# Molecular Identification of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) Using PCR-RFLP[1]

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**ABSTRACT** The fall armyworm, *Spodoptera frugiperda*, as with many noctuid moths, is a serious agricultural pest in the United States. Researchers often use pheromone traps to monitor for the presence of economically important noctuid pests. Pheromone traps may attract more than one species and samples often degrade, making morphological identification of some adult noctuid species nearly impossible. A molecular diagnostics protocol using polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) was developed in order to distinguish the fall armyworm from six other noctuid species commonly found in Arkansas. A 611-bp region of the mtDNA COI, COII genes was amplified using PCR and then sequenced. The restriction enzymes *Dra* I, *Alu* I and *Nla* III had specific restriction sites that distinguished the seven noctuid species. This proved to be a reliable, quick and economical technique for identifying the fall armyworm as well as six other noctuid species.

**KEY WORDS** Fall armyworm, Noctuidae, PCR-RFLP, molecular diagnostics

Noctuid moths, including the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), are well-known agricultural pests of economical importance. Fall armyworms are known to attack at least 60 varieties of crops, most notably corn, rice, peanuts, cotton, soybeans, alfalfa and forage grasses (Knipling 1980, Pashley 1986, Lu & Adang 1996). In Arkansas, estimates of cotton losses alone from fall armyworm damage were $2.7 million for insecticide treatments in 2003 and $2.6 million in cotton bale losses (Williams 2003). On the national level, the FAW ranked 8th as the most important insect pest of cotton in 2003 and it was the 3rd most important pest to cotton in Arkansas (Williams 2003). The preferred host plants of FAW came under scrutiny in 1986 when Pashley proposed that the fall armyworm consists of two morphologically undistinguishable strains, a corn strain that prefers corn, cotton and sorghum, and a rice strain that prefers rice and bermudagrass (Pashley 1986, 1988). Recent studies on *S. frugiperda* genetic variation have revealed that populations can be divided two morphologically identical, but genetically distinct strains using mtDNA cytochrome oxidase sequences (Lewter et al. 2006, Nagoshi et al. 2007).

Pheromone traps are a very easy and popular method for collecting adult male noctuid moths in areas where it is prudent to monitor for destructive insects.

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Although it is relatively easy to use pheromone traps, the insects captured in this manner can survive for several days and adult moths typically remove a large portion of their scales while fluttering around inside the metal traps. Unless a pheromone trap is emptied on a daily basis, the moths trapped inside may become difficult to identify to species. FAW and yellow striped armyworm, *S. ornithogalli* (Guenee), species are particularly difficult to distinguish, even with properly preserved specimens. A molecular diagnostics protocol may be very useful in distinguishing the fall armyworm from other noctuids commonly found in northwest Arkansas, particularly when dealing with specimens that are in poor condition.

The polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) technique offers a very affordable and accurate method for the identification of insect species, and it can be used on the insect during any developmental stage from egg to adult (Taylor & Szalanski 1999). The technique can verify proper identification of moths before subjection to costly DNA sequencing analysis. Although PCR-RFLP has already been used on the fall armyworm (Levy et al. 2002), Levy's research was focused on distinguishing between the two strains of FAW. The objective of this study was to apply PCR-RFLP to distinguish the fall armyworm from other noctuid moths.

## Materials and Methods

Adult and larval FAW, beet armyworm *S. exigua* (Hubner), yellow striped armyworm *S. ornithogalli* (Guenee), true armyworm *Mythimna unipuncta* (Halworth), corn earworm *Helicoverpa zea* (Brodie), soybean looper *Pseudoplusia includens* (Walker), bent-line dart *Choephora fungorum* (Grote and Robinson) and tobacco budworm *Heliothis virescens* (F.) were obtained from lab colonies maintained at the University of Arkansas, lab colonies at the University of Mississippi, lab colonies from the USDA-ARS CMAVE lab in Gainesville, FL and from pheromone traps operated at the University of Arkansas Agricultural Research Farm (Table 1). Larval species identification was confirmed with morphological keys of Peterson (1962), and voucher specimens have been deposited in the Arthropods Museum, University of Arkansas, Fayetteville, AR.

DNA was extracted from the thorax using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). PCR was conducted using 2 µl of extracted genomic DNA and the primers C1-J-2797 (5′- CCTCGACGTTATTCAGATTACC-3′) (Simon et al. 1994) and C2-N-3400 (5′-TCAATATCATTGATGACCAAT-3′) (Taylor et al. 1997) with the following thermalcycler profile: 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 45 s per Szalanski et al (2000). These primers amplify a 3′ portion of COI, tRNA-leu and a 5′ portion of COII. The mitochondrial DNA amplicon (PCR product) was sequenced at the University of Arkansas Medical School DNA Sequencing Facility in Little Rock, AR. Accession numbers for DNA sequences submitted to GenBank from this study are provided in Table 1, as well as accession numbers from Lewter et al. (2006).

The DNA sequence data were used to predict restriction sites with Neb Cutter 2.0 (www.tools.neb.com/NEBcutter2). The digest was performed using the protocol outlined by Cherry et al. (1997) using 3 µl of the PCR product and the restriction enzymes *Dra I*, *Alu I* and *Nla III*. An overnight digest was performed at 37°C for each of the enzymes, and the fragments were visualized using

**Table 1. Noctuid moths used in polymerase chain reaction - restriction fragment length polymorphism analysis (PCR-RFLP).**

| Species/strain | number (n) | GenBank |
|---|---|---|
| *S. frugiperda* corn 1 haplotype | 32 | AY714298 |
| *S. frugiperda* corn 2 haplotype | 2 | AY714299 |
| *S. frugiperda* corn 3 haplotype | 1 | AY714300 |
| *S. frugiperda* rice 1 haplotype | 4 | AY714301 |
| *S. frugiperda* rice 2 haplotype | 29 | AY714302 |
| *S. frugiperda* rice 3 haplotype | 1 | AY714303 |
| *S. frugiperda* rice 4 haplotype | 1 | AY714304 |
| *S. ornithogalli* | 15 | EU918931 |
| *S. exigua* | 25 | EU812749-EU812751 |
| *Mythimna unipuncta* | 5 | EU918926 |
| *Choephora fungorum* | 5 | EU918927 |
| *Helicoverpa virescens* | 5 | EU918928 |
| *Heliothis zea* | 5 | EU918929 |
| *Pseudoplusia includens* | 5 | EU918930 |

electrophoresis with a 2% agarose gel stained with ethidium bromide and a 50 base pair DNA size marker. The agarose gel was photographed in a UVP BioDoc-it system (Upland, CA).

## Results and Discussion

The mtDNA region amplified by PCR was 611 base pairs long. A total of four rice and three corn strain haplotypes were observed for *S. frugiperda* (Lewter et al. 2006). No genetic variation was observed for the other species used for the study, with the exception of *S. exigua* which had three distinct haplotypes. The *Alu* I digest distinguished 5 patterns among the seven noctuid moths (Table 2, Fig. 1). The *Dra* I digest yielded 4 different patterns (Table 3, Fig. 2), and the *Nla*

**Table 2. Restriction sites, fragments, and patterns for noctuid PCR-RFLP using *Alu* I.**

| Species / Strain | Restriction site | Fragments | Pattern |
|---|---|---|---|
| *Spodoptera frugiperda** | 182, 420 | 238, 191, 182 | A |
| *S. frugiperda* Rice 3 haplotype | 182 | 429, 182 | B |
| *S. ornithogalli* | 182 | 429, 182 | B |
| *S. exigua* | 182 | 429, 182 | B |
| *Mythimna unipuncta* | 71, 182, 496 | 314, 115, 111, 71 | C |
| *Choephora fungorum* | 71, 182, 328 | 280, 146, 111, 71 | D |
| *Helicoverpa virescens* | 71 | 540, 71 | E |
| *Heliothis zea* | 71 | 540, 71 | E |
| *Pesudoplusia includens* | 182 | 429, 182 | B |

*All FAW haplotypes from Lewter et al. (2006) except for haplotype Rice 3.

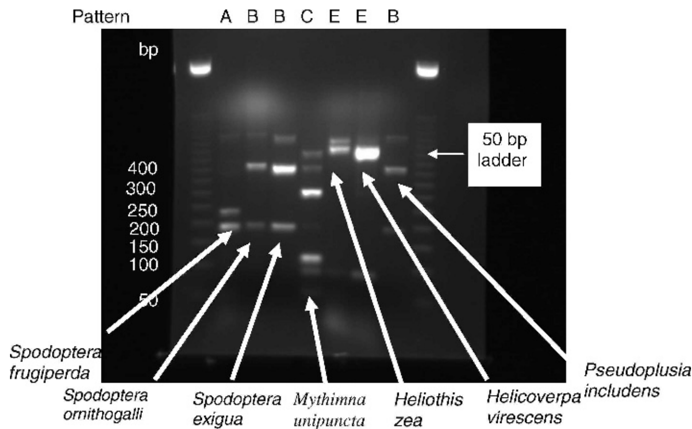


**Fig. 1.** PCR-RFLP agarose gel for fall armyworm and other noctuids using *Alu* I.

III digest yielded 3 patterns (Table 3). By combining the patterns from all 3 digests, each of the seven species of noctuids were distinguished from one another (Tables 4 and 5) as well as the two FAW strains. The rice R3 haplotype of the fall armyworm could also be distinguished.

This is the first PCR-RFLP technique to distinguish the fall armyworm from other noctuid species commonly found in Arkansas. This technique is quick, reliable and costs less than two dollars per sample. It is also particularly useful when trying to identify adults that have been caught in pheromone traps, where specimens often have a large portion of their scales missing.

One advantage to using the COI, COII mtDNA region for the PCR-RFLP technique is that the DNA fragments created by cleaving this area are relatively large and clearly separate during electrophoresis. It is not necessary to use high percentages of agarose gels in order to detect size differences in the cleaved DNA, so this could be cost-efficient when dealing with large numbers of samples (Roehrdanz 1997). The COI, COII region is also a useful PCR-RFLP marker because it is not as hypervariable as some noncoding regions are (ie, nuclear

**Table 3. Restriction sites, fragments, and patterns for noctuid PCR-RFLP using *Dra* I.**

| Species | Restriction site | Fragments | Pattern |
|---|---|---|---|
| *Spodoptera frugiperda** | 138, 252 | 359, 138, 114 | A |
| *S. ornithogalli* | 138, 252 | 359, 138, 114 | A |
| *S. exigua* | 252 | 359, 252 | B |
| *Mythimna unipuncta* | 252, 578 | 326, 252, 33 | C |
| *Choephora fungorum* | 138, 252 | 359, 138, 114 | A |
| *Helicoverpa virescens* | 252 | 359, 252 | B |
| *Heliothis zea* | 138, 252 | 359, 138, 114 | A |
| *Pseudoplusia includens* | 252, 309 | 299, 252, 57 | D |

*All *S. frugiperda* haplotypes from Lewter et al. (2006).

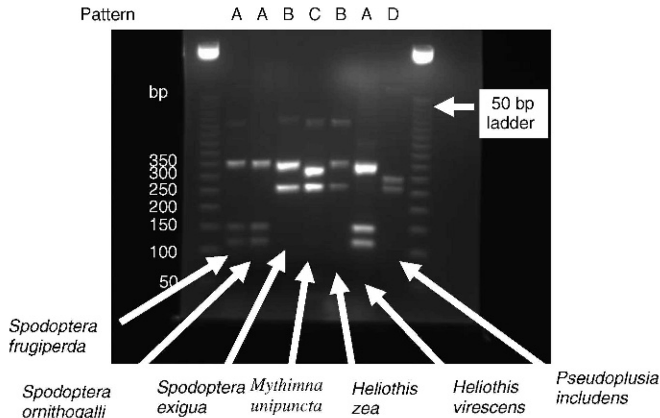


**Fig. 2.** PCR-RFLP agarose gel for fall armyworm and other noctuids using *Dra* I.

rDNA intergenic spacer region.) When intraspecific polymorphisms are at a minimum, there is a lesser likelihood that the restriction enzymes will cleave DNA at the same place in different species. In other words, the patterns designated to each species are truly species-specific.

Another major advantage of the PCR-RFLP technique is that there are no false negatives; the restriction patterns are based on DNA sequences and are specific (Roehrdanz 1997). Also, results can be obtained from PCR-RFLP within one single working day. Other researchers have published RFLP protocols for use on the fall armyworm, but they have been aimed at distinguishing between the two strains of the fall armyworm and not at distinguishing the FAW from other noctuids. The *Nla* III digest is able to identify the R3 haplotype from the other six FAW haplotypes that we have described. This technique is useful for identifying samples that will undergo DNA sequencing and it works as well at identifying the six other noctuid species as it does for the FAW.

**Table 4. Restriction sites, fragments, and patterns for noctuid PCR-RFLP using *Nla* III.**

| Species / Strain | Restriction site | Fragments | Pattern |
|---|---|---|---|
| *Spodoptera frugiperda* corn* | 31, 301, 362 | 270, 246, 61, 31 | A |
| *S. frugiperda* rice* | 31, 301 | 307, 270, 31 | B |
| *S. ornithogalli* | 31, 301, 362 | 270, 246, 61, 31 | A |
| *S. exigua* | 31, 301, 362 | 270, 246, 61, 31 | A |
| *Mythimna unipuncta* | 31, 301, 362 | 270, 246, 61, 31 | A |
| *Choephora fungorum* | 301, 362 | 307, 270, 31 | B |
| *Helicoverpa virescens* | 301, 362 | 307, 270, 31 | B |
| *Heliothis zea* | 301 | 307, 301 | C |
| *Pseudoplusia includens* | 31, 301, 362 | 270, 246, 61, 31 | A |

*All *S. frugiperda* haplotypes from Lewter et al. (2006).

**Table 5. Patterns for noctuid PCR-RFLP using *Alu* I, *Dra* I, *Nla* III.**

| Species / Strain | Pattern* |
|---|---|
| *Spodoptera frugiperda* corn 1,2,3 haplotypes | AAA |
| *S. frugiperda* rice 1,2 haplotypes | AAB |
| *S. frugiperda* rice 3 haplotype | BAB |
| *S. ornithogalli* | BAA |
| *S. exigua* | BBA |
| *Mythimna unipuncta* | CCA |
| *Choephora fungorum* | DAB |
| *Heliocoverpa virescens* | EBB |
| *Heliothis zea* | EAC |
| *Pseudoplusia includens* | BDA |

**Alu* I, *Dra* I, *Nla* III digest patterns.

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