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# GENETIC VARIATION WITHIN AND BETWEEN STRAINS OF THE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

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## ABSTRACT

Limited information exists on molecular genetic variation and distribution of the corn and rice strains of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). This study was conducted to investigate the genetic structure of *S. frugiperda* across a part of its range in the United States. A 608-base-pair portion of the mitochondrial cytochrome oxidase I and II genes was sequenced from 71 individuals resulting in three corn and four rice strain haplotypes. Genetic divergence between the two strains ranged from 0.66 to 0.99%. A 562-base-pair region of the nuclear ITS-1 gene was also amplified and sequenced from 17 individuals representing both corn and rice strains. No variation was detected in any of the samples for the ITS-1 region. Analysis of molecular variance was conducted on the resulting mtDNA haplotypes from the Arkansas and Florida populations and as a hierarchical analysis between populations in the two states. Results indicate a significant overall  $\Phi_{ST}$  for all populations with the hierarchical analysis revealing that this significant  $\Phi_{ST}$  is due to structuring of the populations between states. The observed genetic structure is possibly due to the distribution of fall armyworm strains.

Key Words: COI, COII, ITS-1, DNA sequence, genetic variation, population genetics, *Spodoptera frugiperda*

## RESUMEN

Existe información limitada sobre la variación genética molecular y distribución de las razas del cogollero, *Spodoptera frugiperda* (J.E. Smith) de maíz y de arroz. Este estudio fue realizado para investigar la estructura genética de *S. frugiperda* a través de una parte de su rango de distribución en los Estados Unidos. Una porción de los 608 pares de bases de los genes I y II del citocromo—c-oxidasa mitocondrial fueron secuenciados de 71 individuos resultando en tres haplotipos de la raza de cogollero en el maíz y cuatro haplotipos de la raza de cogollero en el arroz. La divergencia genética entre las dos razas de cogollero fue de 0.66 a 0.99%. Una región de 562 pares de bases del gene nuclear ITS-1 también fue amplificada y secuenciada de 17 individuos representando ambas razas de maíz y de arroz. Ningún variación fue detectada en las muestras para la región ITS-1. Un análisis de variancia fue realizado usando los haplotipos resultantes de ADNmt de las poblaciones de Arkansas y de Florida, al igual que un análisis de jerarquía entre las poblaciones de los dos estados. Los resultados indican una  $\Phi_{ST}$  total significativa para todas las poblaciones con el análisis de jerarquía revelando que esta  $\Phi_{ST}$  significativa es debido a la estructura de las poblaciones entre los dos estados. La estructura genética observada posiblemente es debido a la distribución de las razas de cogollero.

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a major pest on corn, sorghum, and bermudagrass in the southeastern United States (Knipling 1980; Pashley 1986; Sparks 1979). The preferred host plants of the fall armyworm came under new 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, 1988a). The range of *S. frugiperda* is known to cover most of the Western hemisphere, and the range of each strain, however, has been examined from Louisiana down

through Central America and in the Caribbean to Brazil (Pashley et al. 1985; Pashley 1986, 1988b).

Despite the possible benefits that population genetic analysis of the fall armyworm may provide towards understanding dispersal, monitoring the spread of insecticide resistance, and the implementation of area-wide control programs, relatively little research in this area has been conducted. A survey of 22 allozyme loci by Pashley et al. (1985) indicated significant heterogeneity between populations at five of 11 polymorphic loci, due in large part to the distinctness of a single Puerto Rican population collected from rice. The phylogenetic relationships between the two

strains were further examined with three of these polymorphic allozymes (Hbdh, PepF, and Est3; Pashley 1988b). The majority of the genetic studies have focused on differentiating the rice and corn strains with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), strain specific PCR, RFLP, amplified fragment length polymorphism (AFLP) and allozyme markers (Meagher & Gallo-Meagher 2003; Levy et al. 2002; Nagoshi & Meagher 2003; McMichael & Prowell 1999; Pashley et al. 1985; Lu et al. 1992; Adamczyk 1993; Lu & Adang 1996; Pashley 1989). A genetic variation study by Lu et al. (1992) involving RFLP of a random genomic library from six populations (five of which were lab colonies) from Louisiana, Mississippi, and Georgia revealed high levels of genetic variation within and among populations. However, no population genetic analysis was conducted in that study, which focused on finding diagnostic markers for the corn and rice strains.

Mitochondrial-DNA (mtDNA) analysis is generally assumed to be more powerful than allozyme analysis for revealing population structure, and has been used for numerous population genetic studies (Avise 1994). The cytochrome oxidase I (COI) and cytochrome oxidase II (COII) regions of the mtDNA genome have proved useful for measuring genetic variation in numerous insect taxa (Szalanski & Owens 2003; Austin et al. 2002; Taylor et al. 1997; Brower & Jeansonne 2004). Comparison of mtDNA variation with a nuclear genetic variation can provide insight into current versus historical gene flow in a species. For example, high levels of mtDNA variation combined with a lack of nuclear DNA variation may indicate unidirectional mating between strains.

We investigated the extent of genetic variation within and between races of fall armyworm using DNA sequences of a portion of the mitochondrial

COI and COII genes, and the nuclear rRNA first internal transcribed spacer (ITS-1) region.

MATERIALS AND METHODS

Larval fall armyworm samples were collected from sorghum and cotton in Raymond, MS and Colfax, LA, respectively (Table 1). Additional larval samples were obtained from southern Florida and Altheimer, Arkansas, and larval and pupal samples from lab colonies maintained at the University of Mississippi and the University of Florida also were obtained. Larval species identification was confirmed with morphological keys of Peterson (1962), and samples were designated as corn or rice strain based on the host from which they were collected (Table 1). Fall armyworm adults were collected with pheromone traps through summer and fall of 2001 to 2003 from three locations in Arkansas: Tillar, Foreman, and Fayetteville (Table 1). The traps at Tillar were located on the border of experimental research plots of different field crops (cotton, corn, soybean, and sorghum). The adjacent landscape was predominantly cotton with limited acreages of soybean, rice, and corn. A large commercial field of coastal bermudagrass was located within ¼ mile of the traps. The location at Foreman was on a grain farm and the predominant crops were corn, soybean, peanuts, and sorghum. Some limited areas of commercial pasture were near the sample areas. The location at Fayetteville was on an agricultural research farm located in an urban/suburban area. Diverse crops and grasslands were located nearby. Adult fall armyworm identification was confirmed by comparing DNA sequences to larval fall armyworm and other noctuid DNA sequences (unpublished data).

DNA was extracted from individual moths, larvae, and pupae with the Puregene DNA isolation

TABLE 1. SAMPLING LOCATIONS AND FREQUENCY OF FALL ARMYWORM CORN “C” AND RICE “R” HAPLOTYPES.

| Location                         | Strain* | C1 | C2 | C3 | R1 | R2 | R3 | R4 | n  |
|----------------------------------|---------|----|----|----|----|----|----|----|----|
| Fayetteville, Washington Co., AR | —       | 11 | 2  |    |    | 3  |    |    | 16 |
| Tillar, Drew Co., AR             | —       | 6  |    | 1  |    | 3  |    |    | 10 |
| Foreman, Little River Co., AR    | —       | 7  |    |    |    | 3  | 1  |    | 11 |
| Altheimer, Jefferson Co., AR     | R       |    |    |    |    | 4  |    |    | 4  |
| Starkville, Oktibbeha Co., MS    | C       | 2  |    |    |    |    |    |    | 2  |
| Raymond, Hinds Co., MS           | C       | 2  |    |    |    |    |    |    | 2  |
| Colfax, Grant Parish, LA         | C       | 2  |    |    |    |    |    |    | 2  |
| Gainesville, Alachua Co., FL     | C       | 2  |    |    |    |    |    |    | 2  |
| Ona, Hardee Co., FL              | R       |    |    |    | 4  | 3  |    |    | 7  |
| Miami-Dade Co., FL               | R       |    |    |    |    | 6  |    | 1  | 7  |
| Collier Co., FL                  | R       |    |    |    |    | 5  |    |    | 5  |
| Broward Co., FL                  | R       |    |    |    |    | 2  | 1  |    | 3  |
| n                                |         | 32 | 2  | 1  | 4  | 29 | 1  | 1  | 71 |

\*Strain designation based on host from which larvae were collected.

kit D-5000A (Gentra, Minneapolis, MN). Voucher specimens are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR. DNA vouchers, preserved on filter paper according to Owens & Szalanski (2005), are maintained at the Insect Genetics Laboratory, Department of Entomology, University of Arkansas, Fayetteville, AR.

PCR reactions were conducted with 1  $\mu$ l of the extracted DNA with New England Biolabs (Ipswich, MA) *Taq* DNA polymerase with thermopol buffer. Approximately 608 bp of a mtDNA region containing the COI, tRNA leucine, and COII genes was amplified with the primers C1-J-2797 (5'-CCTCGACGTTATTCAGATTACC-3') (Simon et al. 1994) and C2-N-3400 (5'-TCAATATCAT-TGATGACCAAT-3') (Taylor et al. 1997). The mtDNA marker was amplified with a thermal cycler profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 45 s according to Szalanski et al. (2000). A 562-bp section of the nuclear 3' portion of 18S rDNA, all of ITS-1, and the 5' portion of 5.8S were amplified with the primers rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') (Vrain et al. 1992) and rDNA 1.58S (5'-GCCAC-CTAGTGAGCCGAGCA-3') (Cherry et al. 1997) with a thermal cycler profile consisting of 40 cycles of 94°C for 45 s, 53°C for 1 min and 72°C for 1 min as described by Szalanski & Owens (2003). Amplified DNA from individual moths was purified and concentrated with minicolumns according to the manufacturer's instructions (Wizard PCRpreps, Promega). Samples were sent to The University of Arkansas Medical School DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions.

Consensus sequences were derived from both of DNA sequences from an individual with Bioedit 5.09 (Hall 1999) to verify nucleotide polymorphisms, and sequences were aligned by CLUSTAL W (Thompson et al. 1994) for both mtDNA and nDNA sequences. Mitochondrial DNA haplotypes were aligned by MacClade v4 (Sinauer Associates, Sunderland, MA). GenBank accession numbers were AY714298 to AY714304 for the different fall armyworm haplotypes. Genealogical relationships among mtDNA haplotypes were constructed with TCS (Clement et al. 2000) and the method described by Templeton et al. (1992). The distance matrix option of PAUP\* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Tests for population differentiation were conducted by AMOVA as implemented in Arlequin v. 2.0 (Schneider et al. 2000). An analog of  $F_{ST}$ ,  $\Phi_{ST}$  was calculated from the haplotypes frequencies and Tajima and Nei (1984) genetic distances (Excoffier et al. 1992). Initially, AMOVA was used to test mtDNA genetic differentiation among all Arkansas and Florida populations sampled ( $\Phi_{ST}$ ).

Subsequently, a hierarchical AMOVA was conducted in which populations were grouped into states to determine differentiation between states ( $\Phi_{CT}$ ) and among populations within states ( $\Phi_{ST}$ ). Pairwise comparisons, calculated independently for all Arkansas and Florida population pairs of  $\Phi_{ST}$  also were calculated. Permutations of the data set were used to determine statistical significance of the pairwise comparisons ( $P < 0.05$ ).

## RESULTS

Mitochondrial DNA sequencing of 71 fall armyworm samples revealed an amplicon size of 608 bp. Nucleotide positions 1 to 222 were COI, 223 to 289 tRNA-leu, and 290 to 608 COII. The average base frequencies were A = 0.36, C = 0.13, G = 0.09, and T = 0.42. Corn haplotype C1 was the most common haplotype for the corn strain and occurred in all of the sampled locations where the corn strain occurred (Table 1). The other two corn strain haplotypes were found only in Arkansas. Rice strain haplotype R2 was the most common haplotype and occurred in every location where the rice strain was found. Rice strains R1 and R4 were found only in Florida, while strain R3 was found in both Arkansas and Florida.

Nine nucleotide sites were variable among the observed three corn and four rice strain haplotypes (Table 2). Three variable nucleotide sites were located in the COI gene and the remainder were located in the COII gene. Tajima-Nei distances (Tajima & Nei 1984) among the fall armyworm haplotypes ranged from 0.164 to 0.329% for the corn strain, 0.164 to 0.329% for the rice strain, and 0.658 to 0.987% between strains. Fig. 1 shows the 95% parsimony network for the seven haplotypes (Posada & Crandall 2001). Missing haplotypes probably represent sampling gaps.

DNA sequencing of the nuclear ITS-1 region from 17 FAW samples (Table 3) revealed an amplicon size of 562 base pairs. No sequence variation was detected in any of the 17 individuals and the base frequencies were A = 0.23, C = 0.24, G = 0.26, and T = 0.27.

AMOVA detected a significant overall  $\Phi_{ST}$  (0.493,  $P < 0.001$ ) when comparing mtDNA genetic variation among populations (Table 4). The amount of variation was almost equal within versus among populations (within 50.74%, among 49.26%). Hierarchical AMOVA conducted between Arkansas and Florida populations detected a significant  $\Phi_{CT}$  (0.387,  $P < 0.005$ ) between the two states (Table 4). The comparison among groups accounted for 38.70% of the observed variation.

## DISCUSSION

This genetic investigation of the fall armyworm mtDNA revealed significant levels of genetic differentiation among populations both

TABLE 2. GENETIC VARIATION AT NINE NUCLEOTIDE SITES AMONG FALL ARMYWORM HAPLOTYPES.

| Haplotype | 55 | 76 | 79 | 361 | 367 | 403 | 421 | 511 | 529 |
|-----------|----|----|----|-----|-----|-----|-----|-----|-----|
| Corn 1    | A  | C  | A  | T   | T   | A   | C   | T   | C   |
| Corn 2    | T  | .  | .  | .   | .   | .   | .   | .   | .   |
| Corn 3    | .  | .  | C  | .   | .   | .   | .   | .   | .   |
| Rice 1    | .  | T  | .  | C   | C   | G   | .   | .   | A   |
| Rice 2    | .  | T  | .  | C   | C   | .   | .   | .   | A   |
| Rice 3    | .  | T  | .  | C   | C   | .   | T   | .   | A   |
| Rice 4    | .  | T  | .  | C   | C   | .   | .   | C   | A   |

within and between the two fall armyworm strains. This research also represents the first attempt to determine the geographical distribution of fall armyworm haplotypes from mtDNA sequence data as well as determining the extent of genetic variation within each strain. A haplotype or allele is defined by one unique form of the gene and differs from any other gene by at least one nucleotide. Haplotype diversity or gene diversity quantifies the number of haplotypes in relation to their relative frequency to each other, and haplotype diversity is described as the probability that two sequences randomly selected from a population are different (Nei 1987).

Four haplotypes were observed for the rice strain and three haplotypes were found for the corn strain, although it is likely more haplotypes may be discovered for each strain. Observed genetic variation between strains was approxi-

mately 0.66%. Estimated time of divergence between corn and rice strain is approximately 287,000 years based on a molecular clock rate of 2.3% divergence per million years (Brower 1994). Populations of nearly all species, social or otherwise, exhibit at least some degree of genetic differentiation among geographic locales (Ehrlich & Raven 1969). This observation becomes more difficult to accurately discern when dealing with a migratory species such as the fall armyworm; however, more studies such as this one could help determine the migratory paths of the insect.

One of the purposes of the research presented herein was to estimate the baseline genetic variation which occurs both within and between fall armyworm strains. As with other animal populations, additional genetic structure normally is to be expected over increasing spatial scales, where populations can show additional differentiation due to spatial habitat structure, isolation by distance, or other factors (Avice 1994). There may be temporal differences in the occurrence of the rice and corn. Temporal data, obtained by sampling the same area throughout a season and over a period of years, also may provide insight into the specific migratory patterns of the fall armyworm.

Comparing mtDNA sequences with nuclear markers can provide evidence of inter-strain mating within a species. The lack of variation in the nuclear rDNA ITS-1 region combined with previously conducted laboratory-based mating studies (Pashley & Martin 1987; Whitford et al. 1988; Nagoshi & Meagher 2003) suggests that inter-strain mating does occur in the field. However, the lack of genetic variation in the rDNA ITS-1 region

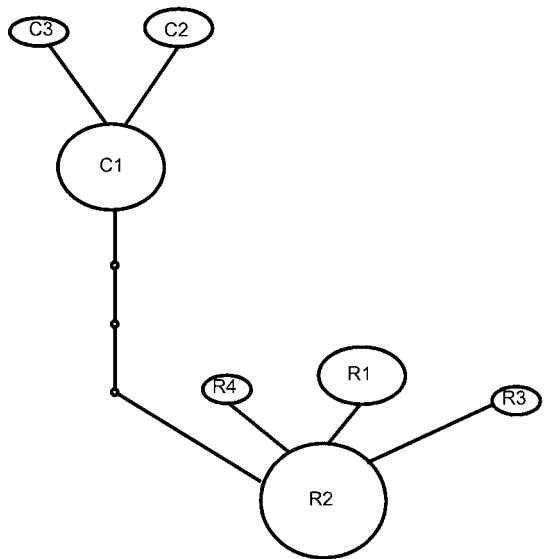


Fig 1. Genealogical relationships among 7 haplotypes of fall armyworm estimated by TCS (Clement et al. 2000). The size of the ovals corresponds to haplotype frequency, and a unit branch represents one mutation. Small ovals indicate haplotypes that were not observed.

TABLE 3. SAMPLING LOCATIONS OF FALL ARMYWORM WITH NUCLEAR rDNA ITS-1 MARKER.

| County       | State | mtDNA haplotype (n) |
|--------------|-------|---------------------|
| Washington   | AR    | C1(3), C2(1), R2(1) |
| Little River | AR    | C1(3)               |
| Drew         | AR    | C1(5)               |
| Jefferson    | AR    | R2(2)               |
| Collier      | FL    | R2(1)               |
| Broward      | FL    | R3(1)               |

TABLE 4. ESTIMATES OF GENETIC DIFFERENTIATION CALCULATED AMONG ARKANSAS AND FLORIDA POPULATIONS AS ONE HIERARCHICAL GROUP (ONE GROUP) AND WITH AN ADDITIONAL HIERARCHICAL GROUP BETWEEN STATES (TWO GROUPS). IN BOTH, THE AMOUNT OF VARIATION OCCURRING AMONG CATEGORIES AND THE ESTIMATE OF GENETIC DIFFERENTIATION IS PROVIDED. AN ASTERISK DENOTES STATISTICAL SIGNIFICANCE AT  $\alpha = 0.05$ .

| Hierarchy  | Categories         | %Variation | $\Phi$ estimate       |
|------------|--------------------|------------|-----------------------|
| One Group  | Among Populations  | 49.26      | $\Phi_{ST} = 0.493^*$ |
|            | Within Populations | 50.74      |                       |
| Two Groups | Among Groups       | 38.70      | $\Phi_{CT} = 0.387^*$ |
|            | Among Populations  | 19.45      | $\Phi_{ST} = 0.582^*$ |
|            | Within Groups      |            |                       |
|            | Within Populations | 41.85      |                       |

must be approached with caution, because this marker has no power to detect gene flow and this invariant region may be ancestral to strain subdivision. Prowell et al. (2004) also reported a lack of variation in the ITS-1 region of the fall armyworm, but it was cited as unpublished data.

Based on this study, there appears to be sufficient genetic variation both within and between populations to substantiate a more comprehensive population genetics study on this species, and we would recommend also that temporal data be taken into consideration.

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