Effects of Fall Armyworm (Lepidoptera: Noctuidae) Interstrain Mating in Wild Populations

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ABSTRACT Fall armyworm is a significant agricultural pest in the United States, affecting most notably sweet corn and turfgrass. Two morphologically identical strains, rice strain (R-strain) and corn strain (C-strain), exist that differ physiologically and behaviorally and can be identified by mitochondrial haplotyping. Recent studies of overwintering populations in Florida indicate that the mitochondrial lineage associated with the R-strain itself consists of two genetically distinct subgroups, with one having molecular markers consistent with interstrain hybridization between R-strain females and C-strain males. To test this possibility and examine the ramifications of interstrain mating on population behavior and strain fidelity, larvae and adult males were tested for genetic marker combinations representative of the host strains and potential hybrids. These studies showed a sexually dimorphic distribution pattern for a sex-linked marker that is a predicted result of interstrain mating. Despite evidence of substantial interbreeding in the overwintering sites, both *FR* and the strain-diagnostic mitochondrial markers still showed the plant host and habitat biases associated with the host strains, indicating that strain integrity was largely maintained. However, there is evidence that the two R-strain subpopulations differ in habitat distribution in a manner suggestive of the "hybrid" genotype being less specific in its plant host preference. The existence of a genetically distinct hybrid subpopulation must be taken into account when evaluating fall armyworm population dynamics and infestation patterns in overwintering areas.

KEY WORDS *Spodoptera frugiperda*, host strains, interstrain mating, *FR*

SYMPATRIC SPECIATION DESCRIBES THE divergence of a single lineage into two species in the absence of geographical isolation (reviewed in Berlocher and Feder 2002). One putative example of such a process is the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), where it is proposed that adaptation to different plant hosts has led to the evolution of premating barriers (Thomas et al. 2003). In this study, we investigated the population dynamics of another Lepidopteran species, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) or fall armyworm, that may be undergoing a similar process of sympatric divergence of two strains that also differ in their preferred hosts (Pashley 1986, 1988, Pashley et al. 1992, Prowell 1998).

Fall armyworm is a significant and periodic pest of several crops, including maize, sorghum, forage grasses, turf grasses, rice, sugarcane, cotton, and peanuts (Luginbill 1928, Sparks 1979, Hall 1988). The species overwinters in southern Florida and southern Texas, which serve as sources of the springtime populations that migrate northward into the central and eastern United States and Canada (Barfield et al. 1980, Mitchell et al. 1991). Analysis of electrophoretic protein variants revealed a strong correlation between plant host and the presence of particular allozymes, identifying one strain (designated corn strain or C-strain) primarily associated with large grasses, such as corn and sorghum, and another (rice strain or R-strain) that fed on smaller grasses, including rice and Bermuda grass (Pashley 1986, 1988b, Pashley et al. 1987).

Strain-specific morphological characters have yet to be found so strain identification remains dependent on molecular markers. Restriction fragment length polymorphism (RFLP) analysis of genomic DNA identified two groups generally consistent with the R-strain and C-strain classifications (Lu et al. 1992), as

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did comparing variations in mitochondrial DNA haplotypes (Pashley 1989, Pashley and Ke 1992, Lu and Adang 1996). In particular, an *Msp*I restriction enzyme polymorphism was identified in the mitochondrial *cytochrome oxidase I* (*COI*) gene that is diagnostic of strain identity and for which a polymerase chain reaction (PCR)-based method of detection is available that allows the analysis of single specimens (Lu and Adang 1996, Levy et al. 2002, Meagher and Gallo-Meagher 2003). We have previously used this marker as the primary indicator of strain identity, with the R-strain–specific polymorphism noted as mt^R and the C-strain as mt^C (Nagoshi and Meagher 2003a, 2004, Meagher and Nagoshi 2004).

The persistence of two genetically distinct subpopulations that display substantial geographical overlap suggests that productive mating between fall armyworm strains must be limited. However, reports examining interstrain mating in the laboratory are conflicting, with some observing mating restrictions that others have not been able to verify (Pashley and Martin 1987, Whitford et al. 1988, Quisenberry 1991, Pashley et al. 1992). Overall, these studies showed that the strains are reproductively compatible, but there may be factors that negate or limit interstrain mating in the field that are compromised by artificial rearing or experimental conditions (Pashley et al. 1992). Field studies measuring the discordance between strain-specific allozyme and mitochondrial markers suggested that as many as 16% of individuals isolated from wild populations could be interstrain hybrids (Prowell 1998).

We examined the likelihood of interstrain mating in wild populations using a tandem repeat sequence called *FR* (for *F*all armyworm *R*ice strain) that was reported to be always and only present in fall armyworms carrying the mt^R mitochondrial marker in Georgia populations (Lu et al. 1994, Lu and Adang 1996). In fall armyworm, the female is the heterogametic sex, with a ZZ (male)/ZW (female) sex determination system. *FR* clusters map to the sex chromosomes (both Z and *W*) and can be detected by PCR amplification, providing a convenient genomic DNA marker to complement the mitochondrial mt^R/mt^C polymorphism (Nagoshi and Meagher 2003a, b). We defined the "parental" marker combinations as mt^R FR^+ for the R-strain and mt^C FR^0 for the C-strain, and showed in the laboratory that the "hybrid" configurations of mt^R FR^0 and mt^C FR^+ can be produced within two generations of interstrain crosses (Nagoshi and Meagher 2003b). Therefore, if interstrain mating commonly occurs in the wild, a substantial proportion of captured fall armyworm should display one or both hybrid combinations. Our survey of fall armyworms in Florida found that the hybrid configuration mt^C FR^+ was rare (<5% of samples) in all habitats tested (Nagoshi and Meagher 2003b), indicating that mating between C-strain females and R-strain males was infrequent or unproductive. In contrast, about one half of the mt^R males collected from pheromone traps were FR^0, the marker combination predicted if R-strain females mate to C-strain males, suggesting substantial levels of interstrain mating in this direction.

These results suggest that the R-strain population in Florida differs substantially with respect to the level of *FR* polymorphism and the proportion of interstrain hybrids estimated in previous studies (Lu et al. 1994, Prowell 1998). This may reflect the fact that the two strains are present throughout the year in Florida, providing prolonged opportunities for interbreeding. However, interstrain mating should rapidly compromise any strain-specific behaviors originally exhibited by the two mitochondrial lineages, bringing into question whether these markers (and *FR*) can accurately identify strains in populations endogenous to overwintering sites. We also cannot preclude the alternative explanation that the *FR* repeat element was introduced relatively recently into the R-strain population, in which case its asymmetric distribution would reflect random segregation rather than interstrain hybridization.

In this paper, we studied the distribution of fall armyworm subpopulations by combining larval collections with pheromone trap captures. The objectives were to test whether overwintering populations in Florida still retained strain-specific distributions despite substantial opportunity for interbreeding. Evidence is presented that, although interbreeding likely occurs, wild populations display sex-specific and habitat-specific marker distributions consistent with two strains. Possible explanations and ramifications of these observations are discussed.

Materials and Methods

Collection Methods and Sites. Larval collections were made in sweet corn (*Zea mays* L.) fields located in Avon Park ($n = 215$) and Belle Glade, FL ($n = 220$). All were in large agricultural areas where both spring and winter corn crops are grown. The most northern location (Avon Park) was located ≈170 km north of Belle Glade (Fig. 1). Larvae were collected at 1- to 2-wk intervals as soon as infestations were visible, typically during the whorl stage of plant development. Adult males were captured during the larval collection periods at the Avon Park and Miami-Dade Co. sites using pheromone traps as previously described (Meagher and Gallo-Meagher 2003). Standard plastic Unitraps were baited with a commercially available fall armyworm pheromone (Scenturion, Clinton, WA) and contained insecticide strips (Hercon Environmental Co., Emigsville, PA). Trapped moths were counted, and a subset (13–15) randomly selected for PCR analysis. Specimens were stored at −20°C after collection.

Male adult moths also were captured in pheromone traps placed adjacent to a large agricultural area of sweet corn production in southern Miami-Dade Co., a sod farm (235 ha) in northcentral Collier Co., and in the vicinity of sugarcane in the Everglades Agricultural Area near Clewiston (Hendry Co.). The Collier Co. site contained year-round production of primarily St. Augustine grass [*Stenotaphrum secundatum* (Walt.)

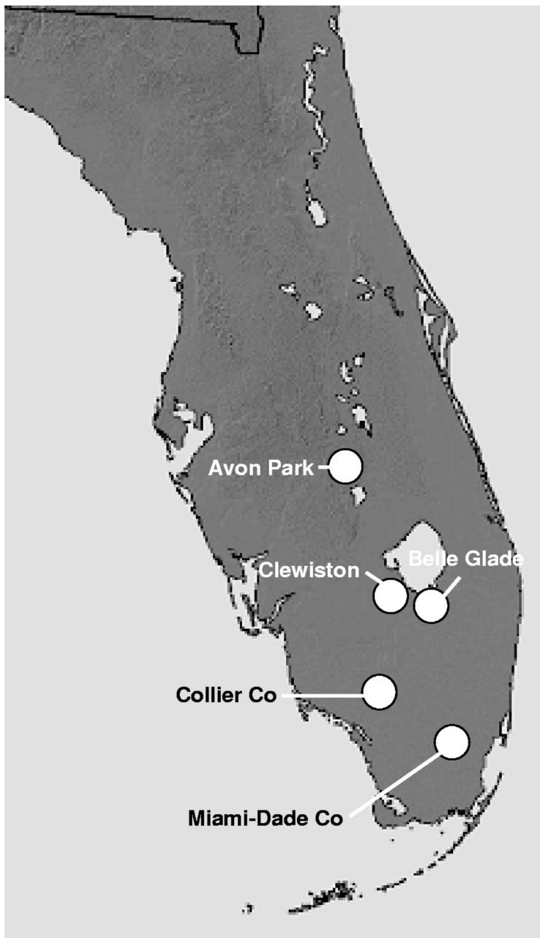

Fig. 1. Map of Florida showing locations of fall armyworm collections.

Kuntze] and sections of bahiagrass (*Paspalum notatum* Flugge). Traps were placed in Miami-Dade and Collier Cos. on 2 January 2003, and collections were made at ≈2-wk intervals until 27 May 2003, which overlapped the winter and spring growing seasons. Collections in sugarcane were made during April, June, and October 2003.

DNA Preparation. Individual adult moths were homogenized in 1 ml of homogenization buffer (0.03 M Tris-HCl at pH 8.0, 0.1 M NaCl, 0.2 M sucrose, 0.01 M EDTA at pH 8.0, 0.5% Triton X-100) in a 5-ml Dounce homogenizer using either a hand pestle or a motorized mixer. To remove large debris, the homogenate was filtered through a 5-ml plastic syringe plugged with cheese cloth (prewet with sterile water) into a 1.5-ml microfuge tube. Cells and nuclei were pelleted by centrifugation at 12,000*g* for 10 min at 4°C, and the supernatant was removed by aspiration. The pellet was resuspended in 600 μl nuclei buffer (0.01 M Tris-HCl at pH 8.0, 0.35 M NaCl, 0.1 M EDTA, and 1% *N*-lauryl sarcosine) and extracted with 400 μl phenol-chloroform (1:1). The supernatant was transferred to a new 1.5-ml tube, precipitated with 400 μl isopropanol for 1 h at room temperature, and centrifuged at 12,000*g* for 10 min. The DNA pellet was washed with 70% ethanol and dried. The pellet was resuspended in 50 μl distilled water, followed by purification using DNA Clean and Concentrator-5 columns (Zymo Research, Orange, CA) according to the manufacturer's instructions. We used 1 μl of the DNA preparation (between 0.05 and 0.1 μg) for each PCR reaction.

PCR Analysis. To determine strain identity from individual moths, each DNA preparation was tested by PCR for the mitochondrial *COI* gene RFLP as described previously (Levy et al. 2002, Nagoshi and Meagher 2003a). We had previously determined empirically that PCR amplification of genomic DNA was most consistent when performed in a 40-μl reaction mix containing 4 μl 10× reaction buffer with $MgCl_2$ (Promega, Madison, WI), 1 μl 10 mM dNTP (New England Biolabs, Beverly, MA), 0.5 μl 20 μM primer mix, 1 μl DNA template, and 2 U *Taq*DNA polymerase (Promega, Madison, WI). Amplification of the *COI* gene used primers *JM76* and *JM77* and began with an initial incubation at 94°C (1 min) followed by 33 cycles of 94 (30 s), 56 (45 s), 72 (1 min), and a final segment of 72°C for 2 min. On completion of the PCR, 2 U of *Msp*I was added to each reaction mixture and incubated at 37°C for 1 h. Samples were examined by gel chromatography on a 1.5% agarose gel stained with ethidium bromide. The R-strain (mt^R) pattern is a 569-bp PCR band, while the C-strain fragment (mt^C) is cut by *Msp*I to produce two fragments of 497 and 72 bp (Levy et al. 2002). Confirmation of these results were made by digestion of the PCR fragment with 2 U of *Sac*I, which produces two bands of 311 and 258 bp in the R-strain and a single 569-bp fragment in the C-strain (unpublished results).

Identification of *FR* clusters was performed using two primer sets for PCR analysis. The initial survey used primers *FR-C* and *FR-2.* The results were confirmed by a set of primers, *FR-D* and *FR-3,* which lie internal to *FR-C/2.* In most cases, a third amplification using one of these primer sets was performed as an additional confirmation. Primers *FR-C/2* are predicted to amplify a 186-bp sequence from the *FR* repeat. In actuality, FR^+ samples produce a DNA ladder associated with a higher molecular weight smear. These result from overlapping amplification of multiple *FR* units organized in clusters of tandem repeats (Nagoshi and Meagher 2003a). In contrast, FR^0 samples produce zero to three relatively faint bands. These amplified fragments result from either the presence of a few *FR* units as described by Nagoshi and Meagher (2003a) or primer self-annealing. The presence of bands >500 bp is diagnostic of FR^+ samples. For PCR, the same conditions were used as with the *JM-76/77* primers except that an annealing temperature of 52°C was used. The primer *FR-D/3* or *FR-D/2* is predicted to amplify an internal sequence in the *FR* repeat. These also produce a DNA ladder with FR^+ genomic DNA as template and were used to confirm the *FR-C/2* results. The same conditions were used as with the *JM-76/77* primers. PCR products were visualized on a 2% agarose gel labeled with ethidium bromide.

Primers were synthesized by DNAgency (Malvern, PA) or Integrated DNA Technologies (Coralville, IA). They included *JM-76,* 5′-GAGCTGAATTAGG(G/A)ACTCCAGG-3′, and *JM-77,* 5′-ATCACCTCC(A/T)CCTGCAGGATC-3′, that span the mitochondrial *COI* gene. The *FR*-specific primers used were *FR-C* (5′-TCGTGTAAAACGTACTTTCTT-3′), *FR-2* (5′-GACATAGAAGAGCACGTTT-3′), *FR-D* (5′-TGTGAGAAGACATTGGTTGACC-3′), and *FR-3* (5′-TGATTTCCGACAAGAATTGC-3′).

Statistics. Analysis of variance (ANOVA; PROC MIXED; Littell et al. 1996) was used to compare the proportion of the population that was either the parental or hybrid genotype (mt^R FR^+, mt^C FR^0, mt^R FR^0, or mt^C FR^+). All means were separated using contrasts, so the comparison between proportions were constructed according to significance. For the larval collection data, the contrast statement was used to individually compare genotypes for female or male larvae. For the pheromone trap data across the corn and turf habitats, contrasts compared genotypes within habitats, and another set of contrasts were used to compare genotypes across habitats. Similarly, contrasts compared genotypes within months and compared genotypes among months for the sugar cane data. In all cases, data were subjected to arcsine-square-root transformation before analysis.

Results

Strain-specific Sexual Dimorphism of *FR*. Mitochondria and the *W*-chromosome both display a strictly maternal pattern of inheritance in that daughters inherit the *W*-chromosome only from their mothers and all progeny carry only the maternal mitochondria. In contrast, the maternal *Z*-chromosome can segregate to either sex. Based on these inheritance patterns, if the two host strains are defined by the mt^C FR^0 and mt^R FR^+ genotypes as we suspect, the hybrid combinations (mt^C FR^+ and mt^R FR^0) will arise from interstrain crosses and will display the distinctive sexual distribution patterns of mt^R FR^0 present only in males and mt^C FR^+ found in both sexes (Fig. 2). We tested this possibility by examining field-collected larvae from corn plants for strain and sexual identity. The mt^C specimens made up the majority of the sample population and were polymorphic for *FR* in both sexes (Fig. 3). Within the mt^C larval subpopulation, 10.2% of males (16/156) and 16.6% of females (24/145) were FR^+, findings consistent with previous observations from pheromone trapping indicating the relative scarcity of the mt^C FR^+ genotype (Nagoshi and Meagher 2003b). In comparison, the R-strain displayed a sexually dimorphic distribution of *FR*. All mt^R females tested were FR^+ (73/73) compared with 32.8% (20/61) of mt^R males collected contemporaneously from the same group of plants. Therefore, the mt^R FR^0 subpopulation was limited to male samples.

Distribution of the Hybrid mt^R FR^0 Genotype. To test whether the genotypes differed in habitat distribution, pheromone trapping was performed in sweet corn and turfgrass dominated habitats during the spring. This is the only season when substantial numbers of C-strain males were consistently collected by pheromone trapping (Meagher and Nagoshi 2004, Nagoshi and Meagher 2004). As anticipated, the two habitats differed in the proportions of the parental mt^R FR^+ and mt^C FR^0 subpopulations present, confirming the observation of strain-dependent habitat preferences (Fig. 4, corn-spring versus turf-spring). In the turf habitat, 53.7% of the total capture population was mt^R FR^+, compared with 25.4% in the spring cornfield ($F_{1,91} = 15.1$, $P = 0.0002$). Even stronger habitat bias was exhibited by the C-strain parental (mt^C FR^0) group, with a five-fold higher proportion (31.5 versus 6.1%) in the spring cornfield than the turf traps ($F_{1,91} = 26.5$, $P < 0.0001$). In contrast to the two parental genotypes, the mt^R FR^0 subgroup showed no habitat bias, comprising ≈40% of the capture population in both locations ($F_{1,91} = 0.08$, $P = 0.7772$).

Similar strain-dependent differences were observed when comparing spring and fall pheromone trap populations from the same cornfield (Fig. 4, corn-spring versus corn-fall). The proportion of C-strain parental mt^C FR^0 males declined from 31.5% in the spring to 7.2% in the fall ($F_{1,91} = 16.1$, $P = 0.0001$), while the proportion of R-strain parental mt^R FR^+ males nearly doubled from 25.4 to 45.5% ($F_{1,91} = 6.7$, $P = 0.0115$). Once again, no significant change was observed in the percentage of the R-strain hybrid mt^R FR^0 population across the different seasons (39.6–45.2%; $F_{1,91} = 0.95$, $P = 0.3324$), consistent with reduced habitat preference compared with the parental mt^R FR^+ group. The reciprocal hybrid class, mt^C FR^+, was only rarely found in all the trap collections, never exceeding 3.5% of the total population with no significant differences among the collections ($F_{1,91} = 0.07$, $P = 0.7980$).

Differences in the distributions of the mt^R FR^+ and mt^R FR^0 subpopulations were also observed in surveys of sugarcane fields in southcentral Florida (Fig. 5). While the R-strain generally predominated, there was a subset of traps in April and June where the C-strain made up a significant fraction (>20%) of the collections. Pooled data from these traps showed C-strain percentages (both FR^0 and FR^+) of 31.3% in April and 35.0% in June, which declined to 2.1% in October captures from the same subset of traps. The latter decline was caused by a significant increase in mt^R FR^+ (October 60.8% versus June 29.9%, $F_{1,40} = 11.4$, $P = 0.0017$; October versus April 28.8%, $F_{1,40} = 12.1$, $P = 0.0013$), indicating a seasonal shift to a more R-strain dominated habitat. However, this increase was not observed with the hybrid mt^R FR^0 subgroup, whose percentages remained relatively unchanged and therefore showed a different dynamic than their FR^+ siblings derived from mt^R mothers.

Discussion

The fall armyworm R-strain can be identified by several molecular markers, including the mt^R polymorphism in the mitochondrial *COI* gene and the presence of the sex-linked *FR* repeat element (Lu et

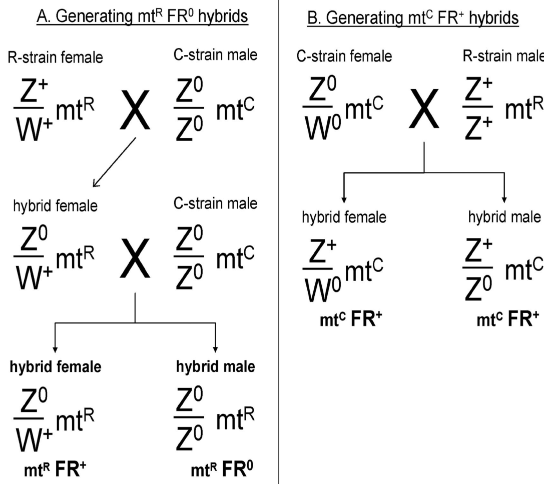

Fig. 2. Schematic of interstrain matings required to generate moths displaying the mt^R FR^0 and mt^C FR^+ phenotypes. (A) mt^R FR^0 hybrids require two generations of interstrain crosses. (B) mt^C FR^+ hybrids are observed after a single generation because FR^+ is a dominant marker.

al. 1994, Levy et al. 2002). While males of the mt^R lineage in Florida were highly polymorphic for the presence of *FR* (Nagoshi and Meagher 2003b), we showed that all mt^R females were FR^+ (Fig. 3). This sexually dimorphic pattern can be explained if the FR^+ sex chromosomes segregated with mt^R mitochondria into the R-strain lineage early enough in the divergence of the two strains such that most, if not all, of the ancestral R-strain population carried the mt^R FR^+ genotype. The strictly maternal inheritance pattern of both mitochondria and the *W*-chromosome would guarantee the continued linkage of the two markers in females even with the occurrence of interstrain mating (Fig. 2). In contrast, successive rounds of cross-hybridization will result in males polymorphic for *FR* because of the segregation pattern of the Z-chromosome. A different *FR* distribution was expected for the mt^C lineage, where interstrain matings of mt^C females with mt^R males should produce mt^C progeny polymorphic for *FR* in both sexes (Fig. 2). The correspondence of the strain-specific and sex-specific patterns of *FR* distribution found in wild populations with that predicted provides strong support that the observed polymorphisms result from interstrain crosses.

We previously showed by pheromone trap surveys in turfgrass habitats that *FR* polymorphism in males occurs more frequently in the mt^R lineage than the mt^C (Nagoshi and Meagher 2003b). This study extends this observation to include larval collections from corn and pheromone trap surveys in both corn and sugarcane dominated habitats in the spring and fall (Figs. 3–5). Male hybrids of the mt^C FR^+ genotype were rare relative to their mt^R FR^0 siblings regardless of collection method, season, or the predominant plant host in the survey area. This indicates that the asymmetry of the hybrid classes is not strongly influenced by environmental factors, even those that affect the distribu-

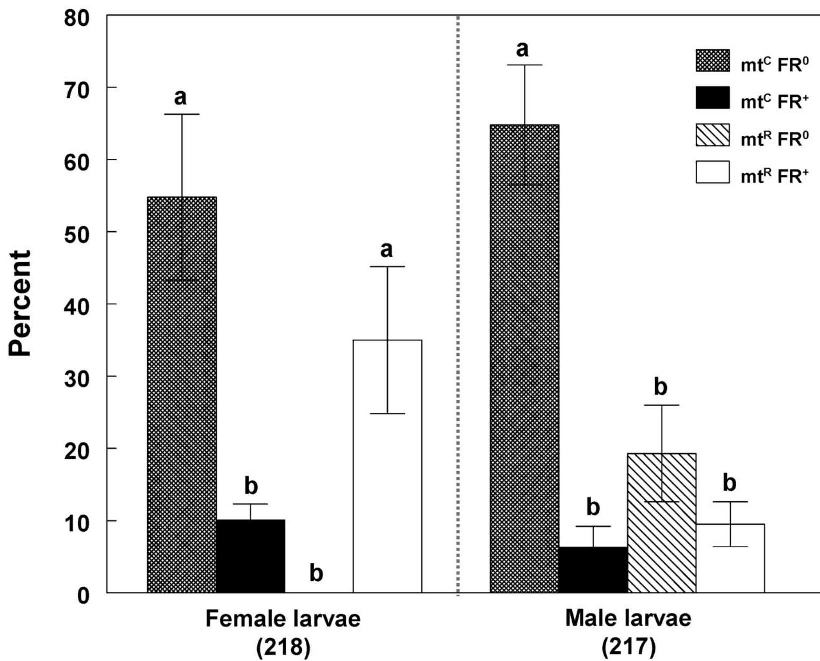

Fig. 3. Percentages of different genotypes in male and female larvae collected from corn plants in Florida during fall/winter 2003. Columns represent each genotype with respect to the total of each sex sampled (number in parentheses). Bars indicate SD. Comparisons were performed among genotypes within each sex, with different letters denoting significant differences ($P < 0.05$).

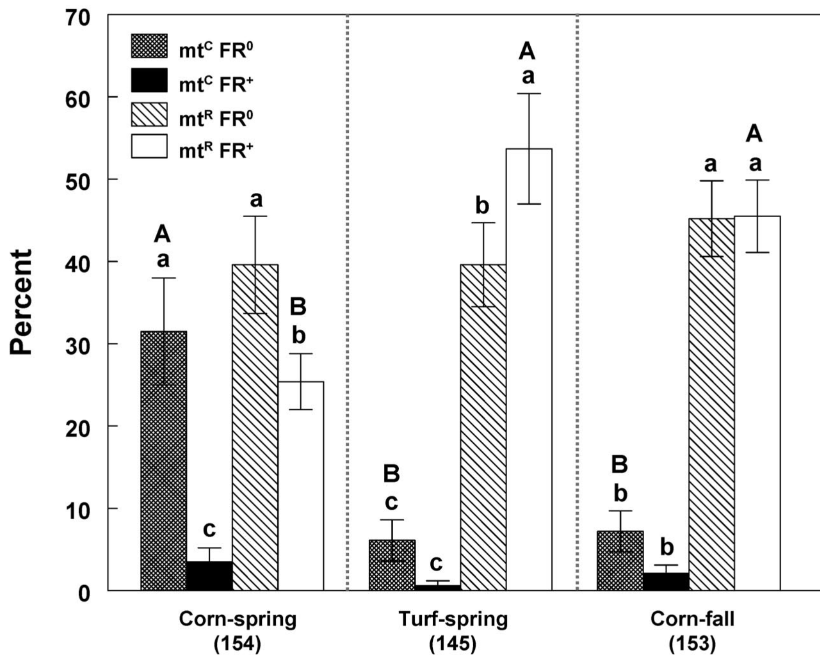

Fig. 4. Percentages of different genotypes of adult males collected from pheromone traps in a cornfield and managed turf habitat in Florida during the spring and fall of 2003. Columns represent each genotype with respect to the total from each collection site (number in parentheses). Bars indicate SD. Different lowercase letters denote significant differences ($P < 0.05$) among genotypes within a collection. Different capital letters denote significant differences ($P < 0.05$) among the same genotypes from different collections.

tions of the two host strains. Given these observations, it seems unlikely that the hybrid ratios can be explained by habitat-dependent differences in fitness, particularly because the two hybrid types differ only in the direction of the parental cross. Instead, we believe it more likely that the interstrain matings that give rise to the $mt^C\ FR^+$ genotype occur infrequently compared with those producing $mt^R\ FR^0$ progeny.

An earlier study also found evidence of hybrid formation occurring primarily by interstrain matings between R-strain females and C-strain males (Prowell et al. 2004). However, in this case, the majority of the presumptive hybrids were found in corn-dominated habitats, which was attributed to their being more opportunities for interstrain matings in corn (where both strains tended to be present in substantial numbers). Our results did not show a similar bias in the distribution of the $mt^R\ FR^0$ hybrids. Instead, the percentage of hybrids remained the same regardless of whether the collection period was dominated by one or the other strain. This absence of bias is most consistent with plant host preference being compromised by the mixing of R-strain and C-strain genomes, resulting in either a loss of specificity or a genetically complex hybrid population comprised of subgroups with different preferences. The latter possibility reflects the complications inherent when using a small number of molecular markers to estimate genealogy (Nason and Ellstrand 1993). While hybrid marker combinations indicate the occurrence of at least one interstrain cross, they do not delineate the number of such crosses or whether backcrosses to the parental strains might also have occurred. Therefore, individuals within the same hybrid subgroup could vary substantially in the proportion of different strain-specific alleles present and consequently with respect to their strain-specific behaviors. Similarly, a subset of those carrying the parental $mt^R\ FR^+$ and $mt^C\ FR^0$ marker configurations could at least potentially arise from crosses involving hybrids and therefore be of mixed genotype. At best we can say that if some restrictions to interstrain mating exist, nonhybrids should be preferentially found in the populations displaying the parental markers and these should therefore be more prone to behave as expected of the host strains. The differences in the geographical and seasonal distributions observed between the different genotypes are consistent with such mating restrictions and also indicate the existence of a substantial hybrid population whose behaviors are largely undefined.

Previous studies on interstrain hybridization estimated that the hybrid genotypes approximated 16% of the wild population (Prowell 1998, Prowell et al. 2004). This was substantially lower than we observed, particularly with respect to pheromone trap collections (Fig. 4). However, the significance of this difference is unclear because the detection of hybrids could be affected by a number of factors, including the method of collection. For example, the percentage of the putative $mt^R\ FR^0$ hybrids observed with larval

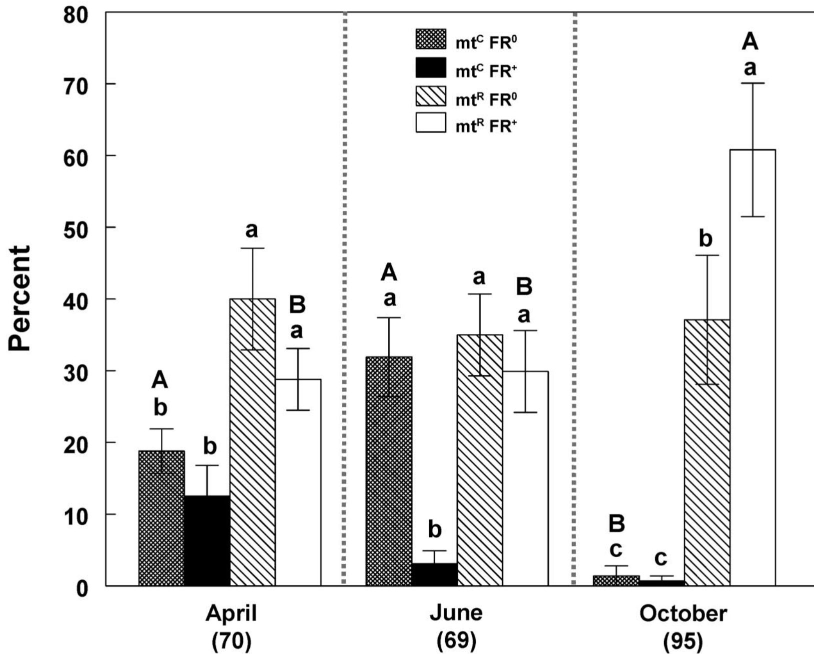

Fig. 5. Percentages of different genotypes of adult males collected from pheromone traps in a sugarcane-dominated habitat in Florida during 2003. The April and June columns represent pooled data from trap collections with >20% carrying the mt^C marker during those periods. The October data are collections from the same traps represented in the April and June groups. Columns represent each genotype with respect to the total from each collection site (number in parentheses). Bars indicate SD. Different lowercase letters denote significant differences ($P < 0.05$) among genotypes within a collection. Different capital letters denote significant differences ($P < 0.05$) among the same genotypes from different collections.

males isolated from corn plants (Fig. 3) was about one half that found in pheromone traps from cornfields (Fig. 4). In addition, hybrids are more likely to occur in overwintering areas, where both strains are present throughout the year, than sites dependent on migratory populations. Considerably more information concerning the behavior and fitness of hybrids and their temporal and geographical distribution in the field is needed before an accurate estimate of hybridization frequency can be made. Defining the behavioral characteristics of the different genetic subgroups and better understanding the processes driving their differential geographical and temporal distribution will be critical to developing methods for controlling fall armyworm infestations, as well as understanding the mechanisms of strain divergence and sympatric speciation.

Substantial interstrain mating in overwintering areas should result in increased discordance between the expected and observed distribution of strain-specific markers in different habitats. Instead, we found strain integrity was still preserved in the tested areas. Pooled data from larval and pheromone trap collections found that 93.0% (475/511) of FR^+ males carried the mt^R marker, whereas 85.9% (366/426) of mt^C males were FR^0. Therefore, the FR^+ Z-chromosome remains largely confined to the mt^R mitochondrial lineage, although a substantial proportion of mt^R males are FR^0 (45.7%, 356/779). In addition, a survey of male larvae collected from corn plants, which should be heavily biased to the C-strain, found that 83.4% (181/217) were FR^0, comparing favorably with the C-strain mt^C marker, which was found in 71.9% (156/217) of this sample population. Finally, a comparison of pheromone trap collections from corn and turf habitats found that a majority of the males from turf traps were FR^+ (53.1%, 77/145) compared with only 29.2% (45/154) in spring cornfields. A similar difference was observed with the mt^R marker, where 93.1% (135/145) of the turf trap collection was mt^R, declining to 66.2% (102/154) in spring cornfields (the higher percentage of mt^R compared with FR^+ males in the traps reflects the substantial numbers of mt^R FR^0 captured). Taken together, these observations indicate that adult males carrying the FR^+ Z-chromosome displayed habitat biases in the field similar to that of the mt^R lineage and generally characteristic of the R-strain.

In conclusion, we showed the sexual dimorphic distribution of the *FR* tandem repeat in wild populations and confirmed the use of this marker as an indicator of strain-specific characteristics. Evidence is provided that the mt^R FR^0 hybrid genotype most likely arises from interstrain mating between R-strain females and C-strain males and that this subgroup represents a substantial proportion of trap captures in the corn and turf habitats in the overwintering areas of Florida. Differences in distribution between mt^R FR^0 and their parental R-strain siblings, mt^R FR^+, suggest that interstrain crosses can have significant effects on such strain behaviors as plant host choice, with po-

tentially important ramifications on biological control and other management strategies.

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