	29	1.00	1.00	1.00	0	0
8	CHA[so1]	Sorghum	23	22	22	1.00	1.00	1.00	0	0
9	CHA[so2]	Sorghum	27	16	16	1.00	1.00	1.00	0	0
10	COR[ri]	Rice	30	29	29	0.03	0.00	1.00	0	0
		Total	373	327	320				2	59
Average frequency in corn hosts						0.74	0.90	0.79		
Average frequency in corn hosts with *COI*-CS majority						0.87	0.95	0.89		

[a] *COI*-CS *Tpi*-C or *COI*-RS *Tpi*-R.

Table 4. Comparison of corn-strain haplotypes (CS-h) derived from polymorphic loci 1164 and 1287 in the *COI* gene for fall armyworms from various locations

Location	CS-h1	CS-h2	CS-h3	CS-h4	CS-h4/ CS-h2
Argentina (2010–11)	22	83	0	0	0.00
Puerto Rico 2007, 2009[a]	13	42	13	161	3.83
Brazil 2005–2007[b]	56	172	0	9	0.05
Florida 2006–2007[c]	7	53	1	154	2.91
Texas 2006–2007[c]	52	211	2	49	0.23

[a] Nagoshi et al. 2010.
[b] Nagoshi et al. 2007.
[c] Nagoshi et al. 2008.

were tested for these corn-strain haplotypes (Table 4). Only CS-h1 and CS-h2 were found, with CS-h2 being the more common overall. The absence of CS-h4 gives the pooled Argentine sampling a CS-h4/CS-h2 haplotype ratio of 0.00, more similar to what has been observed in Brazil (0.05) and Texas (0.23) than in Florida (2.91) or Puerto Rico (3.83).

Discussion

This study took advantage of the availability of multiple colonies to assess the presence of the fall armyworm host strains in Argentina. While the colonies provided a convenient source of specimens, it is questionable how accurately they represented the genetic diversity of the source populations. This stems from practical limitations in the number of parents that can be used to initiate each generation under artificial rearing conditions. Despite efforts to maintain the genetic diversity of the wild populations in the laboratory colonies, the possibility of genetic bottlenecks and subsequent founder effects cannot be discounted. In addition, the artificial rearing protocol could generate unforeseen selection for or against different genotypes. Such effects would tend to reduce genetic diversity and could even skew the frequency distribution of genetic markers away from that present in the field populations. Given these considerations we assumed that while the colonies may generally reflect the predominant haplotypes at each collection site, they would trend toward homogeneity, with individual colonies potentially displaying idiosyncratic deviations. To accommodate these concerns several colonies were examined and the results compared for consistency. The primary focus was on the fall armyworm infesting corn, where 11 independently derived colonies were obtained from six different provinces. Three additional colonies, two derived from larvae feeding on sorghum and one from rice, were available for comparison.

The results provide strong evidence that the two strains as defined by genetic haplotypes are present in northern Argentina and display the expected behaviors with respect to plant host preference and mating choice. These conclusions are based on three observations. First, the consensus *COI* and *Tpi* haplotypes for the fall armyworm strains are defined by the pattern of specific nucleotides present at multiple polymorphic sites. All the Argentine specimens examined carried the DNA sequences representative of *COI*-CS/*COI*-RS as determined by two regions of the *COI* gene. This was indicated by phylogenetic analyses where all the Argentine samples clustered with the fall armyworm strain-specific haplotypes (Figs. 3 and 4). Similarly, all 148 specimens tested displayed either the consensus *Tpi*-C or *Tpi*-R haplotypes (Table 2). These results indicate that the strain-specifying polymorphisms in the *COI* and *Tpi* genes from Argentina populations are identical to those found in fall armyworm sampled from the rest of the Western Hemisphere.

Second, in the great majority of the colonies derived from corn hosts, the predominant haplotypes were those associated with the corn-strain. *COI*-CS was the majority haplotype in nine of 11 corn-derived colonies (Table 3). The *Tpi*-C haplotype was equally predominant, found in an average of 93% of the samples in 10 of 11 corn colonies. The two colonies derived from sorghum, another corn-strain preferred host, also showed a strong *COI*-CS and *Tpi*-C bias, while the single colony derived from rice was predominantly *COI*-RS. Therefore, despite the potential artifacts associated with sampling colonies, the results obtained were generally consistent with strain-biased plant host preference. It is important to note from past studies that the linkage of strains to specific host plants is more a bias than an absolute, with several instances noted when strain proportions in a given habitat conflicted with expectations (Nagoshi and Meagher 2004a, Prowell et al. 2004). This may at least in part be because of variability in the linkage of the genetic markers to strain identity, which appears to be the case with the corn-derived BUE[cn] colony. Here the aberrant *COI*-RS majority is contradicted when examined for the *Tpi*-C haplotype, suggesting that this colony could represent a corn-strain population where the association with *COI*-CS has been compromised. It is also possible that the behavior of the strains themselves can be variable. There are observations of times and locations when a high proportion of the *COI*-RS haplotype is repeatedly found in corn-dominated habitats, suggesting that there are conditions when the rice-strain will use its normally less preferred host (Nagoshi and Meagher 2004a). This appears to be the case represented by the SDE[cn2] colony, where both *COI* and *Tpi* analysis suggest a rice-strain majority even though the founder larvae were collected from corn.

Third, it was found in 13 of the 14 colonies tested strong concordance between the *COI* and *Tpi* haplotypes. The mitochondrial *COI* and Z-linked *Tpi* genes are not physically joined and so are capable of independent segregation. Therefore, the observed linkage between the *COI* and *Tpi* strain haplotypes is an indication of behavioral or physiological restrictions that favor reproduction within rather than between strains. Specifically, matings between females of a given *COI* cytotype and males of the corresponding *Tpi* haplotype must be more frequent or successful than the discordant pairing.

The exception was the colony BUE[cn] from the Buenos Aires province, the most southern of the collection sites, where over 90% of the sampled population was of the discordant genotype *COI*-RS *Tpi*-C. Whether this combination was an accurate representation of the local population or an artifact of artificial rearing is unclear and can only be resolved by additional and more direct surveys of field collections. Interestingly, mating studies using specimens from the Buenos Aires colony gave unusual results (Murúa et al. 2008). When females from colonies derived from northern Argentine provinces were mated to Buenos Aires males, they were found to carry significantly fewer spermatophores than occurred in the reciprocal cross or when mated to local males. This suggests unidirectional incompatibility between the Buenos Aires colony and other populations. However, a subsequent study examining heterotypic crosses between another Buenos Aires colony (derived from larvae found on corn) with a colony (from fall armyworm feeding on rice) from the Corrientes province showed no evidence of incompatibility (M. G Murúa, unpublished data). The Buenos Aires region is distinct from the other sites tested in this study in that the climate in the Pampean region does not support overwintering of fall armyworm populations (Murúa and Virla 2004). Therefore this area is repopulated annually from migratory populations whose origins have yet to be determined. The actual consequences of this are unknown, but it could result in higher levels of mixing between genetic subgroups, including the host strains, and therefore increased variability in genotype and reproductive behaviors.

The comparison of haplotype proportions within the corn-strain has provided a useful tool for identifying the natal origins of migratory fall armyworm populations (Nagoshi et al. 2008b). It has also identified two groups in the Western Hemisphere in which genetic exchange is at least partially restricted (Nagoshi et al. 2010). One group (FL-PR) is associated with permanent populations in Florida, Puerto Rico, and transient populations along the U.S. eastern coast that result from annual migrations from Florida. The other group (TX-BZ) has established populations in Texas and Brazil with annual migrations into sections of North America west of the Appalachian mountain range. The two groups differ in the ratio of the CS-h4/CS-h2 haplotypes, with the FL-PR ratio >1.5 (CS-h4>CS-h2) and TX-BZ <0.5 (Table 4). This ratio difference has been observed since 2002 (Nagoshi et al. 2009), indicating that population exchanges between the two groups are not large enough to significantly alter the frequency distributions of the relevant haplotypes. The observation that the Argentine haplotype ratios are more similar to Brazil than the Caribbean is not surprising given that Argentina and Brazil are neighboring countries. But the absence of CS-h4 in Argentina suggests only minimal interactions with fall armyworm originating from Puerto Rico. These discontinuities in haplotype distributions suggest that a detailed hemispheric genetic mapping of fall armyworm populations would provide insight into the pattern of fall armyworm movements in the Western Hemisphere.

In summary, the availability of colonies from different host plants and provinces in Argentina made it possible to establish that the strain-specifying haplotypes characterized in fall armyworm from other locations are present in Argentina and are generally distributed in a manner consistent with strain-specific plant host preferences. Strain-specific mating behavior is also preserved as indicated by the linkage between the *COI* and *Tpi* haplotypes. However, more detailed descriptions of strain behavior and distributions in the wild will require the direct analysis of specimens collected from the field. While the majority of the colonies displayed the haplotype proportions expected from their host plant origins, there were exceptions that illustrate the need for genetic markers to characterize the colony population for its predominant strain. Fortunately, methods are now available using PCR and restriction mapping that simplify this procedure and do not require DNA sequencing analysis.

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