Puerto Rico Fall Armyworm Has Only Limited Interactions With Those From Brazil or Texas but Could Have Substantial Exchanges With Florida Populations

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ABSTRACT Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an important agricultural pest that is endemic to Puerto Rico and the rest of the Caribbean islands. Relatively little is known about the population movements of fall armyworm in the Caribbean and the magnitude of genetic interactions, if any, with populations from North, South, and Central America. To address this issue, a novel method involving mitochondrial haplotype ratios currently being used to study the migration of fall armyworm in North America was applied to populations in Puerto Rico. The results indicate limited interactions between Puerto Rico fall armyworm and those from Brazil or Texas but the potential for significant exchanges with populations in Florida.

KEY WORDS *Spodoptera frugiperda*, migration, haplotypes

Noctuid moths include several species that are significant economic pests to a variety of agricultural products. Because many of these exhibit seasonal, long-range migratory behaviors, this group is capable of rapid dispersion and genetic interactions with even distant populations An example is fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), an important economic pest in the Western Hemisphere, responsible for significant economic costs to sweet corn, *Zea mays* L.; sorghum, *Sorghum vulgare* Pers.; cotton, *Gossypium hirsutum* L.; field corn; sugarcane (hybrids of *Saccharum* L.); and several turfgrass varieties, particularly in the sporadic "outbreak" years associated with abnormally high population levels (Sparks 1979, Pashley 1988, Foster 1989). In South America, fall armyworm has long been considered one of the primary pests of sweet corn and cotton (Martinelli et al. 2007).

Fall armyworm is not known to diapause and does not survive freezing winters (Sparks 1979, Horner et al. 2003). Although this limits where it can become permanently established, its capacity for long-range migration allows for widespread seasonal infestations and colonization. The best studied example of this behavior is the annual northward migration of fall armyworm from overwintering areas in southern Texas and Florida that is responsible for infestations in much of the central and eastern United States and Canada (Luginbill 1928, Rose et al. 1975, Young 1979, Pair et al. 1987). A variety of methods have been used to study this migration pattern, including comparing chemical or viral susceptibility of fall armyworm from different locations (Young 1979, Fuxa 1987, Pitre 1988), monitoring adult moths by pheromone trapping and radar (Rose et al. 1975, Pair et al. 1987), and correlating population changes with wind and weather patterns (Mitchell 1979, Westbrook and Sparks 1986, Mitchell et al. 1991, Westbrook 2008). These studies provided a broad and not always consistent approximation of the annual migration (summarized in Nagoshi and Meagher 2008).

Even less is known about the pattern of fall armyworm movements in Central America, South America, and the Caribbean, where documented instances of seasonal migrations have yet to be reported. Recent attempts to measure genetic variation between geographically distant sites from this region (including samples from Argentina, Brazil, Mexico, and Puerto Rico) led to the conclusion that fall armyworm populations are genetically heterogeneous with interbreeding occurring generally throughout the Western Hemisphere (Clark et al. 2007). However, a detailed analysis of moth capture and meteorological data found no conclusive evidence of a significant contribution from the Caribbean to fall armyworm populations in the temperate regions of North America (Mitchell et al. 1991). This issue is of topical interest as it was reported that fall armyworm in Puerto Rico were largely unaffected by the *Bacillus thuringiensis* (Bt) endotoxin Cry1 F when expressed in transgenic corn (A. Reynolds, unpublished data, as described in

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Table 1. Source locality and host information

Location	Collection	Plant/habitat	Date	Reference
Isabela (Is), Puerto Rico	Pheromone	Corn	July–Dec. 2007–2009	This study
Juana Diaz (JD), Puerto Rico	Pheromone	Corn	July–Nov. 2007–2009	This study
Miami-Dade Co. (MD), FL	Pheromone	Corn	June 2007	This study
Miami-Dade Co. (MD), FL	Pheromone	Corn	2003–2006	Nagoshi et al. (2007a)
Alachua Co. (Al), FL	Pheromone	Corn	July–Oct. 2007	This study
Alachua Co. (Al), FL	Pheromone	Corn	May–Oct. 2004–2005	Nagoshi et al. (2007a)
Levy Co., FL	Pheromone	Corn	June 2003	Nagoshi et al. (2007a)
Gadsden Co., FL	Pheromone	Corn	Aug. 2006	This study
Lowndes Co., GA	Pheromone	Corn	Aug. 2007	This study
Hidalgo Co. (Hi), TX	Pheromone	Corn	2006–2007	Nagoshi et al. (2008b)
Tom Green Co. (TG), TX	Pheromone	Corn	Sept. 2006	Nagoshi et al. (2008b)
Brazos Co. (Br), TX	Pheromone	Corn	May–Nov. 2004	Nagoshi et al. (2008b)
Mato Grosso (MG), Brazil	Larva	Corn, sorghum	2005, 2007	Nagoshi et al. (2007a)
Parana (Pn), Brazil	Larva	Corn, sorghum	2005	Nagoshi et al. (2007a)

Moar et al. 2008; Matten et al. 2008; Tabashnik 2008). Describing fall armyworm movements to and from Puerto Rico is critical to predicting the likely dispersal patterns of resistance traits that might arise in this area, and more generally for understanding the contributions of Caribbean populations to infestations in the rest of the hemisphere.

Fall armyworm can be subdivided into two subgroups designated as "rice-strain" or "corn-strain" based on plant host use (Pashley 1986, Prowell et al. 2004). These strains are morphologically identical, making an unambiguous determination of strain identity limited to molecular techniques, such as mitochondrial DNA haplotyping (Pashley 1989, Lu and Adang 1996, Levy et al. 2002, Nagoshi et al. 2007b). A method using such haplotypes was recently developed that could distinguish between corn-strain populations in Florida and Texas, and Florida and Brazil (Nagoshi et al. 2007a, 2008b). Sequence analysis of a portion of the mitochondrial cytochrome oxidase subunit I (*COI*) gene identified two sites that were polymorphic within the corn-strain population and generated four haplotype classes: CS-h1, CS-h2, CS-h3, and CS-h4. Although each of these were found in fall armyworm collected from Brazil, Texas, and Florida, the haplotype proportions varied by location, best seen by calculating the ratio between CS-h4 and CS-h2 frequencies. Sampling of almost 500 specimens from pheromone traps or larval collections in Florida consistently displayed CS-h4/CS-hs2 values >1.5 that were stable over a 5-yr period and independent of location. In comparison, corn-strain populations from Brazil and Texas routinely gave ratios <0.5. The ability to distinguish fall armyworm from Texas and Florida provided a method for defining the migration pathways from these areas. As a proof of concept, it was shown that corn-strain isolated from Georgia closely resembled those from FL, whereas those in Alabama, Mississippi, and Louisiana were similar to the Texas profile (Nagoshi et al. 2008b), a result consistent with the migratory pattern proposed by Luginbill (1928).

The above-mentioned results demonstrate the utility of the haplotype ratios to describing the long-range movements of fall armyworm populations. In this article, this strategy was applied to the fall armyworm corn-strain in Puerto Rico to investigate the likelihood of substantial interactions with populations in Brazil to the south, Texas to the west, or Florida to the north. These studies using *COI* haplotypes were complemented by analysis of the same collections using a second molecular marker. It was recently shown that polymorphisms in the *triose phosphate isomerase* gene (*Tpi*) could be used to discriminate between fall armyworm strains (Nagoshi 2010). *Tpi* lies on the Z-chromosome and therefore displays a different inheritance pattern than that of the mitochondrial *COI* gene. This difference can be exploited to make inferences about the frequency and pattern of interstrain mating behavior, as was previously done with another sex-linked strain marker (Nagoshi and Meagher 2003). The implications of these results to the likely migration patterns of fall armyworm in the Caribbean are discussed.

Materials and Methods

Specimen Collections and Sites. The specimens tested were either adult males obtained from pheromone-based traps using the method described by Meagher (2001) or field-collected larvae (Table 1). Standard plastic Universal moth traps (Unitraps) were baited with a commercially available fall armyworm pheromone (Suterra LLC, Bend, OR) and contained insecticide strips (Hercon Environmental Co., Emigsville, PA). After collection, specimens were typically stored at −20°C. The collected specimens were identified as fall armyworm by morphological criteria before molecular analysis. Most of the specimens from Brazil, Florida, and Texas were archived genomic DNA preparations whose collections were described in previous studies and stored at −20°C (Table 1; Nagoshi et al. 2007a,b, 2008b). Additional specimens were collected from three corn growing areas in Florida (Miami-Dade, Alachua, and Levy counties and a cornfield in Lowndes Co., GA. Collections from Puerto Rico were from corn or sorghum fields near the municipalities of Isabela (northwestern coast) and Juana Diaz (southern coast).

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 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 precipitated by the addition of 100 μl of 8 M potassium acetate and pelleted by centrifugation at 10,000 rpm in a microcentrifuge for 3 min. 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. Genomic DNA preparations of fall armyworm samples from previous studies were stored at −20°C (Table 1).

Characterization of the COI Haplotypes. polymerase chain reaction (PCR) amplification of the mitochondrial *COI* gene was performed in a 30-μl reaction mix containing 3 μl of 10× manufacturer's reaction buffer, 1 μl of 10 mM dNTP, 0.5 μl of 20 μM primer mix, 1 μl of DNA template (between 0.05 and 0.5 μg), and 0.5 U of *Taq*DNA polymerase (New England Biolabs, Ipswich, MA). The thermocycling program was 94°C (1 min), followed by 33 cycles of 92°C for 30 s, 56°C for 45 s, 72°C for 45 s, and a final segment of 72°C for 3 min. Typically, 96 PCR amplifications were performed at the same time using either 0.2-ml tube strips or 96-well microtiter plates. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Amplification of the *COI* region used the primer pair *COI-893F* (5′-CACGAGCATATTTTACATCWGCA-3′) and *COI-1472R* (5′-GCTGGTGGTAAATTTT-GATATC-3′) to produce a 600-bp fragment.

For fragment isolations 6 μl of 6× gel loading buffer was added to each amplification reaction and the entire sample run on a 1.8% agarose horizontal gel containing GelRed (Biotium, Hayward, CA) in 0.5× Tris-borate buffer (TBE; 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. The isolated fragments were analyzed by DNA sequencing using primer *COI-893F* performed by or the University of Florida ICBR center. Strain identity and CS-h4/CS-h2 haplotype ratios were determined by the presence of specific nucleotides at strain-specific polymorphic sites as described in the text.

Strain Designation Using *Tpi* Polymorphisms. PCR amplification with *Tpi* primers 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. PCR-amplified fragments were isolated from agarose gels using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, CA). The isolated fragments were directly analyzed by DNA sequencing performed by the University of Florida ICBR center. PCR amplification and DNA sequencing used the primers *Tpi*-282F (5′-GGTGAAATCTCCCCTGC-TATG-3′) and *Tpi*-850gR (5′-AATTTTATTACCT-GCTGTGG-3′). Because *Tpi* is on the fall armyworm Z-chromosome, males carry two copies of the gene. Therefore, direct sequencing of male PCR products can produce ambiguous data if the specimen is heterozygous for polymorphisms (Nagoshi 2010). This is associated with DNA sequencing chromatographs showing either overlapping patterns at a single site, indicative of a single-base polymorphism, or large regions of ambiguous signals representing the presence of a heterozygous frame-shifting insertion or deletion. Only samples where an unambiguous sequence was obtained for the relevant strain-diagnostic sites were used in the analyses.

DNA Sequence Analysis. The isolated fragments were analyzed by DNA sequencing performed by or the University of Florida ICBR center. DNA comparisons, alignments, and restriction site mapping were performed using the DS Gene program (Accelrys, San Diego, CA) and the CLUSTAL algorithm. Statistical analyses were performed using GraphPad InStat version 5.1 (GraphPad Software Inc., San Diego, CA; www.graphpad.com). In Fig. 1, comparisons of haplotype ratio means were performed using one-way analysis of variance (ANOVA), with a post hoc Tukey-Kramer multiple comparison test to calculate *P* values. See Fig. 3 comparisons used a paired *t*-test.

Results

Fall Armyworm Mitochondrial Haplotypes in Puerto Rico. A 320-bp portion of the *COI* gene was sequenced from Puerto Rico corn-strain ($n = 67$) and rice-strain ($n = 66$) specimens. The consensus sequences derived from these data were identical to that obtained from similar analyses of samples from Florida, Brazil, and Texas (data not shown). An earlier study of fall armyworms from Florida and Brazil characterized eight sites (coordinates: 1044, 1125, 1164, 1176, 1182, 1197, 1216, and 1287) within this region associated with strain-specific, single-base polymorphisms (Nagoshi et al. 2007b). In seven of the eight sites the polymorphism was generally (>90%) a choice between two nucleotides, each representative of a host strain. The exception was at site 1164, where the rice-strain was associated with a thymine (T) and the corn-strain either an adenine (A) or guanine (G).

The most direct way to demonstrate the strain-specificity of a particular haplotype is to collect larvae from plant hosts associated with specific strains and show that they carry the expected genetic markers. However, such collections can be difficult to obtain for many plant hosts and were not available for Puerto Rico. Instead, we tested whether the *COI* polymorphisms found in Puerto Rico fall armyworm clustered in a pattern consistent with the existence of the two host strains. In this study, the polymorphisms at site 1164 were used to identify host strain, and the frequency of finding the rice-strain associated nucleotide at five other strain-defining sites calculated relative to that identification (Table 2).

Table 2. Frequency of rice-strain (RS) specifying polymorphisms in the *COI* gene relative to strain identity defined by the presence (RS) or absence (corn-strain, CS) of a T at site 1164

Location-strain	*n*	Polymorphic sites [RS-specifying nucleotide]				
		1125 [C]	1176 [C]	1182 [T]	1197 [A]	1216 [A]
Puerto Rico-RS	65	1.00	1.00	1.00	1.00	1.00
Brazil-RS	29	1.00	1.00	1.00	1.00	1.00
Texas-RS	24	1.00	1.00	1.00	1.00	1.00
Florida-RS	49	0.98	0.98	0.98	1.00	1.00
Puerto Rico-CS	88	0.00	0.00	0.05	0.05	0.00
Brazil-CS	126	0.00	0.00	0.01	0.06	0.00
Texas-CS	320	0.00	0.00	0.00	0.04	0.00
Florida -CS	103	0.00	0.00	0.00	0.07	0.00

There was a strong similarity in the distributions of the *COI* strain markers observed in Puerto Rico fall armyworm with those from other areas where both strains are known to exist. Rice-strain populations from Texas, Florida, and Brazil carried the expected nucleotide at site 1125 (C), 1176 (C), 1182 (T), 1197 (A), or 1216 (A) at least 98% of the time, a consistency similar to that observed with the Puerto Rico population (Table 2). Conversely, the rice-strain polymorphisms were rarely found in specimens designated as corn-strain by the 1164 marker. The highest variability was observed at sites 1182 and 1197, but even here the presence of the rice-strain marker never exceeded 7%. These observations demonstrate that fall armyworm in Puerto Rico can be separated into two major subpopulations based on the strain-specific *COI* markers.

Comparisons of CS-h4/CS-h2 Haplotype Ratios. The polymorphisms at sites 1164 and 1287 in the corn-strain population were used to calculate the CS-h4/CS-h2 ratio for Puerto Rico fall armyworms categorized by date and location and compared with samples from Texas, Brazil, and Florida (Fig. 1). It had been shown previously that the average haplotype ratio for Florida populations were significantly different from that calculated from Brazil and Texas collections (Nagoshi et al. 2007b, Nagoshi et al. 2008b). The Puerto Rico specimens had a mean ratio of 4.0 that was statistically indistinguishable from Florida fall armyworm (ratio = 3.1; $P > 0.05$), but was significantly different ($P < 0.001$) from Texas and Brazil populations, for which the ratios were 0.2 and 0.06, respectively.

In 2007, the highest CS-h4/CS-h2 ratio was found in Puerto Rico (3.2), followed by southern Florida (Miami-Dade Co., 2.5), and southern Georgia (Lowndes Co., 1.9) (Fig. 2). This south to north gradation was also observed in four of the 5 yr from 2003 to 2007 in a comparison of archived samples from northern and southern Florida. Specimens from Miami-Dade Co. showed higher ratios than collections in the same year from the more northern Alachua, Levy, and Gadsden counties, with 2005 being the one exception (Fig. 3). There was considerable year-to-year variability, however, so the observed differences were not statistically significant by standard two-tailed *t*-test ($t = 1.97$, df = 4, $P = 0.12$).

Distribution of Tpi Haplotypes in Puerto Rico. We used the combination of the *Tpi* and *COI* markers to compare the frequency of potential interstrain hybrids

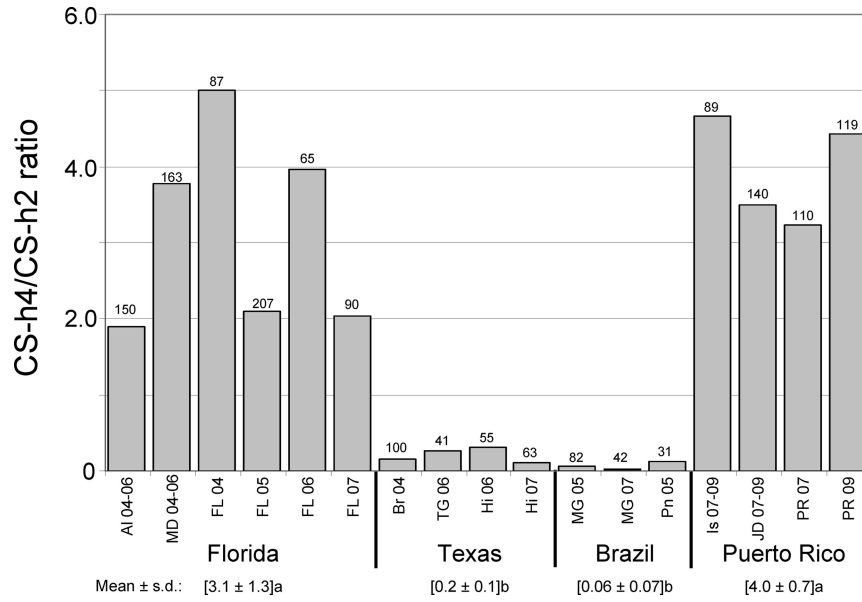

Fig. 1. CS-h4/CS-h2 ratios calculated from collections from Puerto Rico, Florida (FL), Texas (TX), and Brazil. Ratios >1.5 are considered similar to the Florida corn-strain population and those <0.5 are defined to the same category as Texas fall armyworm. Numbers adjacent to columns indicate the number of specimens analyzed. Numbers in brackets represent mean ± SD. Means with different letters (a or b) are statistically significant different ($P < 0.05$) based on post hoc Tukey–Kramer multiple comparisons test ($q_{FL\text{-}TX} = 7.3$; $q_{FL\text{-}BZ} = 7.0$; $q_{FL\text{-}PR} = 2.1$, $q_{TX\text{-}BZ} = 0.3$; $q_{TX\text{-}PR} = 8.6$; $q_{BZ\text{-}PR} = 8.3$). FL, Florida; PR, Puerto Rico; BZ, Brazil; TX, Texas. Other abbreviations are listed in Table 1.

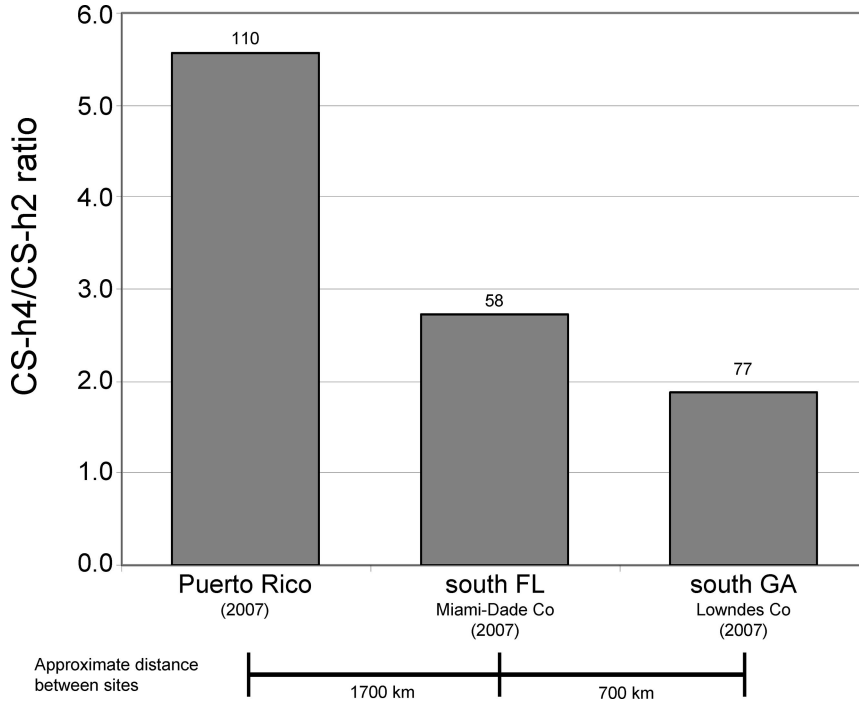

Fig. 2. Comparison of CS-h4/CS-h2 ratios from collection sites along an approximate north–south axis from southern Georgia (GA) to Puerto Rico. Numbers adjacent to columns indicate the number of specimens analyzed and the approximate distance in kilometers from each site provided along the *x*-coordinate.

in the different fall armyworm populations. The ten strain-specific sites in the *Tpi* gene previously identified for samples from Florida, Brazil, and Texas also were found to be polymorphic in the Puerto Rico collections (Table 3; Nagoshi 2010). Strain-identity using *Tpi* was defined by a strong majority (≥70%) of the polymorphic sites carrying the nucleotides preferentially associated with a particular strain. More

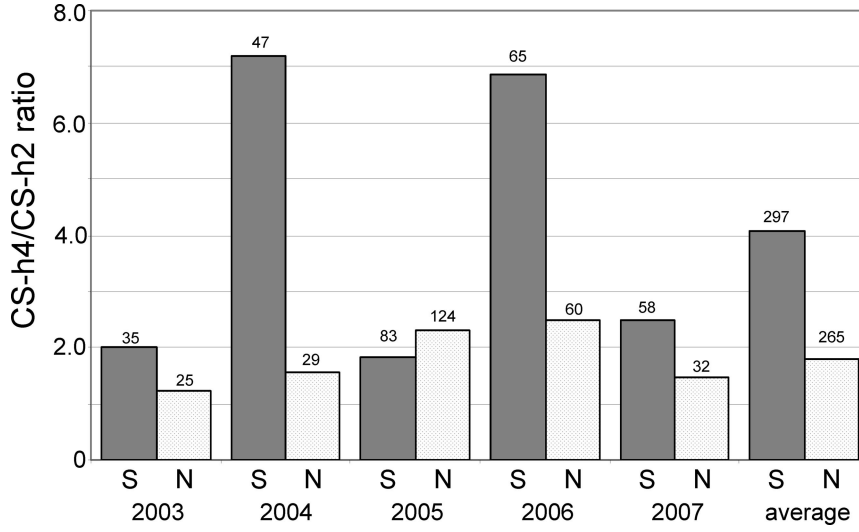

Fig. 3. Comparison of CS-h4/CS-h2 ratios from collection sites in northern Florida (N) to southern Florida (S) for different years. All southern Florida collection sites were in Miami-Dade Co. during March–June 2003, Feb.–June 2004, Nov.–Dec. 2005, June 2006, and June 2007. Northern Florida specimens were collected from Gadsden Co. (June 2003), Alachua Co. (April–May 2004, Sept.–Oct. 2005, Oct. 2007), and Levy Co. (Aug. 2006). Numbers adjacent to columns indicate the number of specimens analyzed. Approximate distances to Miami-Dade Co: Levy Co., 520 km; Gadsden Co., 810 km; Alachua Co., 560 km.

Table 3. Specimens from Puerto Rico, Florida, Texas, and Brazil categorized by the number of corn-strain specific polymorphisms found in the *Tpi* gene

No. CS-specifying polymorphisms	Strain designation[a]	Puerto Rico	Forida	Texas	Brazil
10	CS	34	110	34	26
9	CS	5	24	16	10
8	CS	1	20	5	14
7	CS	0	0	0	0
6	int	1	0	1	0
5	int	0	2	0	1
4	int	1	14	3	3
3	RS	8	11	9	6
2	RS	9	18	18	6
1	RS	8	13	16	12
0	RS	3	18	2	3
Total specimens		70	230	104	81

[a] Based on number of corn-strain polymorphisms present (≤3 denotes rice-strain, ≥7 denotes corn-strain).

than 97% (84/86) of the Puerto Rico samples could be categorized to a strain by this criterium, a frequency equivalent to that found in collections from Florida (93%), Texas (96%), and Brazil (95%) (Table 3). This strongly suggests that the Puerto Rico populations share a similar distribution of *Tpi* polymorphisms with respect to the two host strains as found in these other areas.

We next examined the extent to which the strain identity as defined by *Tpi* corresponded to that determined by *COI* haplotypes. In Puerto Rico, 44% of the specimens carrying *COI*-R were found to be *Tpi*-C (Fig. 4). We call this the "RS hybrid" configuration as it can be produced by repeated mating of rice-strain females to corn-strain or hybrid males (Nagoshi 2010). This high level of discordance was in the same range as that found in collections from cornfields in Texas (31%) and Florida (65%) analyzed during the same year (2007) and is consistent with earlier studies using different marker combinations that suggested significant interstrain hybridization of this type can occur in corn-dominated habitats (Nagoshi and Meagher 2003, Prowell et al. 2004). A different result was obtained with the reciprocal "CS hybrid" configuration (*COI*-CS *Tpi*-R), which can be produced by matings between corn-strain females and rice-strain males. Consistent with previous studies (Nagoshi and Meagher 2003, Nagoshi et al. 2008a), the *COI*-CS *Tpi*-R configuration was rarely found in the Texas and Florida collections, only making up 3 and 5% of the *COI*-CS specimens in 2007, respectively (Fig. 4). In contrast, 31% of the Puerto Rico *COI*-CS population carried the rice-strain *Tpi*-R marker.

Discussion

The sequence variations associated with the CS-h1–4 haplotypes do not affect the *COI* protein sequence and are therefore unlikely to have fitness con-

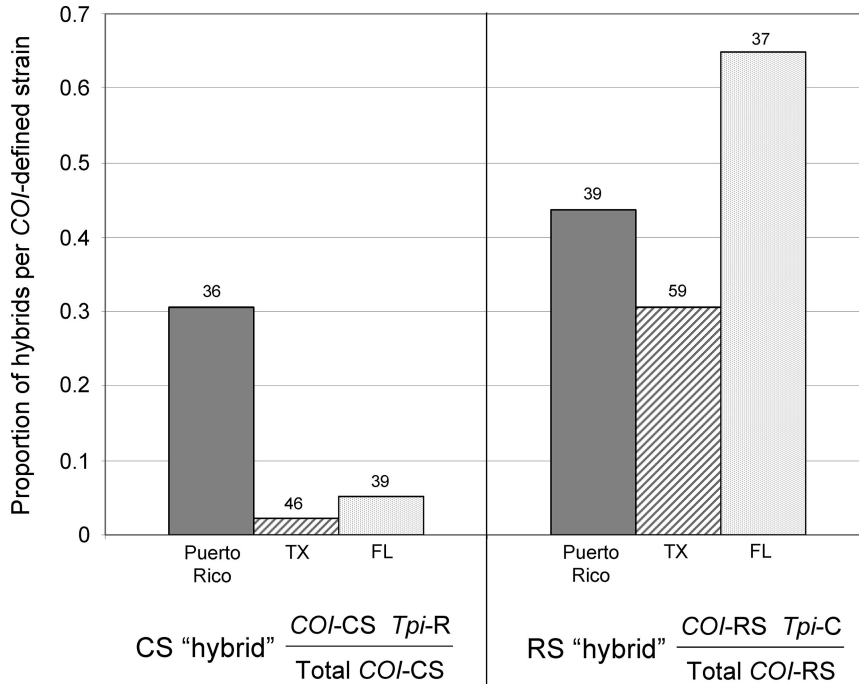

Fig. 4. Proportion of specimens displaying the "hybrid" *COI-Tpi* configuration in collections from Puerto Rico, Texas, and Florida. Corn-strain (CS) hybrids were defined as those specimens with the corn-strain *COI*-CS mitochondrial cytotype and a rice-strain *Tpi*-R allele. The rice-strain (RS) hybrid is the reciprocal configuration of *COI*-RS combined with the *Tpi*-C marker. Proportions are calculated by dividing the number of each type of hybrid by the total specimens with the same *COI* cytotype. Numbers adjacent to columns indicate the number of specimens analyzed.

sequences. If we accept this as true, then the CS-h4/CS-h2 ratio of a given population is likely the result of genetic drift and once established will remain stable as long as the population remains sufficiently large, and exchanges with females with different ratios are minimized (reflecting the maternal inheritance of mitochondrial haplotypes). Therefore, stable differences in the *COI* haplotype ratio are an indicator of limited exchanges between the two populations.

The corn-strain fall armyworm from Puerto Rico displayed the high CS-h4/CS-h2 ratios associated with Florida populations and therefore can easily be distinguished from populations endemic to Texas or Brazil. This has two important implications. The first is that exchanges between the fall armyworm from Puerto Rico and those from Texas or Brazil are not significant enough to alter the haplotype ratios at these locations. This was surprising given that the closeness of the Lesser Antilles islands to the Venezuelan coast would seem to make possible a periodic influx of South American fall armyworm into Puerto Rico through a northward migration along this island chain. Either such movements do not occur at a significant level, or the haplotype ratio along the northern South American coast is markedly different from those found in central Brazil. The latter seems unlikely given the migratory propensity of fall armyworm and the lack of obvious physical barriers between this region and Brazil but indicates that a survey of fall armyworm populations in Venezuela and surrounding areas is warranted.

A second implication is that the similarity in the haplotype ratios of the Florida and Puerto Rico corn-strain populations is consistent with significant interactions. An attractive feature of this possibility is that the periodic influx of Puerto Rico fall armyworm into southern Florida would provide a simple explanation for the haplotype ratio differences typically observed between northern and southern Florida (Fig. 3). The most likely reason for the lower haplotype ratio in the north is mixing with Texas migrants that become established in Georgia and northern Florida. This presumably should lead to the homogenization of the Florida and Texas populations. Therefore, if this north/south ratio difference persists, as seems to be the case from 2003 to 2007 (Fig. 3), it would suggest some process exists that maintains or restores a high haplotype ratio in southern Florida. One possibility would be a periodic and unidirectional movement of fall armyworm from Puerto Rico, and presumably the rest of the Caribbean, into Florida.

Potentially complicating this scenario are the findings from the analysis of *Tpi-COI* marker combinations. In previous studies of populations from Brazil, Texas, and Florida, polymorphisms within the *Tpi* gene showed strong correlations with the distribution of the strain-specific *COI* haplotypes, indicating their utility as markers of strain identity (Nagoshi 2010). This correlation was not as apparent in Puerto Rico populations, however, as the *Tpi* polymorphisms were more randomly distributed relative to the *COI* strain-specific markers. Specifically, the frequency of the discordant marker configuration was substantially higher in Puerto Rico fall armyworm than those in Florida. Although we consider this observation preliminary because of the relatively small numbers tested, if confirmed it would not be consistent with a large and general influx of moths from Puerto Rico into Florida.

It may be possible to reconcile these observations by assuming a more limited movement of fall armyworm populations. For example, it was previously suggested that the most likely cause of the discordant *Tpi-COI* configurations was interstrain hybridization that would produce progeny carrying the *COI* cytotype of one strain and the *Tpi* allele of the other (Nagoshi 2010). If this is the case, then we only need assume that the interstrain hybrids are nonmigratory to explain why the influx of fall armyworm into Florida is limited to "pure" strains carrying the concordant marker configuration. Although highly speculative, such a linkage between migration and strain-identity should be testable and could explain how the integrity of the two strains is maintained despite repeated evidence of substantial hybridization in the field (Nagoshi and Meagher 2003, 2008a; Prowell et al. 2004).

In summary, the comparison of *COI* haplotype ratios indicate that corn-strain fall armyworm in Puerto Rico are genetically more similar to populations in Florida than to those in Brazil or Texas. Therefore, Florida is the most likely destination for migrant fall armyworm from Puerto Rico, and by extension, the rest of the Caribbean. Whether such movements occur on a regular basis or are significant is not clear, but additional surveys of the region using existing genetic markers should be able to address that issue.

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