# Comparison of Haplotype Frequencies Differentiate Fall Armyworm (Lepidoptera: Noctuidae) Corn-Strain Populations from Florida and Brazil

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J. Econ. Entomol. 100(3): 954–961 (2007)

**ABSTRACT** Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a major economic pest throughout the Western Hemisphere. Populations can be subdivided into two morphologically identical but genetically distinct strains (corn-strain and rice-strain) that differ in their host plant preferences. These strains can be distinguished by using polymorphisms in the mitochondrial *cytochrome oxidase I* gene. Additional sequence analysis of this locus identified two sites that were highly polymorphic in the corn-strain population and that produced four different haplotype subgroups. Comparisons of the frequency distribution of these haplotypes found no seasonal or plant host specificities, but they did demonstrate that the Brazil corn-strain population is different from corn-strain fall armyworm found in Florida. The development of a rapid means of distinguishing fall armyworm populations originating from Brazil versus Florida provides an opportunity for investigating and comparing the genetic complexity and long-range movements of this important agricultural pest.

**KEY WORDS** *Spodoptera frugiperda*, *cytochrome oxidase I*, armyworm

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a major agricultural pest of most of the Western Hemisphere, with a host range extending from southern Canada to central Argentina (Ashley 1986). In the United States, fall armyworm is a consistent pest of corn, *Zea mays* L., sorghum (*Sorghum* spp.), and turfgrass (Sparks 1979, Pashley 1988a, Foster 1989), and it sporadically causes significant damage to cotton, *Gossypium hirsutum* L., and sugarcane (interspecific species of *Saccharum*) (Hall 1988, Pashley 1988b). In South America, fall armyworm is one of the primary pests of maize and cotton (Martinelli et al. 2006).

A contributor to the widespread distribution of fall armyworm is its capacity for long-distance movements. Because fall armyworm does not diapause, its appearance in areas with freezing winters arises from populations that overwinter in milder climates and migrate in the spring. This movement has been studied in some detail in North America where fall armyworm infesting central and eastern United States and southern Canada are part of an annual migration from sites in Mexico, Texas, and Florida (Luginbill 1928, Rose et al. 1975, Young 1979, Pair et al. 1987). Movements of fall armyworm in Central and South America have not been studied extensively, but presumably they also could occur in response to seasonal changes in rainfall, temperature, and agricultural plantings.

Studies primarily on North American and Caribbean populations indicated the existence of two strains that differ in host preference, physiology, behavior, and pesticide susceptibility (Leuck et al. 1968; Lynch et al. 1983; Pashley 1986, 1988a; Pashley et al. 1987, 1995; Whitford et al. 1988; Jamjanya et al. 1990; Quisenberry 1991; Whitford et al. 1992; Veenstra et al. 1995; Prowell et al. 2004). The corn-strain is associated with maize and sorghum, whereas the rice-strain is found preferentially in rice and turfgrass. Fall armyworm has been reported to be able to colonize >60 different plant hosts, but whether host strain preferences exist for most of these is unknown (Luginbill 1928). The two host strains are morphologically identical, and they can only be reliably 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).

Studies in South America identified two biotypes that displayed plant host preferences and physiological, developmental, and pesticide susceptibility differences that seemed analogous to the two North American host strains (Busato et al. 2004, 2005a, 2005b, 2006). More recently, we demonstrated that the mitochondrial haplotypes diagnostic of the North

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**Table 1. Source locality and host information. *S. frugiperda* were collected as noted from the listed plant type or plant dominated habitats and during the given dates**

| Location | Collection | Plant/habitat[a] | Date | No.[b] | Source |
|---|---|---|---|---|---|
| Key West, FL | Pheromone | Turf | Aug. 2002 | 15 | R.L.M. |
| Miami-Dade Co [1], FL | Pheromone/larvae | Corn | 2003–2006 | 54 | R.L.M. |
| Miami-Dade Co [2], FL | Pheromone | Corn | 2002–2003 | 40 | R.L.M. |
| Hague, FL | Pheromone | Corn | 2005–2006 | 43 | R.L.M. |
| Quincy, FL | Pheromone | Corn | June 2003 | 24 | R.L.M. |
| Avon Park, FL | Larvae | Corn | Nov. 2003 | 72 | [c] |
| Belle Glade, FL | Larvae | Corn | Nov. 2003 | 26 | [c] |
| Okeechobee, FL | Larvae | Sorghum hybrid | Oct.–Nov. 2003 | 14 | [c] |
| Homestead, FL | Larvae | Sorghum hybrid | July 2006 | 20 | R.L.M. |
| Palotina, Parana, Brazil | Larvae | Corn | Feb.–Dec. 2005 | 29 | P.S. |
| Campo Verde, Mato Grosso, Brazil | Larvae | Corn | Jan.–Nov. 2005 | 18 | P.S. |
| Campo Verde, Mato Grosso, Brazil | Larvae | Sorghum hybrid | Mar.–April 2005 | 16 | P.S. |
| Primavera do Leste, Mato Grosso, Brazil | Larvae | Sorghum hybrid | Oct.–Nov. 2005 | 15 | P.S. |
| Primavera do Leste, Mato Grosso, Brazil | Larvae | Cotton | Dec. 2005 | 14 | P.S. |
| Primavera do Leste, Mato Grosso, Brazil | Larvae | Corn | Oct.–Nov. 2005 | 23 | P.S. |

[a] Turf, turfgrass field with various species; corn, *Z. mays.*; sorghum, *S. bicolor;* and cotton, *G. hirsutum.*
[b] Number of samples.
[c] Nagoshi et al. (2006).

American host strains exist in South America and display similar plant host specificities (Nagoshi et al. 2007). This suggests either that the divergence of the two host strains preceded the dispersion of fall armyworm into North and South America or that there is substantial interaction between these geographically distant populations. Supporting the latter are genetic studies suggesting substantial gene flow between populations in the Caribbean region and mainland North America (Pashley 1986), and among fall armyworm populations in Argentina, Mexico, and Mississippi (Clark et al. 2007).

However, there is also evidence of physiological differences between populations that are consistent with reproductive isolation caused by geographical separation in the tested areas. Studies comparing susceptibility to pesticides found evidence that fall armyworm populations sampled from several sites east of the Mississippi River were significantly different from those on the western side (Young 1979). This suggests that overwintering populations in Florida (Mitchell et al. 1991), the likely source of fall armyworm in the eastern United States, may be isolated and diverging from fall armyworm arising in Texas and Mexico, the presumed source of more western populations. In addition, comparisons of female pheromones from fall armyworm populations in Brazil, the Caribbean, Mexico, and the United States. identified differences in either composition or response that seemed specific to geography (Andrade et al. 2000, Batista-Pereira et al. 2006). It therefore remains unclear the extent to which the dispersed populations of fall armyworm in the Western Hemisphere genetically interact.

The objective of this study was to identify molecular methods for distinguishing between geographically distant fall armyworm populations that might in the longer term be used to follow their relative distributions and interactions. Sequence analysis of the mitochondrial *cytochrome oxidase I* (*COI*) gene was performed to find haplotype variants within the corn-strain population in Florida. The distributions of the identified variants were tested for distribution patterns that might suggest geographical, seasonal, or plant host specificities. These results were compared with fall armyworm populations from Brazil to assess whether behavioral and genetic differences exist between the corn-strain from these two geographically distant areas.

## Materials and Methods

**Specimen Collections and Sites.** Fall armyworm specimens were obtained at several locations in Florida (larvae and adults) and Brazil (larvae) (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 lures, Suterra, Bend, OR), and contained insecticide strips (Hercon Environmental Co., Emigsville, PA). Collections from traps were made at various intervals, ranging from 1 to 14 d. After collection, specimens were stored frozen 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 (Florida specimens; Guy et al. 1985; Brazil specimens; Heliothis Premix, Stonefly Industries, Bryan, TX) to complete development. DNA was isolated from either adults or late (post-fourth) instars.

**DNA Preparation.** Individual specimens were homogenized in 4 ml of phosphate buffered saline (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 at 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

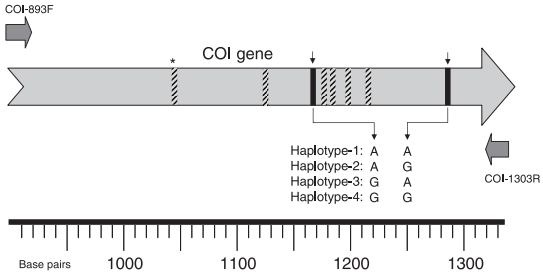


**Fig. 1.** Diagram of the portion of mitochondrial *COI* gene amplified by PCR and the relevant primers. The putative translational start site of the *COI* gene was arbitrarily designated as coordinate 0. Vertical lines within the gene indicate sites of the strain-specific nucleotide substitutions described in Table 2. Horizontal arrows indicate location and direction of primers used for the PCR amplification and DNA sequencing. Vertical arrows indicate positions of the sites used to generate the four haplotype subgroups. Asterisk identifies site previously described in Nagoshi et al. (2006).

precipitated by the addition of 100 μl of 8 M potassium acetate. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Orange, CA) and 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 Amplification and DNA Analysis.** PCR amplification of the mitochondrial *COI* gene was performed in a 30-μl reaction mix containing 3 μl of 10× manufacturer's reaction buffer, 1 μl of 10 mM dNTP, 0.5 μl of 20 μM primer mix, 1 μl of DNA template (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. Typically, 96 PCR amplifications were performed at the same time by using either 0.2-ml tube strips or 96-well microtiter plates. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Amplification of the *COI* region used the primer pair COI-893F (5′-CACGAGCATATTTTACA TCWGCA-3′) and COI-1303R (5′-CAGGATAGTCA GAATATCGACG-3′) to produce a 410-bp fragment (Fig. 1). Corn-strain samples were identified by digestion of the PCR product with EcoRV (New England Biolabs). 0.5 μl of EcoRV was added to each reaction and incubated at 37°C for 1 h. Five microliters of gel loading buffer was added to each sample, and the entire reaction was loaded on a 1.8% agarose horizontal gel containing a 1:10,000 dilution of GelRed (Biotium, Hayward, CA) in 0.5× Tris-borate buffer (45 mM Tris base, 45 mM boric acid, and 1 mM EDTA pH 8.0). Fragments were visualized on a long-wave UV light box. The corn-strain product is uncut, leaving a single 410-bp band, whereas the rice-strain product is cut once to produce two bands of 289 and 121 bp. The corn-strain products were cut out from the gel, and the fragments were isolated using Zymo-Spin I columns (Zymo Research) according to manufacturer's instructions. The purified fragments were analyzed by DNA sequencing performed by Northwoods DNA, Inc. (Bemidji, MN). All other DNA sequences were obtained from National Center for Biotechnology Information GenBank. DNA comparisons, alignments, and restriction site mapping were performed using the DS Gene program (Accelrys, San Diego, CA).

**Statistical Analysis.** Haplotype subgroups for each category (location, host plant, or time of season) were compared in contingency tables using Fisher exact test (PROC FREQ, SAS Institute 2003).

## Results

Our previous sequence analysis of a portion of the mitochondrial *COI* gene identified several nucleotide polymorphisms that were diagnostic of fall armyworm host strain identity (Nagoshi et al. 2006). We extended the analysis to sequences immediately downstream of this region and identified seven additional strain-specific sites (Fig. 1). At five of the sites the polymorphic nucleotide was >99% fixed in each strain, making these useful diagnostic markers of strain identity (Table 2). In particular, the polymorphism at site 1182 results in an EcoRV restriction site present in rice-strain DNA but not in the corn-strain, allowing the identification of strains without DNA sequencing. The remaining two strain-specific loci were fixed in the rice-strain, but they displayed relatively high polymorphism within the corn-strain population. At site 1164, all rice-strain samples carried a thymidine (T), whereas the Florida corn-strain population was split between those with either guanosine (G; 66/105 = 63%) or adenosine (A; 37%). In comparison, the Brazil corn-strain samples were 95% (18/19) of the A haplotype. At site 1287, the Brazil corn-strain population showed

**Table 2. Strain-specific polymorphic sites and the number of *S. frugiperda* carrying each nucleotide (A, adenosine; C, cytosine; G, guanosine; T, thymidine) in Florida and Brazil samples**

| Coordinates | 1044[a] | | 1125 | | 1164 | | | 1176 | | 1182 | | 1197 | | 1216 | | 1287 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | G | C | T | T | G | A | C | T | T | C | A | G | A | T | A | G |
| FL-R | 5 | 24 | 28 | 0 | 29 | 0 | 0 | 29 | 0 | 29 | 0 | 29 | 0 | 29 | 0 | 10 | 0 |
| FL-C | 102 | 3 | 0 | 105 | 0 | 66 | 39 | 0 | 105 | 0 | 105 | 1 | 104 | 0 | 105 | 7 | 98 |
| BR-R | 6 | 14 | 18 | 2 | 20 | 0 | 0 | 20 | 0 | 20 | 0 | 20 | 0 | 20 | 0 | 15 | 0 |
| BR-C | 18 | 1 | 0 | 19 | 0 | 1 | 18 | 0 | 19 | 0 | 19 | 0 | 19 | 0 | 19 | 6 | 12 |

FL-R, Florida, rice-strain; FL-C, Florida, corn-strain; BR-R, Brazil, rice-strain; BR-C, Brazil, corn-strain.
[a] Previously described in Nagoshi et al. (2006).

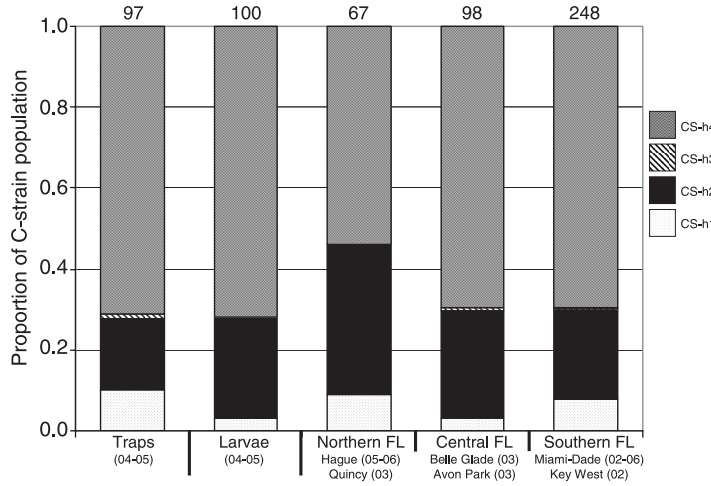


**Fig. 2.** Proportions of *S. frugiperda* corn strain haplotypes present in samples collected from different locations in Florida. Haplotypes were determined by analysis of the COI gene sequence as described in Fig. 1. Numbers above bars indicate number of samples tested.

substantial polymorphism with 33% (6/18) carrying the A nucleotide. This contrasted with 93% (98/105) of the Florida corn-strain samples carrying a G at this site. The observed polymorphisms at sites 1164 and 1287 generate four possible haplotype subgroups that we have designated CS-h for Corn-Strain Haplotype subgroup. These subgroups are defined as CS-h1 (A [1164] A [1287]), CS-h2 (A G), CS-h3 (G A), and CS-h4 (G G) (Fig. 1).

**Geographical and Seasonal Distribution of Haplotypes in Florida Fall Armyworm.** Specimens were obtained from pheromone trapping or larval collections in several locations and times throughout Florida and confirmed as corn-strain by sequence examination of the five diagnostic sites (1125, 1176, 1182, 1197, and 1216) (Table 2). The two polymorphic sites (1164 and 1287) were used to categorize the corn-strain samples into the four haplotype subgroups. In contemporaneous sampling over a 2-yr period, the larval and pheromone trap collection methods both produced the same general haplotype distribution pattern of CS-h4 > CS-h2 > CS-h1, with only a small number of the CS-h3 haplotype (Fig. 2). Variation at borderline statistical significance ($P = 0.073$) was observed that primarily reflected differences in the levels of the CS-h1 haplotype relative to CS-h2.

Newly collected and archived specimens were used to compare haplotype distribution patterns between geographically distant areas in Florida, extending from Key West as the southernmost collection site to Quincy in the Florida panhandle, a distance of 1,066 km (Table 1). The results were pooled into three groups representing collections from "southern" (south of the Everglades), "central" (between the Everglades and Orlando), and "northern" (north of Orlando) Florida (Fig. 2). All three groups showed the same general distribution pattern as the pooled traps and larval collections, even though some sampling dates differed by as many as 4 yr. The CS-h4 haplotype was the majority subgroup, followed by CS-h2, with the two accounting for >85% of the corn-strain population in each area. The CS-h1 haplotype was present at variable and low levels, making up 4–13% of corn-strain captures. Again, the CS-h3 haplotype was rarely observed. The variation among locations was of borderline statistical significance ($P = 0.075$).

Archived samples were used to examine the seasonal distribution of the haplotypes over a 3-yr period from 2003 to 2006 (Fig. 3). Only fall armyworm collected from corn-dominated habitats in Miami-Dade Co. were examined to limit variation due to geographic factors and differences in plant hosts. In this region, fall armyworm normally displays two population peaks, one peak during the spring (February–June) and the other peak in the fall (October–December) that generally coincides with the sweet corn growing seasons (Nagoshi and Meagher 2004). The pooled data show very little difference between the haplotype distributions during the spring and fall seasons ($P = 0.795$). There was greater variation with the yearly comparisons, not surprising given the relatively small sample sizes for individual seasons. Nevertheless, the distribution pattern of CS-h4 > CS-h2 > CS-h1 was still consistently observed, with the CS-h1 haplotype again showing a minor and sporadic distribution and the CS-h3 haplotype rarely found.

**Distribution of Corn-Strain Haplotypes in Brazil.** Larvae from Brazil were obtained from several plant hosts in the southern state of Parana and the more central state of Mato Grosso. Both sites displayed similar haplotype proportions ($P = 0.460$) that differed markedly from that observed in Florida ($P < 0.001$; Fig. 4). The Brazil populations displayed a haplotype relationship of CS-h2 > CS-h1 > CS-h4, with no CS-h3 haplotype collected. The CS-h4 haplotype, the dominant subgroup in Florida, was found in <10% of the samples from either Brazilian state.

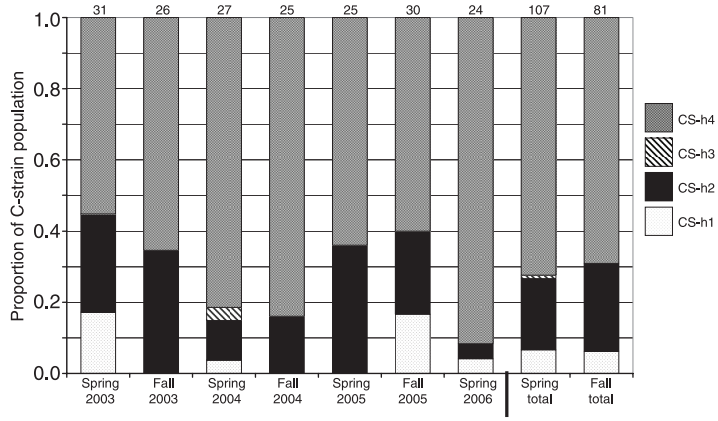


**Fig. 3.** Proportions of *S. frugiperda* corn strain haplotypes present in samples collected from corn growing sites in Miami-Dade Co., FL. Haplotypes were determined by analysis of the COI gene sequence as described in Fig. 1. Spring represents the period between February and June, where fall is from September to December. Numbers above bars indicate number of samples tested.

**Distribution of Haplotypes among Plant Types.** The two host strains of fall armyworm were originally identified by the finding of molecular differences between samples isolated from different plant types. We performed a similar analysis to determine whether our observed haplotype distribution pattern could arise from the four genotypes having different plant host specificity. Comparisons between larvae isolated for *Sorghum* spp. and corn in Florida from 2003 to 2006 identified some differences in haplotype distributions (Fig. 5). Larvae with the CS-h3 haplotype were more frequent in sorghum, making up 15% (5/34) of the sample population. There was also more than a twofold increase in the proportion of CS-h1 in sorghum-derived larvae compared with those in corn (12 to 5%). However, these differences arose almost entirely from larval samples obtained in 2003, when all the CS-h3 haplotype from sorghum were collected, producing a distribution pattern significantly different from that produced by larvae collected from corn ($P < 0.001$; Fig. 5). In comparison, more recent sampling in sorghum in 2006 gave a distribution pattern statistically indistinguishable to corn-derived larvae ($P = 0.522$), with no CS-h3 observed and equivalent CS-h1 levels.

Larvae were collected from three plant types in Brazil and all showed the same general distribution of haplotypes. There was a higher proportion of CS-h1 observed in sorghum than in cotton or corn collections (39% compared with 18 and 27%, respectively), but this difference was not statistically significant ($P = 0.267$). The CS-h3 haplotype, which was found in unusually high proportions in 2003 Florida sorghum, was not identified in any plant host in Brazil. The

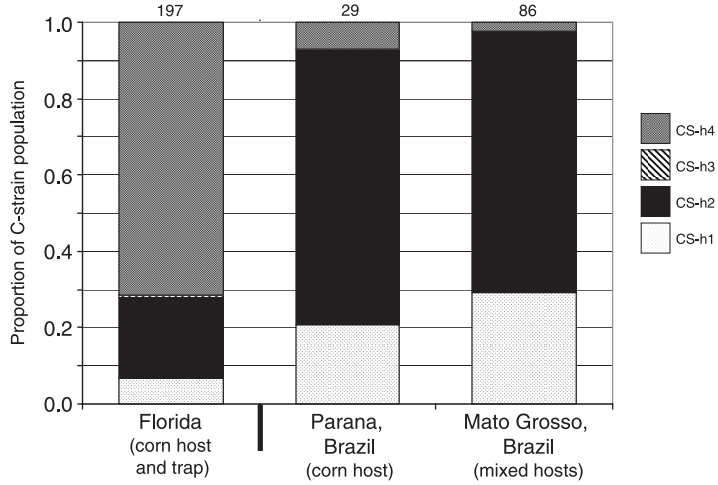


**Fig. 4.** Proportions of *S. frugiperda* corn strain haplotypes present in samples collected from sites in Florida and Brazil. "Mixed larvae" indicate samples were obtained from corn, sorghum, and cotton. "Trap" denotes analysis of adult males collected from pheromone traps. Numbers above bars indicate number of samples tested by DNA sequencing of the COI gene. Data for Florida is pooled numbers from collections in 2004–2005 as described in Fig. 2.

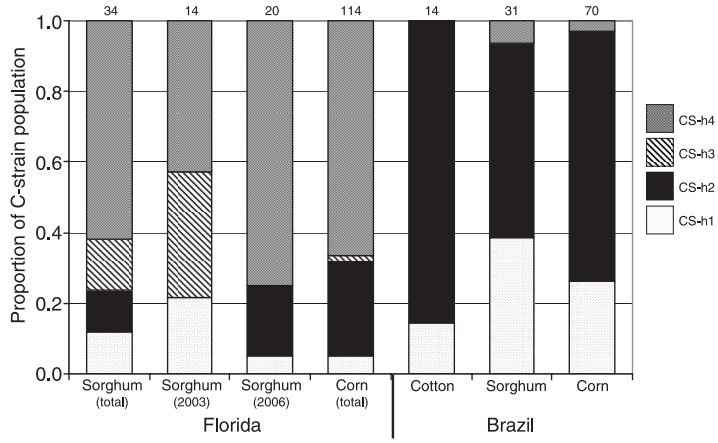


**Fig. 5.** Proportions of *S. frugiperda* corn-strain haplotypes present in larval samples collected from different plant hosts in Florida and Brazil. Haplotypes were determined by analysis of the COI gene sequence as described in Fig. 1. Numbers above bars indicate number of samples tested.

majority subgroup in Florida sorghum and corn plants (CS-h4) was found in only 7% of the Brazil sorghum and corn fall armyworm samples and was absent in the cotton samples. Together, these results indicate that the haplotype proportion differences reflect geographical rather than plant host specificities.

## Discussion

We used polymorphic sites in the mitochondrial DNA to subdivide the Florida fall armyworm corn-strain population into four haplotype subgroups. Comparison of seasonally and geographically distinct sample collections showed a relatively consistent haplotype distribution pattern. This was characterized by a majority of specimens with the CS-h4 haplotype, with most of the remainder coming from CS-h2, and low, more variable levels of CS-h1 individuals. The one exception to this pattern was the 2003 collection of larvae isolated from sorghum where about a third of the samples were of CS-h3 haplotype (Fig. 5). However, the sample size for this collection was small (14) and was not reproduced in a collection of 20 larvae collected from sorghum in 2006. In several hundred samples from pheromone traps and larval collections in Florida made from 2003 to 2006, including 57 samples from 2003, only three other CS-h3 specimens were identified. This haplotype was never found in samples from Brazil, which included 28 samples collected from sorghum. Therefore, although the CS-h3 haplotype might be biased toward development on sorghum, it does not seem to currently be an important contributor to the corn-strain populations in the tested areas.

The corn-strain haplotype distribution pattern in Florida was generally unaffected by differences in collection method, season, or plant host. Although some fluctuations in proportions were observed, in every case the general pattern of CS-h4 > CS-h2 > CS-h1 was preserved. These results suggest a generally homogeneous corn-strain population in which the haplotypes are in equilibrium, observations consistent with the long-range mobility of fall armyworm that should facilitate the rapid mixing of populations. Variations approaching statistical significance were observed for comparisons between collection methods and geographical areas within Florida, leaving open the possibility for more subtle behavioral or fitness differences between the haplotypes (Fig. 2). Particularly variable were the levels of CS-h1, and it is possible that more extensive surveys focusing on this haplotype might identify correlations with geography, season, or plant host.

The examination of Brazilian populations was based on surveys in the two nonadjacent states of Parana and Mato Grosso. The similarity of the distribution patterns observed suggests a homogeneous corn-strain population in this region. In particular there seemed to be no plant host specificity between the three haplotypes present with respect to cotton, corn, or sorghum, the latter two results consistent with that of analogous studies performed in Florida.

Of interest was the observation that the haplotype profile in Brazil was different than that observed in Florida. The Florida populations were ≈95% fixed for the presence of a G at site 1287 and polymorphic for G or A at site 1164, with a bias toward G. The Brazilian corn-strain shows the reciprocal pattern. It is ≈95% fixed for an A at site 1164 and polymorphic for an A or G at site 1287. It has been pointed out that the use of mitochondrial DNA for phylogenetic studies is problematic in many insects because maternally inherited symbionts can rapidly skew the distribution and diversity of haplotypes (Hurst and Jiggins 2005). Nevertheless, our data indicate that genetic exchange between fall armyworm from Brazil and Florida is sufficiently limited that rapid homogenization of the two populations does not occur. This could in part be due to the geographical distance between the populations as well as possible mating pheromone differences (Batista-Pereira et al. 2006). Regardless of cause, the observed asymmetry in haplotype distribu-

tions provides an opportunity to determine whether corn-strain populations in more northern areas of South America, Central America, and the Caribbean islands display the Brazil or Florida haplotype profile, and thereby map the long-range movements and dispersion of these two populations.

The methodology we developed to examine and identify fall armyworm populations has several advantages that allow relatively rapid analysis of large numbers of samples. The four haplotype subgroups are defined by two polymorphic sites that are separated by only 123 bp. This means that a single fragment containing both sites can be efficiently amplified in a single PCR reaction and sequenced in a single DNA sequencing reaction. By focusing only on specific base changes occurring at two specified sites, rather than on all polymorphisms in the region, the confounding effects of errors in DNA sequencing or amplification can be discounted. This is because artifactual base changes that coincidentally occur at the two marker sites should be sufficiently rare that they will not significantly alter the results. Therefore, in most cases confirmatory DNA sequencing reactions are not necessary. We are still in the early stages of exploring the usefulness of this experimental approach with fall armyworm, but our ability to genetically distinguish Florida populations from those in Brazil is suggestive that molecular tools are now available to study the annual long-range movements of this economically important lepidopteran pest.

## Acknowledgments

We thank Jane Sharp for excellent technical support. The statistical assistance of M. C. Christman (Department of Statistics, IFAS Statistical Consulting Unit, University of Florida) is appreciated. We thank Steve Valles (USDA–ARS), Ryan Jackson (USDA–ARS), and Howard Fescemyer (The Pennsylvania State University) for helpful comments on the manuscript.

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*Received 14 December 2006; accepted 19 February 2007.*