Multilocus Genetic Analysis of Host Use, Introgression, and Speciation in Host Strains of Fall Armyworm (Lepidoptera: Noctuidae)

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ABSTRACT Two genetically differentiated forms of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), use different graminaceous host plants, coexist sympatrically throughout their ranges, yet seem to hybridize. To address the taxonomic status of the two forms, determine extent and directionality of hybridization, and examine host specificity, we compiled multilocus genotypes consisting of mitochondrial DNA (mtDNA) haplotypes, an esterase locus, and eight amplified fragment-length polymorphism (AFLP) loci in moths collected across a broad geographic range. Multilocus analyses indicated 16% of individuals sampled were potentially hybrids with a minority being F_1 in origin. Analysis of host use indicated asymmetries in host specificity with one strain specific to corn, *Zea mays* L., and the other strain predominating on pasture grasses and rice, but occasionally using corn. Location of hybrids in nature was biased toward cornfields, the habitat used by both strains. To assess genetic divergence of each gene, we calculated their relative strain discriminating ability. Eight AFLP loci collectively had the greatest discriminating power (98%), followed by a single AFLP locus (93%) and mtDNA (91%). Esterase exhibited 89% discrimination. Esterase is X-linked along with an assortative mating trait, suggesting esterase differentiation may be maintained by association with strain-specific fitness genes. Despite strong discrimination of these genes, most of the genome surveyed was not distinct. Cytonuclear comparisons provided evidence for unidirectional matings consistent with mate preference studies. Collectively, these data support introgressive hybridization between recently evolved species that are not completely reproductively isolated. Genetic divergence in the presence of gene flow may be a common phase in the speciation process, especially in taxa whose ranges have been altered dramatically by humans.

KEY WORDS hybridization, host specificity, *Spodoptera frugiperda*, AFLP, mtDNA, allozymes

STUDIES OF INTERSPECIFIC HYBRIDIZATION have contributed significantly to advances in our understanding of the speciation process (Harrison 1993a, Howard and Berlocher 1998, Jiggins and Mallet 2000). They provide opportunities for examining the nature and development of reproductive barriers (Howard et al. 1998, Richie and Phillips 1998), and, consequently, arenas for addressing concepts associated with species status. Such taxa are challenging to categorize because of varying degrees of differentiation, uncertainty about introgression, and conflicting species definitions (De Queiroz 1998, Hey 2001). More importantly, hybrid zones provide ideal settings for examining the genetic architecture of divergence (Harrison and Bogdanowicz 1997, Wu and Hollocher 1998). They allow insights into the types of genes that diverge and, ultimately, mechanisms responsible for divergence of specific gene regions.

From a spatial perspective, hybridization is not always restricted to a zone. Often, it occurs in a mosaic pattern where habitats abut (Harrison and Rand 1989). Moreover, hybridization occurs more commonly between closely related taxa than previously thought, but not all genes introgress freely (Howard et al. 1997, Howard 1998, Beltran et al. 2002, Machado et al. 2002, Crochet et al. 2003, Emelianov et al. 2004). For example, loci associated with assortative mating and habitat fitness often remain distinct. In Lepidoptera, for unexplained reasons, many traits that distinguish strains and species seem to be localized on the X chromosome (Sperling 1994, Prowell 1998). Understanding how these genes and others resist recombination and introgression provides important insights into speciation.

Several features of the biology and ecology of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), make this species complex ideally suited for speciation studies. First, a host shift has accompanied divergence of two strains such that traits facilitating habitat adapta-

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tion can be studied along with those influencing reproductive isolation (Pashley 1986). Second, the two strains are very closely related genetically and seem to be in the early stages of speciation (Prowell 1998, Dres and Mallet 2002). Third, fall armyworm strains are not distributed in a typical hybrid zone pattern. Their habitats are intermingled and pure parental populations do not occupy consistent, predictable zones. Finally, apparent X-linkage of traits associated with mating behavior and an esterase gene offers an opportunity to address the interaction between the X chromosome and speciation in Lepidoptera (Prowell 1998).

Almost two decades ago, two genetically differentiated forms of fall armyworm were detected using allozymes (Pashley et al. 1985). One set of genotypes was found feeding on corn, *Zea mays* L. (=maize, referred to as the corn strain) and another set was found feeding on rice and forage grasses (referred to as the rice strain). Cross-rearing studies indicated differences were likely due to reduced gene flow between host-associated strains (Pashley 1986). Subsequently, differences were detected in behavioral and physiological responses to insecticides (Pashley et al. 1987), developmental rates (Pashley 1988, Pashley et al. 1995), feeding efficiency (Veenstra et al. 1995), and several other traits (Prowell 1998). Despite these differences, genetic markers remain the only reliable means of distinguishing strains. The esterase allozyme locus is highly divergent (Pashley 1986) and strains contain different mitochondrial DNA (mtDNA) haplotypes (Pashley 1989, Pashley and Ke 1992, Lu and Adang 1996, Meagher and Gallo-Meagher 2003). Other genetic differences have been reported for nuclear DNA restriction fragment-length polymorphisms (Lu et al. 1992) and a nuclear DNA repeat (Lu et al. 1994, Nagoshi and Meagher 2003).

Assortative mating within stains may be mediated by several factors, including differences in seasonality, host use, nocturnal mating activity times, and mate attraction and choice (Pashley and Martin 1987, Pashley et al. 1992). Despite these differences, indirect data from natural populations suggest that interbreeding may be occurring (Prowell 1998). In a genetic analysis based on mtDNA and allozymes, ≈11% of individuals with an allozyme genotype that predominated in one strain had an mtDNA genotype of the other.

In this study, we address hybridization in nature further by examining variation in both cytoplasmic and nuclear genes in fall armyworm from the two host assemblages across a latitudinal transect of its range in the Western Hemisphere. We present data from an esterase allozyme locus, mtDNA, and eight amplified fragment-length polymorphism (AFLP) loci. Our goals were to 1) determine the degree and directionality of hybridization between fall armyworm strains, 2) contrast the discriminating power and introgression of genes, 3) examine patterns of host use of each strain, and 4) evaluate the taxonomic status of the two strains.

Materials and Methods

Populations Sampled. For esterase analyses, samples of fall armyworm were collected on corn, rice, or pasture grasses from 26 geographic locations throughout the Western Hemisphere from 1983 through 1992. Locations included Louisiana (Baton Rouge, Hammond, St. Gabriel), Georgia (Tifton), Florida (Dade County), and Texas (College Station) in the United States, and Puerto Rico (Isabela and Vega Baja), Dominican Republic (Santo Domingo), Jamaica (May Pen), Guadeloupe (Gardel and Godet), Mexico (Poza Rica, Santiago Tuxtla, Tampico, and Tikinimal), Honduras (Zamorano), Costa Rica (Manuel Antonio and San Jose), Ecuador (Guayaquil), and French Guiana (Matoury and Trou Poisson). Multilocus analyses of esterase, mtDNA, and AFLPs were performed on a subset of individuals collected in Louisiana (LA), FL (FL), Puerto Rico (PR), Guadeloupe (GU), and French Guiana (FG) in 1990–1992. Individuals were collected as larvae on hosts, reared to adulthood, and frozen at −70°C. Voucher specimens have been deposited in the Louisiana State Arthropod Museum.

Esterase Methods. Individuals were screened for allozyme genotypes at the esterase locus because it was the most distinctive allozyme locus between fall armyworm strains (Pashley 1986). Four other allozyme loci exhibit significant frequency differences between strains but have limited use as strain markers and were not included here. Methods were the same as those described in Pashley et al. (1985).

mtDNA Methods. mtDNA restriction fragment-length polymorphism profiles were examined in a 650-bp region of the ND1 gene where strain differences have been documented previously (Pashley 1989, Pashley and Ke 1992). An extraction method used to isolate total DNA from individual moths was described in McMichael and Prowell (1999). DNA was amplified with mtDNA primers specific to the ND1/lrRNA region (primer FAW16S, 5′-TTCAAACCGGTGTAAGCCAGG-3′; primer FAWND1, 5′-TAGAATTAGAAGATCAACCAG-3′). mtDNA haplotypes were generated by digestion of amplification product with restriction endonuclease *Hinf* I (10 u/20 ml of digest).

AFLP Methods. In a pilot study, we identified 10 AFLP loci that exhibited strain-biased frequencies (McMichael and Prowell 1999), eight of which were surveyed in the current study. DNA isolation methods were the same as for mtDNA. Five primer pair combinations were used to generate AFLPs. Procedures followed those of Vos et al. (1995) and are described in detail elsewhere (McMichael and Prowell 1999). Individuals were scored for presence (1) or absence (0) of a band at each locus. Because AFLP phenotypes exhibit dominance, heterozygotes cannot be distinguished from the dominant homozygote. A character state matrix was produced for all individuals at all loci.

Strain Assignment Methods for Markers. Previous work indicated esterase alleles B, C, and D predominated in the corn strain, whereas E and F characterized the rice strain (Pashley 1986). Individuals were

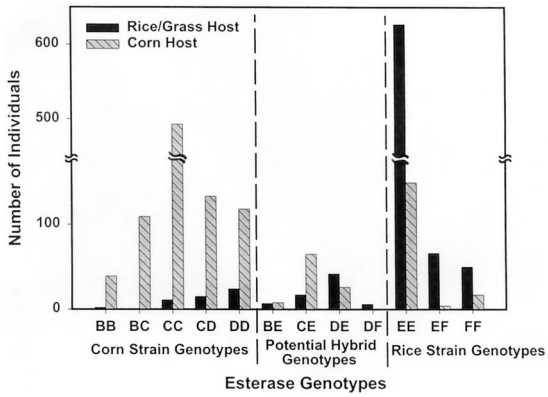

Fig. 1. Genotypic frequencies at esterase in two habitats, corn (n = 1161) and rice/pasture grasses (n = 867), for pooled fall armyworm collections from the United States, Carribbean Islands, and Central and South America.

given strain designations based on the following genotypes: corn strain, BB, BC, BD, CC, CD, and DD; rice strain, EE, EF, and FF; and hybrid, BE, BF, CE, CF, DE, and DF. Because the esterase locus is sex-linked and females are the heterogametic sex in Lepidoptera, only hybrid males can be detected.

Two mtDNA haplotypes were produced by the *Hinf* I restriction enzyme (Pashley 1989). One haplotype, an uncut 650-bp fragment, predominated in individuals collected in corn, and the other haplotype, two fragments of 590 and 60 bp, predominated in rice and pasture grass samples. Strain designations for mtDNA were based on these two haplotype categories.

Individual strain assignments by using AFLP data were based on banding patterns at all loci. Corn-collected individuals were characterized by the presence of a band at a majority of the AFLP loci, whereas rice- and grass-collected individuals lacked a band at most loci. Individuals were designated corn strain when they contained five or more of eight bands. Rice strain assignments were made for four or fewer bands. This numerical score is referred to as the character index score (Howard et al. 1997).

Data Analysis. Sex-linkage results in different allele frequencies in males and females but does not affect genotypic assignments. The hemizygous sex (females in this case) lacks the heterozygous condition and is, for practical purposes, placed in the homozygous genotypic class, although females only contain one of the alleles in that class. All analyses used genotypic scores as opposed to allele frequencies to overcome the affects of sex-linkage with one exception. Allele frequency data were presented where tests were conducted for departure from Hardy–Weinberg equilibrium, but only males were used in the analysis as inclusion of females was inappropriate given the lack of a heterozygous class.

To determine agreement among markers and relative ability of markers to place individuals within the correct strain, individuals with data for host plant, mtDNA, esterase, and AFLPs (n = 162) were assigned a multiletter character code based on strain assignments for each marker. For example, individuals collected on corn, with mtDNA haplotype, esterase genotype, and AFLP character index score assignments of corn strain were designated CCCC. Two-, three-, and four-letter character codes were used in several analyses.

To quantitatively assess relative marker discrimination, we adapted allele diagnostic value calculations described in Bert et al. (1996) to the marker level (host, esterase, mtDNA, and AFLP character index scores) and refer to these calculations as marker diagnostic values (MDVs). We also calculated an MDV for each AFLP locus to assess their relative ability to diagnose strains. MDVs were calculated as follows: $F_C - F_R$, where F_C and F_R are frequencies of a particular marker in the corn and rice strains, respectively. Using the data set with four marker scores described above (n = 162), majority consensus of markers were used to assign each individual to a strain. In other words, if three or more markers received a rice strain score, the individual was recorded as a member of the rice strain. Only one individual did not have a majority consensus (i.e., CRCR), and it was not used in calculations. Upon this strain assignment backdrop, each marker was surveyed independently for corn or rice strain concordance to generate F_C and F_R frequencies. As a hypothetical example, if say 100 individuals were designated as corn strain and 90% (F_C = 0.90) contained the mtDNA fragment typical of the corn strain and if 100 were rice strain individuals and 5% (F_R = 0.05) contained the corn strain mtDNA fragment, the MDV would be 0.85 (or 0.90–0.05). Nine individuals were not used in the MDV calculation for esterase because they were heterozygous and could not be assigned to a strain. MDVs can range from 1 to −1. A value of zero indicates no diagnostic ability. The closer the value is to 1 or −1, the better the marker is at diagnosing individuals to strain or the greater the frequency difference of the marker between strains.

Fixation indices (F) were calculated for a subset of collections from Louisiana, Florida, and Puerto Rico containing sexed individuals scored for both mtDNA and esterase to determine the direction of departures from random mating (BIOSYS-1, Swofford and Selander 1981). χ^2 was used to test genetic frequency data for biased proportions, indicating unidirectional matings between strains or associations among loci or markers.

Results

Esterase Strain and Host Associations. Highly significant differences in genotypic composition at the esterase locus existed between habitats consistent with previous reports (X^2 = 1144, df = 11, $P < 0.0001$; Fig. 1). The B, C, and D alleles predominated in the corn habitat in association with the corn strain, whereas the E and F alleles occurred in rice and pasture grasses in association with the rice strain. De-

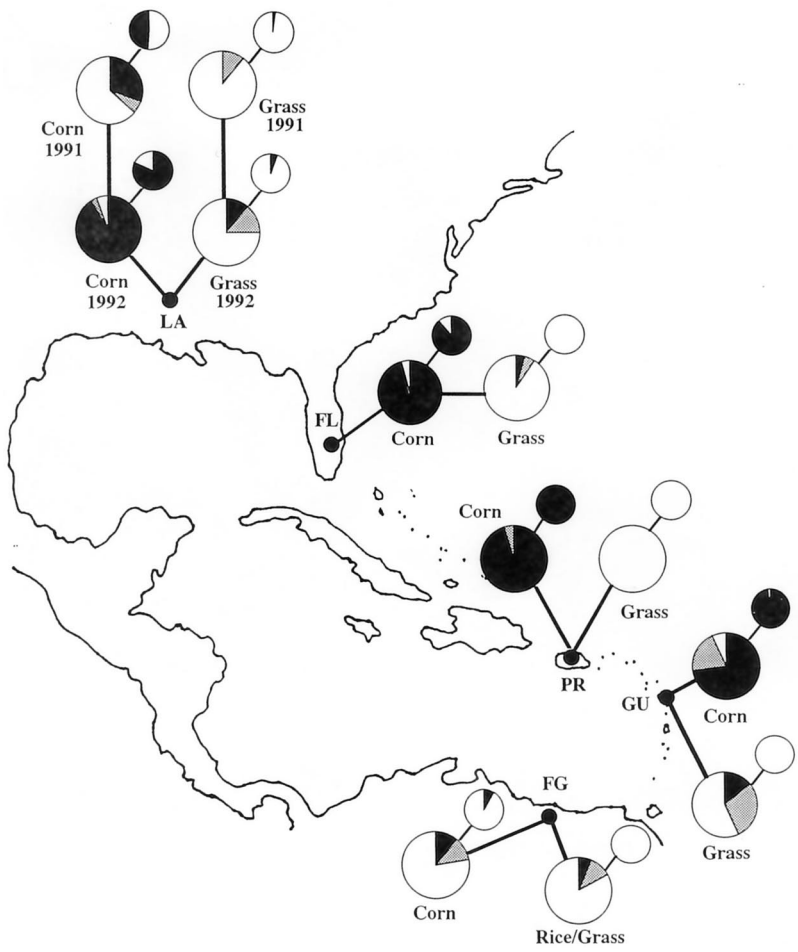

Fig. 2. Fall armyworm strain frequencies in two habitats from five geographic sites (LA, Louisiana; FL, Florida; PR, Puerto Rico; GU, Guadeloupe; and FG, French Guiana) based on esterase genotypes (large pie) and mtDNA haplotypes (small pie). Corn strain genotypes indicated by black shading, rice strain by white, and potential hybrids by hatching. See text for description of strain assignments for each locus. Dates of collection and sample sizes (n_e and n_m, esterase and mtDNA, respectively) are LA corn May-July 1991, $n_e = 33$, $n_m = 35$ and May-September 1992, $n_e = 208$, $n_m = 216$; LA pasture grasses September 1990 and September 1991, $n_e = 174$, $n_m = 160$ and July-September 1992, $n_{e,m} = 36$; FL corn May 1991, $n_e = 26$, $n_m = 25$; FL pasture grasses June 1991, $n_{e,m} = 22$. PR corn February 1991, $n_e = 64$, $n_m = 72$; PR pasture grasses February 1991, $n_e = 9$, $n_m = 7$; GU corn January-June 1992, $n_e = 103$, $n_m = 84$; GU pasture grasses June 1992, $n_e = 14$, $n_m = 9$. FG corn December 1991, $n_e = 45$, $n_m = 23$; FG rice and pasture grasses December 1991 and June 1992, $n_e = 145$, $n_m = 81$.

spite a bimodal distribution, 8% ($n = 171$) of the individuals were heterozygous for alleles characterizing each habitat and represent potential hybrids. X-linkage of esterase resulted in detection of only male heterozygotes. Females are hemizygous for their male parental allele but are scored as homozygotes. If male and female hybrid progeny have equivalent survivorship, the percentage of hybrid individuals in this sample was double that observed (i.e., closer to 16%) because hybrid origin females are hidden in parental strain genotypic classes.

Host fidelity differences between strains were indicated by the esterase data. Assuming allelic designations given above are strain-specific, relatively high numbers (19%) of rice strain individuals (EE, EF, and FF) used corn as a host. Corn strain individuals (BD, BC, CC, DC, and DD) fed on pasture grasses or rice but in lower relative numbers (5%). There was a geographic pattern to variation in host use with greater use of corn by the rice strain in certain regions (Fig. 2, large circles). Most notably, individuals collected from corn in French Guiana had genotypes predominately found in the rice strain. Likewise, summer collections in Louisiana corn in 1991 were mostly rice strain individuals. The 1991 sample was significantly different from the 1992 sample that was mostly corn strain ($\chi^2 = 24.2$, df = 4, $P < 0.001$). In contrast, corn strain genotypes are less common on the rice strain hosts. These results suggest that although host fidelity is generally strong, asymmetrical temporal and spatial patterns of host use exist, with a wider range of host use in the rice strain.

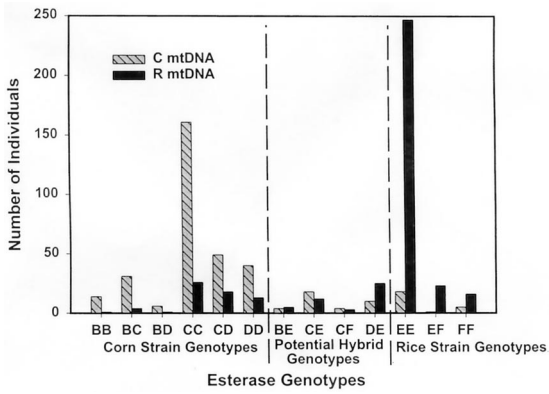

Fig. 3. Associations between esterase genotypes and mtDNA haplotypes (n = 756, 434 collected in corn and 322 in rice/pastures) for fall armyworm collections pooled across geographic sites.

mtDNA Covariation with Esterase and Host Use. mtDNA analyses revealed almost no corn mtDNA haplotypes (2%) in rice strain habitats but a fairly high percentage (18%) of rice strain mtDNA haplotypes in the corn strain habitat (Fig. 2, small circles). This corroborates esterase data presented above that indicated broader host use in the rice strain.

A strong association existed between esterase genotypes and mtDNA haplotype consistent with the two strains ($\chi^2 = 392$, df = 12, $P < 0.0001$; Fig. 3). The rice mtDNA haplotype was most often associated with E and F alleles, and the corn mtDNA haplotype was most often associated with B, C, and D alleles. However, a number of individuals contained composite genotypes that were not concordant (i.e., mtDNA of one strain and esterase genotype of another). Sixteen percent of the rice mtDNA haplotypes were associated with corn strain esterase genotypes, and 6% of the corn mtDNA haplotypes were associated with rice strain esterase genotypes. If this discordance results from introgression between the strains, more interstrain matings involving rice strain females than corn strain females were indicated because of the higher percentage of rice mtDNA (obtained maternally) in the corn nuclear background. Of 86 individuals with discordant composite genotypes, 68 were collected in the corn habitat, whereas only 18 were collected in pastures and rice fields. This indicates a greater presence of potential hybrids in the corn habitat ($\chi^2 = 29$, df = 1, $P < 0.001$).

By using strain or hybrid assignments for esterase genotypes and mtDNA haplotypes in a subset of sexed individuals, mating biases were more apparent (Fig. 4). Categories other than CC and RR represent potential male (CH and RH) and female (RC and CR) F_1 hybrids, although they may contain other hybrid types as well (F_2, backcrosses, etc.). Again, 16% of the sample were potential hybrids. Significantly more potential hybrids resulted from matings involving rice strain females and corn strain males (66%; $\chi^2 = 12.00$, df = 1, $P < 0.001$). Most of these hybrids occurred in the corn habitat (77%). The mtDNA haplotype was associated with the correct host type in 89% of the individuals and esterase with 88%, indicating equivalent associations with host use.

To assess random mating in these samples, tests for departures from Hardy–Weinberg proportions were conducted on males using esterase data alone. Significant departures were observed for most populations and departures were caused by deficiencies of heterozygotes (positive F values in Table 1). Heterozygote deficiencies most likely resulted from population samples containing mixtures of the two strains, each of which was assortatively mating (i.e., a Wahlund effect). In all three locations, highest F values were found in pooled samples (e.g., LA-C&G) as expected. Significant values probably reflected the degree of strain mixtures in samples. For example, PR-C had no rice strain genotypes and was in Hardy–Weinberg equilibrium.

AFLP Strain and Host Associations. In total, 168 individuals from the southern United States., the Caribbean, and South America were examined in the AFLP analysis (Table 2). Band frequencies at the eight AFLP loci exhibited strong host associated differences across all geographic locations. As with esterase and mtDNA, AFLP genotypes in the French Guiana collection from corn indicated rice strain individuals were using corn as a host. MDVs for individual AFLP loci ranged from 0.93 to 0.74 (Table 2).

Table 1. Esterase allele frequencies and mtDNA haplotype frequencies for fall armyworm males collected in Louisiana (LA), Florida (FL), and Puerto Rico (PR) from corn (C) and pasture grasses (G)

Allele	Location-host								
	LA-C	LA-G	LA-C&G	FL-C	FL-G	FL-C&G	PR-C	PR-G	PR-C&G
n	133	125	258	14	10	24	34	5	39
B	0.10	0.01	0.06	0.11	0.00	0.06	0.03	0.00	0.03
C	0.54	0.01	0.28	0.75	0.05	0.46	0.72	0.00	0.60
D	0.20	0.05	0.13	0.07	0.05	0.06	0.25	0.00	0.22
E	0.13	0.82	0.47	0.07	0.90	0.42	0.00	1.00	0.15
F	0.03	0.11	0.06	0.00	0.00	0.00	0.00	0.00	0.00
F	0.42*	0.22*	0.55*	0.14	0.46*	0.59*	0.29		0.50*
Haplotype									
M_C	0.77	0.02	0.41	0.93	0.00	0.54	1.00	0.00	0.87
M_R	0.23	0.98	0.59	0.07	1.00	0.46	0.00	1.00	0.13

F, fixation index (Swofford and Selander 1981); *, significant at the 0.05 level.

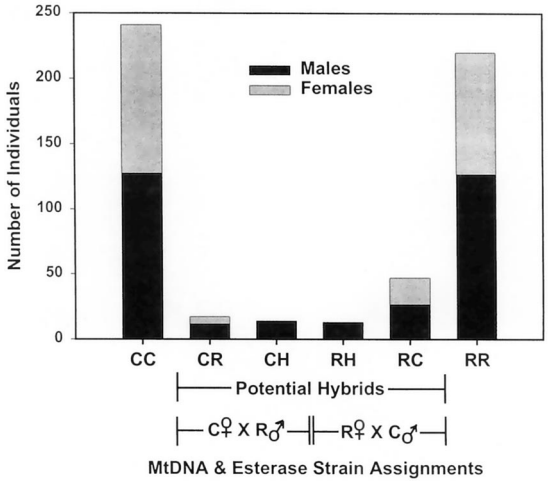

Fig. 4. Frequency of multilocus categories by sex based on strain designations at mtDNA and esterase, respectively, in fall armyworm from Louisiana, Florida, and Puerto Rico (male, $n = 321$; female, $n = 231$; C, corn strain; R, rice strain; and H, hybrid). Mating directionality for potential hybrid categories indicated below the x-axis.

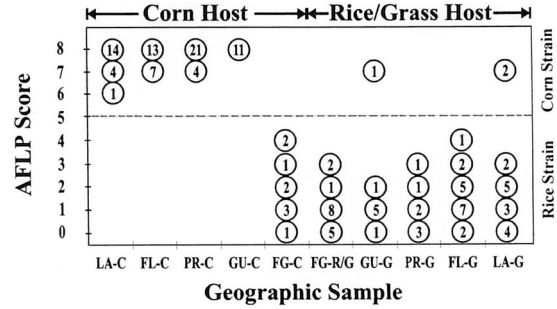

Fig. 5. Character index scores for eight AFLP loci in individuals (numbers within circles, $n = 168$) from five geographic collections of fall armyworm. AFLP scores refer to the number of bands present in an individual summed across eight loci. Abbreviations refer to site of collection (same as Fig. 3) and host of origin (C, corn; R, rice; and G, pasture grasses). Strain assignments on the right y-axis are based on AFLP scores.

The distribution of AFLP character index scores indicated distinction of the two strains (Fig. 5). Other than the French Guiana corn collection, discordance between AFLP score and host or strain occurred in only three individuals or 2% of the sample. One individual from Guadeloupe and two from Louisiana, all feeding on grasses, seemed to be members of the corn strain by AFLP score. The two other genetic markers supported a corn strain designation for one of the Louisiana samples, but not the other two. The Guadeloupe individual had an mtDNA haplotype and esterase genotype typical of the rice strain. The other Louisiana sample had a corn strain mtDNA haplotype and rice strain esterase genotype. Thus, two potential hybrids (1% of sample) and one corn strain individual were detected in the grass habitat, and nine rice strain individuals (5%) were detected in corn (FG-C).

Multilocus Comparisons. Multicharacter codes for three genetic markers were assigned to 162 individuals to determine agreement among markers and associations of markers with host use and to identify potential hybrids. In the majority of individuals, all characters were concordant (*a*, 84%, in Fig. 6). In 26 samples (16%), at least one marker was discordant with the other two. In 18 individuals (*b*, 11%), the discordant marker was the esterase genotype, and in seven (*c*, 4%), the mtDNA haplotype was discordant with the others. The AFLP score was discordant in only one individual (*d*, <1%).

In this sample, 6% (10 individuals) were possible F_1 hybrids and 10% (16 individuals) were second or later generation hybrids (Fig. 6; all classes except *a*). Hybrid origins were slightly biased toward rice strain

Table 2. AFLP band frequencies in five geographic collections of fall armyworm from two host-plant categories

Primer[a]/fragment size (bp)	Frequency of AFLP fragment										MDV[b]
	Corn host				Rice/pasturegrass host						
	LA	FL	PR	GU	FG	FG	GU	PR	FL	LA	
CGA/AGG-250	1.00	1.00	1.00	1.00	0.20	0.00	0.10	0.00	0.05	0.25	±0.93
n	20	20	28	12	11	18	10	7	20	20	
CT/ACT-215	0.90	0.95	0.96	0.92	0.55	0.22	0.22	0.12	0.06	0.20	±0.88
n	20	20	25	12	11	18	9	8	18	20	
CGC/AAG-265	1.00	1.00	0.93	1.00	0.27	0.06	0.10	0.25	0.30	0.35	±0.76
n	20	20	28	12	11	17	10	8	20	20	
CGC/AAG-125	1.00	0.85	0.96	1.00	0.27	0.06	0.00	0.12	0.05	0.11	±0.90
n	19	20	28	12	11	17	10	8	19	19	
CGC/AAG-90	1.00	1.00	1.00	1.00	0.09	0.22	0.30	0.25	0.45	0.18	±0.75
n	20	20	28	12	11	18	10	8	20	17	
CGC/ACA-320	0.95	0.85	1.00	1.00	0.00	0.00	0.20	0.00	0.15	0.20	±0.88
n	20	20	28	12	11	18	10	8	20	20	
CGC/ACA-150	0.90	1.00	1.00	1.00	0.36	0.33	0.10	0.12	0.25	0.40	±0.74
n	20	20	28	12	11	18	10	8	20	20	
CGA/ACG-200	0.97	1.00	1.00	0.92	0.36	0.22	0.67	0.00	0.25	0.15	±0.78
n	20	19	28	12	11	18	9	8	20	20	

[a] Letters before diagonal refer to nucleotide extension on *Eco*RI primer; letters after diagonal refer to nucleotide extension on *Mse*I primer.
[b] MDV, marker diagnostic value calculated using host of origin, mtDNA, and esterase for strain assignments.

females crossed with corn strain males (54% compared with 46% originating from the reciprocal cross). Nearly two-thirds (62%) of these presumptive hybrids occurred in the corn habitat.

Discussion

Hybridization in Nature. In this study, we were interested in the genetic architecture of divergence between strains and whether multilocus genotypic patterns could be used to infer interstrain hybridization. In general, our data indicated fall armyworm strains are independent cohesive entities. The strains were readily distinguishable from each other throughout their ranges at the three types of genes examined. They are different from traditional hybrid zone taxa in that they are highly mobile, use intermingled habitats, and contain no consistent known allopatric populations. Nevertheless, genetic heterogeneity of strain differences was similar to hybridizing species (Harrison 1993b, Jiggins and Mallet 2000, Machado et al. 2002) and races (Emelianov et al. 2004). The majority of individuals in all populations were parental types with some individuals containing alleles predominating, to different degrees depending on the genetic marker, in the other strain.

Collective use of strain-biased markers from esterase, mtDNA, and AFLPs indicated ≈16% of fall armyworm individuals were discordant for at least one marker. Discordance can result from two causes. Individuals with discordant loci may be the product of interstrain matings. Alternatively, individuals are not hybrids, but reflect variation in the ancestor to the two strains at the time of speciation. In other words, loci did not diverge to fixation and alleles are shared between strains due to retention of ancestral polymorphisms. Deciding between these alternatives influences interpretation of our results. Although we cannot know with certainty which is the cause, several lines of evidence favor hybridization as the explanation.

First, and most compellingly, genetic data from natural populations indicated a bias in the direction of interstrain matings consistent with asymmetries in laboratory mate preference studies (Pashley and Martin 1987). Cytonuclear patterns indicated rice strain females mated more frequently with corn strain males than the reciprocal cross, and mating behavior studies indicated the same bias. Another study of cytonuclear comparisons of field collected males obtained the same result, i.e., corn strain females rarely if ever mated with rice strain males (Nagoshi and Meagher 2003). In contrast to these studies, one research group found bidirectional compatibilities in laboratory no-choice crosses (Whitford et al. 1988, Quisenberry 1991), indicating mating behavior can be a labile trait. Second, habitat location of presumptive hybrids was consistent with strain differences in host specificity. Most hybrids, between 62 and 79% depending on the subsample, occurred in the corn habitat. Because the rice strain occasionally uses corn as a host and not visa versa, more opportunities for interstrain matings exist in corn. Finally, the many behavioral, physiological, and genetic differences between strains are suggestive of species level differences (Prowell 1998, Dres and Mallet 2002).

Collectively, these indirect observations give credence to an hypothesis of hybridization between strains. In an ideal situation, a series of strain-biased autosomal codominant markers would be able to definitively resolve this issue. F_1 hybrids would simultaneously exhibit heterozygous genotypes at divergent loci. Unfortunately, even in such cases, and where pure parental populations exist, establishing genealogical origins is problematic. Generally, many independent loci (70 by some estimates) are required for significant confidence in assignments because genotypic arrays of second and later generation hybrids overlap with parentals and first generation hybrids (Nason and Ellstrand 1993, Boecklen and Howard 1997, Epifanio and Philipp 1997).

Assuming that individuals with discordant markers are of hybrid origin, by using three markers together, only 6% of the individuals were potentially F_1 progeny and 10% were other types of hybrids. Given the preponderance of parental types, we expected more individuals in this F_1 hybrid class. F_1 deficiencies have been observed in many hybrid studies and the suggestion is that these hybrids may be at a selective disadvantage (Harrison and Bogdanowicz 1997, Howard et al. 1997, Arnold and Emms 1998, Jiggins and Mallet 2000). Although laboratory studies on fall armyworm have not indicated compromised survival of F_1 progeny, mating was compromised in adult F_1 moths (Pashley and Martin 1987). Female F_1 hybrids did not mate with parentals, whereas males did. Only a small number of matings between F_1 individuals produced a viable F_2 generation. These laboratory data support the possibility of aberrant behavior or physiology that reduces hybrid survival or interstrain mating in nature. Until more precise and direct studies of interstrain mating in nature are conducted, our study provides the best support for introgression via a restricted set of hybridization pathways.

Differential Discrimination and Permeability of Genes. One of our objectives was to contrast the evolution of various types of genes and to relate their degree of divergence to introgression of those regions of the genome. Of three types of loci examined, AFLPs as a group had the greatest amount of differentiation and thus the highest diagnostic power. When all eight loci were used, diagnostic ability was almost complete (MDV ±0.98). Using the single most diagnostic AFLP (Table 2), discrimination dropped somewhat (MDV ±0.93) but exceeded that of mtDNA (MDV ±0.91). The esterase locus had the lowest discrimination (MDV ±0.89). For the subsample scored for three genetic markers, larval host plant was indicative of strain for 86% of the individuals.

Despite high divergence of these particular AFLP loci, the majority of loci did not exhibit a strain-biased pattern. To obtain these eight loci, five primer pairs that amplified >1000 loci were screened. Independent cluster analyses of ≈250 of these polymorphic AFLP

loci did not discriminate strains, due to the swamping effect of variable, nonstrain-specific loci (M.M. and D.P.P., unpublished data). In contrast, clustering algorithms of AFLP loci in other organisms have successfully separated races (Emelianov et al. 2004), subspecies (Suazo and Hall 1999, Bensch et al. 2002), and species (Beismann et al. 1997, Beardsley et al. 2003).

As with most interspecific comparisons in Lepidoptera (Sperling 1994, Prowell 1998) and animal taxa in general (Moritz et al. 1987, Simon et al. 1994), significant mtDNA differences exist between fall armyworm strains in the form of two predominant haplotypes. Cytonuclear comparisons indicated mtDNA introgression was biased in one direction consistent with interspecific mating compatibilities. It has been suggested that cytonuclear incompatibility may influence directionality of interstrain or interspecific hybridization (Harrison and Bogdanowicz 1997, Rice 1998). That is, mtDNA of one type is at a selective advantage in the nuclear background of the other taxon, so selection favors transgression in that cross but not the reciprocal cross. This hypothesis is consistent with our results, but concordance among genetic markers and mate preferences suggests mating asymmetries are the more likely explanation for asymmetries in mtDNA.

Although esterase was not as strain-specific as our eight focal AFLPs and mtDNA, it is the most discriminating allozyme locus surveyed. Esterase is X-linked as is mating time (J. McNeil, unpublished data). Because of the widespread occurrence of an X association in traits that distinguish lepidopteran sister-taxa (Sperling 1994, Prowell 1998), association of esterase and mating time with the X chromosome is not likely to be coincidental. Linkage of esterase with the strongest reproductive isolating mechanism likely has contributed to differentiation at esterase (Pashley et al. 1992). Eleven other allozyme loci that are not X-linked exhibited much less or no strain differentiation (Pashley 1986).

Thus, lack of strain-specific variation in the majority of AFLPs, most allozymes, a sodium channel intron (Adamczyk et al. 1996), and ITS-1 (D.P.P., unpublished data) all support the hypothesis that fall armyworm strains are recently evolved. Gene flow may be maintaining similarities in some of these gene regions. The small number of loci that have diverged (esterase and a few AFLPs) may be linked to loci in regions of the genome that are associated with fitness in each habitat or with mate choice. Accordingly, these regions are not free to introgress between strains because of the selective cost to hybrid individuals. Heterogeneous genomic evolution may turn out to be the rule rather than the exception for many closely related species (Machado et al. 2002, Navarro and Barton 2003, Emelianov et al. 2004). Determining genetic regions that are not exchanged during hybridization may provide the most significant clues to the speciation process (Howard 1998, Beltran et al. 2002, Machado et al. 2002).

Host Fidelity. A third objective of this study was examination of host use patterns in the two fall armyworm strains. Because the strains can only be identified using genetic markers, determining host specificity relied on the association between genotype and larval host plant. We associated only parental genotypes for each strain at each marker to assess host use. Esterase genotypes in the largest survey of individuals ($n = 1857$) indicated 19% of the rice strain used corn and 5% of the corn strain used pasture grasses and rice. mtDNA data ($n = 756$) produced a similar result of 18 and 2%, respectively. These two loci combined ($n = 461$) gave lower estimates 6 and 1%, respectively. However, the data set containing all three markers ($n = 136$) raised the estimate again to 17 and 2%, respectively. Together, these data indicated broader host use in the rice strain, at least with respect to corn, rice, and pasture grass hosts.

Although our research was not aimed at quantifying factors affecting host use, seasonal, temporal, and geographic components were indicated. In Louisiana, where the most extensive surveys have been conducted, there was a greater tendency to find the rice strain feeding on corn late in the corn growing season (late June). Both strains probably migrate to Louisiana from subtropical regions, but the rice strain apparently arrives in June and the corn strain in April (Pashley et al. 1992). However, this pattern is not always consistent from year to year. For some unknown reason, May and June collections from Louisiana corn in 1991 contained a majority of rice strain individuals. Corn is harvested or becomes an unacceptable host due to age by the end of June. After June, the only acceptable hosts are pasture grasses that do not seem to be used successfully by the corn strain. The corn strain probably migrates north where corn is in a younger developmental stage. For some reason, rice is rarely used as a host for the rice strain except in the tropics. Pasture grasses are the primary host of the rice strain in temperate regions.

Host use is more complicated in tropical regions. Collections in both southern Florida and Puerto Rico indicated almost no overlap in host use by the two strains. In Guadeloupe, there was some overlap, and in French Guiana, the rice strain predominated in corn. At this French Guiana site, a 40-ha cornfield was surrounded by 140 ha of rice. These data indicated that single collections may not be sufficient to determine host use patterns, because they can vary in both space and time.

From ecological and behavioral perspectives, there are two explanations for differential host specificity in the two strains. Either females exhibit differences in ovipositional specificity (i.e., the rice strain oviposits in both habitats, whereas the corn strain does not) or differential immature survival occurs after random egg laying. Although fall armyworm females will oviposit on host plants, they also lay eggs on various other substrates, including trees and houses (Luginbill 1928). Several attempts of determine differences in oviposition preferences in caged and greenhouse settings failed because females laid eggs on a variety of substrates (D.P.P., unpublished data). Another study with caged plants found some evidence of preferences

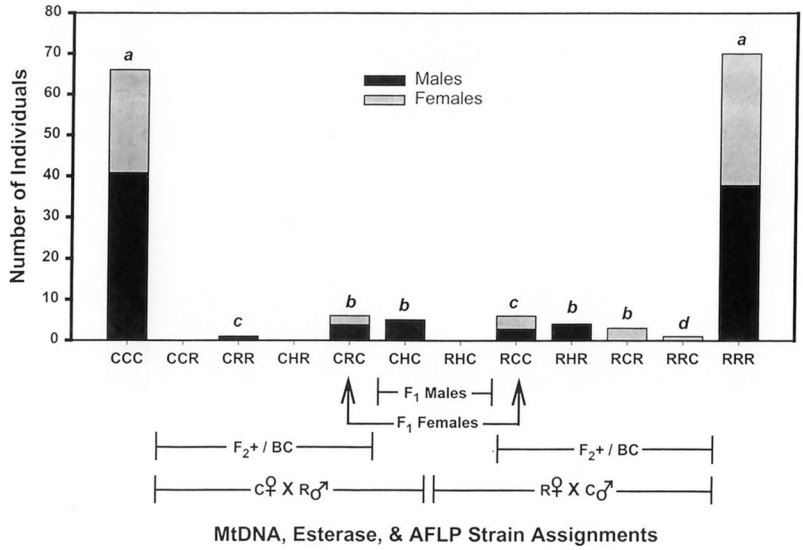

Fig. 6. Frequency of multilocus categories by sex based on strain designations at mtDNA, esterase, and AFLP character index scores, respectively, in fall armyworm from Louisiana, Florida, Puerto Rico, Guadeloupe, and French Guiana (male, $n = 96$; female, $n = 66$). See text for description of strain assignments for each locus. Letters above bars indicate the following groupings: *a*, all characters concordant; *b*, esterase discordant; *c*, mtDNA discordant; and *d*, AFLP score discordant. Potential sources for hybrid categories indicated below the x-axis.

in each strain but results were not upheld in a second trial (Whitford et al. 1988). Presumably females locate hosts, lay egg masses on or near hosts, and larvae find hosts via dispersal. This strategy could result in some ovipositional specificity, but it would be challenging to document experimentally. In the alternative case, if larval survival differences in each habitat maintain differential host use, differences are not likely to be due to nutritional factors. Nutritional studies indicated the corn strain develops at least as well on pasture grasses as corn (Pashley 1988, Pashley et al. 1995, Veenstra et al. 1995). Larval survival differences would more likely to be due to ecological or behavioral differences between strains, such as parasite or predator avoidance differences. For example, corn strain larvae may be preyed upon more readily in pasture environments than rice strain larvae because they lack behaviors associated with crypsis in a pasture environment. Feeding on corn occurs in the whorl where larvae are hidden from predators until they seek a pupation site in the soil. We lack evidence to support either of these hypotheses, but it is more efficacious for selection to operate earlier in the life cycle, on oviposition sites, than later, on larval survival.

Speciation and Taxonomic Status. Molecular systematic studies of *Spodoptera* support a sister-taxa position of two fall armyworm strains (Adamczyk et al. 1996; J.-F.S., unpublished data). A companion study of sequence divergence from four mtDNA genes (12S, 16S rDNA, cytochrome *b*, and cytochrome oxidase subunit 1) indicated that fall armyworm strains were similar in divergence to *Spodoptera latifascia* (Walker) and *Spodoptera descoinsi* Lalanne-Cassou & Silvain, two Western Hemisphere sister-taxa with distinct morphologies and habitats (J.-F.S., unpublished data). Although molecular distances are not necessarily appropriate measures of taxonomic rank, a level of divergence consistent with differentiation in other species supports species status of fall armyworm strains (Dres and Mallet 2002, Sperling 2003).

Despite strong evidence of distinct evolutionary histories, barriers to gene exchange do not seem to be absolute. By most species definitions, the two strains are species, but by some they are not (Harrison 1998, Hey 2001). Species status is complicated by the fact that there are no morphological differences and, consequently, a species description has not been written. Such cryptic species may be common in organisms transported around the globe by humans, causing juxtaposition of forms that are not completely reproductively isolated. This problem becomes acute when species are frequent targets of research due to their economic importance.

Finally, the origin of fall armyworm species is not clear. When a traditional hybrid zone exists, the spatial component of divergence often provides evidence for past geographic separation. In fall armyworm, because isolated, pure populations have not been consistently found, reconstructing the geography of divergence is difficult. Fall armyworm may have diverged in allopatry when ranges were different and more restricted before human cultivation of their hosts. Alternatively, divergence could have occurred parapatrically via an ecological shift from one host to another, a speciation mode that is receiving increasing support (Rolan-Alvarez et al. 1997, Feder 1998, Jiggins and Mallet 2000, Berlocher and Feder 2002, Dres and Mallet 2002, Funk et al. 2002). Indeed, there is growing support for a

sympatric phase of genetic divergence in the presence of gene flow during the speciation process (Machado et al. 2002, Crochet et al. 2003, Navarro and Barton 2003, Emelianov et al. 2004). Fall armyworm seems to fit this model.

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