

Demonstration and Quantification of Restricted Mating Between Fall Armyworm Host Strains in Field Collections by SNP Comparisons

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Abstract

Gene introgression between related pest populations is an important component in the assessment of how rapidly economically important traits, such as pesticide resistance, can spread within a region. An example of this is provided by the noctuid moth *Spodoptera frugiperda* (J.E. Smith), or fall armyworm, which is composed of two ‘host strains’ that differ in their host plant preferences. Resistance to a *Bacillus thuringiensis* toxin has been observed in some populations and there is concern about its spread throughout the Western Hemisphere. If this trait is easily transmitted between strains, it would expand the range of plants affected and make control efforts more difficult. A complicating factor is that the strains are morphologically indistinguishable and can only be identified by a small number of genetic markers. As a result, little is known about the frequency of interstrain hybridization in the wild. This study uses a novel strategy involving comparisons between two single-nucleotide polymorphisms (SNPs) to quantify the frequency of interstrain mating in field populations. The results demonstrate that hybridization between strains is 4- to 5-fold reduced compared to that within strains. In addition, it appears that directional interstrain mating biases observed in laboratory studies are probably not a major factor in determining the distribution of hybrid genotypes in field populations. The differential SNP technique is a significant improvement over current methods for identifying interstrain hybrids and should facilitate our understanding of fall armyworm strain and hybrid distributions in the field and the frequency of genetic exchanges between strains.

Key words: *Spodoptera frugiperda*, heterozygosity, hybridization

A continuing challenge for agriculture is the introgression of alleles between populations that produce altered and often more damaging pest behavior, an example of ‘transgressive segregation’ (Rieseberg et al. 1999). Even limited hybrid success can result in the introgression of new traits that, if they alter critical behaviors, could have serious economic consequences. Meaningful risk assessments of such events require a description of interbreeding metrics in the wild, as laboratory studies can only provide an approximation of what can occur.

The noctuid moth, *Spodoptera frugiperda* (Lepidoptera:Noctuidae) (J.E. Smith), commonly called the fall armyworm, is an example of this phenomenon. Two subgroups have been identified that exhibit reproducible biases in host plant distributions in the field. These groups were designated ‘strains’ and named for the host plant from which they were originally collected (Pashley 1986). The rice-strain (RS) is consistently associated with pasture grasses and alfalfa (Juárez et al. 2012, Murúa et al. 2015), whereas

the corn-strain (CS) is predominantly found in corn and cotton hosts (Pashley 1988, 1989; Nagoshi et al. 2007b). The two strains are generally sympatric, therefore mating barriers of some type must exist and several mechanisms have been reported. These include strain-directed assortative mating (Pashley and Martin 1987, Pashley et al. 1992) that may be associated with differences in female pheromone composition (Groot et al. 2008, Lima and McNeil 2009), timing of mating (Pashley et al. 1992, Schoff et al. 2009, 2011), or genetic incompatibilities affecting the viability or fertility of hybrid progeny (Pashley and Martin 1987, Kost et al. 2016).

In 2009, resistance to a genetically modified corn line expressing the *Bacillus thuringiensis* toxin Cry1F was identified in fall armyworm populations in Puerto Rico (Storer et al. 2010). Similar phenotypes have subsequently been reported in Brazil and the southeastern United States, suggesting either multiple origins or rapid dispersion of the resistance trait (Niu et al. 2013, Velez et al. 2013, Farias et al. 2016, Li et al. 2016, Santos-Amaya et al. 2016). Currently, it is not

known the degree to which the resistance trait is present in both strains, although its infestation of corn suggests it to be widespread in the CS. Significant introgression of the trait between strains would substantially increase the number of plant hosts potentially affected, exacerbating the economic threat of the resistant population and complicating efforts to mitigate the problem. Measuring the frequency of interstrain mating in wild populations is necessary for an accurate assessment of this risk.

However, as they are morphologically indistinguishable, the only method for identifying strains and any potential hybrids is through the use of genetic markers. Specifically, the strains are defined by the observation that certain markers are non-randomly associated among the fall armyworm larvae found on different host plants. A complicating factor is that the correspondence between marker and plant host is not absolute. This could be due to a number of factors, including incomplete fixation of the markers to the two strains, behavioral plasticity in host plant usage, or hybridization between strains. Most commonly used for population studies are mitochondrial haplotypes, with those defined by polymorphisms in the *Cytochrome oxidase subunit I* gene (*COI*) the best characterized (Pashley 1989, Lu and Adang 1996, Nagoshi et al. 2006b). Genomic strain markers have been difficult to isolate and are currently limited to a small number of polymorphic loci located on the Z sex chromosome (Lu et al. 1994, Prowell et al. 2004a, Nagoshi 2010). Given these limitations in identifying strains, attempts to study interstrain hybridization in wild populations have depended upon extrapolations from disagreements between marker and host plant or between two different strain markers (Nagoshi and Meagher 2003a, b; Prowell et al. 2004b; Nagoshi 2010; Juarez et al. 2012; Murúa et al. 2015). However, such estimates are obviously complicated by the uncertainties in the accuracy of the markers.

In this article, we describe a new approach with the potential for more conclusive and quantitative estimates of hybridization frequency between strains. The *Triosephosphate isomerase* gene (*Tpi*) is highly conserved in noctuid moths and is located on the Z sex chromosome in all Lepidoptera species so far examined (Nagoshi 2010). Although the *Tpi* product is thought to have a general housekeeping

function (Helfert et al. 2001), it appears to be linked to one or more loci generally involved in speciation. Polymorphisms in the *Tpi* gene have been found that can distinguish between closely related populations, including the sibling species *Helicoverpa armigera* and *Helicoverpa zea*, the two ‘races’ of *Ostrinia nubilalis* (Hübner), and the two fall armyworm host strains, indicating a tendency for *Tpi* variants to become fixed in diverging populations (Dopman et al. 2004, Nagoshi 2010, Nagoshi et al. 2016). Neutral single-nucleotide polymorphisms (SNPs) within the fall armyworm *Tpi* coding region were identified that differ in their degree of strain-specificity. We reasoned that the occurrence of heterozygosity at the SNP with high strain-specificity depends primarily on hybridization between strains, whereas the frequency at the site with low specificity will be more reflective of total mating. Therefore, comparisons between the two should provide a direct indicator of the frequency of interstrain relative to intrastrain hybridization.

Materials and Methods

Specimen Collections and Sites

Larval collections were used to measure the strain-specificity of the two SNPs used in this study, and were previously described (Table 1). Larvae were collected by hand from host plants in Florida, Mississippi, and Texas. Host plants preferred by the RS included pasture grasses (primarily of the *Cynodon* genus) and millet. CS hosts were sweet corn or forage corn. To avoid complications due to heterozygosity at the *Tpi* locus, only specimens that gave unambiguous sequence information (and so were homozygous males or hemizygous females for the two *Tpi* SNP sites) were included in this portion of the study.

Because of their Z-chromosome linkage, the analysis of heterozygosity frequency at the two SNP sites could only be done with male specimens, which were obtained by pheromone trapping at six locations. In each case, one or more traps were located at the same site and collections were made for a single period of no more than 10 d, thereby providing a ‘snapshot’ of the local fall armyworm population for a discrete spatial and temporal frame. Four collections,

Table 1. Source information for specimens

Country/State	County or Municipality	Collection date	<i>n</i>	Host plant	Coordinates (N, W)	Reference
Larvae						
FL	Hardee	8/2003	7	RS	27.4820°, 81.9190°	A
FL	Miami-Dade	1/2005	9	CS	25.4687°, 80.4776°	B
FL	Highlands	10/2003	9	CS	27.5959°, 81.5062°	B
FL	Palm Beach	10/2003	23	CS	26.6845°, 80.6676°	B
MS	Washington	7/2006	4	CS	33.3001°, 90.9438°	C
MS	Washington	7/2006	16	RS	33.3001°, 90.9438°	C
MS	Washington	8/2006	5	CS	33.3001°, 90.9438°	C
MS	Washington	8/2006	11	RS	33.3001°, 90.9438°	C
TX	Brazos	7/2004	17	CS	30.6504°, 96.3226°	B
Adult males						
FL-05	Alachua	9/26/2005*	178	CS [†]	29.7705°, 82.4212°	D
FL-12	Orange	1/6/2012*	53	CS [†]	28.4845°, 81.2519°	This study
GA-07	Thomas	9/11/2007*	62	CS [†]	30.8417°, 83.8473°	E
PA-15	Erie	9/22/2015*	43	CS [†]	42.1292°, 80.0851°	This study
PR-09	Juana Diaz	3/31/2009*	101	CS [†]	18.0534°, 66.5075°	F
DR-16	La Vega	8/8/2016*	48	CS [†]	19.2212°, 70.5289°	G

DR, Dominican Republic; FL, Florida; GA, Georgia; MS, Mississippi; PA, Pennsylvania; PR, Puerto Rico; TX, Texas. References: A, Nagoshi et al. 2006a; B, Nagoshi et al. 2007b; C, Nagoshi et al. 2008; D, Nagoshi et al. 2007a; E, Nagoshi et al. 2014; F, Nagoshi et al. 2010; and G, Nagoshi et al. 2017.

*Start of 3- to 14-d collection period.

[†]Pheromone trapping in corn-dominated habitat.

Puerto Rico, the Dominican Republic, and two in Florida, were in areas that can support a permanent fall armyworm population, whereas the remaining collections from Georgia and Pennsylvania represent seasonal migrants. All but two of the collections were previously described (Table 1). The new locations were at Orange County, Florida, Erie County, and Pennsylvania. All pheromone trap collections were performed using standard (green top, yellow funnel, and white bucket) or all-green Universal moth traps (Unitraps) (Great Lakes IPM, Vestaburg, MI) baited with a commercially available fall armyworm pheromone (Scentry Biologicals, Inc., Billings, MT; Trécé, Inc., Adair, OK; and Suterra LLC, Bend, OR). Each trap contained insecticide strips containing 10% 2,2-dichlorovinyl dimethyl phosphate (Hercon Environmental, Emigsville, PA) to kill moths. All specimens were stored at -20°C until DNA preparation.

DNA Preparation

Individual specimens were homogenized in 1.5 ml of phosphate-buffered saline (20 mM sodium phosphate, 150 mM NaCl, pH 8.0) using a tissue homogenizer (PRO Scientific Inc., Oxford, CT) and the homogenate transferred to a 2-ml microcentrifuge tube. Cells and tissue were pelleted by centrifugation at $6,000 \times g$ for 5 min at room temperature. The pellet was resuspended in 800 μl Genomic Lysis buffer (Zymo Research, Orange, CA) by vortexing and incubated at 55°C for 5 min. Debris was removed by centrifugation at $6,000 \times g$ for 3 min. The supernatant was transferred to a Zymo-Spin III column (Zymo Research) and processed according to manufacturer's instructions. The DNA preparation was increased to a final volume of 100 μl with distilled water. Genomic DNA preparations of fall armyworm samples from previous studies were stored at -20°C .

Polymerase Chain Reaction (PCR) Amplification of the CO1 and Tpi Gene Segments

PCR amplification for each gene segment was done separately, using a 30- μl reaction mix containing 3- μl $10\times$ manufacturer's reaction buffer, 1 μl 10-mM dNTP, 0.5 μl 20- μM primer mix, 1 μl DNA template (between 0.05 and 0.5 μg), 0.5 unit Taq DNA polymerase (New England Biolabs, Beverly, MA) and water. The thermocycling program was 94°C (1 min), followed by 33 cycles of 92°C (30 s), 56°C (45 s), 72°C (45 s), and a final segment of 72°C for 3 min. Typically, 96 PCR amplifications were performed at the same time using either 0.2-ml tube strips or 96 well microtiter plates. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Amplification of CO1 used the primer pair CO1-891F (5'-TACACGAGCATATTTTACATC-3') and CO1-1472R (5'-GCTGGTGGTAAATTTTGATATC-3') to produce a 603-bp fragment, or CO1-101F (5'-TTCGAGCTGAATTAGGGACTC-3') and CO1-857R (5'-GCTGATGTAAATATGCTCGTG-3') to produce a 814-bp fragment. Amplification of the Tpi region was done with the primers Tpi412F (5'-CCGACTGAAGGTTATCGCTTG-3') and Tpi1140R (5'-GCGGAAGCATTGCTGACAACC-3') that spans a variable length intron to produce a fragment with a mean length of 500 bp.

Analysis of CO1 Haplotypes

The determination of CO1-CS and CO1-RS was determined by two methods. In one, the PCR product produced by the CO1-891F/1472R combination was digested with the EcoRV restriction enzyme (New England Biolabs). The amplified CO1 segment is polymorphic for an EcoRV recognition sequence that is present in the RS (CO1-RS), but absent in the CS (CO1-CS) (Nagoshi et al. 2012). In the second method the PCR product from the CO1-101F/857R primer pair was digested by the restriction enzyme MspI (New England Biolabs). The

CO1-CS haplotype is associated with two MspI digestion fragments of 499 and 315 bp, whereas the CO1-RS haplotype was defined by the presence of fragments of 636- and 178-bp (Nagoshi et al. 2008a, Meagher and Nagoshi 2010).

Analysis of Tpi Polymorphisms at the Two SNPs

The coding region of the fall armyworm Tpi gene is contained in five exons separated by four introns (Nagoshi 2010). The strain-specific SNP used in this study was initially designated as C370. To simplify comparisons with other nearby SNPs, we renamed this site as $e4_{183}$, which indicates its location within the fourth exon of the coding region, 183-bp from the 5' splice site. The SNP $e4_{192}$ is located on the same exon 9 bp downstream of $e4_{183}$ (Fig. 1). The polymorphisms at both SNPs are synonymous. The PCR amplified fragment produced by the primer pair Tpi412F/1140R contains both SNP sites and can be simultaneously read from a single sequencing run using Tpi412F as primer.

The frequencies of the C-allele and T-allele for each SNP were estimated using Hardy-Weinberg equilibrium analysis. The allele frequencies for C and T are given by p and q , respectively, such that $p + q = 1$. Homozygotes for each allele are designated as CC and TT, whereas heterozygotes are indicated by CT or as Y in chromatographs with overlapping C and T peaks as per IUPAC convention. For each collection, p was calculated by the equation $p = \text{freqCC} + 0.5[\text{freqCT}]$, where freqCC is the frequency of CC homozygotes and freqCT is the frequency of CT heterozygotes. The frequency of the T-allele (q) is then given by the equation $q = 1 - p$. The local expected heterozygote frequency, H_e , is equal to the equation $H_e = 2pq$. The local observed heterozygote frequency, H_o , is given by the empirically determined freqCT. Wright's local inbreeding coefficient, $F = (H_e - H_o)/H_e$, was calculated for SNPs $e4_{183}$ and $e4_{192}$.

DNA Sequence and Statistical Analysis

PCR amplified products were purified by gel electrophoresis. Each PCR reaction was run on a 1.8% agarose horizontal gel containing GelRed (Biotium, Hayward, CA) in 0.5 \times Tris-borate buffer (45 mM Tris base, 45 mM boric acid, 1 mM ethylenediaminetetraacetic acid pH 8.0). Fragments were visualized on a long-wave UV light box and cut out from the gel. Fragment isolation was performed using Zymo-Spin I columns (Zymo Research) according to manufacturer's instructions. DNA sequencing was performed directly from gel-purified PCR fragments by Sanger sequencing at the University of Florida Interdisciplinary Center for Biotechnology (Gainesville, FL) and by Genewiz (South Plainfield, NJ). DNA sequence alignments and comparisons were performed using programs available on the Geneious 10.0.7 software (Biomatters, Auckland, New Zealand). Generation of graphs was done using Excel and Powerpoint (Microsoft, Redmond, WA). Other statistical analyses including t -tests and Fisher's exact test were performed using GraphPad Prism version 7.00 for Mac (GraphPad Software, La Jolla, CA).

Results

SNPs in Tpi With Different Levels of Strain-Bias

Previous studies characterized three SNPs in the 3' half of the fourth exon of the coding region as displaying a statistically significant degree of strain-specificity (Nagoshi 2012). This was confirmed for the most 3' SNP in this cluster, $e4_{183}$, which is polymorphic for nucleotides C ($e4_{183}\text{C}$) and T ($e4_{183}\text{T}$). In total, 101 larvae were examined, 67 from corn and 34 from host plants preferred by the RS. As expected, the CO1 strain-biased haplotypes showed a statistically

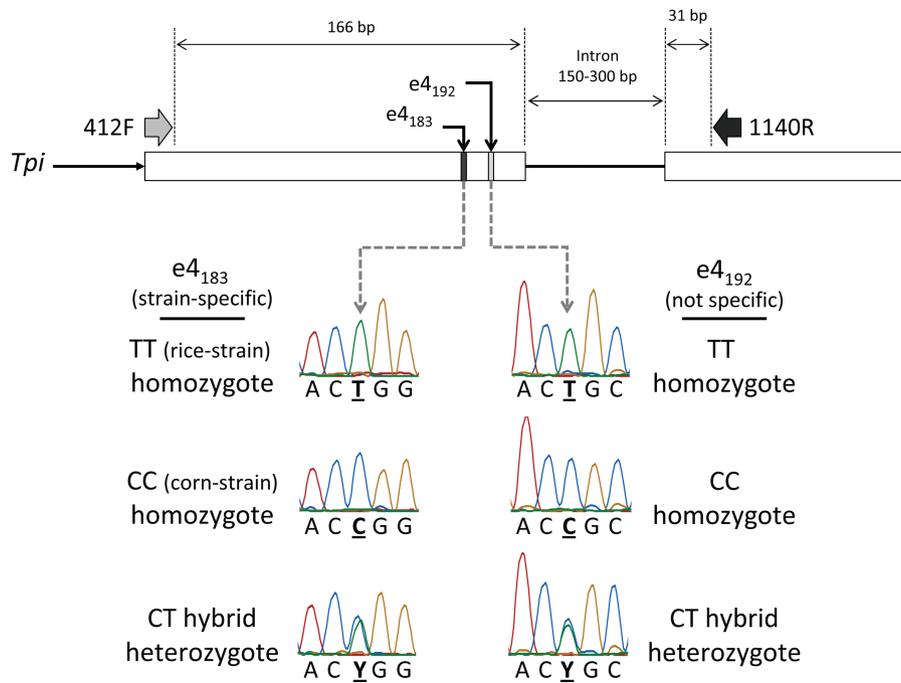


Fig. 1. Map of a portion of the fall armyworm *Tpi* gene used in this study. Block arrows indicate primers used for PCR amplification and DNA sequencing. Rectangles indicate exons separated by a variable length intron. The approximate locations of the $e4_{183}$ and $e4_{192}$ SNPs are indicated by vertical bars. Below the map are DNA sequencing chromatographs indicating the patterns associated with each genotype. The CT heterozygote gives an overlapping C and T chromatograph designated 'Y' as per IUPAC code convention.

significant distribution, with *CO1*-CS and *CO1*-RS primarily found with CS and RS host plants, respectively (Fig. 2A). The $e4_{183}$ SNP showed a similar level of strain-specificity; with in both cases, the $e4_{183}$ C allele significantly associated with CS preferred host plants (Fig. 2B) and the *CO1*-CS haplotype (Fig. 2C).

The $e4_{192}$ SNP is located only 9-bp downstream of $e4_{183}$ in the same exon and is also polymorphic for nucleotides, C and T. The majority of larvae were either homozygotes or hemizygotes of the $e4_{192}$ C allele, with this genotype making up over 75% of the tested samples. Consistent with their physical proximity is the observation of genetic linkage between the two SNPs, with $e4_{192}$ C significantly associated with the $e4_{183}$ T allele (Fig. 2D). Despite this physical linkage, the $e4_{192}$ SNP exhibits much less strain-specificity than $e4_{183}$, with no statistically significant bias found with respect to host plant (Fig. 2E) or *CO1* haplotypes (Fig. 2F).

This arrangement of two SNPs in close proximity displaying a significant difference in strain-specificity provides an opportunity to quantify the frequency of hybridization between strains relative to that within strain. This is shown by Punnett square analysis that predicts the SNP genotypes of the male progeny produced by random and strain-specific hybridization (Fig. 3). If $e4_{183}$ defines strain identity ($e4_{183}$ C = CS and $e4_{183}$ T = RS) then there are two CS gamete genotypes with respect to the two SNPs, $e4_{183}$ C $e4_{192}$ C and $e4_{183}$ C $e4_{192}$ T, and two RS gamete genotypes, $e4_{183}$ T $e4_{192}$ C and $e4_{183}$ T $e4_{192}$ T. If mating is random, heterozygotes will be produced at an equal frequency at the two SNPs (Fig. 3A), whereas complete strain-specificity in mating will result in heterozygosity only at $e4_{192}$ (Fig. 3B). Therefore, the difference in heterozygosity frequency between the two SNPs is an indicator of reproductive restrictions between the two strains. As a test of the methodology, a single female from a *CO1*-RS laboratory colony was mated to a single male from a *CO1*-CS colony (Fig. 4A). Sequence analysis demonstrated as expected that the female parent was hemizygous for $e4_{183}$ T while the male was homozygous for $e4_{183}$ C. All 10 female progenies tested showed only $e4_{183}$ C, consistent

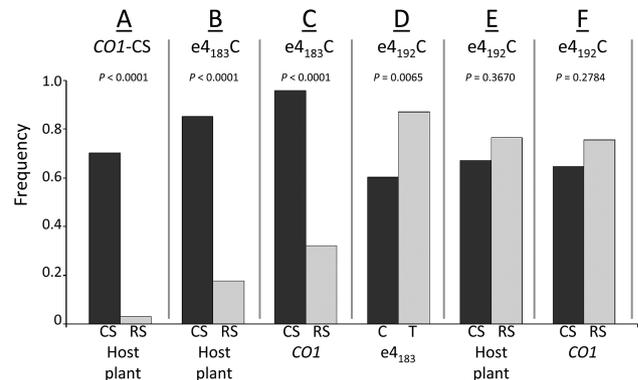


Fig. 2. Frequencies of the $e4_{183}$ and $e4_{192}$ SNP polymorphisms and *CO1* haplotypes were compared relative to each other and to host plants from the larval collection data ($n = 101$). P -values are from Fisher's exact test analysis. (A) The *CO1*-CS haplotype frequency found in CS and RS preferred host plants. (B) The frequency of $e4_{183}$ C in each strain-specific host plant group. (C) The frequency of $e4_{183}$ C in the *CO1* haplotype groups. (D) The $e4_{192}$ C frequency relative to the $e4_{183}$ polymorphism. (E) The frequency of $e4_{192}$ C in the strain-specific host plant groups. (F) The frequency of $e4_{192}$ C in the *CO1* haplotype groups.

with that being hemizygous for this allele, whereas all 10 male progenies were CT heterozygotes (Fig. 4A). These patterns are consistent with sex-linked segregation within the WZ/ZZ sex determination system characteristic of Lepidoptera (Traut 1999), and demonstrate that the expected SNP heterozygotes can consistently be identified by overlapping signals in DNA sequence chromatographs.

Frequency of SNP Heterozygotes in Field Collections

Adult males obtained from pheromone trapping were analyzed for their SNP genotypes. At SNP $e4_{183}$, there were 284 specimens

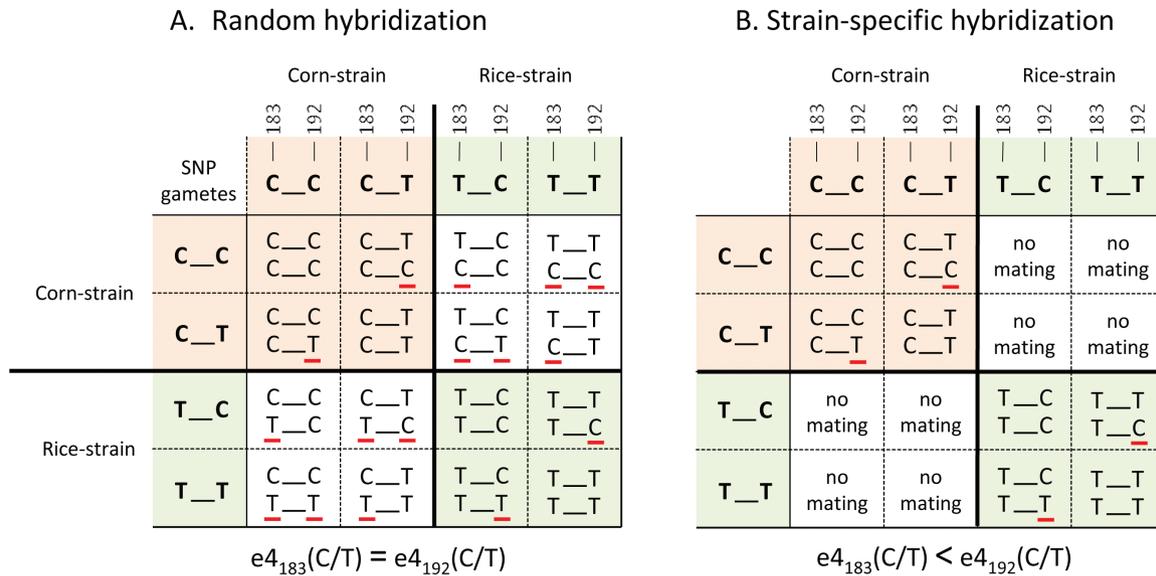


Fig. 3. Punnett square analysis of the SNP genotypes produced in random and strain-specific hybridization scenarios. Strain identity is determined by $e_{4_{183}}$, with CS denoted by $e_{4_{183}}C$ (orange boxes) and RS by $e_{4_{183}}T$ (green boxes). Interstrain hybrid genotypes indicated by white boxes. Red line identifies predicted heterozygosity. (A) Predicted SNP progeny genotypes from random mating with no strain restrictions. The frequencies of heterozygosity at both SNPs are equal. (B) Predicted SNP progeny genotypes if hybridization is strain specific. Heterozygosity at $e_{4_{183}}$ is reduced relative to $e_{4_{192}}$. This difference increases as interstrain hybridization frequency declines.

A. Generating $e_{4_{183}}$ hybrid males.

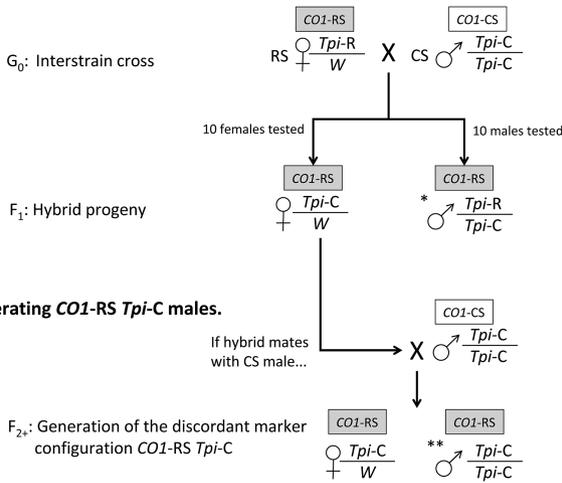


Fig. 4. Mating patterns that produce hybrid and discordant genotypes. (A) An interstrain cross between a RS female and a CS male will produce male progeny heterozygous for C and T at $e_{4_{183}}$. The *CO1* haplotype is maternally inherited and indicated by boxes, shaded if *CO1-RS*, white if *CO1-CS*. The CS (*Tpi-C*/*Tpi-C* and *Tpi-C*/*W*) and RS (*Tpi-R*/*Tpi-R* and *Tpi-R*/*W*) genotypes are defined by the presence of either $e_{4_{183}}C$ or $e_{4_{183}}T$, respectively. The hybrid (*Tpi-C*/*Tpi-R*) is indicated by the presence of both $e_{4_{183}}C$ and $e_{4_{183}}T$. Single asterisk identifies $e_{4_{183}}$ male hybrid. (B) Mating of F_1 female with CS male will produce the discordant genotype *CO1-RS* *Tpi-C*. Double asterisks identifies *CO1-RS* *Tpi-C* discordant male.

homozygous for C (CC), 136 TT, and 65 heterozygous for C and T (CT). SNP $e_{4_{192}}$ also displayed a majority C-allele distribution with 265 CC, 62 TT, and 158 Y. There were five outliers at $e_{4_{192}}$, three of which were homozygous for an A-allele, one heterozygous AT, and another heterozygous AC. Because the A-allele was rare (found in 5 of 490 samples), these specimens were not included in the analysis.

The frequency of CT heterozygotes for each SNP is equal to the observed heterozygosity, H_o , and was calculated for each collection.

The mean H_o at $e_{4_{183}}$ was significantly reduced from $e_{4_{192}}$ in the combined strain and *CO1-CS* groups, but was statistically indistinguishable from $e_{4_{192}}$ in the *CO1-RS* specimens (Fig. 5A). A more accurate accounting of heterozygosity adjusts for the influence of the allele frequencies of the SNPs in the population. This was done using Wright’s local inbreeding coefficient (F), which provides a simple metric for quantifying the degree of observed heterozygosity (H_o) relative to what would be expected (H_e) from a population at Hardy-Weinberg equilibrium. An $F = 0$ indicates a state of equilibrium where $H_e = H_o$, with values becoming increasingly positive if heterozygosity is suppressed (high inbreeding) and more negative if the observed heterozygosity is greater than expected.

For the $e_{4_{183}}$ SNP, H_o was always less than H_e for each collection as indicated by the consistently positive F scores that ranged from 0.48 to 0.82 with a mean of 0.63 (Table 2A). In comparison, at $e_{4_{192}}$ the mean was near zero (−0.04) with a range from −0.14 to 0.21. Generally, similar results were found for the *CO1-CS* and *CO1-RS* groups, where the $e_{4_{183}}$ F score was consistently more positive than the $e_{4_{192}}$ (Table 2B and C). The mean F for all collections indicated that the difference between SNPs was statistically significant for all categories (Fig 5B), confirming that fewer heterozygotes were consistently recovered at the more strain-specific $e_{4_{183}}$ SNP relative to $e_{4_{192}}$. This was even the case in the *CO1-RS* group, where no significant difference was found with the H_o metric (Fig. 5A).

RS Females Are More Likely to Mate With the Opposite Strain

Specimens that were not hybrid at $e_{4_{183}}$ are characterized as carrying either the CS (*Tpi-C*) or the RS (*Tpi-R*) allele based on the presence of $e_{4_{183}}C$ or $e_{4_{183}}T$, respectively. Comparison of this $e_{4_{183}}$ categorization with that produced by the mitochondrial *CO1* markers produces two strain concordant configurations where the strain markers are consistent (*CO1-CS* *Tpi-C* and *CO1-RS* *Tpi-R*) and two discordant or inconsistent combinations of (*CO1-CS* *Tpi-R* and *CO1-RS* *Tpi-C*). The majority of our specimens had a concordant genotype, as would be expected if the strains are reproductively restricted

such that hybridization within strains are preferred. However, there was a large difference in the frequency of the concordant configurations. Among the *CO1*-CS specimens, concordance occurred at a significantly higher frequency (94%) than discordance (Fig. 6A). In

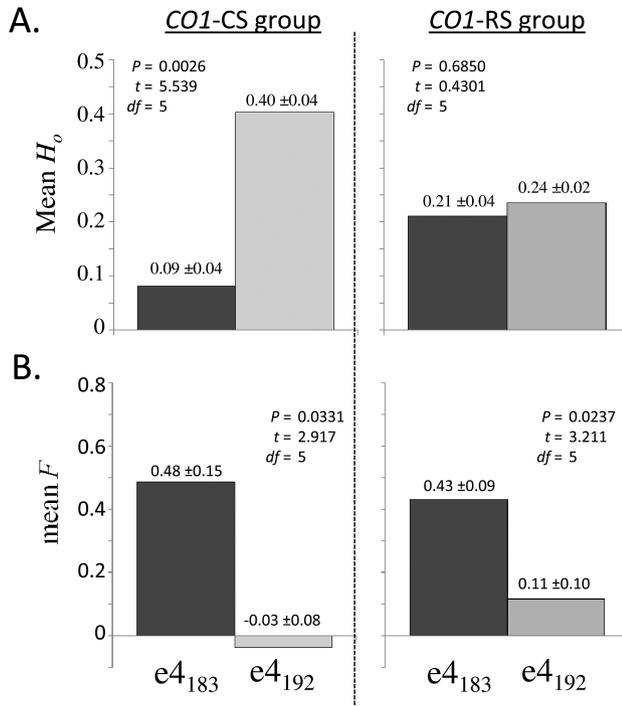


Fig. 5. Observed heterozygosity and inbreeding coefficient F values for the $e4_{183}$ and $e4_{192}$ SNPs in the *CO1*-RS and *CO1*-CS groups. Specimens were adult males from pheromone traps. Statistics are from two-tailed paired t -test comparisons and mean values \pm SEM are indicated above the columns. (A) Mean H_o values from the six collections were compared for each *CO1* group. (B) Mean F values from the six collections were compared for each *CO1* group.

comparison, only 64% of *CO1*-RS specimens were of the concordant configuration, which was larger than, but not statistically different than, the discordant frequency. A possible explanation for this observation comes from a comparison of expected heterozygosity (H_e). The mean $e4_{183}$ *CO1*-RS H_e was significantly higher than the H_e for the *CO1*-CS group (Fig. 6B), an indication that for our dataset an excess of hybrids with the *CO1*-RS haplotype was to be expected based solely on allele frequencies.

Discussion

Quantifying the Suppression of Interstrain Hybridization

The fall armyworm host strains represent two subpopulations that appear to be in the process of species divergence (Pashley 1986, Prowell 1998), which would be expected to involve restrictions in mating between strains. To determine whether and quantify the degree to which interstrain hybridization is suppressed in wild populations, we identified two SNPs that differed in their strain-specificity but were otherwise similar in structure and location. The $e4_{183}$ and $e4_{192}$ polymorphisms are present in the same exon and are associated with the same polymorphic base alternatives (C or T). Because the two SNPs are treated identically, comparisons between the two should control for unexpected influences such as Wahlund effects, leaving the frequency of strain-specific hybridization as the primary determinant of heterozygosity differences. Such differences are quantified by comparing the mean F inbreeding coefficient, which was substantially more positive for $e4_{183}$ than for $e4_{192}$ in each *CO1* sub-group (Fig. 5B). The suppression of heterozygosity at $e4_{183}$, both in absolute terms and relative to $e4_{192}$, is consistent with at least a four-fold reduction in interstrain compared with intrastrain hybridization.

Is There a Directional Bias in Interstrain Mating in the Field?

We applied the SNP method to investigate the biological relevance of a previously described directional bias in interstrain mating behavior.

Table 2. Description of polymorphisms at the $e4_{183}$ and $e4_{192}$ SNPs

SNP	Metric	DR-16	PR-09	PA-15	FL-05	FL-12	GA-07
Total	n	48	101	43	53	178	62
$e4_{183}$	H_o	0.08	0.06	0.09	0.25	0.13	0.23
	H_e	0.46	0.15	0.27	0.47	0.49	0.48
	F	0.82	0.59	0.66	0.48	0.73	0.53
$e4_{192}$	H_o	0.31	0.36	0.47	0.34	0.30	0.24
	H_e	0.29	0.50	0.45	0.35	0.38	0.21
	F	-0.07	0.29	-0.02	0.03	0.21	-0.14
B. <i>CO1</i> -CS	n	14	72	34	33	63	16
$e4_{183}$	H_o	0.00	0.06	0.03	0.21	0.05	0.13
	H_e	0.13	0.10	0.03	0.28	0.13	0.30
	F	1.00	0.47	-0.01	0.24	0.64	0.59
$e4_{192}$	H_o	0.43	0.39	0.53	0.44	0.38	0.25
	H_e	0.34	0.50	0.48	0.40	0.46	0.22
	F	-0.27	0.22	-0.09	-0.10	0.18	-0.14
C. <i>CO1</i> -RS	n	34	29	9	20	115	46
$e4_{183}$	H_o	0.12	0.07	0.33	0.30	0.18	0.26
	H_e	0.21	0.24	0.40	0.38	0.47	0.50
	F	0.43	0.71	0.17	0.20	0.61	0.48
$e4_{192}$	H_o	0.26	0.28	0.22	0.15	0.26	0.24
	H_e	0.27	0.49	0.20	0.22	0.32	0.21
	F	0.02	0.44	-0.13	0.31	0.18	-0.14

Observed heterozygosity (H_o), expected heterozygosity (H_e), and Wright's local inbreeding coefficient ($F = [H_e - H_o]/H_e$) were calculated for each SNP.

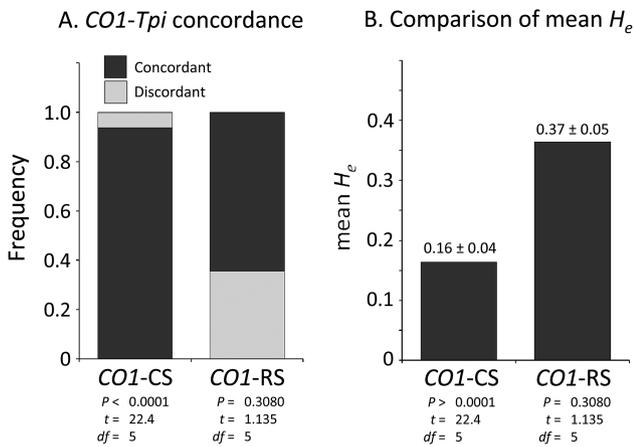


Fig. 6. Interstrain hybrids are more frequent in the CO1-RS group. Statistics are from two-tailed paired t -test comparisons. (A) Frequency of CO1-Tpi concordant and discordant configurations was calculated for each CO1 group. (B) Mean H_e values compared for the CO1 groups with mean values \pm SEM above the columns.

It was reported that under laboratory conditions, RS females have a higher propensity for mating with the opposite strain than CS females (Pashley and Martin 1987). This finding has been indirectly supported by field studies using combinations of mitochondrial and genomic strain-specific markers, which found that the majority of putative hybrids had a RS maternal origin (Nagoshi and Meagher 2003a, Prowell et al. 2004b, Nagoshi 2010). We found the same pattern in this study, where the great majority of CO1-Tpi discordant configurations were of the CO1-RS Tpi-C type (Fig. 6A). This pattern could initiate from the mating of RS (CO1-RS) females to CS males, and the subsequent backcross of the female hybrids to CS males (Fig. 4B). Therefore, the consistent preponderance of the CO1-RS Tpi-C discordant type is suggestive that a directional bias in interstrain mating favoring CO1-RS females may be a significant contributor to the distribution of fall armyworm genotypes found in the wild. However, an alternative explanation is suggested by the comparison of the mean H_e for the CO1 groups (Fig. 6B), which shows that when allele frequencies are taken into consideration, the majority of interstrain hybrids are expected to come from RS females. This probably reflects the tendency for fall armyworm collections to occur in cornfields, habitats predominated by the CS. In such a situation, a CO1-RS female will have substantially greater opportunities for mating with a male of the opposite strain than a CO1-CS female, resulting in a higher frequency of hybrids with the CO1-RS mitochondrial haplotype.

In conclusion, we describe and demonstrate the utility of a new method for quantifying mating between two closely related populations in the field that is based on combining a population-specific SNP with a less specific SNP acting as an internal control. There was at least a four-fold reduction in the recovery of heterozygotes of strain-specific alleles, indicating a suppression of fall armyworm interstrain hybridization of similar magnitude. We further found that similar levels of hybrid suppression was observed for both strain-specific CO1 haplotypes, suggesting that the directional biases in interstrain mating observed in laboratory studies may not be the primary determinants of asymmetries in hybrid genotypes consistently found in field studies. Instead, these asymmetries could simply reflect the allele frequencies found in the habitats typically sampled, reflecting more the distribution of strains in those locations rather than a directional bias in interstrain hybridization behaviors. These results demonstrate the advantages of the differential SNP method

over current strategies as it provides additional information that substantially improves our understanding of how the two strains interact in the wild.

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