Insect Molecular Biology (2003) **12**(5), 453–458

Fall armyworm *FR* sequences map to sex chromosomes and their distribution in the wild indicate limitations in interstrain mating

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Abstract

The fall armyworm, *Spodoptera frugiperda*, consists of two host strains (rice and corn) that differ in developmental, physiological and behavioural characteristics. However, because the strains are morphologically indistinguishable the investigation of strain-specific behaviour, particularly in the wild, is very difficult. This has spurred the isolation of diagnostic molecular markers. *FR* sequences are tandem-repeat genetic elements found in large clusters only in the rice strain. To facilitate their use as a strain marker we genetically mapped *FR* clusters and found they localized to the sex chromosomes. This represents one of the first examples of chromosome mapping in fall armyworm. The *FR* sequence and a strain-specific mitochondrial marker were then used to examine the distribution of different marker combinations in field specimens. These studies identified significant barriers to interstrain mating in the wild, specifically that corn strain females rarely, if ever, mate with rice strain males. The data also suggest that only a genetically distinct subset of the overwintering rice strain population in Florida annually migrate to Georgia. These studies demonstrate that the availability of genetically characterized molecular markers for strain identity makes possible studies on fall armyworm biology in the wild previously considered unfeasible.

Keywords: *Spodoptera frugiperda*, fall armyworm, rice strain, corn strain, *FR* sequence.

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is a major agricultural pest of corn, forage grasses, rice, cotton and peanuts. This noctuid moth overwinters in southernmost Florida and southern Texas, migrating northward during the spring and summer (Luginbill, 1928; Pair *et al*., 1986). Two sympatric strains have been identified by their plant host preferences (Pashley, 1986). The rice (R) strain is associated with rice and bermudagrass, while the corn (C) strain predominates on corn and cotton, though this host specificity is not absolute (McMichael & Prowell, 1999). The two strains are morphologically identical, but differ in certain physiological characteristics, including rates of development, larval weight, and pupal weight (Pashley, 1988a; Quisenberry & Whitford, 1988; Whitford *et al*., 1992; Whitford *et al*., 1988). R-strain development is particularly sensitive to the type of plant host (Pashley, 1988a), which may be due to lower inherent oxidase activity than the C-strain (Veenstra *et al*., 1995). There is also evidence for strain-specific behavioural differences in plant host ovipositional preference and mating preference (Pashley *et al*., 1992; Whitford *et al*., 1988).

The R-strain and C-strain differ in certain diagnostic molecular characteristics. These include electrophoretic variations in allozymes (Pashley, 1986), mitochondrial DNA polymorphisms (Lu & Adang, 1996; Pashley, 1989), nuclear restriction fragment-length polymorphisms, or RFLPs (Lu *et al*., 1992), amplified fragment-length polymorphisms (McMichael & Prowell, 1999), and differences in repetitive DNA sequences (Lu *et al*., 1994). Two genetic markers are of particular relevance to this study. The first is a polymorphic *Msp*I restriction site in the mitochondrial cytochrome oxidase subunit I (*COI*) gene that can be detected by polymerase chain reaction (PCR) methodology (Lu & Adang, 1996; Levy *et al*., 2002). Several studies demonstrated that this marker is a consistent indicator of strain identity, showing a strong correlation with the preferred plant host (Levy *et al*., 2002; Lu & Adang, 1996; Meagher & Gallo-Meagher, in press).

The second strain-specific marker is a repeated DNA sequence called *FR* (fall armyworm Rice strain; Lu *et al*., 1994). The 189 bp *FR* unit is abundant in R-strain females, accounting for an estimated 0.05% of the genomic DNA, and is organized in large tandem repeat arrays. The *FR* sequence is also present and repetitive in males, though there is an approximately 120-fold reduction in copy number.

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This sex-specificity suggested a sex chromosomal location (Lu *et al*., 1994), but chromosomal mapping is difficult in this organism and has not been performed. Several hundred FAW from laboratory colonies were tested by Southern blot hybridization and in all cases *FR* was specific to the R-strain, making it a potentially useful nuclear marker for strain identity (Lu *et al*., 1994). We recently developed a PCR-based method for detecting *FR* sequences and found that they are present at low copy number in at least a subset of the C-strain population (Nagoshi & Meagher, 2003). However, large tandem-repeat clusters were only detected in individuals from R-strain laboratory cultures.

Sustainable genetic differences between sympatric strains suggest significant barriers to the formation of interstrain hybrids in the wild. To test for such barriers we first mapped *FR* clusters to the sex chromosomes, thereby allowing us to define their expected pattern of inheritance. We then surveyed males collected from several south Florida sites and found that the distribution of *FR* clusters with respect to the mitochondrial *COI* marker was asymmetric and strain-specific, indicating significant limitations in interstrain mating. Specifically the data showed that C-strain females rarely if ever mated with R-strain males. The ramifications of our findings on strain-specific behaviour in the wild and on the migration of the R-strain population are discussed.

Results

Genetic mapping of FR repeat clusters

FR clusters were mapped by a series of crosses between individuals from laboratory populations derived either from larvae collected from corn (*Zea mays* L.) plants (C-strain) or bermudagrass (*Cynodon dactylon* L., R-strain). The strain identity of each colony was confirmed by analysis of randomly selected individuals for the strain-specific RFLP in the mitochondrial *COI* gene (Levy *et al*., 2002). All individuals tested from each population ($n > 15$) displayed the appropriate polymorphism (designated mt^C or mt^R for the C-strain or R-strain, respectively). The crosses are outlined in Fig. 1 with the assumption that *FR* clusters are located on the sex chromosomes. Two matings were performed to generate FR^+/FR^0 hybrids (Fig. 1A). In Cross-A, C-strain females were mated to R-strain males, while the reciprocal mating was performed in Cross-B. In both cases several hundred eggs were laid, confirming that interstrain matings can occur (at least between inbred laboratory cultures) as reported previously (Whitford *et al*., 1988). After several days of laying eggs, the parents from each cross were tested by PCR to confirm that the C-strain parents used were mt^C and lacked *FR* clusters (FR^0), while the R-strain parents were mt^R and carried at least one chromosome with *FR* clusters (FR^+).

The progeny from Cross-A all carried the mt^C marker, as expected given the maternal inheritance of mitochondria. The presence of *FR* clusters in the hybrids can be unambiguously identified by the generation by PCR amplification of a higher molecular mass DNA ladder, while FR^0 samples produce at best 1–3 faint bands of a few hundred base pairs (Nagoshi and Meagher, 2003). We found that both daughters and sons of Cross-A were polymorphic (3/7 and 2/5, respectively) for *FR* clusters, indicating that the single male R-strain parent was heterozygous for an FR^+ chromosome (Fig. 2A). In comparison, *FR* clusters were present in all progeny from Cross-B, indicating that the female R-strain parents were most likely homozygous for the FR^+ chromosome (Fig. 2B).

The hybrid progeny were then used to chromosomally map *FR*. If *FR* clusters map to the sex chromosomes, then the F_1 female progeny from Cross-A that were FR^+ (Fig. 1,

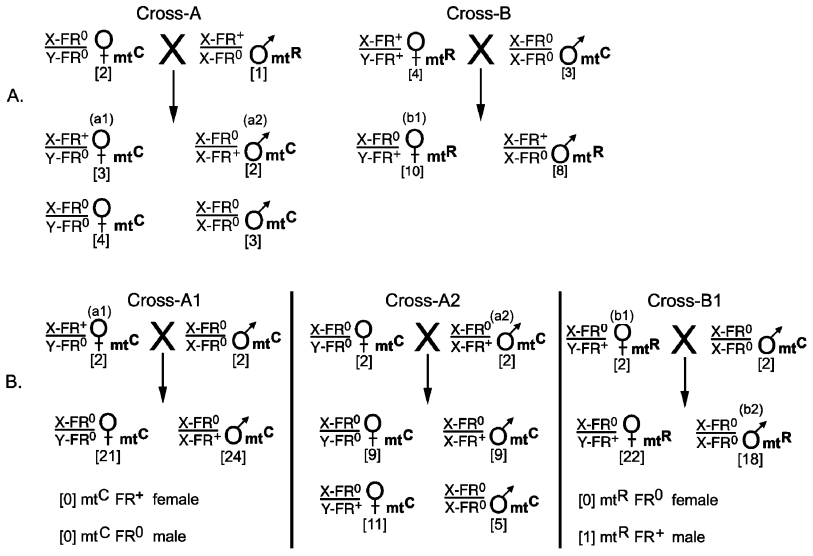

Figure 1. FAW crosses to map the chromosome carrying *FR* clusters. In this diagram it is assumed that *FR* clusters are located on the sex chromosomes. (A) In Cross A, females from the C-strain Tifton colony were mated to R-strain males from the Ona colony. Subsequent analysis (see text) demonstrated that the male parent was heterozygous for the FR^+ chromosome. In Cross-B, females from the Ona colony were mated to males from the Tifton colony. Progeny designated a1, a2 and b1 were used in subsequent crosses. (B) In cross A1, hybrid females (a1 from Cross-A) were mated to C-strain males from the Tifton colony. In Cross-A2, C-strain females from the Tifton colony were mated to hybrid males (a2 from Cross-A). In Cross-B1, hybrid females (b1 from Cross-B) were mated to C-strain males from the Tifton colony. Progeny class b2 represents a hybrid marker configuration. The mitochondrial marker and presence/absence of *FR* clusters were confirmed by PCR analysis. Expected F1 progeny are shown as well as the numbers obtained experimentally (in brackets). FR^0 indicates no *FR* clusters as seen by PCR; FR^+ indicates that *FR* clusters are present.

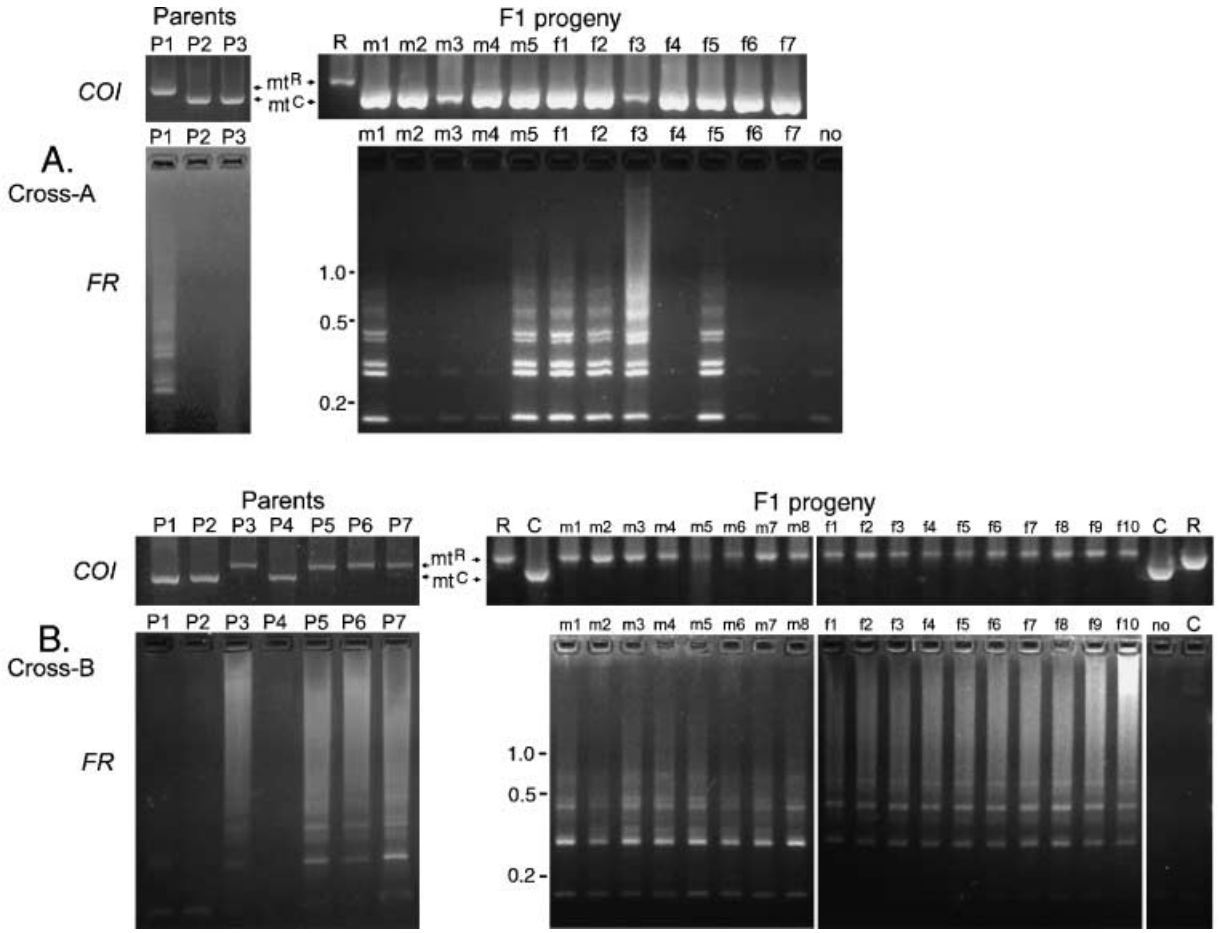

Figure 2. Agarose gels of PCR reactions showing COI polymorphism and *FR* clusters. All gels are stained with ethidium bromide and photographed under ultraviolet illumination. In the upper *COI* gels, the *JM-76/JM-77* primer combination amplifies a fragment from the mitochondrial *COI* gene that contains a strain-specific *Msp*I site. After digestion with *Msp*I, a larger band defines the mt^R marker while a lower band denotes mt^C. In the *FR* gels, the presence of bands greater than 0.5 kb is indicative of the presence of an FR^+ chromosome. One to three faint lower molecular weight bands are occasionally seen in FR^0 lanes and in no DNA controls. Low copy numbers of *FR* elements have been identified in the C-strain and the primers used can self-anneal, however, FR^0 and negative controls never produce PCR bands greater than 0.5 kb (Nagoshi and Meagher, 2003). (A) PCR analysis of Cross-A parents and progeny. Parents: P1, male R-strain parent; P2–3, female C-strain parent. F1 progeny: R, R-strain control; m1–5, male progeny; f1–7 female progeny; 'no', no DNA template control. We considered m1, m5, f1, f2, f3, f5 to be FR^+. (B) PCR analysis of Cross-B parents and progeny. Parents: P1,2,4: male C-strain parents; P3, 5–7: female R-strain parents. F1 progeny: R, R-strain control; C, C-strain control. We considered all progeny to be FR^+. Sizes are in kilobases.

a1) will be heterozygous for an FR^+ X-chromosome and an FR^0 Y-chromosome. Upon mating with C-strain males as depicted in Cross-A1, the resulting progeny should show the sex-specific pattern of FR^+ males and FR^0 females (Fig. 1B). Our results were consistent with these expectations. When progeny from Cross-A1 were tested for the presence of *FR* clusters by PCR, all males ($n = 24$) were FR^+ while all females examined ($n = 21$) were FR^0 (Fig. 1B).

In Cross-B1, F_1 females from Cross-B (designated b1) were mated to C-strain males. Assuming that the b1 females had an FR^+ Y-chromosome and FR^0 X-chromosome, then the cross should produce FR^+ males and FR^0 females, the reciprocal to the pattern observed Cross-A1. This was the case, as all female progeny (22/22) were FR^+, while nearly all male progeny (18/19) were FR^0 (Fig. 1B). Therefore, *FR* clusters display sex-specific segregation patterns that are dependent on the direction of the cross, a property indicative of exclusive linkage to the sex chromosomes. The origin of the exceptional FR^+ male is unclear. One explanation is that there was genetic exchange between the X-chromosome and Y-chromosome in one of the b1 hybrid mothers such that *FR* elements were transferred to a previously FR^0 chromosome. Examples of genetic recombination between X- and Y-chromosomes have been described in other organisms, though these occur at low frequency (Cooke *et al.*, 1984; Polanco *et al.*, 2000).

Final confirmation came from Cross-A2, where hybrid males (a2) were mated to virgin C-strain females (Fig. 1B). In this case, *FR* clusters should not segregate in a sex-specific pattern. Once again this prediction was confirmed, as both sexes were polymorphic for FR^+ (9/14 males and 11/20 females).

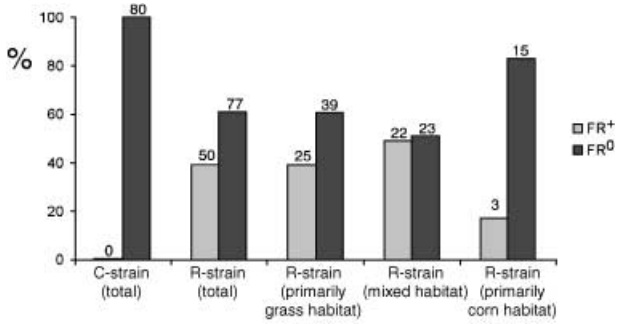

Figure 3. Distribution of FR^+ and FR^0 FAW in field collected male adults. Adult FAW males were collected from pheromone traps placed in several locations in south Florida. Each individual was tested for the COI polymorphism (mt^R or mt^C) and for the presence of *FR* clusters by PCR. All mt^C individuals were FR^0. R-strain males (mt^R) were polymorphic for *FR* clusters. The total R-strain distribution is shown as well as those from different 'habitats' (described in the text). Numbers above columns indicate number of samples tested.

Strain-specific distribution of FR clusters in field populations

If interstrain matings are common in the wild, the distribution of FR^+ and FR^0 X-chromosomes should become randomized with respect to the mitochondrial marker. This can be tested by the examination of field-isolated FAW males. Pheromone traps were used to capture adult males from 18 locations in south and central Florida. Collectively we identified 135 C-strain and 160 R-strain males based on the *COI* RFLP. A subset from each group was then tested for the presence of the *FR* clusters by PCR. We found that 39% (50/127) of R-strain individuals were FR^+ (Fig. 3). To confirm these results, we retested a subset of the samples by PCR analyses with a second *FR*-specific primer pair. A total of 41 individual samples were tested and the results were all consistent with the first study (data not shown). In contrast, we did not identify a single FR^+ individual ($n = 80$) in the C-strain population. This result indicates that C-strain females rarely, if ever, productively mate with R-strain males in the wild (Fig. 1A; Cross-A, a2).

Whether R-strain females mate with C-strain males is less clear. The polymorphism exhibited by the mt^R population for *FR* clusters could be a naturally occurring variation or the result of interstrain mating (Fig. 1B; Cross-B1, b2). If the mt^R FR^0 class represents genetic hybrids, then we might expect to see differences in plant host preference from their mt^R FR^+ siblings, the genotype expected of the 'pure' R-strain. To test this possibility we examined the proportions of the R-strain subpopulations in three different habitats. 'Primarily corn' was arbitrarily defined as one where over 70% of the FAW collected were mt^C, indicating that the plants in this habitat were attractive primarily to the C-strain. Traps containing over 70% mt^R males were defined as 'primarily grass habitats', with the remainder forming the 'mixed habitat' category. We found that the proportion of FR^0 males increased to 83% (15/18) of the total R-strain population trapped in primarily corn habitats, compared to 61% (39/64) in areas preferred by the R-strain, and 51% (23/45) in mixed habitats (Fig. 3). Although the differences are only of borderline statistical significance (see Experimental procedures for description), the results provide a preliminary suggestion that mt^R FR^0 males may be less specific in their plant host preference than mt^R FR^+ males. This would be consistent with this genotype representing interstrain hybrids.

Discussion

The existence of two host strains of fall armyworm is well established, but surprisingly little is known about the behaviour of these strains in the wild. Of particular interest is whether and to what degree interstrain mating occurs. Pashley & Martin (1987) performed laboratory matings studies to test for reproductive incompatibility between the two host strains. They found that R-strain females mated readily with males of either strain. In comparison, C-strain females never mated with R-strain males. Hybrid males could mate with C-strain females, but were significantly less productive than within-strain matings with respect to the transfer of spermatophores and female fertility. However, another study reported near normal fertility rates with laboratory interstrain crosses, including those between C-strain females and R-strain males (Whitford *et al.*, 1988). The reason for this disagreement with Pashley & Martin (1987) is not clear, but may indicate that strain mating bias can be confounded by artificial culture conditions or may disappear after multiple generations in the laboratory. In any case, the frequency of interstrain matings in the wild was unclear.

Our findings support those of Pashley & Martin (1987). The absence of individuals with the mt^C FR^+ marker combination strongly suggests that matings between C-strain females and R-strain males are rare. Furthermore, the presence of a substantial population carrying the mt^R FR^0 marker combination are consistent with the observation of near-normal hybridization between R-strain females and C-strain males. It therefore appears that the limited and directional interstrain mating behaviour observed in the laboratory is likely occurring in the field. The basis for this mating limitation is not known but there is evidence for strain-specific differences in the preferred time of mating and for strain-specific biases in pheromone response (Pashley, 1988b; Pashley *et al.*, 1992).

Lu *et al.* (1994) provided evidence for even more extreme strain mating bias in fall armyworm populations examined in Georgia. Using Southern blot analysis to detect *FR* clusters, they reported complete correspondence with the presence of *FR* sequences and the mt^R marker. This indicates that in the field populations tested, productive interstrain matings in either direction are rare, if they occur at all. These results may suggest that the R-strain population of south

Florida differs from that of Georgia, even though the latter are derived from yearly migrations of FAW overwintering in Florida. The data could be explained if interstrain hybrids are less likely to migrate, in which case the 'pure' R-strain subpopulation will be the predominant genotype in more Northern climes. This possibility is being tested by field surveys of migratory FAW populations.

Experimental procedures

Strains and collections

Male adult fall armyworm (FAW, *Spodoptera frugiperda*) were obtained from field traps and laboratory-reared colonies. The C-strain colony (designated Tifton) was obtained from the USDA/ARS at Tifton, Georgia. The R-strain (Ona) colony was started from larvae isolated from grass pasture land in Ona, Florida, and has been maintained by inbreeding for almost one year. The C-strain colony was reared on a pinto bean-based artificial diet (Guy *et al.*, 1985). The R-strain colony was reared on greenhouse grown bermudagrass.

Field collection sites were in south Florida and represented a variety of habitats. Adult males were collected using pheromone traps as described in Meagher & Gallo-Meagher (2003). Standard plastic Unitraps were baited with a commercially available FAW pheromone (Scenturion Inc., Clinton, WA), and also contained insecticide strips (Hercon Environmental Co., Emigsville, PA). Collections were made throughout the year at empirically determined intervals (depending on number of trapped specimens). Specimens were stored at –20 °C until analysis.

DNA preparation

Individual adult FAW were homogenized in 1 mL of homogenization buffer (0.03 M Tris/HCl pH 8.0, 0.1 M NaCl, 0.2 M sucrose, 0.01 M EDTA 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 distilled water) into a 1.5 mL microfuge tube. The Dounce homogenizer was washed with 800 μL of buffer, which was filtered and added to the homogenate. 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 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, v/v). 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 manufacturer's instructions. Each PCR reaction used 1 μL of the DNA preparation (between 0.1 and 0.5 μg).

PCR analysis

Genomic DNA was tested by PCR for the mitochodrial *COI* gene RFLP to confirm strain identity. PCR amplification of genomic DNA was performed in a 50 μL reaction mix containing 5 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, 0.5 μL *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 38 cycles of 94 °C (1 min), 56 °C (1 min), 72 °C (1 min), and a final incubation at 72 °C for 5 min. Upon completion of the PCR, 0.5 μL of *Msp*I was added to each reaction and incubated at 37 °C for 1 h. Five microlitres of gel loading buffer were added to each sample, and 20 μL were loaded on a 1.5% agarose gel. The R-strain (mt^C) pattern is a 569 bp PCR band, while the C-strain fragment is cut by *Msp*I to produce two fragments of 497 and 72 bp.

Amplification of the *FR* and other fragments (unless otherwise noted) used an initial incubation at 94 °C (1 min), followed by 38 cycles of 94 °C (45 s), 56 °C (45 s), 72 °C (1 min), and a final incubation of 72 °C for 5 min. Five microlitres of gel loading buffer were added to each sample, and 20 μL were loaded on a 2% agarose gel. FR^+ samples produce a DNA ladder associated with a higher molecular mass smear. These results from overlapping amplification of multiple *FR* units due to their organization in clusters of tandem repeats (Nagoshi & Meagher, 2003). FR^0 samples produce 0–3 relatively faint bands. The amplified fragments result from either the presence of a few *FR* units as described by Nagoshi & Meagher (2003), or primer self-annealing. The presence of bands greater than 500 bp is diagnostic of FR^+ samples.

Primers were synthesized by DNAgency (Malvern, PA). They included *JM76*, 5′-GAGCTGAATTAGG(G/A)ACTCCAGG-3′, and *JM77*, 5′-ATCACCTCC(A/T)CCTGCAGGATC-3′, which span the mitochondrial cytochrome oxidase C subunit I gene (*COI*). The *FR*-specific primer combination used was *FR*-c (5′-TCGTGTAAAACGTACTTTCTT-3′) and *FR*-2 (5′-GACATAGAAGAGCACGTTT-3′). Confirmation of the presence of *FR* clusters used the primer combination *FR-a* (5′-TTTTACACCGGTCACAACGA-3′) and *FR-2*.

Acknowledgements

We thank J. Sharp for excellent technical support and C. Dillard for the maintenance of FAW cultures. We thank Dr James Carpenter (USDA-ARS, Tifton, GA) for supplying Tifton moths. We also thank Dr M. Gallo-Meagher (University of Florida) and Dr A. Handler (USDA-ARS, CMAVE, Gainesville, FL) for helpful comments on the manuscript. The use of trade, firm or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

References

Cooke, H.J., Brown, W.A. and Rappold, G.A. (1984) Closely related sequences on human X and Y chromosomes outside the pairing region. *Nature* **311**: 259–261.

Guy, R.H., Leppla, N.C., Rye, J.R., Green, C.W., Barette, S.L. and Hollien, K.A. (1985) *Trichoplusia ni*. In *Handbook of Insect Rearing* (Singh, P. and Moore, R.F., eds). Elsevier, Amsterdam, the Netherlands.

Levy, H.C., Garcia-Maruniak, A. and Maruniak, J.E. (2002) Strain Identification of *Spodoptera Frugiperda* (Lepidoptera: Noctuidae)

insects and cell line: PCR-RFLP of cytochrome oxidase subunit I gene. *Florida Entomol* **85**: 186–190.

Lu, Y.J. and Adang, M.J. (1996) Distinguishing fall army worm (Lepidoptera: Noctuidae) strains using a diagnostic mitochondrial DNA marker. *Florida Entomol* **79**: 48–55.

Lu, Y., Adang, M.J., Eisenhour, D.J. and Kochert, G.D. (1992) Restriction fragment length polymorphism analysis of genetic variation in North American populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Mol Ecol* **1**: 199–208.

Lu, Y.J., Kochert, G.D., Isenhour, D.J. and Adang, M.J. (1994) Molecular characterization of a strain-specific repeated DNA sequence in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insect Mol Biol* **3**: 123–130.

Luginbill, P. (1928) The fall armyworm. *US Department Agric Techn Bull* **34**: 1–91.

McMichael, M. and Prowell, D.P. (1999) Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera: Noctuidae) host strains. *Ann Entomol Soc Am* **92**: 175–181.

Meagher, R.L. Jr and Gallo-Meagher, M. (2003) Host strains of fall armyworm (Lepidoptera: Noctuidae) in Florida. *Florida Entomol* (in press).

Nagoshi, R.N. and Meagher, R. (2003) *FR* tandem-repeat sequence in (Lepidoptera: Noctuidae) host strains. *Ann Entomol Soc Am* **96**: 329–335.

Pair, S.D., Raulston, J.R., Sparks, A.N., Westbrook, J.K. and Dounce, G.K. (1986) Fall armyworm distribution and population dynamics in the southeastern states. *Florida Entomol* **69**: 468–487.

Pashley, D.P. (1986) Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? *Ann Entomol Soc Am* **79**: 898–904.

Pashley, D.P. (1988a) Quantitative genetics, development, and physiological adaptation in host strains of fall armyworm. *Evolution* **42**: 93–102.

Pashley, D.P. (1988b) Current status of fall armyworm host strains. *Florida Entomol* **71**: 227–234.

Pashley, D.P. (1989) Host-associated differentiation in armyworms (Lepidoptera: Noctuidae): an allozymic and mitochondrial DNA perspective. In *Electrophoretic Studies on Agricultural Pests* (Loxdale, H.D. and der Hollander, J., eds), pp. 103–114. Clarendon Press, Oxford, UK.

Pashley, D.P. and Martin, J.A. (1987) Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). *Ann Ent Soc Am* **80**: 731–733.

Pashley, D.P., Hammond, A.M. and Hardy, T.N. (1992) Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera: Noctuidae). *Ann Entomol Soc Am* **85**: 400–405.

Polanco, C., Gonzalez, A.I. and Dover, G.A. (2000) Patterns of variation in the intergenic spacers of ribosomal DNA in Drosophila melanogaster support a model for genetic exchanges during X–Y pairing. *Genetics* **155**: 1221–1229.

Quisenberry, S.S. and Whitford, F. (1988) Evaluation of bermudagrass resistance to fall armyworm (Lepidoptera: Noctuidae): Influence of host strain and dietary conditioning. *J Econ Entomol* **81**: 1463–1468.

Veenstra, K.H., Pashley, D.P. and Ottea, J.A. (1995) Host-plant adaptation in fall armyworm host strains: Comparison of food consumption, utilization, and detoxication enzyme activities. *Ann Entomol Soc Am* **88**: 80–91.

Whitford, F., Quisenberry, S.S. and Moellenbeck, D.J. (1992) Nutritional response by rice and corn fall armyworm (Lepidoptera: Noctuidae) strains to dietary component substitution in artificial diets. *J Econ Entomol* **85**: 1491–1496.

Whitford, F., Quisenberry, S.S., Riley, T.J. and Lee, J.W. (1988) Oviposition preference, mating compatibility, and development of two fall armyworm strains. *Florida Entomol* **71**: 234–243.