Identification and Comparison of Fall Armyworm (Lepidoptera: Noctuidae) Host Strains in Brazil, Texas, and Florida

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ABSTRACT Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a major economic pest throughout the Western Hemisphere. Studies of populations in the southern United States and the Caribbean demonstrated the existence of two morphologically identical but genetically distinct host strains. These races can be distinguished by using polymorphisms in the mitochondrial *cytochrome oxidase I* gene that define two distinct maternal lineages that correlate with strain-specific behaviors in Florida populations. Although there is evidence of different biotypes in Brazil, it has not been demonstrated that these biotypes are equivalent to the U.S. strains. Sampling from Brazil indicates that its fall armyworm populations consist of the two strain-specific haplotypes found in Florida and also display the expected biases in plant host distribution. The same genetic markers also were present in samples from Texas, a major source of migrating fall armyworm in North America. These results indicate that the biology and behaviors of Brazilian fall armyworm populations are similar to those found in North America.

KEY WORDS fall armyworm, *Spodoptera frugiperda*, haplotype

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) is a pest of a number of agricultural crops. In the United States, fall armyworm is responsible for substantial economic damage to field and sweet corn, *Zea mays* L., sorghum, *Sorghum vulgare* Pers., and several turfgrass varieties (Sparks 1979, Pashley 1988b, Foster 1989). It also is considered a sporadic, but important, late-season pest of cotton, *Gossypium hirsutum* L., and sugarcane, *Saccharum* spp. L. (Hall 1988, Pashley 1988b). In Brazil, fall armyworm has long been considered one of the primary pests of maize and has more recently been observed infesting cotton (Martinelli et al. 2006).

The highly polyphagous behavior of fall armyworm in the United States and the Caribbean is in part due to the presence of two strains that differ in host preference (Pashley 1986, Prowell et al. 2004). The corn (C) strain is associated with maize and sorghum, whereas the rice (R) strain is preferentially found in rice and turfgrass. The two strains are morphologically identical, so they can only reliably be distinguished by molecular methods, most notably allozyme polymorphisms (Pashley 1986), genetic polymorphisms (Lu et al. 1992, McMichael and Prowell 1999, Prowell et al. 2004), and mitochondrial haplotyping (Pashley and Ke 1992, Lu and Adang 1996). The molecular differences correlate with differences in physiology, development, and behavior that are consistent with genetically distinct populations (Pashley 1988a, Whitford et al. 1988, Pashley et al. 1995, Veenstra et al. 1995). An important consideration for pest management is that the two strains differ in their susceptibility to chemical and biological agents. R-strain larvae were more susceptible to transgenic *Bacillus thuringiensis* (Bt) Berliner cotton and to the insecticides diazinon and carbaryl, whereas the reverse was true for carbofuran (Pashley et al. 1987, Adamczyk et al. 1997). In addition, several Bermuda grass, *Cynodon dactylon* (L.) Pers., cultivars showed differential resistance to the two strains (Leuck et al. 1968, Lynch et al. 1983, Pashley et al. 1987, Quisenberry and Whitford 1988, Jamjanya et al. 1990).

Evidence that the same two strains exist in South America is suggestive but inconclusive. Amplified fragment length polymorphism (AFLP) analysis of larvae collected from maize and rice plants in several locations in Brazil was consistent with the existence of two distinct populations specific to each plant host (Busato et al. 2004). Fall armyworm lines derived from larvae collected from either maize or rice also differed in physiology, development, and insecticide susceptibility (Busato et al. 2005a,b, 2006). However, another AFLP study comparing populations from Brazil, Argentina, Mexico, and the

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Table 1. Source locality and host information

Location	Collection	Plant/habitat	Date	Source	Sample (in Fig. 2)
Gainesville, FL	Colony	Artificial	Oct. 2004	R.L.M.	GN-FL1-4
Homestead, FL	Pheromone	Corn	July 2002	R.L.M.	HM-FL1
Homestead, FL	Pheromone	Corn	Feb. 2004	R.L.M	HM-FL2
Homestead, FL	Pheromone	Corn	Oct.–Nov. 2004	R.L.M	HM-FL3-6
Homestead, FL	Pheromone	Corn	Nov. 2005	R.L.M	HM-FL7-10
Homestead, FL	Larva	Corn	Jan 2005	R.L.M.	HM-FL11
Ona, FL	Pheromone	Pasture	April–May 2005	R.L.M.	ON-FL1-4
Avon Park, FL	Larva	Corn	Nov. 2003	—[a]	AP-FL1-2
Belle Glade, FL	Larva	Corn	Nov. 2003	—[a]	BG-FL1-2
Okeechobee, FL	Larva	Sorghum (sorghum/ sudan hyb.)	Oct.–Nov. 2003	R.L.M.	
Weslaco, TX	Pheromone	Corn	April 2004	B. Warfield	WL-TX1-3
College Station, TX	Pheromone	Corn	April–Oct. 2004	J.L.	CS-TX1-13
College Station, TX	Larva	Corn	Sept. 2004	J.L.	CS-TX14-16
Palotina, Parana, Brazil	Larva	Corn	Feb. 2005	P.S.	PN-BR1-7
Palotina, Parana, Brazil	Larva	Corn	Dec. 2005	P.S.	
Campo Verde, Mato Grosso, Brazil	Larva	Pasture	Oct. 2005	P.S.	MT-BR1-5
Campo Verde, Mato Grosso, Brazil	Larva	Poaceae	Nov. 2005	P.S.	MT-BR6-9
Campo Verde, Mato Grosso, Brazil	Larva	Millet	Nov. 2005	P.S.	MT-BR10-12
Campo Verde, Mato Grosso, Brazil	Larva	Corn	Jan. 2005	P.S.	MT-BR13-14
Campo Verde, Mato Grosso, Brazil	Larva	Sorghum (BF80)	Mar.–April 2005	P.S.	MT-BR15-23
Primavera do Leste, Mato Grosso, Brazil	Larva	Sorghum (SARA)	Oct.–Nov. 2005	P.S.	
Primavera do Leste, Mato Grosso, Brazil	Larva	Amaranthus	Nov. 2005	P.S.	
Primavera do Leste, Mato Grosso, Brazil	Larva	Millet	Nov. 2005	P.S.	MT-BR24-26
Primavera do Leste, Mato Grosso, Brazil	Larva	Cotton	Dec. 2005	P.S.	
Primavera do Leste, Mato Grosso, Brazil	Larva	Corn	Oct.–Nov. 2005	P.S.	
Rio Grande do Sul, Brazil	Larva	Rice, corn	Mar. 2005	V.M.	

[a] Nagoshi and Meagher (2004).

United States found high genetic variability in larvae collected from corn, with some samples showing close genetic similarity to larvae collected from turfgrass in the United States (Clark 2005). Allozyme and mitochondrial haplotype analyses of larvae from French Guiana identified the two strains, but there was no evidence of plant host specificity as the R-strain predominated in both corn- and turf-dominated habitats (Prowell et al. 2004). The seeming inconsistencies in the results from the different groups, the wide geographical distribution of the tested populations, and the different methodologies used to identify strains make it difficult to assess whether the strains identified in U.S. populations are genetically comparable with the biotypes described in South America.

In this study, we used the mitochondrial *cytochrome oxidase I* (*COI*) gene to identify and compare haplotypes present in Brazil with those in Texas and Florida. Fall armyworm in these two states are the primary source of the migratory populations that infest central and eastern North America (Sparks 1979), but relatively little is known about the haplotypes present in the Texas population. We further tested whether the haplotypes present in Brazil showed comparable biases in host plant distribution to that observed for the two U.S. host strains. The implications of these results on the use of the strain-specific haplotype markers to study geographically separated fall armyworm populations were discussed.

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 Unitraps were baited with a commercially available fall armyworm pheromone (Scenturion Inc., Clinton, WA), 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 identified by morphological criteria. These were then preserved in 100% ethanol until DNA isolation or were placed individually in 22.5-ml (0.75-oz.) plastic cups with artificial diet (Heliothis Premix, Stonefly Industries, Bryan, TX) to complete development. DNA was isolated from either adults or late (postfourth) 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 6000 × *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-

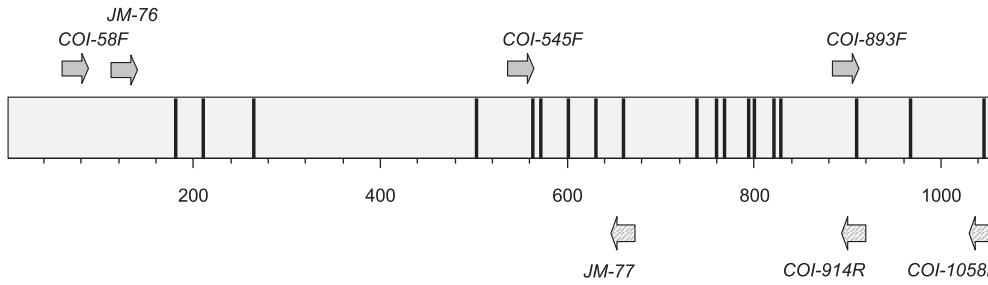

Fig. 1. Diagram of the portion of the mitochondrial *COI* gene analyzed along with relevant primers. The putative translational start site of the *COI* gene was arbitrarily designated as coordinate 0. Vertical lines within the gene indicate sites of the strain-specific nucleotide substitutions described in the text. Arrows indicate location and direction of primers used for the PCR amplification and DNA sequencing. Primers *JM-76* and *JM-77* were described previously (Levy et al. 2002).

Spin III column (Zymo Research, Orange, CA) and processed according to 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.

PCR Analysis and Cloning. PCR amplification of the mitochondrial *COI* gene was performed in a 30-μl reaction mix containing 3 μl of 10× reaction buffer, 1 μl of 10 mM dNTP, 0.5 μl of 20 μM primer mix, 1 μl of DNA template (0.05–0.5 μg), and 0.5 μl of *Taq*DNA polymerase (New England Biolabs, Beverly, MA). The thermocycling program was 94°C (1 min), followed by 33 cycles of 92°C (45 s), 56°C (45 s), 72°C (1 min), and a final segment of 72°C for 3 min. The appropriate restriction enzyme was diluted in 1× of the manufacturer's reaction buffer to a concentration of 1 U/μl, and 5 μl was added to 8 μl of each PCR reaction. Digestions were at 37°C for 1–2 h. Two microliters of 6X gel loading buffer was added to each sample, which was run on a 1.8% PCR grade agarose (Fisher, Hampton, NH) horizontal gel. Typically, 40 or 96 PCR amplifications and restriction digests 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 *JM76* (5′-GAGCTGAATTAGGRACTCCAGG-3′) and *COI-1058R* (5′-ACACCTGTTAATCCTCCTACAG-3′) to produce a 1.0-kb fragment (Fig. 1).

For fragment isolations 6 μl of 6X 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 0.5X 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 cut out from the gel. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research, Orange, CA) according to manufacturer's instructions. When necessary, fragments were subcloned into pGEM-T-Easy vector (Promega, Madison, WI) according to manufacturer's instructions. DNA was isolated from subclones using the GenElute 5-min Plasmid Miniprep kit (Sigma-Aldrich, St. Louis, MO) according to manufacturer's instructions.

DNA Sequence Analysis. DNA sequencing was performed by Northwoods DNA, Inc. (Bemidji, MN). Templates were either the fragment-isolated PCR product (0.5 μg) or the fragment subcloned into pGEM-T-Easy (1.0 μg). Direct sequencing of the fragment isolations was initially performed using *JM-76*, *COI-1058R*, and *COI-914R* (5′-ACACCT GTTAATCCTCCTACAG-3′) (Fig. 1). Sequencing of the subclones used the SP6 Promoter (5′-GAT TTAGGTGACACTATAG-3′), the T7 Promoter (5′-TAATACGACTCACTATAGGG-3′), and *COI-914R*. The sequences obtained were then aligned, and a consensus sequence was derived for each strain haplotype.

To optimize the accuracy of the DNA sequences the following strategy was used. The sequence of each sample was compared with the consensus sequences of both the R-strain and C-strain COI regions. If variations from the consensus were found, the region in question was sequenced again using one or more of these primers as needed: *COI-545 F*, 5′-TTTGAGCT GTAGGTATTACYG-3′; *JM77*, 5′-ATCACCTCCWC CTGCAGGATC-3′; and *COI-914R*, 5′-GCWGATG TAAAATAWGCTCGWG-3′. If the variation from consensus was confirmed, a second PCR reaction was performed from the sample genomic DNA, and the DNA sequence was analyzed. It is highly unlikely that the same random amplification error will occur in two independent reactions. If discrepancies between the two sequences were found, a third PCR reaction was performed and compared. By this strategy, each variation from the consensus sequence was confirmed by two independent PCR reactions, and the DNA sequence was analyzed. 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). Phylogenetic trees were generated using neighbor-joining analysis and Tajima-Nei distance by using the DS Gene program (Tajima and Nei 1984, Felsenstein 1985). Trees were unrooted, and nodes were supported by 10,000× bootstrap analysis.

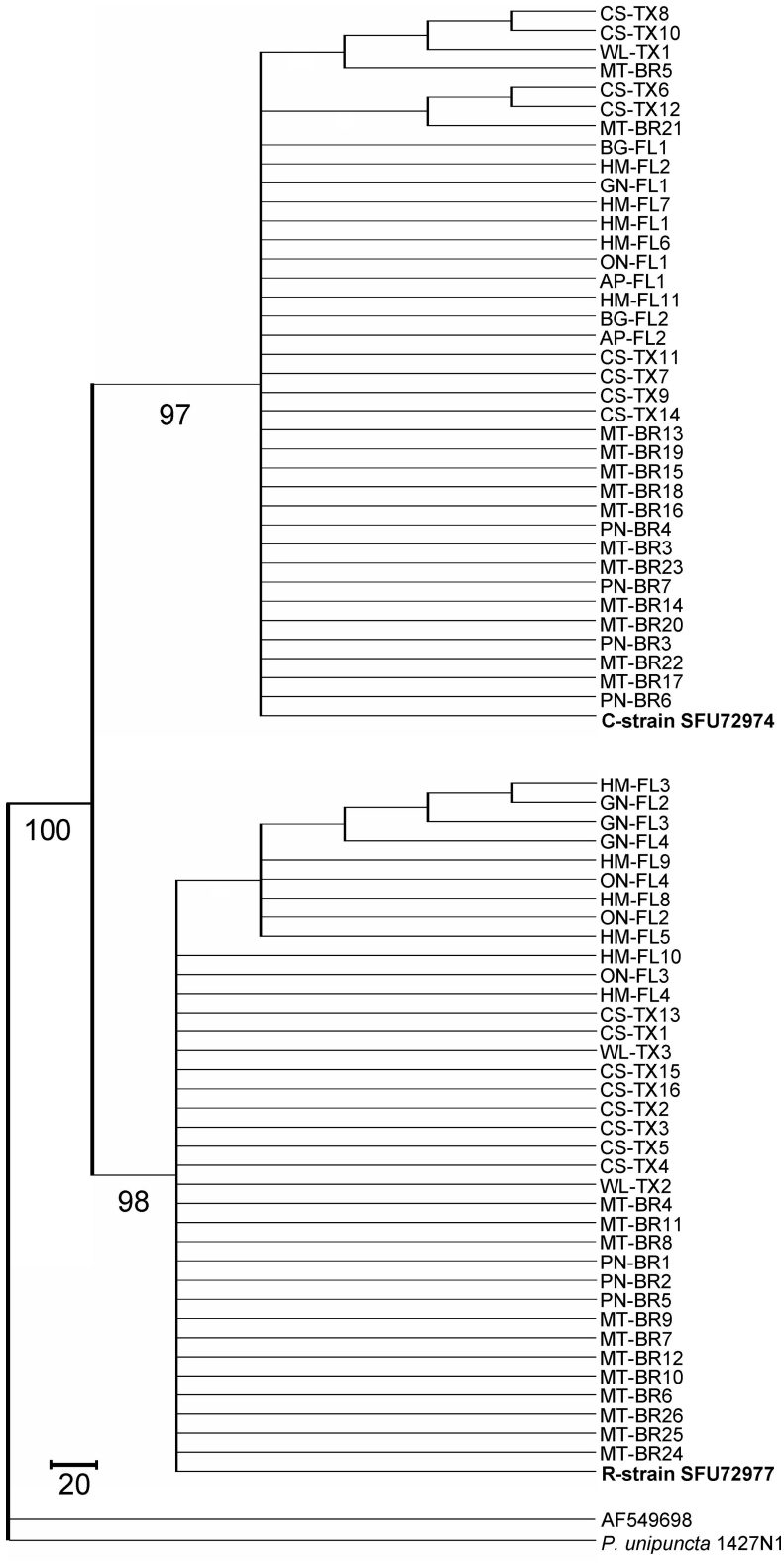

Fig. 2. Strict consensus tree based on neighbor-joining analysis of 73 *COI* sequences representing fall armyworm populations from Florida (FL), TX (TX), and Brazil (BR). The R-strain (SFU72977) and C-strain (SFU72974) sequences were obtained from GenBank and used to identify the host strain haplotypes. The COI sequences from *Pseudaletia unipuncta* (Haworth) were used as an outgroup. The sequences were obtained from GenBank (AF549698) and from a specimen isolated from the field (our unpublished data). Numbers under branches denote bootstrap values (%) based on number per 10000 replicates. Only confidence values above 75% are indicated and nodes below 50% are collapsed. Scale bar equals 0.2 substitutions per site.

Table 2. Distribution of polymorphisms at the strain-specific loci in the mitochondrial *COI* gene for fall armyworm strains collected from Florida, Texas, and Brazil

	171		207		*Sac*I [R] 258		489		*Aci*I [R] *Bsm*I[C] 564		570		*Msp*I [C] 600		634		663		*Msp*I [R] 738	
	C[a]	T	A	T	T	C	C	T	C	T	T	C	T	C	C	T	A	T	G	A
FL-R	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0
FL-C	0	11	0	11	0	11	0	11	0	11	0	11	0	11	0	11	0	11	0	11
BR-R	14	0	14	0	14	0	14	0	14	0	14	0	14	0	14	0	14	0	14	0
BR-C	0	16	0	16	0	16	0	16	0	16	0	16	2	14	0	16	0	16	0	16
TX-R	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0
TX-C	0	9	0	8[b]	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9

	759		768		795		798		816		823		912		987		1044	
	C	T	T	C	T	C	A	G	T	C	T	C	T	C	C	T	A	T
FL-R	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	7	5
FL-C	0	11	0	11	0	11	0	11	0	11	0	11	0	11	0	11	0	11
BR-R	14	0	14	0	14	0	14	0	14	0	14	0	14	0	14	0	11	3
BR-C	0	16	0	16	0	16	0	16	0	16	0	16	0	16	0	16	1	15
TX-R	9	0	9	0	9	0	9	0	9	0	9	0	9	0	9	0	5	4
TX-C	0	9	1	8	0	9	0	9	0	9	0	9	0	9	0	9	0	9

[a] C, T, A, and G represent the cytosine, thymine, adenine, and guanine nucleotides, respectively.
[b] In one sample, a C was present.

Results

A 937-bp portion of the mitochondrial *COI* gene was isolated and sequenced from a total of 73 individuals from Brazil, Florida, and Texas (Fig. 1). The samples were obtained by either pheromone trapping or larval collection from a variety of plant types, including maize, cotton, sugarcane, millet, sorghum, and pasture grasses (Table 1). To determine whether the sequences could be partitioned into two groups associated with the known host strains, neighbor-joining reconstruction analysis was performed, and a phylogenetic tree was constructed (Fig. 2). The sequences from all areas could be subdivided into two major groups that corresponded with the consensus R-strain and C-strain sequences (GenBank accession nos. U72977 and U72974, respectively). Subsets from the Texas and Brazil samples were present in each, indicating that the strain-specific haplotypes characterized in Florida populations are also present in fall armyworm from these other locations.

Sequence Characterization of the Strain-Specific Sites. The strain-specific subpopulations were defined by 19 polymorphic nucleotide sites (Table 2). Three of the sites (their coordinates of 258, 564, and 600 indicate nucleotide distance from the presumptive translational start site of the COI gene) were described previously in sequence, and restriction enzyme analyses of an internal 568-bp region was defined by the *JM76/JM77* primers (Levy et al. 2002, Nagoshi et al. 2005). An additional strain-specific *Msp*I site was observed at site 738 that is present in the R-strain but not the C-strain. In 16 of the 19 sites, there was 100% strain specificity for fall armyworm samples from all locations (Table 2). Two sites showed single exceptions; at site 207 one C-strain sample from Texas had a C instead of the expected T (found in the rest of the C-strain), and at site 768 one C-strain sample carried the R-strain–associated A instead of the expected T. The most polymorphic site was at coordinate 1044, which was specific for the C-strain (with one exceptional sample from Brazil) but variable in the R-strain for populations from all three locations. In the latter case, the exceptions resulted from the substitution of the normally C-strain–specific T instead of the expected A. None of the base changes in these strain-specific sites alter the conceptual COI amino acid sequence.

Additional polymorphisms were observed in 24 other nucleotide loci that were not specific to a strain. In combination with the variations in the strain-specific sites these identified a total of 28 different haplotypes, 14 each in the C-strain and R-strain subpopulations. We designated the RS1 and CS1 haplotypes as the consensus sequences for the R-strain (Table 3) and C-strain (Table 4), respectively, and these differed by no more than three nucleotides from the other variants within each strain. Six of these polymorphisms alter the conceptual peptide sequence by a single base substitution.

RS1 was the most common R-strain haplotype overall, representing 42% (15/36) of the R-strain population (Table 5). Similarly, CS1 was predominant in the C-strain, making up 51% (19/37) of this subgroup. However, there was substantial variability in the distribution of the haplotypes between geographical areas. For example, in the Florida samples the proportion of the RS3 haplotype approximated that of the RS1, whereas RS3 was not found in Brazil or Texas. In addition, although the C-strain samples from Brazil consisted of nine different haplotypes, each present in roughly equal proportions, CS1 was the predominant C-strain haplotype in Texas and Florida. The majority

Table 3. Haplotypes identified from fall armyworm collected from Brazil, Texas, and Florida for the R-strain

Haplotype	132	133	143	204	268	357	430	444	570	596	606	633	666	839	848	879	891	1044
RS1	A	A	A	A	G	A	A	T	T	T	T	T	A	T	T	A	T	G
RS2																		A
RS3												C						A
RS4														C[a]				
RS5		G															A	
RS6			G[a]															
RS7				G														
RS8						G	T											A
RS9									C									A
RS10											C							A
RS11													G					
RS12																G		
RS13	G							C							C[a]			
RS14					A[a]					G[a]								

[a] Causes an amino acid substitution.

of haplotypes of either strain, 22 of 28, were represented by only a single specimen.

Brazilian Strains Display Plant Host Preferences Similar to U.S. Populations. In the Rio Grande do Sul state, contemporaneous larval collections from corn and rice plants showed a definitive strain-specific host preference, with the C-strain predominating in corn and absent in rice (Fig. 3). The C-strain also predominated in corn from the Mato Grosso state throughout the year, making up >80% of the larvae collected as well as in spring-grown cotton (in Brazil, spring is approximately from September to November). The R-strain was the predominant population found in millet and in two common pasture plants, one of the *Amaranthus* genus, the other of the Poaceae family. A more variable pattern was observed in sorghum. During two collection periods in March and April, almost 90% of the larvae were of the C-strain, consistent with the C-strain bias reported previously for Honduras and Georgia (U.S.) sorghum (Pashley 1988b, Lu and Adang 1996). However, as the year progressed, the proportion of C-strain larvae declined (Fig. 3). To investigate whether a similar pattern could be found in U.S. populations, we analyzed larvae collected from sorghum in the Okeechobee region of Florida in 2003 (Fig. 4). Variability in strain proportions was observed between the two collection periods, with the C-strain percentage changing from 0 to 60 in a 2-wk period.

Discussion

Fall armyworm populations in Brazil consist of the same two mitochondrial lineages that define the two host strains in North American populations. The Brazilian lineages also displayed similar asymmetries in plant host use, with the C-strain predominating in corn and cotton and the R-strain in millet and a pasture grass. The specificity of C-strain larvae to corn and R-strain to turf/pasture grasses was demonstrated previously for populations in the Caribbean, Florida, and Louisiana (Pashley 1986, Meagher and Gallo-Meagher 2003, Meagher and Nagoshi 2004, Prowell et al. 2004). That cotton is preferred by the C-strain was demonstrated for fall armyworms collected in Louisiana and the Mississippi delta (Pashley 1988a; our unpublished data). These data indicate that the same two strains exist in both Brazil and the United States and display similar behaviors with respect to host plant choice.

A more complicated behavior is exhibited with respect to sorghum, which was presumed to be preferred by the C-strain based on studies of samples from Honduras and Mississippi (Pashley 1988b, Lu and

Table 4. Haplotypes identified from fall armyworm collected from Brazil, Texas, and Florida for the C-strain

Haplotype	173	207	600	666	711	717	768	788	851	858	925	974	1020	1044
CS1	A	T	T	A	T	A	T	A	T	T	A	A	A	A
CS2				G										
CS3					C									
CS4													G	
CS5								G[a]						
CS6									G					
CS7							T							
CS8		C												
CS9										C				
CS10						G								
CS11			T											
CS12											G			G
CS13												G		
CS14	G													

[a] Causes an amino acid substitution.

Table 5. Distribution of haplotypes in different locations

Haplotype	Locality			n
	Florida	Texas	Brazil	
RS-1	3	6	6	15
RS-2	0	3	3	6
RS-3	4	0	0	4
RS-4	1	0	0	1
RS-5	1	0	0	1
RS-6	1	0	0	1
RS-7	1	0	0	1
RS-8	1	0	0	1
RS-9	0	1	0	1
RS-10	0	0	1	1
RS-11	0	0	1	1
RS-12	0	0	1	1
RS-13	0	0	1	1
RS-14	0	0	1	1
Total	12	10	14	36
CS1	8	9	2	19
CS2	0	2	1	3
CS3	0	3	1	4
CS4	1	0	0	1
CS5	1	0	0	1
CS6	1	0	0	1
CS7	0	1	0	1
CS8	0	1	0	1
CS9	0	0	1	1
CS10	0	0	1	1
CS11	0	0	1	1
CS12	0	0	1	1
CS13	0	0	1	1
CS14	0	0	1	1
Total	11	16	10	37

Adang 1996). However, larvae collected from sorghum in Brazil showed substantial variation in strain proportions, with C-strain values ranging from 13 to 92% (Fig. 3). Similar differences were observed in collections from sorghum in Florida, suggesting that the strain preference for this plant type is significantly influenced by environmental factors (Fig. 4). The role of seasonality was indicated by the observation that the C-strain predominated (89%) in Brazil sorghum during the autumn (March–June), but it declined to 50% of the collections in the spring (September–November), with substantial variation in strain proportions between the three spring collections (Fig. 3). Interestingly, we also observed seasonality in strain use of corn in southern and central Florida (Nagoshi and Meagher 2004). Although the proportion of larvae found in corn during the Florida spring season (February–April) was heavily biased to the C-strain, there were periods in the fall (September–November) when R-strain larvae were the majority collected (Fig. 4). The reason for this seasonality is unknown, but it could reflect migration behaviors, seasonal differences in the availability of alternative plant hosts, or other season-specific environmental factors that can overrule strain-specific physiological preferences.

These observations demonstrate that conclusions of plant host preferences based on sampling over a limited time can be misleading. This is particularly true for the R-strain, which seems to be able to use host plants normally associated with the C-strain, at least during certain times of the year. This flexibility is consistent with laboratory studies showing that although the two strains can differ in their use of, and development on, specific plant hosts, they are both able to develop to adulthood on these hosts with relatively small differences in viability and no re-

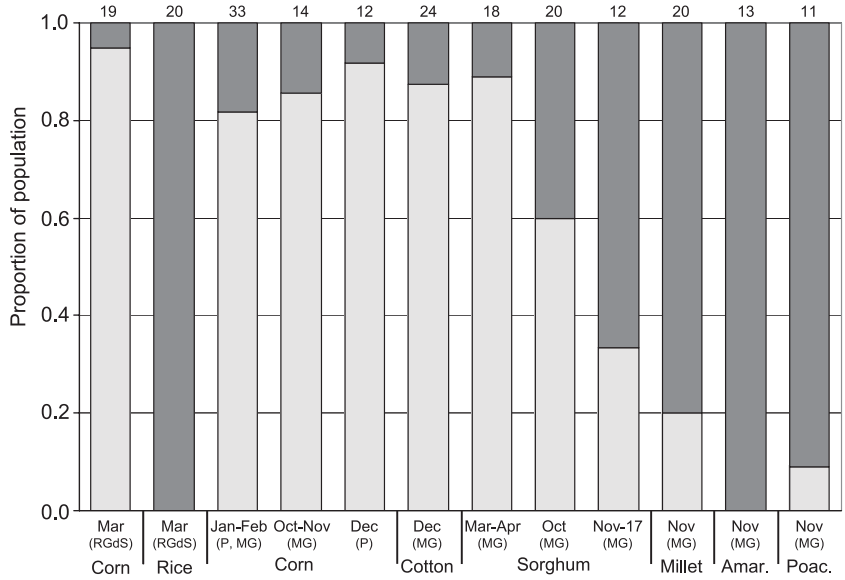

Fig. 3. Strain proportions in larvae collected from different plant hosts in Brazil. Solid bar denotes R-strain, striped bar denotes C-strain. Numbers above bars indicate number of samples tested. All dates are from 2005. Note that seasons in Brazil are reversed from that observed in Florida such that spring occurs during September–November and fall during March–June. RGdS, Rio Grande de Sol; P, Parana; MG, Mato Grosso; Amar., Amaranthus; and Poac., Poaceae.

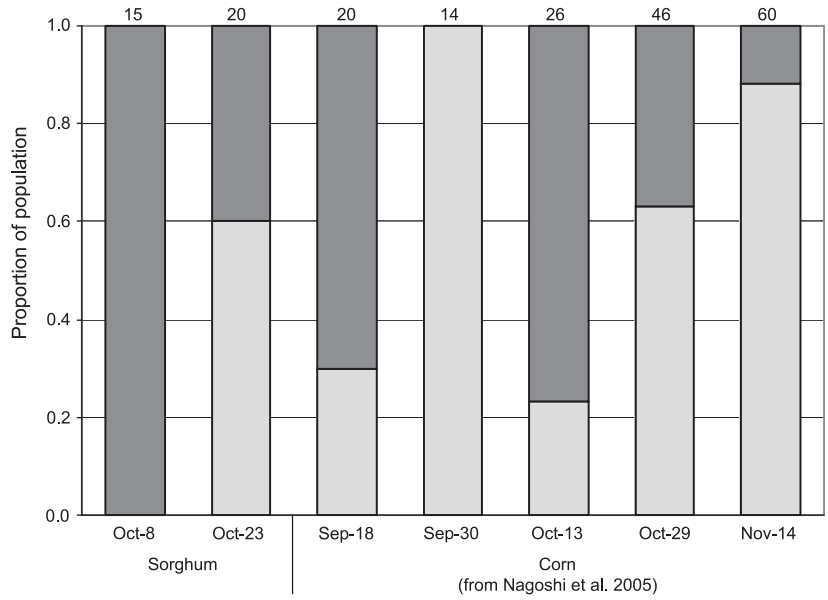

Fig. 4. Strain proportions in larvae collected from corn and sorghum in Florida. Solid bar denotes R-strain, striped bar denotes C-strain. Numbers above bars indicate number of samples tested. The sorghum collections were from Okeechobee, FL. Larval collections from corn were from Belle Glade, FL. All samples were collected in 2003.

ported differences in adult fitness (Pashley 1988a, Veenstra et al. 1995).

Our analysis of the *COI* region showed >99% identity between the Brazil, Texas, and Florida mitochondrial sequences for each strain haplotype. Furthermore, the strain specificity of the 19 sites was conserved in all three populations, as was the high rate of polymorphism in site 1044 for the R-strain. Therefore, it seems that either the divergence of the two mitochondrial lineages into the two strains occurred before the dissemination of fall armyworm into Texas, Florida, and Brazil, where they are now reproductively isolated, or there is substantial gene flow occurring that has essentially homogenized these geographically distant populations. Geographical isolation would be supported by the existence of genetic polymorphisms that are specific to the geographical areas. Three candidate polymorphisms were observed. Site 633 is present in one third of the Florida R-strain tested, but it was not found in Brazil or Texas samples. Site 717 and site 666 were found in one third and two ninths of the Texas C-strain, respectively, but not in the Florida C-strain. More extensive surveys of these polymorphisms are being performed to more accurately assess their geographical distribution.

The demonstration that the same strain-specific mitochondrial markers identify equivalent strains in both Brazil and U.S. fall armyworm populations should facilitate research on this important agricultural pest in South America. Relatively simple and rapid methods are available for characterizing these markers, allowing for more extensive sampling of population distributions, and the commonality in strains and mitochondrial lineages legitimatize comparisons with North American surveys. Comparisons of fall armyworm behavior in such different environments should provide new insight into the biology underlying host plant choice and migration, and further characterization of genetic differences between strains and geographically distant populations will potentially identify new markers for investigating these issues.

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