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Haplotype Identification within *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) Corn and Rice Strains from Colombia

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Abstract

The fall army worm *Spodoptera frugiperda* (Smith) is a migratory important pest of corn, sorghum, rice, grass and bermudagrass in North and South America. This species has diverged into two genetically differentiated but morphologically identical strains, "the rice" and "the corn". They have been analyzed by sequencing the genes *cytochrome oxydase I*, *II* and *ITS1* from populations from the United States and Brazil. However, no such studies were performed in Colombia. In here, we identified 43 haplotypes by sequencing a fragment of the *COI* gene from 102 individuals, of which 40 had already been identified as the "corn" and "rice" strains or to their hybrids from Tolima, and the rest were collected from corn, cotton, sorghum, grass and rice fields in other regions of Colombia. The corn strain haplotype H1 was the most frequently found in this country, representing the main target for FAW monitoring programs. AMOVA analysis confirmed the population structure between Colombian and North American *S. frugiperda* haplotypes (F_{ST} = 0.76812, P < 0.001), but not within the different Colombian regions, suggesting high gene flow within the country. The ML trees obtained for Tolima and for Colombia as a whole did not generate clustering amongst *S. frugiperda* sequences, neither via host-plant association nor by geographical areas. The minimum spanning network for Colombia corroborated our finding that the haplotype H1 has the highest frequency in the country. Our data suggest that haplotype frequency determination will be useful in the establishment of a monitoring system for this species.

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), is a tropical insect that is endemic to the Western Hemisphere (López-Edwards *et al* 1999), and is distributed from the United States to Argentina (López-Edwards *et al* 1999, Prowell *et al* 2004, Murúa *et al* 2008). In Colombia, FAW is a primary pest of corn (*Zea mays*), and a secondary pest in sorghum (*Sorghum* spp.), cotton (*Gossypium hirsutum*), and pasture grasses (García *et al* 2002). Likewise, in the United States (Nagoshi & Meagher 2004, Nagoshi *et al* 2006, 2007a,b), México (López-Edwards *et al* 1999), Argentina (Murúa *et al* 2008) and Brazil (Busato *et al* 2004), FAW larvae are a costly pest for all these crops, particularly for corn.

The widespread distribution of FAW populations is thought to be due to long distance movement and a lack of diapause in adults (Luginbill 1928, Rose *et al* 1975). In North America, studies on the movement of FAW have shown that populations from central and eastern

United States and south of Canada disperse to Mexico, Texas and Florida as part of the annual migration of this moth (Luginbill 1928, Rose *et al* 1975, Young 1979, Pair *et al* 1987, Nagoshi *et al* 2007a). This wide distribution makes this pest difficult to control (Nagoshi & Meagher 2004, 2008) and such detailed dispersal studies have never been carried out in Central and South America. However, Nagoshi *et al* (2007a) argue that in this part of the continent, movement of FAW can also occur as a response to seasonal changes in rainfall, temperature and agricultural plantings. In addition, Pashley (1988) suggests the possibility of gene flow between FAW populations from the Caribbean region and North America.

Analyses based on feeding behavior, physiology and molecular biology have identified two morphologically identical, but genetically differentiated *S. frugiperda* strains, named the "corn" and the "rice" strains, which display host plant preferences (Pashley 1986, Levy *et al* 2002, Prowell *et al* 2004, Nagoshi *et al* 2006). The corn strain has also been found in cotton and sorghum, while the rice strain in Bermuda grasses in the United States (Nagoshi & Meagher 2004) and Colombia (Vélez-Arango *et al* 2008). These strains also exhibit reproductive isolation under laboratory conditions, at behavioural (Pashley & Martin 1987), temporal (Pashley *et al* 1992) and chemical levels (Groot *et al* 2008). Both have a widespread distribution throughout America, including the Caribbean islands (Prowell *et al* 2004). In Brazil, they also display host plant preferences, physiological, developmental, and pesticide susceptibility differences that seem equivalent to the two North American strains (Busato *et al* 2004, 2005a,b, 2006).

In Colombia, the presence of FAW was confirmed by Vélez-Arango *et al* (2008) in the region of Tolima (centre of Colombia), where the corn strain was mainly found in corn and cotton crops and the rice strain in rice, with very low frequency in corn and cotton. The existence of strains for FAW complicates the management of this moth (Levy *et al* 2002) as both have shown differential dispersal capabilities, as the corn strain seems to disperse for longer distances than the rice strain (Nagoshi *et al* 2007a). In addition, both strains differ in susceptibility to *Bacillus thuringensis* (particularly to the endotoxin Cry1AC) and to the insecticides diazinon and carbaril; the rice strain is more susceptible than the corn strain to both biological and chemical controls (Adamczyk *et al* 1997).

The identification of FAW strains has largely been via the use of molecular markers, where the majority of the studies have focused on differentiating strains with PCR-RFLP and AFLP (McMichael & Prowell 1999, Levy *et al* 2002, Prowell *et al* 2004). Population genetic analyses performed so far have demonstrated little gene flow between the strains (Saldamando & Vélez-Arango 2010) and substantial genetic differentiation within rather than between populations from samples from corn, lemon tree, princess tree and Bermuda grass from Argentina, Brazil, Puerto Rico and the United States (Clark *et al* 2007). However, the genetic analyses conducted by Clark *et al* (2007) did not concentrate on the identification of FAW strains or on the use of the most appropriate marker to analyze the migration of the pest.

Mitochondrial DNA (mtDNA) analysis is generally assumed to be more powerful than allozyme and nuclear DNA markers for revealing historical gene flow versus current gene flow (Lewter *et al* 2006). The cytochrome oxidase I (*COI*) and II (*COII*) genes of the mtDNA are useful for the measurement of genetic variation, haplotype identification, construction of phylogenies and population genetic studies in insects (Avise 1994, Freeland 2005), and these mitochondrial genes have been mainly used for analysis of migratory patterns of FAW populations in the United States and Brazil (Nagoshi *et al* 2007a,b, Nagoshi & Meagher 2008).

Lewter *et al* (2006) sequenced a 608 bp fragment from a *COI*, tRNA leu and *COII* regions of the mitochondrial DNA of 71 individuals collected in Arkansas, Mississippi, California and Florida, and found significant levels of genetic differentiation in all these populations. Nagoshi *et al* (2007b) analyzed a 937 bp *COI* fragment from 73 individuals collected in Brazil, Texas and Florida; in corn, sorghum, pasture, amaranthus, millet, cotton and rice, and found 28 haplotypes with similar host plant association in these two countries, but with differences in the host plant distribution between localities, with a seasonal pattern. Nagoshi *et al* (2007a) analyzed sequences of FAW larvae and adults identified as the "corn strain", to determine the level of haplotype differentiation within and between Florida and Brazil and observed four subgroup - haplotypes (CS-h1, CS-h2, CS-h3 y CS-h4) with a remarkable differentiation in distribution patterns across localities and host plants within each country. Similarities in haplotype compositions across several states of the US suggest migration of the species from Central to Southern US, as that Georgia was infested by corn-strain populations with the same haplotype distribution of southern Florida, and corn-strain populations in Louisiana, Mississippi, and Alabama were indistinguishable (Nagoshi *et al* 2008, Nagoshi & Meagher 2008).

Given that mtDNA is a useful tool to study migratory pattern in insects, particularly in FAW, the objective of this study was to carry out a molecular identification of *S. frugiperda* haplotypes from 102 larvae collected from five regions of Colombia between 2006 and 2009, from corn, rice, grass, sorghum and cotton. We compare these sequences to the US sequences obtained from GenBank in order to a) identify haplotype variants in Colombia, b) determine the level of genetic differentiation within and between these two countries and, c) obtain nucleotide

polymorphism and effective population size estimators. We discuss our results in relation to the implementation of DNA sequencing for the FAW monitoring system and the improvement of the Integrated Pest Management (IPM) in Colombia.

Material and Methods

Larvae collection

FAW larvae were collected from corn, cotton, rice, sorghum and grass fields from the regions of Antioquia, Córdoba, Meta, Tolima and Valle del Cauca in Colombia (Fig 1). In Tolima, collections were made during late 2006 and early 2007, and in the other regions during late 2008 and early 2009. Following collection, larvae were stored in 2.5 ml plastic tubes with 70% ethanol, while larvae were stored at -70°C until processing. The larvae sequenced for the region of Tolima were genotyped in a previous study by Vélez-Arango *et al* (2008) and were used in here in order to detect whether both FAW strains have haplotypes in common and whether these haplotypes showed host plant association (Table 1).

Fig 1 Map of Colombia representing the five regions were larvae of *Spodoptera frugiperda* were collected (Valle = Valle del Cauca).

Table 1 Number of *Spodoptera frugiperda* larvae analyzed in Colombia with sampling localities and host plant where the samples were collected.

Region	Crop	N° individuals
Tolima	Cotton	18
	Rice	12
	Corn	6
	Sorghum	4
Meta	Corn	19
	Rice	4
	Sorghum	4
Antioquia	Grass	2
	Corn	6
Córdoba	Cotton	12
	Corn	7
	Sorghum	4
Valle del Cauca	Cotton	3
	Corn	1
Total		102

DNA preparation

Spodoptera frugiperda genomic DNA was extracted using the CTAB method (Black & Duteau 1997) with some modifications. Larvae were homogenized in 400 µl extraction buffer (100 mM Tris-HCL pH 8.0, 1.4M NaCl, 0.02m EDTA, 2x CTAB), and 4 µL β-mercaptoethanol and 20 µl Proteinase K were added to the homogenate for 1h; each tube was mixed by inversion every 10 min. The homogenate was centrifuged (3,000 *g* for 6 min at 10º C), the supernatant was collected and mixed with 500 µl of chloroform isoamyl - alcohol (24:1), and centrifuged (3,000 *g* for 30 min at 10ºC). The aqueous phase was transferred into a 1.5 ml vial and the chloroform isoamyl - alcohol step was repeated. The aqueous phase was transferred into a new tube, an equivalent volume of chloroform (100%) was added and the phases were separated by centrifugation (3,000 *g* for 20 min at 10ºC). The final aqueous phase obtained was transferred into a new tube, 400 µl isopropanol were added, and sample was incubated at -20º C for 1h before centrifugation (3,000 *g* for 30 min at 4ºC). The obtained DNA pellet was washed with 500 µl 100% of ethanol and centrifuged (3,000 *g* for 6 min at 4ºC) twice before left to dry at room temperature for 45 min. The obtained DNA was re-suspended in 50 µl TE buffer (1x) (TRIS HCL 100 mM, EDTA 10 mM, pH 8.0). Finally, 1 µl RNAse (1 mg/ml) was added to each tube for RNA removal and then incubated for 1h at 37ºC. Each sample yielded an approximately 22.2 ng/µl of genomic DNA.

PCR analysis

PCR amplification of the mitochondrial *COI* gene was performed in a 50 µl reaction mixture containing 5 µl of reaction buffer (1x), 1 µl of dNTP (0.2 mM), 2 µl of each primer (0.4 µM), 2 µl of DNA template and 1 µl of Taq polymerase (0,5 U/ µl) (Invitrogen). The thermocycling program was 94ºC (3 min), followed by 30 cycles of 94ºC (1 min), 59ºC (1 min), 72ºC (1 min), with a final extension at 72ºC for 10 min. The set of primers used were JM76 (5' GAGCTGAATTAGGRACTCCAGG 3') and COI-1483 (5' GCTGGTGGTAAATTTTGATAT 3') (Nagoshi *et al* 2007a,b).

DNA sequence analyses

DNA sequencing was performed by Macrogen Inc. (Korea). The sequences obtained were edited by hand with Bioedit (Hall 1999) and aligned with the algorithm Clustal W (Cheena *et al* 2003, Larkin *et al* 2007). The estimations for nucleotide polymorphism, nucleotide divergence, segregant sites, number of polymorphic sites and number of haplotypes were obtained using DNAsp V5 software (Librado & Rozas 2009). The software jModelTest was used (Guindon & Gascuel 2003, Posada 2008) to determine the nucleotide model of substitution (Nei & Kummar 2000).

ML trees were chosen to visualize the genetic similarity within and between Colombian haplotypes with US haplotypes. These phylogenies were obtained with the online software Phylogeny.fr (www.phylogeny.fr), which uses MUSCLE for multiple alignments, Gblocks for alignment curation, PhyML for cladogram construction based on maximum likelihood and 100 bootstrapping and TreeDyn for the visualization of the tree (Dereeper *et al* 2008).

To determine whether FAW haplotypes produced structured populations amongst the five regions of Colombia and between Colombia and the US, an AMOVA test was performed using Arlequin 3.11 (Excoffier *et al* 2005). This latter software was also used to obtain the estimator of gene neutrality Tajima-Nei (1984). The Tajima-Nei test was performed to determine: a) whether FAW Colombian population's size is in expansion (or positive selection pressure), b) whether FAW populations are under balancing selection (or negative selection pressure) and finally c) whether FAW they under a selective neutral hypothesis (Nei & Kummar 2000). In addition, dendrograms based on *Fst* values were constructed in Mega 4.0 (Kummar *et al* 2008) and used to compare the genetic differentiation across the five Colombian regions and the US samples. Finally, a minimum spanning tree was obtained for all five regions of Colombia in TCS v. 1.21 (Clement *et al* 2000).

Nine sequences from the US were used for genetic comparisons between Colombian and US FAW populations. The GenBank access number and origin (Country/ State, crop) are: SFU72974 (US, corn), SFU72977 (US, rice), AY714298 (Florida, Arkansas, Mississippi and California, corn), AY714299 (Florida, corn), AY714300 (Florida, corn), AY714301 (Florida, rice), AY714302 (Florida, Arkansas, rice), AY714303 (Florida, Arkansas, rice) and AY714304 (Florida, rice).

Results and Discussion

FAW haplotypes for Tolima region

A 642 bp portion of the 5´ region of the mitochondrial DNA was sequenced from a total of 40 larvae from Tolima, previously identified as the rice or corn strain or as a hybrid strain (Velez-Arango *et al* 2008, Saldamando & Vélez-Arango 2010) (Table 2). The average base (nucleotide) frequencies were A = 41.6%, C = 12.6%, G = 14.3% and T = 31.5%. Ten polymorphic sites were found for the 624 bp fragment: 1st position (T/C), 2nd position (A/C), 6th position (A/C), 10th position (A/T), 14th position (C/T), 20 (A/C), 105th position (T/C), 177 (T/C), 180th position (A/G) and 190th position (A/G). Moreover, the DNA polymorphism found for the sample from Tolima is summarized by the following parameters: π (nucleotide diversity) = 0.14976, Hd (haplotype diversity) = 0.926, S (segregant sites) = 399 and θ (nucleotide polymorphism) = 0.16196.

The number of haplotypes obtained for this region was 18, with H1 being the most frequent (8/40), followed by haplotypes H5 (6/40), H10, H11 (3/40), H6 (2/40), and haplotypes H7-9 and H12-18 were the least frequent (1/40) (Fig 1).

For the Maximum likelihood (ML) tree, we obtained the substitution model HKY (Hasehawa, Kishino and Yano) given by the BIC criteria (BIC = 3872.6662, -lnL = 1677.9257) provided in jModeltest (Posada 2008) (Fig 2). In general, this tree failed to show associations between sequenced samples of FAW and their respective hosts: corn, cotton, rice and sorghum. Moreover, this tree also mixed haplotypes from both FAW strains and their respective hybrids suggesting that genetic differentiation at *COI* gene between "corn" and "rice" strains is low, as early demonstrated by PCR-RFLP analysis (Lu & Adang 1996).

With respect to the frequency of each haplotype and its host, H1 was the most frequent from all samples. This haplotype was collected from corn, sorghum and cotton. Given the distribution on these host plants, H1 could represent the "corn" strain, as Vélez-Arango *et al* (2008) demonstrated significant host plant association of the corn strain with these three crops at the region of Tolima. Haplotype H5 was found in cotton and rice, and haplotypes H2 and H10 in corn, cotton and rice. The other haplotypes were less frequent and their host plant association less defined. It is important to take into

Table 2 FAW haplotypes found in Tolima for each strain and the respective type of hybrid between them (H++ = positive for both mitochondrial and nuclear markers; H-- = negative for both mitochondrial and nuclear markers)

Haplotype	No sampled	Crop	Type (No individuals)
1	8	Cotton	H - - (1)
		Cotton	H ++(3)
		Cotton	Corn strain (1)
		Sorghum	Corn strain (1)
		Corn	Corn strain (1)
		Rice	Rice strain (1)
2	4	Cotton	H - - (1)
		Cotton	Corn strain (2)
		Corn	Corn strain (1)
3	2	Cotton	H -- (1)
		Cotton	H ++ (1)
4	2	Cotton	H -- (1)
		Cotton	Corn strain (1)
5	6	Cotton	H - - (1)
		Rice	Rice strain (2)
		Rice	H - -(1)
		Rice	Corn strain (1)
		Rice	H ++ (1)
6	2	Cotton	Rice strain (1)
		Cotton	Corn strain (1)
7	1	Cotton	Corn strain (1)
8	1	Cotton	Corn strain (1)
9	1	Cotton	H ++ (1)
10	3	Rice	H - -(1)
		Corn	Rice strain (1)
		Rice	Rice strain (1)
11	3	Sorghum	Rice strain (1)
		Corn	Rice strain (1)
		Rice	Rice strain (1)
12	1	Rice	H - - (1)
13	1	Rice	H - - (1)
14	1	Sorghum	Corn strain (1)
15	1	Corn	Corn strain (1)
16	1	Corn	Corn strain (1)
17	1	Sorghum	H - -(1)
18	1	Rice	Rice strain (1)

account that most of the FAW haplotypes were found in cotton and, therefore, this crop was the most sampled.

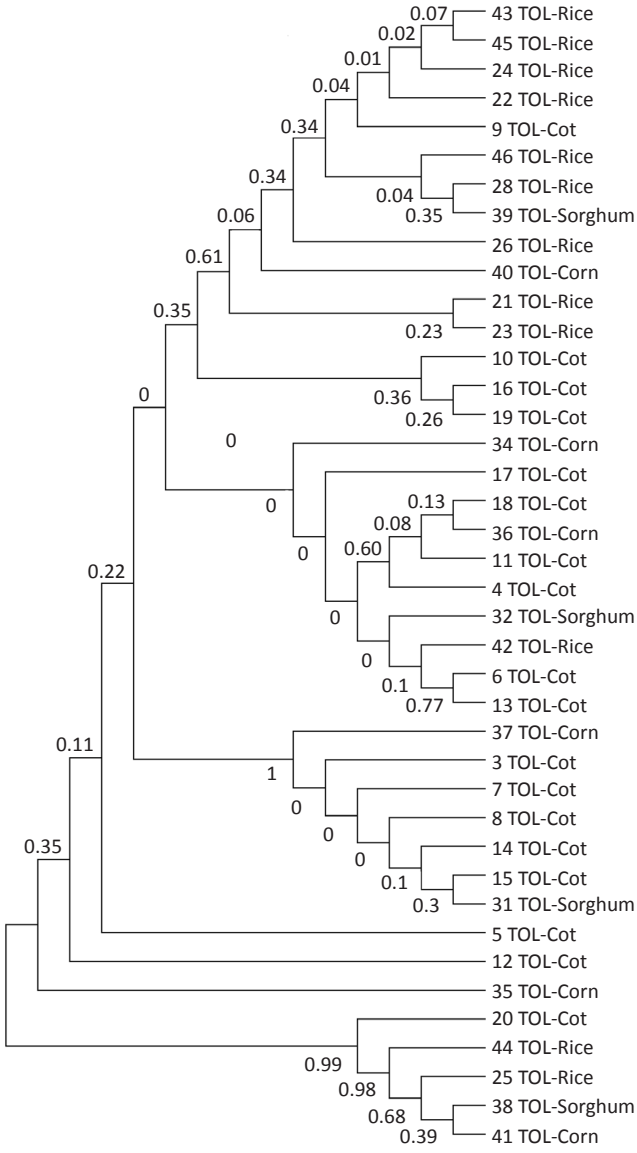

Fig 2 ML tree based on the HKY distance constructed from 40 sequences of the *COI* gene obtained from larvae collected at the region of Tolima and their hosts (RS: Rice strain, CS: Corn strain, H++: hybrid ++, H- -: hybrid - -). Bootstrap values are in black.

FAW haplotypes for Colombia (Antioquia, Córdoba, Meta, Tolima and Valle del Cauca)

A 528 bp portion of the 5´ region of the mitochondrial DNA was sequenced from a total of 102 larvae from the regions: Antioquia, Córdoba, Meta, Tolima and Valle del Cauca (Table 2). The average base frequencies of the fragment found for the five regions were A = 40.5%, C = 14.7%, G = 12.4% and T = 32.4%. Six polymorphic sites were found for the 528 bp fragment: 2 (T/G/C), 60 (A/T), 150 (T/A), 199 (A/G) and 456 (C/T). The DNA polymorphism for Colombia is given by the following parameters: π (nucleotide diversity) = 0.18956, Hd (haplotype diversity) = 0.912, S (segregant sites) = 363

and θ (nucleotide polymorphism) = 0.18956. All these FAW estimators are high if compared to the values obtained for the butterflies *Papilio xuthus* (L.) and *Pieris rapae* (L.) (Lepidopera: Pieridae) in Korea from a *COI* fragment of 658 bp (Jeon *et al* 2009).

Forty-three haplotypes were detected in all areas sampled, with haplotype H1 (29/102) being the most common, and followed by H4 (12/102) and H5 and H9 (8 and 7, respectively). H1 was found in all crops and regions of Colombia, but not in any US sequence. H4 was in cotton, corn and rice in all sampled areas of Tolima, Antioquia and Córdoba. H5 found was in all regions but in Antioquia, only in corn, cotton and rice, and H9 in all regions, but only in corn and sorghum. These haplotypes were used to produce a multifurcated tree that corroborated the previous results, where haplotypes H1 and H4 were the most frequent haplotypes of the species in Colombia (Fig 3). The high number of haplotypes found in Tolima compared to the other sites can be explained by the number of individuals analyzed, since Tolima was the most extensively sampled. H22 from Colombia exhibited an identical sequence to the US (SFU72974). This haplotype represents the only shared sequence between Colombia and the US.

The FAW ML tree based on 61 sequences analyzed here (52 sequences dropped, low bootstrap values) produced a cladogram that did not cluster sequences from both countries, showing no clear differentiation mediated by host plant association (Fig 4), as observed in previously analysis including Brazilian and US sequences of FAW (Nagoshi *et al* 2007a,b). Our results suggest either little genetic differentiation between the corn and the

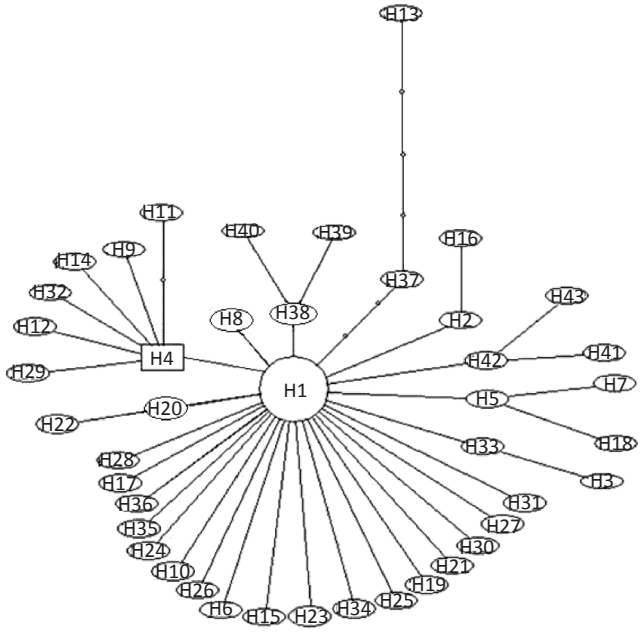

Fig 3 Minimum spanning network of 111 sequences of *COI* gene from Colombian and US samples of *Spodoptera frugiperda*.

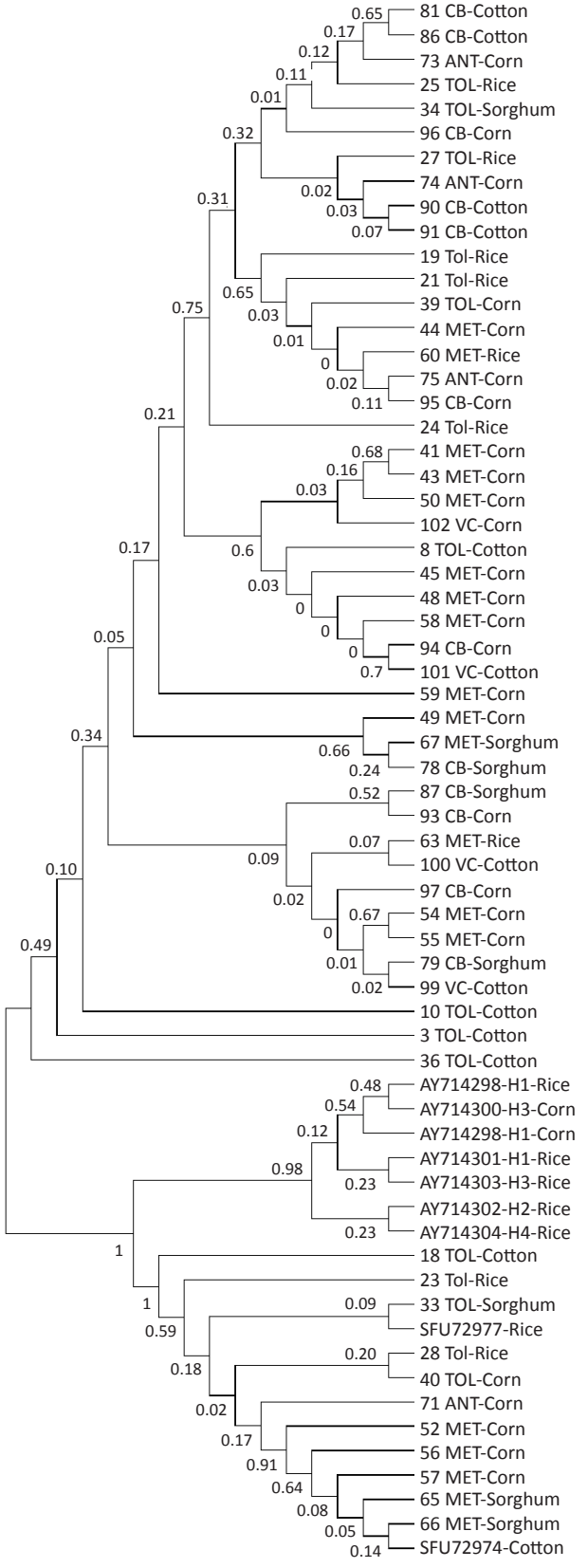

Fig 4 ML tree based on the HKY distance constructed from 102 sequences of the *COI* gene obtained from larvae collected at five regions of Colombia (Ant = Antioquia, CB = Córdoba, Met = Meta, Tol = Tolima, VC = Valle del Cauca). Bootstrap values are in black.

rice strains or a very high mutation rate for the *COI* gene in *S. frugiperda*. In addition, the ML tree clustered most of the US sequences together, demonstrating that FAW populations from US are genetically different from FAW populations from Colombia.

The Tajima-Nei analysis indicated that all sampled areas, with the exception of Antioquia, were neutral, indicating they are not under selection pressure, population expansion, bottleneck, or heterogeneity of mutation rate (Table 3) (Tajima 1989, 1996). The outcome obtained for Antioquia might be due to the low sample size analyzed for this location and also because most of the FAW samples were collected in grass.

The F statistics (Table 4) obtained here (F_{ST} = 0.76, F_{CT} = 0.16 and F_{CS} = 0.20) demonstrate population genetic differentiation between FAW from Colombia and the US; most of the genetic variation (76.37%) was amongst groups (group 1 composed by all five regions vs. group 2 composed by US samples), followed by 23.19% of the variation within groups and only 4.5% of variation amongst groups/within populations. These results imply reduced or non-existent gene flow between populations of both countries, but high gene flow across the five areas of Colombia sampled. Based on our pairwise F_{ST} analysis, we found high genetic differentiation between US FAW samples and the five sampled areas from Colombia, particularly between Córdoba (at the Caribbean of Colombia) and the US (Table 5, Fig 5). Our data also contradicts Pashley (1988) work, given that this author suggests the possible genetic contact between FAW populations from North and South America, particularly with the Caribbean countries.

In general, F statistics obtained for FAW were higher than the F (F_{CT} and F_{CS}) values obtained for *Ostrinia nubilalis* (Hübner) (Malausa *et al* 2007), suggesting that the population structuring in *S. frugiperda* is stronger than in the European corn borer. Thus, the evolutionary biology of both lepidopterans is similar, since both have shown host plant association, but FAW strains are differentiating more rapidly. Nevertheless, it is important to mention that nuclear genes (*Tpi*, *Ket*, *Pbp* and *Mpi*) were used in the case of *O. nubilalis* (Malausa *et al* 2007), whereas we used

Table 3 Tajima-Nei neutrality test performed for the five regions of Colombia and the samples of the United States.

	Tolima	Meta	Antioquia	Córdoba	Valle del Cauca	E.U	Mean
N	40	27	8	23	4	9	18.5
D (Tajima-Nei)	-0.025	0.835	-1.742	0.45	0.591	0.319	0.0714
P	0.52	0.835	*0.003*	0.712	0.822	0.673	0.6058

Table 4 Population structure analysis performed for Colombia and United States sequences of *Spodoptera frugiperda* (Group 1 = Antioquia, Córdoba, Meta, Tolima, Valle del Cauca, Group 2 = United States).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	1672.172	98.90881 Va	76.37
Among populations/ within groups	4	162.464	0.57834 Vb	0.45
Within populations	105	3153.454	30.03290 Vc	23.19
Total	110	4988.090	129.52005 VT	
		F_{CT} = 0.76366 (Va/VT)	F_{SC} = 0.01889 (Vb/Vb+Vc)	*F_{ST} = 0.76812* (Va+Vb/VT)
		P = 0.16617	P = 0.20197	P < 0.0001

Table 5 Pairwise Fst comparisons between *Spodoptera frugiperda* populations from Colombia and the United States.

	Tolima	Meta	Antioquia	Córdoba	Valle del Cauca	USA
Tolima	0.00000					
Meta	-0.01061	0.00000				
Antioquia	-0.05638	-0.03082	0.00000			
Córdoba	0.05902	0.12342	0.17974	0.00000		
Valle del Cauca	-0.04126	-0.02074	0.01944	0.02011	0.00000	
USA	*0.72962*	*0.66114*	*0.68997*	*0.88883*	*0.77249*	0.00000

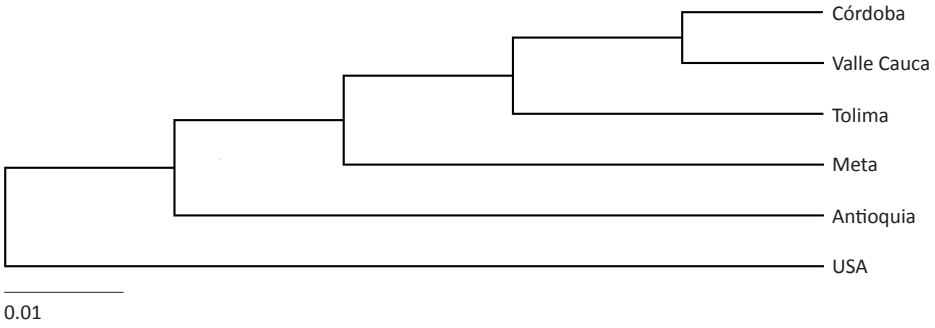

Fig 5 Neighbor Joining (NJ) tree based on pairwise F_{ST} comparisons of five Colombian regions and the United States.

a mitochondrial gene, which has a higher mutation rate and an effective population size lower than that of nuclear DNA (Freeland 2005). However, even though these moth species have been differentiating at different rates, both exhibit significant genetic differentiations, meaning that host plant adaptation is an important cause of divergence and, therefore, speciation.

The effective population size (*Ne*) represents the minimum number of individuals that a population requires to lessen the effects of genetic drift on reducing genetic variation and it represents 10% of a census population (Mallet 2001). For this reason, *Ne* is important for conservation biology (Rieman & Allendorf 2001) and for the insect integrated pest management (IPM) (Pinto *et al* 2002). *Ne* is relevant to IPM as fluctuations in population size over time provide information on the success of the control measures adopted (Pinto *et al* 2002). This estimator can be calculated in various ways (Freeland 2005, Malausa *et al* 2007), and one of them is by using the nucleotide polymorphism value (θ) (Yu *et al* 2004). For mitochondrial DNA, $\theta = 2Ne\mu$ (Freeland 2005, Hedrick 2004), therefore if one assumes a mutation rate (μ) of 1×10^{-7} for the *COI* gene, the *Ne* for *S. frugiperda* will equals 1,889,100 individuals, suggesting that a substantial reduction in population size is necessary for the control of this pest.

The *Ne* value obtained in here is much higher than that estimated for other lepidopterans using different methods (Brakefield *et al* 2001, Malausa *et al* 2007). If compared to the *Ne* value (150,000-200,000 individuals) reported to *O. nubilalis*, we can infer that the actual effective populations size (*Ne*) of *S. frugiperda* is higher than that of *O. nubilialis* or that the mutation rate is higher for mitochondrial than for nuclear genes, and therefore our *Ne* estimation was biased by the type of gene analyzed (*COI*). However, both lepidopterans have provided high values of *Ne* and thus their integrated pest management must be undertaken with care.

General guidelines suggest that effective population sizes between 50 and 500 individuals are essential to minimize inbreeding effects in natural populations (Riemman & Allendorf 2001). This implies that the *S. frugiperda* effective population size is quite high, and indicates that a great effort will be required to decrease the movement of FAW populations (Nagoshi *et al* 2007a,b) within different crops and areas of Colombia in order to reduce the population size of this species. Moreover, previous studies have demonstrated differential resistance of the corn and rice strains to *Bacillus thuringensis* and several insecticides (Adamczyk *et al* 1997). Thus it is important to use the appropriate chemical and biological control strategies for the pest management, depending on the host plant.

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