DISTINGUISHING FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) STRAINS USING A DIAGNOSTIC MITOCHONDRIAL DNA MARKER

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), includes morphologically indistinguishable corn and rice strains. The two strains were surveyed for diagnostic restriction patterns in mitochondrial DNA (mtDNA) using 25 restriction endonucleases. Polymorphic mtDNA restriction patterns were identified for *Bst*NI, *Hin*fI and *Msp*I. The *Msp*I pattern was the most distinctive since the molecular size of each DNA fragment differed between the two strains. Analyses of laboratory and field-collected insects showed the *Msp*I mtDNA pattern to be a diagnostic marker for corn and rice strain insects. Strain identification by the *Msp*I mtDNA profile correlated exactly with nuclear DNA markers. Since no *Hae*III sites are present in fall armyworm mtDNA, a double-digest of total fall armyworm DNA using *Hae*III and *Msp*I allowed the direct detection of mtDNA restriction fragments from total DNA on a stained agarose gel. In contrast to conventional techniques utilizing mtDNA markers, this rapid and simple procedure does not require the isolation of mtDNA, or avoids the use of DNA blots and labeled mtDNA.

Key Words: Sp*odoptera*, fall armyworm, mitochondrial DNA, strain identification.

RESUMEN

El gusano trozador, *Spodoptera frugiperda* (J. E. Smith), posee cepas de maíz y arroz morfológicamente indistiguibles. Esas dos cepas fueron muestreadas para el diagnóstico mediante patrones de restricción en DNA mitocondrial (mtDNA), utilizando 25 endonucleasas de restricción. Los patrones polimórficos del mtDNA de restricción fueron identificados para BstNI, HinfI y MspI. El patrón MspI fue el más distintivo debido a que el tamaño molecular de cada fragmento de DNA difirió entre las dos cepas. Los análisis de insectos de laboratorio y colectados en el campo mostraron que el patrón del MspI mtDNA es un marcador diagnóstico para los insectos de las cepas de maíz y arroz. La identificación del perfil del MspI mtDNA se correlacionó exactamente con los marcadores nucleares de DNA. Debido a la ausencia de sitios HaeIII en el mtDNA del gusano trozador, una doble digestión del total del DNA usando HaeIII y MspI permitió la detección de los fragmentos de restricción del

mtDNA a partir del DNA total en un gel de agarosa teñido. En contraste con las técnicas convencionales que utilizan marcadores de mtDNA, este proceso rápido y simple no requiere del aislamiento del mtDNA, o evita el uso de blots de DNA y de mtDNA marcado.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a major pest on corn (*Zea mays* L.), sorghum (*Sorghum vulgare* pers.) and bermudagrass (*Cynodon dactylon* pers.) in the southeastern United States. The insect is an occasional pest on many other crops, including cotton (*Gossypium hirsutum* L.), peanut (*Arachis hypogea* L.), millet (*Pennisetum glaucum* Pers.), alfalfa (*Medicago sativa* L.), rye (*Secale cereale* L.), rice (*Oryza sativa* L.) and soybean (*Glycine max* Merr.) (Sparks 1979). Difficulties in the control of fall armyworms have been attributed to its wide range of host plants, vast geographical distribution, and rapid and long distance movement (Knipling 1980).

Pashley (1986) discovered by allozyme analyses that fall armyworm populations consist of two host strains. The corn-strain prefers corn, sorghum and cotton, while the rice-strain prefers rice and bermudagrass. Subsequent investigations of the nuclear and mitochondrial genomes using restriction fragment length polymorphism (RFLP) techniques revealed significant differences between the two strains (Pashley 1989, Lu et al. 1992). Further evidence for the genetic separation of the strains was the discovery of a unique repeated DNA sequence in the genome of rice strain insects (Lu et al. 1994). Reproductive isolation mechanisms exist between the two fall armyworm strains (Pashley et al. 1987; Pashley et al. 1992). Genetic separation and barriers to interbreeding support the species status for the fall armyworm strains (Pashley et al. 1992).

The practical impact of the sympatric strains is not clear, but several studies suggest the strain diversity complicates pest management. Quisenberry & Whitford (1988) demonstrated that bermudagrass bred for resistance to the corn strain insects was still susceptible to rice strain. As efforts are directed towards developing fall armyworm-resistant corn (Wiseman & Davis 1979, Williams et al. 1989, Wiseman & Isenhour 1988), the use of characterized fall armyworm strains may be crucial. Other biological differences between the strains, including dispersal pattern (Pashley et al. 1992) and response to pesticides (Pashley et al. 1988), may influence population monitoring studies (Barfield et al. 1980, Pair et al. 1986) and pest control strategies.

The major objective of this study was to identify restriction enzyme patterns for mtDNA that distinguish the corn and rice strain insects. Pashley (1989) reported differences in the number of restriction fragments for several restriction enzymes. We tested additional enzymes and report that *Msp*I digested mtDNA reveals a diagnostic pattern for the strains. This *Msp*I pattern is directly detected on agarose gels when a second endonuclease *Hae*III is included to digest nuclear DNA. Due to its simplicity this *Msp*I/*Hae*III co-digestion method will be useful in the identification of fall armyworm strains.

Materials and Methods

Insect Sources

Both laboratory-reared and field-collected fall armyworms were used. Sources described in Lu et al. (1992) include the corn strain (designated as C) and the rice strain

(R) colonies from Louisiana State University (a gift from S. Quisenberry); a colony (M) from the USDA-ARS laboratory at Mississippi State University (a gift from F. Davis); and colony (I) formerly maintained by D. Isenhour at the University of Georgia, Coastal Plains Experimental Station. Population P was collected from corn plants in Tifton, Georgia, in 1990. Other insect sources included a laboratory colony established with bermudagrass-collected fall armyworms (provided by R. Mcpherson, USDA Tifton, Georgia) and fall armyworms collected from 2 sorghum and 5 corn fields near Athens, Georgia in 1992.

Total DNA Isolation

Total DNA was isolated from individual fifth instar larvae as described in Lu et al. (1992). Isolated DNA samples in TE buffer (10 m*M* Tris, 1 m*M* EDTA, pH 8.0) were stored at -20°C until needed.

Mitochondrial DNA Isolation and ^{32}P-labeling

Fifteen larvae (4.2 g) were homogenized with about 20 strokes in a Dounce tissue homogenizer with 20 ml of homogenization buffer [200 m*M* mannitol, 70 m*M* sucrose, 500 m*M* Tris-HCl pH 7.5, 100 m*M* EDTA, and 0.2 mg/ml proteinase K (Sigma, St. Louis, MO) (Zehnder et al. 1992)]. The homogenate was centrifuged at 1,600 × g for 10 min to pellet the cellular debris. The supernatant was centrifuged again at 17,000 × g for 30 min to pellet mitochondria. The mitochondrial pellet was washed once by resuspending the pellet with 20 ml of homogenization buffer, centrifuged at 1,600 × g for 10 min, and the supernatant decanted and centrifuged at 40,000 × g for 15 min. The final mitochondrial pellet was resuspended in 3 ml of buffer containing 100 m*M* NaCl, 500 m*M* Tris-HCl pH 8.0, 10 m*M* EDTA pH 8.0, and then mixed with 0.375 ml of 10% sodium dodecyl sulfate (SDS). To the above mixture, 6.8 g CsCl and 0.25 ml of ethidium bromide solution (10 mg/ml) were added, and the total volume was adjusted to 7.15 ml with TE buffer. The mixture was centrifuged at 161,000 × g for 20 h. The DNA bands were visualized under UV light and the lower mtDNA band was removed. Isolated mtDNA was extracted with 1-butanol to remove ethidium bromide according to procedures described (Sambrook et al. 1989). Purified mtDNA was labeled with ^{32}P-dCTP using the random primer procedure (Feinberg & Vogelstein 1984), and labeled mtDNA was used as a probe to hybridize with restriction digested total DNA.

Restriction Digestion and Electrophoresis

Complete digestion of total DNA was carried out by using 2 units of enzyme (Boehringer-Mannheim Laboratories, Indianapolis, Ind.) per µg of DNA in the supplied buffer at 37°C for 16 h. In the case of double digestion, the same enzyme/DNA ratio was used for each enzyme. Digested DNA was electrophoretically fractionated in a 1% agarose gel (Bio-Rad, Richmond, Calif) in TAE buffer (40 m*M* Tris-acetate, 1 m*M* EDTA, pH 7.5). After electrophoresis, the agarose gel was stained with ethidium bromide and DNA visualized under UV.

Southern Blot Procedures

Following electrophoresis, DNA was transferred to nylon membranes (GeneScreen Plus, DuPont) by the method of Southern (1975). DNA was immobilized on membranes by baking at 80°C for 2 h.

Hybridizations were carried out as follows: membranes were prehybridized with sheared salmon sperm DNA (100 g/ml) in hybridization buffer consisting of 3X SSC (1X SSC=0.15 *M* NaCl, 0.015 *M* Na citrate) containing 0.1% SDS at 65°C for 6 h. Labeled mtDNA was denatured by boiling for 20 min and then added to the prehybridized filter in hybridization buffer. Hybridization was for at least 6 h at 65°C. Filters were washed once in 2X SSC, 0.1% SDS for 20 min and twice in 1X SSC, 0.1% SDS for 20 min at 65°C. Filters were air-dried and exposed to X-ray film (Kodak) at -80°C with intensifying screens.

RESULTS

We screened for polymorphic mtDNA restriction patterns between the corn and rice strains of fall armyworm by digesting total DNA from both strains with 25 restriction enzymes followed by probing with ^{32}P-labeled mtDNA. The preliminary screening data is not shown. Among the restriction enzymes tested, a single cleavage site was observed for *Eco*RV, *Hin*cII, *Pvu*II, *Sal*I and *Sca*I. The size of fall armyworm mtDNA was estimated as approximately 14.8 kb. This value is slightly smaller than the 16.3 kb size estimated by Ke & Pashley (1992). *Bam*HI, *Hae*III, *Hpa*I and *Sma*I did not digest fall armyworm mtDNA. Restriction enzymes having 2 or more cleavage sites included *Alu*I, *Bst*NI, *Dra*I, *Eco*RI, *Fok*I, *Hha*II, *Hin*fI, *Hin*dIII, *Hpa*II, *Msp*I, *Nde*I, *Nru*I, *Pst*I, *Rsa*I, *Taq*I and *Xba*I. Of the 16 restriction enzymes that generated more than two mtDNA fragments, only three enzymes (*Bst*NI, *Hinf*I and *Msp*I) produced restriction profiles that differed between the corn and rice strains (Fig. 1). The *Msp*I and *Hpa*II patterns are the same because the two enzymes share the same recognition site (designated the *Msp*I pattern). The *Bst*NI and *Hinf*I mtDNA patterns were previously described by Pashley (1989), while the *Msp*I pattern is first described in this study. The *Msp*I pattern is very distinctive between the two strains, with the corn strain pattern consisting of 4 restriction fragments of 5.4, 4.3, 3.8 and 1.3 kb, and the rice strain pattern consisting of 2 fragments of 10.4 and 4.4 kb (Fig. 1).

Further, we explored the *Msp*I mtDNA restriction pattern as a diagnostic marker for corn and rice strain insects. We determined the *Msp*I mtDNA patterns for fall armyworms from five populations identified previously as rice (R) or corn (C, I, M, and P) strain populations (Lu et al. 1992). Fifteen to 20 individual larvae were selected from each population and total DNA extracted. Fig. 2 shows a Southern blot of representative samples of *Msp*I-digested DNA probed with ^{32}P-labeled mtDNA. Each insect belonged to one of two mtDNA haplotypes; one represented by the corn strain and the other by the rice strain (Fig. 2). We also examined the mtDNA from insects of unknown strain status. Larvae were collected from corn and sorghum fields, and from a laboratory colony initiated from bermudagrass-collected insects. The analyses showed that 32 of 36 fall armyworms (over 85%) collected from the corn and sorghum fields had the corn strain *Msp*I restriction pattern (data not shown). All of the 38 insects from the bermudagrass-originated colony had the rice strain pattern (data not shown). These results are consistent with the host preference of strains as reported by Pashley et al. (1988). Corn strain fall armyworms prefer corn and sorghum, while the rice strain insects prefer rice and bermudagrass. However, the above result also showed that a small fraction of rice strain insects can be found on corn and sorghum, indicating some overlap in host usage occurs (Pashley 1989).

Total DNA preparations from the field-collected and bermudagrass colony insects were analyzed using a repeated DNA marker found only in rice strain insects (Lu et al. 1994). All the total DNA samples that reacted with the rice strain-specific marker showed the rice strain mtDNA pattern, while the remainder displayed the corn strain

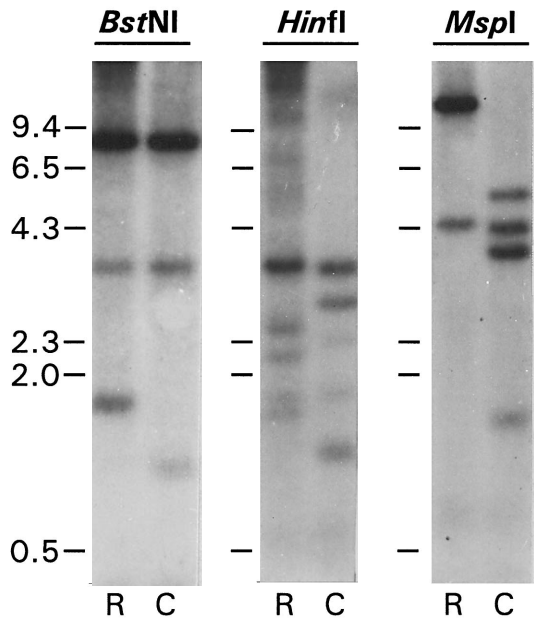

Figure 1. Polymorphic mtDNA restriction patterns between the fall armyworm corn (C) and rice (R) strain generated by *Bst*NI, *Hin*fI and *Msp*I, and detecting by probing total DNA blot with ^{32}P-labeled mtDNA. Molecular sizes (bp) are indicated at the left.

mtDNA pattern (data not shown). These results correlate the *Msp*I mtDNA pattern with known nuclear DNA markers and establish the *Msp*I mtDNA pattern as a diagnostic marker for fall armyworm host strains.

We felt that the *Msp*I strain marker would be more useful if the time consuming and expensive Southern blotting steps could be eliminated. This was accomplished using the following rationale and approach. We observed that fall armyworm mtDNA is uncut by *Hae*III, while genomic DNA is digested by *Hae*III to fragments smaller than 4 kb in molecular size (data not shown). Also, after *Msp*I digestion of total fall armyworm DNA, several mtDNA restriction fragments were partially discernible in an ethidium bromide stained agarose gel (data not shown). These observations led us to postulate that the diagnostic *Msp*I mtDNA pattern might be visible on a stained gel if the molecular size of the background genomic DNA fragments was reduced. Figure 3 depicts a stained agarose gel of fall armyworm DNA samples after treatment with *Msp*I and *Hae*III. As expected the genomic DNA fragments migrated further down the agarose gel and uncovered the mtDNA fragments. The two mtDNA fragments (10.4 and 4.4 kb) of the rice strain pattern, and 3 of the 4 fragments (5.4, 4.3 and 3.8 kb) of the corn strain pattern were easily detected. The 1.4 kb fragment in the corn strain pattern was hidden by the bulk genomic DNA fragments in the lower part of the gel. We have termed this the "3-band pattern" for the corn strain and the "2-band pattern" for the rice strain.

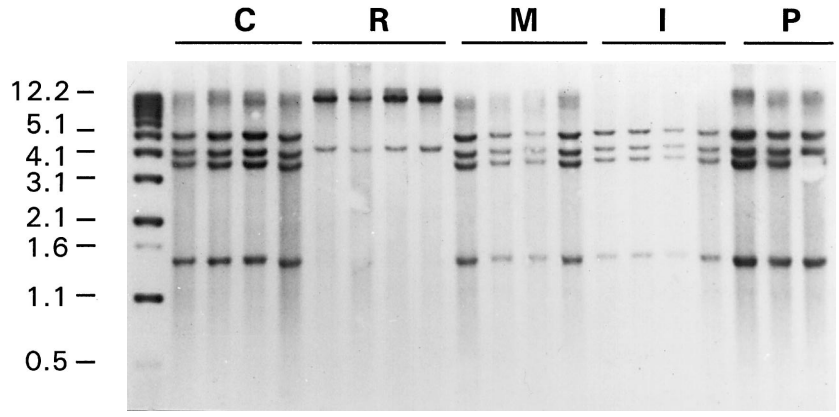

Figure 2. A Southern blot showing the *Msp*I mtDNA pattern of fall armyworms from different populations as indicated. Population R is the rice strain. Populations C, M, I and P were previously identified as corn strain populations (Lu et al. 1992).

DISCUSSION

The results of a previous study (Pashley 1989) and this study show that the corn and rice strains of fall armyworm have distinct mtDNA RFLPs for *Bst*NI, *Hinf*I and *Msp*I. Since the diagnostic *Msp*I mtDNA pattern can be detected by a simple double-

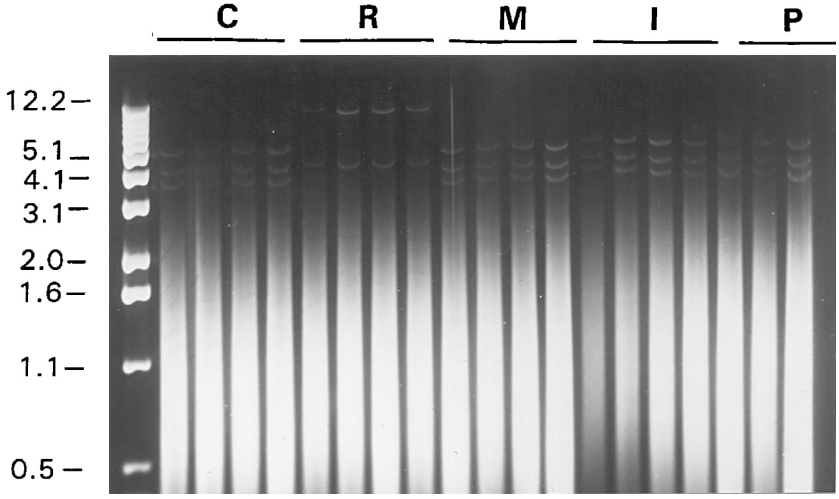

Figure 3. A stained agarose gel of *Msp*I and *Hae*III digested total fall armyworm DNA, showing the diagnostic mtDNA restriction pattern on the upper part of the gel. Fall armyworm populations and DNA samples are the same as in Fig. 2.

digest of total DNA using *Msp*I and *Hae*III, the method does not require the isolation of mtDNA, or the use of Southern blot analysis. Thus, this very straightforward *Msp*I/*Hae*III digestion technique facilitates the rapid detection of fall armyworm strains.

Since mtDNA is maternally inherited, a concern is that mtDNA markers may not correctly identify intraspecific pest populations, particularly when these populations are sympatrically distributed and interbred. In these situations, insect populations with certain mtDNA genotypes may simply represent maternal lineages, but not nuclear genotypes. To address this concern, we analyzed fall armyworms with both mtDNA and nuclear DNA markers (this study, Lu et al. 1992, Lu et al. 1994). Our results demonstrated that the two types of markers agreed completely, indicating that fall armyworm mtDNA genotypes correspond to their nuclear genotypes. These results support Pashley's conclusion that gene flow between the sympatric fall armyworm strains is very limited (Pashley 1989).

Fall armyworm strains were shown to be selective in host usage (Pashley 1986), however, it should not be assumed that all the insects collected from a given host will belong to the host strain. Pashley (1989) reported finding a small number of corn strain insects on bermudagrass and rice strain insects on corn and sorghum plants. By using the mtDNA and nuclear DNA markers, we also identified a small number of fall armyworms taken from corn and sorghum plants to be rice strain insects. Further studies are needed to gain insight into the mechanism of strain differentiation and its impact on pest management.

ACKNOWLEDGMENT

The authors are grateful to Drs. D. Isenhour, G. Kochert, and M. Bass for their support. This research was funded by a HATCH project through the University of Georgia College of Agriculture and Environmental Sciences.

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