Structure and Distribution of a Strain-Biased Tandem Repeat Element in Fall Armyworm (Lepidoptera: Noctuidae) Populations in Florida, Texas, and Brazil

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ABSTRACT Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a major economic pest throughout the Western Hemisphere. There exist 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 polymorphisms in the mitochondrial cytochrome oxidase I gene. There is also a tandem-repeat genetic element called *FR* that is found in large sex-linked clusters primarily in the rice strain, as characterized by sampling of fall armyworm populations in the southeastern United States. It was recently shown that the *FR* element is also present in Brazil, where it exhibits a similar strain-biased distribution. In this article, the analysis of *FR* was extended to populations in southern Texas, one of the principle overwintering locations for fall armyworm that infests the continental United States. DNA sequence analysis and an optimized polymerase chain reaction (PCR)-based method demonstrated that *FR* sequences are present in Texas and show the same distribution pattern as observed in Florida. The distribution of *FR* in Florida has remained relatively unchanged over a 4-yr period, suggestive of polymorphic equilibrium and the existence of at least partial barriers to the generation of interstrain hybrids. The implications of these findings on our understanding of interstrain mating behavior and the utility of the modified detection method to study fall armyworm populations are discussed.

KEY WORDS *Spodoptera frugiperda*, haplotype, polymorphisms

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is composed of two sympatric subpopulations that differ in their distribution on different plant hosts (Pashley 1986). These have been characterized as a rice-strain that is associated with rice, pasture, and turfgrasses, and a corn-strain that predominates on corn, sorghum, and cotton. The two strains are morphologically identical, but they differ in certain physiological characteristics, including rates of development, plant host ovipositional preference, and mating preference, although there is considerable within strain variation (Pashley 1988; Quisenberry et al. 1988; Whitford et al. 1988, 1992; Pashley et al. 1992).

The two strains are best distinguished by molecular characteristics. These include electrophoretic variations in allozymes (Pashley 1986), mitochondrial DNA polymorphisms (Pashley 1989, Lu and Adang 1996), nuclear restriction fragment length polymorphisms (RFLPs) (Lu et al. 1992), and amplified fragment length polymorphisms (McMichael and Prowell 1999). More than 20 different polymorphisms have been identified within the mitochondrial cytochrome oxidase subunit I (*COI*) gene that identify two haplotypes that are highly diagnostic of strain identity based on plant host distribution studies (Nagoshi et al. 2006).

Another purported strain-specific marker is a repeated DNA sequence called *FR* (for *F*all armyworm *R*ice strain; Lu et al. 1994). The 188 bp *FR* unit is abundant in rice-strain females, accounting for an estimated 0.05% of the genomic DNA and is organized in large tandem repeat arrays. The *FR* sequence is also present and repetitive in males, although there is an ≈120-fold reduction in copy number. This sex specificity suggested a sex chromosomal location, which was confirmed by interstrain crossing experiments (Nagoshi and Meagher 2003a). In the original description of *FR*, several hundred fall armyworm from laboratory colonies were tested by Southern blot hybridization, and in all cases the element was specific to the

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Table 1. Source locality and collection information of fall armyworm specimens used in this study

Location	Stage collected	Date	Source
Homestead, FL	Adult male	May 2007	R.M.
Belle Glade, FL	Adult male	Jun 2007	R.M.
Hidalgo Co., TX	Adult male	Nov. 2006–Jan. 2007	J.A.
Campo Verde, Mato Grosso, Brazil	Larva	2005–2006	P.S.[a]
Primavera do Leste, Mato Grosso, Brazil	Larva	2005–2006	P.S.[a]

[a] Nagoshi et al. (2007).

rice-strain, making it a potentially useful nuclear marker for strain identity (Lu et al. 1994).

This asymmetric distribution of *FR* clusters with respect to strains was used to examine the likelihood of interstrain mating in the wild. Individual fall armyworms captured in the field were tested for both their strain-specific mitochondrial haplotype (mt^C and mt^R are haplotypes diagnostic of the corn-strain and rice-strain, respectively) and the presence (FR^+) or absence (FR^0) of *FR* clusters (Nagoshi and Meagher 2003a). It was observed that although the mt^C population was invariably FR^0, the mt^R population was roughly evenly split between the FR^+ and FR^0 phenotypes. This was interpreted as suggesting asymmetric interstrain hybridization in the wild, with corn-strain females tending to mate with mt^C FR^0 males, whereas rice-strain females were less selective. This assumes that *FR* clusters were at one time exclusively present in rice-strain individuals, as was first reported (Lu et al. 1994), and that the more recently observed mt^R FR^0 class is the result of interstrain hybridization with corn-strain males.

These larger population surveys examining the distribution of *FR* clusters were facilitated by the development of polymerase chain reaction (PCR) methodologies that allow rapid analysis of single individuals. The typical PCR pattern observed when amplifying genomic DNA containing *FR* clusters is a ladder of bands with an upper molecular weight smear. This ladder was assumed to be the result of PCR amplification between primers in nonadjacent elements separated by different numbers of *FR* repeats. The generation of larger molecular weight bands of a kilobase or more was considered to be diagnostic for the presence of large *FR* clusters (Nagoshi and Meagher 2003a), but this was never directly demonstrated.

Here, we examined the accuracy of the PCR-based method used to diagnose the presence of *FR* clusters and optimized the procedure to facilitate larger surveys of wild populations. The modified method was combined with DNA sequence analysis to demonstrate the presence of the *FR* element in Texas populations, a major source of migrating fall armyworm in North America, and test the geographical extent and temporal consistency of the strain-biased distribution pattern. These results have potential applications and implications toward the study of fall armyworm population dynamics and mating behavior.

Materials and Methods

Field Collections. 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 (Nagoshi et al. 2007). Standard plastic Unitraps were baited with a commercially available fall armyworm pheromone (Scenturion Inc., Clinton WA), and they 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 examined for morphological characteristics diagnostic of fall armyworm, dried, and stored at −20°C in sealed plastic bags. Fall armyworm larvae were manually collected from maize, cotton, sugar cane, millet, sorghum, and pasture grasses in two locations in Brazil (described in Nagoshi et al. 2007), and they were stored in ethanol (95%) at −20°C until they were processed.

DNA Preparation. Individual specimens were homogenized in 4 ml of phosphate-buffered saline (PBS; 20 mM sodium phosphate, 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. The resuspension was transferred to a Zymo-Spin III column (Zymo Research, Orange, CA), and processed according to the manufacturer's instructions. The DNA preparation was increased to a final volume of 40 μl with distilled water. Each PCR reaction required 1 μl of the DNA preparation.

Strain Determination by PCR Analysis. 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), 0.5 μl of *Taq*DNA polymerase (New England Biolabs, Beverly, MA). The thermocycling program was 94°C (1 min), followed by 30 cycles of 92°C (30 s), 56°C (30 s), 72°C (45 s), and a final segment of 72°C for 3 min. Typically 40 or 96 PCR amplifications were performed at the same time by using either 0.2-ml tube strips or 96-well microtiter plates. Amplification of the *COI* region used the primer pair *COI-58 F* (5′-GGAATTTGAGCAGGAATAGTAGG-3′) and *COI-1058R* (5′-

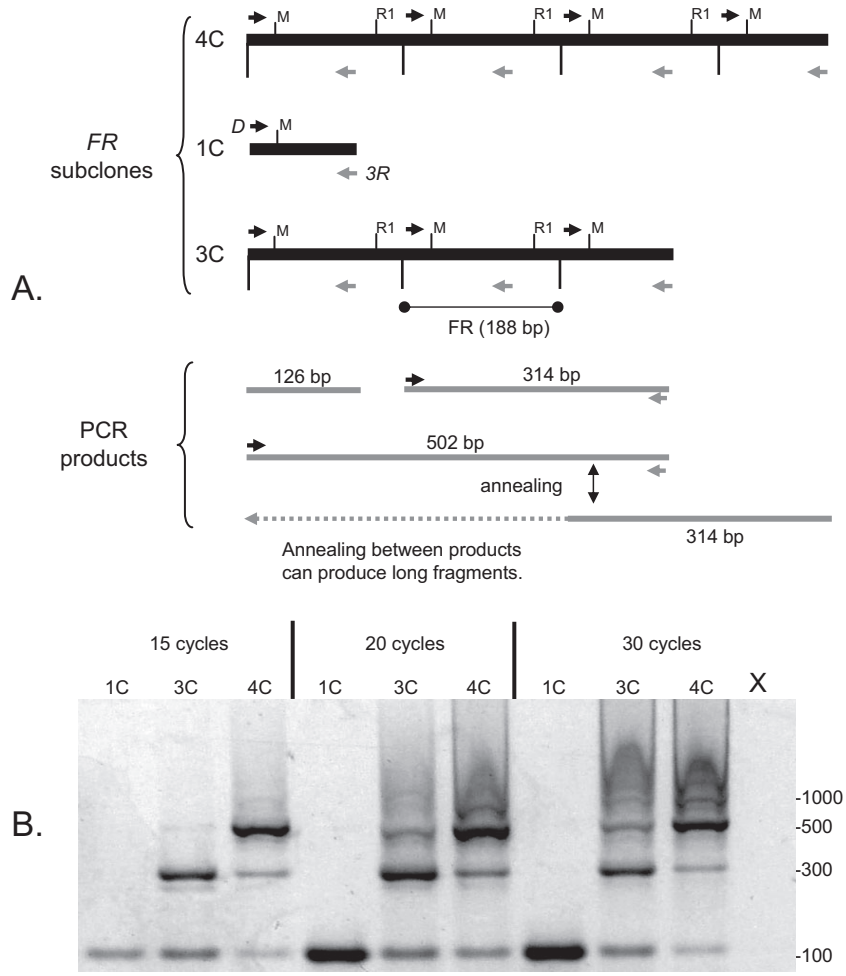

Fig. 1. Map of the 188-bp *FR* element with the location of primers and picture of the resulting DNA ladders produced by PCR. The primer combinations associated with each ladder are listed on top of each lane. Gel picture is a negative image to facilitate detection of bands.

ACACCTGTTAATCCTCCTACAG-3′) to produce a 1.0-kb fragment. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). The fragment contains two MspI sites, one specific to the corn-strain and the other to the rice-strain. Digestion of the PCR product with MspI results in two different restriction patterns that can be identified by agarose gel electrophoresis. The MspI restriction enzyme was diluted in 1× 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 6× gel loading buffer was added to each sample, which was run on a 1.8% PCR grade agarose (Fisher, Hampton, NH) horizontal gel.

Detection and Sequence Analysis of *FR* Clusters. The presence of *FR* clusters was determined by PCR amplification by using the same amplification conditions as for the *COI* gene, unless otherwise noted. The amount of genomic template used for each reaction was estimated from the amount of product produced from the *COI* PCR analysis. Genomic DNA that produced no or aberrant PCR products with the *COI* primers were not further analyzed. Primers were synthesized by Integrated DNA Technologies. The *FR* primers used included *C* (5′-TCGTGTAAAACGTACTTTCTT-3′), *D* (5′-TGAGAGAAGACATTGGTTGACCT-3′), *F* (5′-AAAACGTGCTCTTCTATGTCG-3′), *HfdR* (5′-TGCTCTTCTATGTCCAATTCGTG-3′), *GfdM* (5′-GACCTTTTTACACCCGTCAC-3′), *2R* (5′-GACATAGAAGAGCACGTTT-3′), *3R* (5′-TGATTTCCGACAAAGAATTGC-3′), and *6R* (5′-CGTGATTGCACTTCCACTACAA-3′). PCR-amplified *FR* fragments were isolated from agarose gels using the Zymoclean Gel DNA Recovery kit (Zymo Research) and subcloned using the pGEM-T Easy vector system (Promega, Madison, WI) and selected by β-galactosidase staining and ampicillin resistance according to the manufacturer's instructions. The appropriate subclones were identified by restriction enzyme analysis. DNA was isolated from subclones using the GenElute 5-min Plasmid Miniprep kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. DNA sequencing was

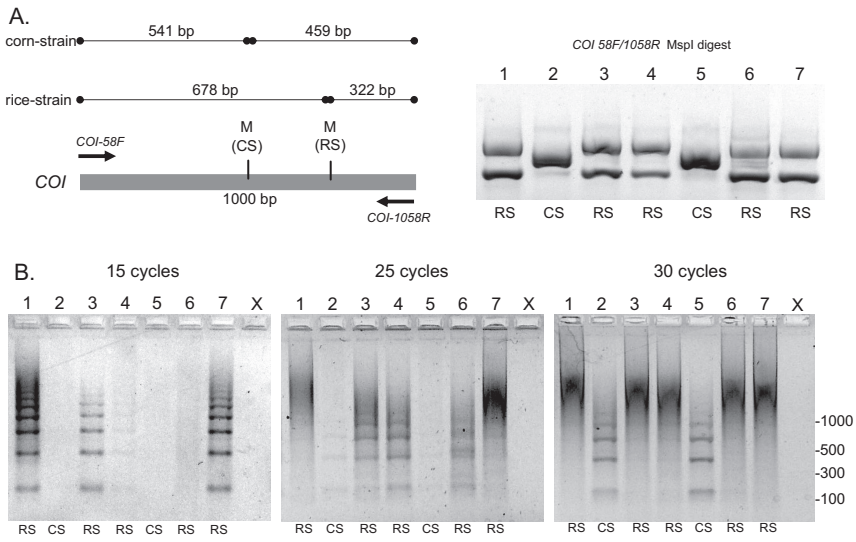

Fig. 2. PCR amplification patterns produced by *FR* subclones carrying variable numbers of repeats and with different numbers of amplification cycles. (A) Map of the *FR* regions inserted into three subclones and a subset of the PCR products predicted from the 3C subclone. Subclone 1C contains the region between primers *D* and *3R*. Subclones 3C and 4C contain multiple copies of the 188-bp *FR* element. Dark arrows indicate locations of primer *D*. Light arrows indicate locations of primer *3R*. Annealing also can occur between PCR products to potentially produce amplification products larger than the starting template. M and R1 represent sites for the restriction enzymes MspI and EcoRI, respectively. (B) Negative image of agarose gel showing banding patterns produced by the *FR* subclones by using different numbers of amplification cycles. Sizes are in base pairs. X indicates control amplifications by using no DNA template.

performed by Northwoods DNA, Inc. (Bemidji, MN), on both strands by using the T7 and SP6 RNA polymerase promoter sequences that flanked the vector insertion site. To optimize the accuracy of the sequence for each element the sequence data from both strands were compared and only those that were completely identical were used. However, aligning the two strands can be difficult with repeated elements. For this reason, we only used data from reactions that extended across the full length of the *FR* insertion. By aligning the two sequenced strands to the vector polylinker restriction sites, it was possible to unambiguously pair each element to its corresponding sequence. The DNA sequencing reaction typically resulted in ≈800 bp of useable DNA sequence, which was, therefore, the upper limit of the insertion size that could be used in this analysis.

Statistical Analysis. Two-tailed paired *t*-test was performed using GraphPad InStat version 5.1 (GraphPad Software Inc., San Diego, CA; www.graphpad.com).

Results

PCR Amplification Patterns Produced by *FR* Repeats. PCR amplification of genomic DNA containing *FR* clusters with any pair of primers specific to *FR* produces a ladder of bands with an upper molecular weight smear (Fig. 1). The "rungs" of the ladder can sometimes occur as doublet bands, as is the case in this example. To test whether this pattern is dependent on the number of *FR* elements, PCR amplification was performed on subclones carrying one (1C), three (3C), or four (4C) copies of the *FR* region defined by the primers *D* and *3R* (Fig. 2A). The 3C and 4C subclones contained two and three intact *FR* elements in tandem repeat array, respectively. After 15 amplification cycles, the three templates produced bands consistent with the different number of priming sites present and the size of the insertion (Fig. 2B). The 1C template generated a single band of ≈100 bp that corresponded to the 126-bp region spanned by the two priming sites. The 3C subclone resulted in two major bands of ≈100 and 300 bp, consistent with the 126-bp *D*/*3R* fragment and a 314-bp product composed of the *D*/*3R* region plus a complete *FR* element (Fig. 2A). The addition of a second *FR* element to the 314-bp fragment would create a 502-bp product. A band of that size occurs as a minor product in the 3C lane, and it was the most prominent species with the 4C template (Fig. 2B). Increasing the PCR amplification to 20 or 30 PCR cycles did not alter the 1C pattern, but produced with the 3C and 4C templates increasing amounts of higher molecular weight bands that ultimately created a "smear" at sizes >1 kb. These larger fragments are the predicted results of priming by the PCR products to themselves or to portions of the template (Fig. 2A).

We tested whether increasing the number of amplification cycles had a similar effect when genomic DNA was used as template. Seven individual samples were analyzed by PCR analysis of the *COI* gene to determine strain identity and estimate the quality of the genomic DNA preparations with respect to PCR amplification (Fig. 3A). Roughly equal amounts of

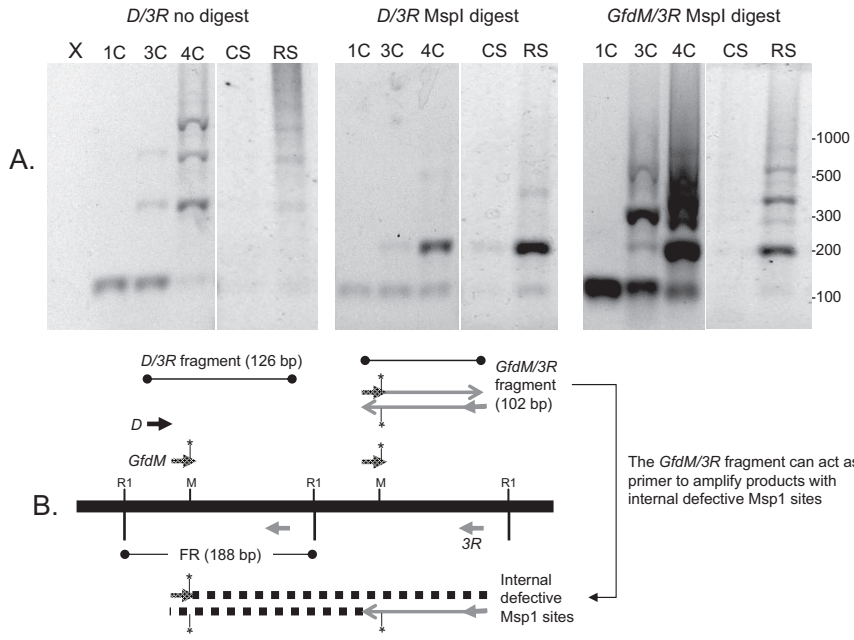

Fig. 3. *FR* PCR patterns produced when using genomic DNA as template and with different numbers of amplification cycles. (A) Diagram and agarose gel (reverse image) of the strain-diagnostic restriction products produced by PCR amplification with the *COI* primers *58 F* and *1058R* followed by MspI digestion. The 1–7 represent individual samples. RS, rice-strain; CS, corn-strain. (B) Negative images of stained agarose gels showing PCR products produced with different numbers of amplification cycles. X indicates control amplifications using no DNA template. The same amount of template DNA was used in the *COI* and each of the *FR* PCR reactions. Sizes are in base pairs.

COI product were produced in each reaction and the same amount of each template DNA was used in the *FR* amplifications. A 15-cycle PCR amplification with *FR* primers produced DNA ladders with rice-strain samples 1, 3, 4, and 7, and no detectable product with the corn-strain samples 2 and 5 or rice-strain sample 6 (Fig. 3B). When the same templates were amplified for 25 cycles, bands became visible in the corn-strain lanes, though remaining <1.0 kb. The sample 6 pattern began to resemble that found in the other rice-strain lanes where background smearing had begun to obscure the individual bands. After 30 cycles, bands in all the rice-strain lanes were almost completely replaced by a high-molecular-weight smear. The corn-strain samples displayed a clear DNA ladder pattern, though the most prominent bands remained <1 kb.

Optimizing Detection of *FR* Clusters by PCR. To facilitate detection of *FR* clusters, we examined whether the higher molecular weight smear could be resolved into discrete bands by digestion of the PCR reaction with a restriction enzyme that cuts once in the consensus *FR* repeat. PCR amplification was performed using the 1C, 3C, and 4C subclones and genomic DNAs from a corn-strain and a rice-strain specimen (Fig. 4A). After 25 cycles, the subclones produced patterns similar to that described in Fig. 2B. The rice-strain genomic DNA produced a ladder pattern that was partially obscured by a higher molecular weight smear, whereas only faint bands were detected with the CS genomic DNA and no product observed in the no template (X) control. Digestion with MspI had little effect on the 1C pattern, but a faint band was observed in the corn-strain genomic DNA lane at about the size of an intact *FR* element, indicating the presence of a low copy number of *FR* repeats. With the 3C PCR reaction, the MspI digestion pattern was consistent with the two larger bands being cut to produce the 126-bp *D/3R* fragment and the 188-bp *FR* element. In the 4C and rice-strain reactions, the most prominent band was one of about the 188-bp size of the single *FR* repeat. This is the expected pattern if the high-molecular-weight smear is made up of fragments containing multiple *FR* repeats that upon digestion with a single-site cutter resolves into individual elements.

Although digestion with MspI simplifies the *FR* amplification pattern by resolving the DNA smear, it also eliminates the DNA ladder, leaving low-molecular-weight (<200-bp) products as the primary markers for the presence of *FR*. Bands of this size can be difficult to detect using traditional agarose gel electrophoresis. To make detection easier, we replaced the *D* primer with *GfdM*, which contains a point mutation that disrupts the internal MspI site in *FR*. The PCR products produced by amplifications with the *GfdM/3R* primer combination are predicted to include a subset with internal defective MspI sites (Fig. 4B). Upon complete digestion with MspI, this should produce a ladder of bands with sizes consistent with varying numbers of *FR* elements and/or *GfdM/3R* fragments. This predic-

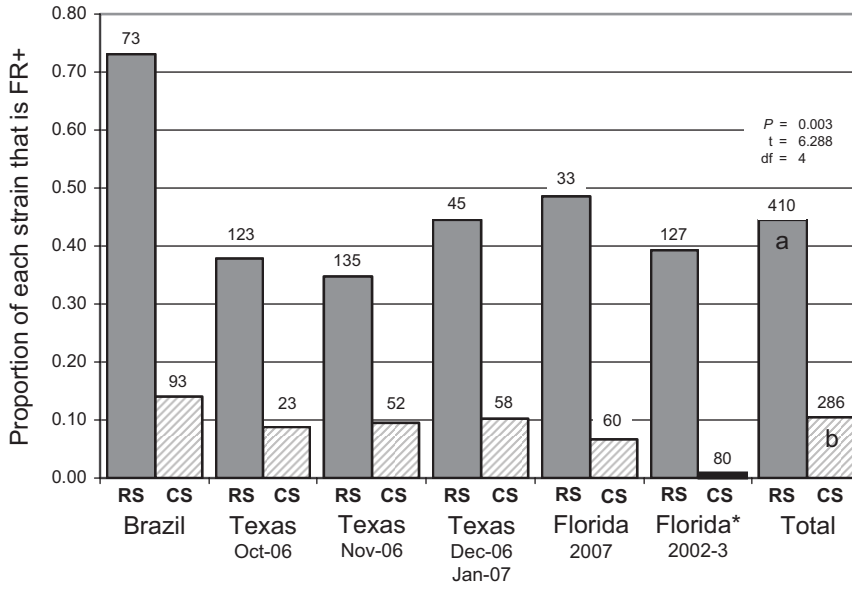

Fig. 4. PCR patterns produced by genomic DNA and *FR* subclones after restriction digests. (A) Negative images of stained agarose gels of PCR patterns by using primer combinations *D/3R* or *GfdM/3R*. 1C, 3C, 4C: PCR using as template the *FR* subclones with one, three, or four copies of the *D/3R* region. CS and RS: PCR using as template corn-strain and rice-strain genomic DNA, respectively. X indicates control amplifications using no DNA template. Sizes are in base pairs. (B) Diagram of the anticipated PCR products produced by *FR*-elements arranged in tandem repeat array. Annealing between PCR products can produce amplification products with defective internal MspI sites. Asterisk: defective MspI site. Dark arrows indicate locations of primer *D*. Light arrows indicate locations of primer *3R*. Cross-hatched arrows indicate locations of primer *GfdM*. M and R1 represent sites for the restriction enzymes MspI and EcoRI, respectively.

tion was confirmed (Fig. 4A). A similar result was obtained when primer *HfdR* (Fig. 1), which carries a defective EcoRI site, was used in combination with primer *3R* (data not shown). Based on these results we modified the *FR* detection methodology to include either the *GfdM* or *HfdR* primers and complete digestion of the amplified products with the appropriate restriction enzyme. The number of amplification cycles was empirically limited to the lowest (typically 25–30) needed to detect bands in the corn-strain sample.

The effectiveness of this modified *FR* detection methodology was tested by reanalysis of DNA that had been previously examined for *FR* clusters (Nagoshi and Meagher 2003a). In that study the identification of FR^+ and FR^0 genotypes required at least two independent PCR reactions using different primer pairs. The results obtained after a single run with the modified method were in agreement with the previous study in 31 of the 32 samples reanalyzed, the exception being a corn-strain sample previously testing as FR^0 that now showed evidence of *FR* clusters. The latter result is likely to be correct as the modified method is potentially more sensitive (due to the concentration of *FR* DNA into fewer bands by the restriction digest). We considered these results a successful test and used the new protocol to analyze fall armyworm populations from Brazil, Texas, and Florida.

Distribution of FR in Brazil and Texas Populations. The Brazil samples were composed of larvae collected in 2005 from corn, sorghum, millet, and native pasture grasses, and they had been analyzed for mitochondrial haplotype, but not *FR* (Nagoshi et al. 2007; Table 1). *FR* clusters were found in 73% of the rice-strain larvae, compared with only 14% of the corn-strain (Fig. 5). This difference between the two strains also was observed in adult males collected by pheromone trapping in the Hidalgo County area of Texas during fall and winter 2006. Each sample was tested for strain identity by mitochondrial haplotype and for the presence of *FR* clusters by PCR analysis. Approximately 40% of the rice-strain population carried *FR* clusters during each of the three collection periods (October 2006, November 2006, December 2006–January 2007), compared with only ≈10% of the corn-strain group (Fig. 5). Approximately the same proportions were observed for fall armyworms collected by pheromone trapping in Florida in 2007, suggesting equilibrium between the *FR* subgroups in the two major U.S. overwintering states. The proportions were also similar to that observed in 2002–2003 in the initial survey of Florida fall armyworm populations (Nagoshi and Meagher 2003a). Data from all areas were pooled (except for the Florida 2002–2003 results), and it was observed that the proportion of rice-strain that were FR^+ was 4.5× greater than in the corn-strain, a highly significant difference as measured by two-tailed, paired *t*-test (see Total in Fig. 5).

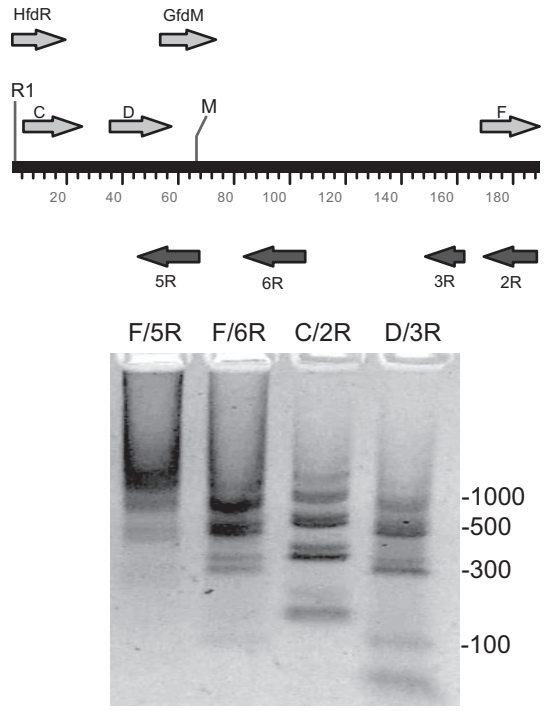

Fig. 5. Distribution of *FR* clusters among fall armyworm strains in different regions. RS, CS: rice-strain, corn-strain. Number of samples tested is indicated on top of each bar. Paired *t*-test was performed on the total (a, b), which showed a highly significant difference in the means of the two strains. Asterisk (*) indicates data from Nagoshi and Meagher (2003a).

Sequence Analysis of *FR* from Texas Fall Armyworm. *FR* sequences had not previously been examined in Texas populations. *FR* elements isolated from eight individuals collected in Texas were sequenced and compared with repeats from Florida and Brazil populations. As reported previously (Machado et al. 2008), *FR* elements from Brazil and Florida shared high similarity and both were homologous to the Texas samples (Fig. 6). Similar numbers of polymorphisms were observed between elements within an individual as were found between individuals. All 37 polymorphic sites involved a single base change, with two sites (between 144–145 and 5–6) associated with a single base insertion. In an earlier study of *FR* elements from Brazil, the same sites were associated with insertion polymorphisms, with an A insertion (in four of 17 elements) between 144–145 and an ACTA insertion (in two of 17 elements) between sites 5–6 (Machado et al. 2008). The presence of size polymorphisms within the *FR* cluster could explain the occasional appearance of doublet bands in the PCR pattern (Fig. 1). The T/G polymorphism at site 84 displays a geographical bias, with nine of 16 of the North American elements carrying a T at this site, whereas all nine sequences from Brazil carried a G. Among the 17 *FR* elements from Brazil analyzed previously, only one carried a T at the site, with the remainder carrying the consensus G (Machado et al. 2008).

Discussion

The tandem repeat organization of *FR* in fall armyworm results in a complex PCR amplification pattern that can be used as a diagnostic marker for the presence of *FR* clusters. PCR amplification of subclones carrying different numbers of *FR* repeats demonstrated that after 15 amplification cycles, the resultant PCR pattern corresponded to the number of repeats present on the template. However, as cycle numbers increased the sizes of the PCR products also increased, with lengths often substantially longer than the template size (Fig. 2). The result was that even a cluster containing only two to three elements can produce a DNA ladder previously considered diagnostic of much larger *FR* clusters. Such ladders were not observed with single element templates or with the *FR* primers alone (no template). These characteristics indicate that although the PCR method is very sensitive for identifying the presence of *FR* tandem array clusters, the ability to accurately distinguish between the large clusters associated with the rice-strain and the smaller arrays often observed in the corn-strain (Nagoshi and Meagher 2003b), will be influenced by the amount of template DNA and the number of amplification cycles.

To account for these issues the amount of template genomic DNA used in the *FR* amplification study was determined by an independent measure of the quality (with respect to the ability to be amplified) and concentration of the template. This was accomplished by PCR amplification of the genomic DNA preparations for a segment of the mitochondrial *COI* gene, which was required in any case to assess strain identity. Simple visual inspection of the stained agarose gel pattern was then used to estimate the amount of template DNA to use in the *FR* experiment (Fig. 3A). Although this method provides only a rough approximation of DNA levels and quality, it was sufficient to reproducibly distinguish between FR^{+} and FR^{0} patterns in samples from Florida, Brazil, and Texas.

Although *FR* clusters were originally identified as being strictly rice-strain specific in tests of laboratory colonies and wild populations from Georgia (Lu et al. 1994), a more polymorphic distribution was observed in Florida populations that could have arisen from limited interstrain matings (Nagoshi and Meagher 2003a). In 2002–2003, 39% of the mt^{R} (rice-strain) specimens tested were FR^{+} compared with none of the 80 mt^{C} (corn-strain) samples (Fig. 5). This asymmetry has remained relatively unchanged after 4 yr, because our 2007 Florida sampling found 48% (16/33) of the rice-strain and only 7% (4/60) of the corn-strain tested were FR^{+}. The *FR* element in Texas fall armyworm also displayed a strain-biased distribution, with proportions in each strain similar to that observed in Florida in 2003 and 2007. These observations suggest that *FR* may be in polymorphic equilibrium in the southeastern United States.

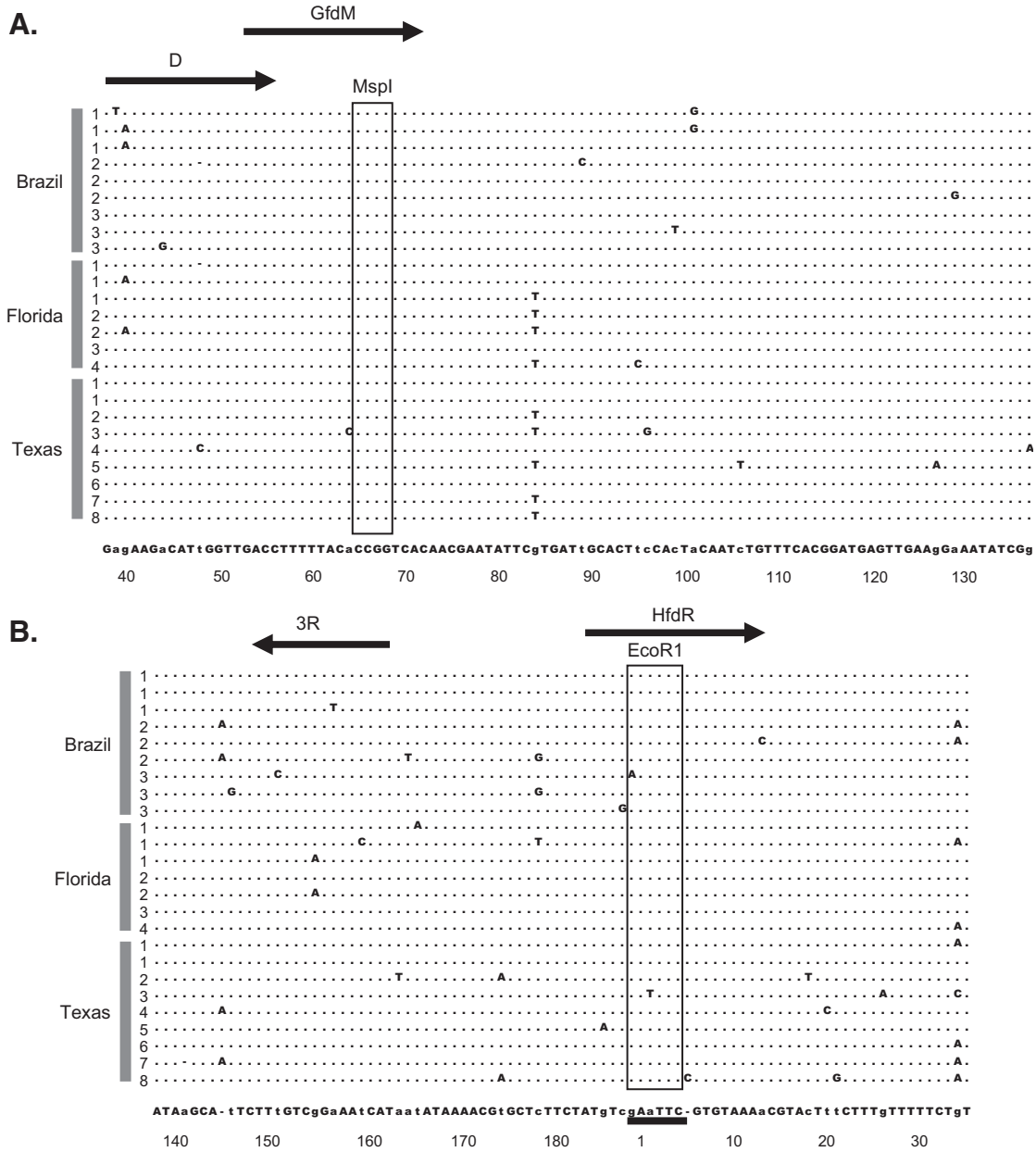

Fig. 6. DNA sequence comparisons between *FR* elements from different locations. Numbers indicate different individual specimens. Sequences with the same number in the same location are different elements in the same individual. Letters indicate nucleotides that differ from the consensus. The coordinates are based on the *FR* sequence of Lu and Adang (1994).

Recently, *FR* elements in Brazil fall armyworm populations were characterized (Machado et al. 2008). Although sample numbers were relatively small, they demonstrated a strain biased distribution of *FR* elements similar to that consistently seen in the United States over multiple years. Therefore, the asymmetric distribution of *FR* between strains is relatively stable across time and between geographically distant regions, with *FR* clusters always preferentially found in the rice-strain. This indicates restrictions in interstrain mating in all the tested populations, because otherwise a more homogeneous distribution of the FR^+ phenotype in the two strains would be expected.

The strain-biased distribution of *FR* clusters makes it a useful marker to examine subpopulations within the individual strains that are likely to have arisen from interstrain mating. The modifications in the *FR* detection protocol described in this article should facilitate these studies by allowing larger sample numbers to be more quickly and accurately analyzed. This was dem-

onstrated by our examination of fall armyworm populations from Texas and Florida, which gave results consistent with earlier surveys. The results indicate that the *FR* sequence is present throughout most of the Western Hemisphere and is behaving similarly in the different geographic locations. We are currently continuing these field studies to determine whether the presence or absence of *FR* clusters identifies genetic subgroups with significantly different behaviors or susceptibilities to pesticides. The identification of such subpopulations is essential for the development of effective Insect resistance management and integrated pest management strategies for fall armyworm.

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