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GENETIC VARIATION OF THE SOUTHERN CORN ROOTWORM, (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Corn rootworms of the genus *Diabrotica* (Coleoptera: Chrysomelidae) are among the most important insect pest of crops in the United States. The southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber is an economically important pest of corn, cucurbits and peanuts. Genetic analysis of southern corn rootworms, collected from South Dakota, Nebraska, and Arkansas was undertaken using DNA sequences of the nuclear ribosomal first internal transcribed spacer region (ITS1), and a portion of the mitochondrial DNA (mtDNA) cytochrome oxidase I and II genes. Among the 22 beetles subjected to DNA sequencing analysis, no polymorphic nucleotide sites were observed for the ITS1 marker and one variable nucleotide site was observed for the mtDNA marker. The lack of genetic distinction observed in southern corn rootworm populations suggests either high levels of dispersal or a recent geographical expansion from a relatively small base.

Key Words: Southern corn rootworm, population genetics, mitochondrial DNA, ribosomal DNA

RESUMEN

Los gusanos de la raíz del maíz del género *Diabrotica* (Coleoptera: Chrysomelidae) están entre las plagas insectiles más importantes en los cultivos en los Estados Unidos. El gusano sureño de la raíz del maíz, *Diabrotica undecimpunctata howardi* Barber es una plaga del maíz, los cucurbitos y de maní económicamente importante. Se llevó a cabo un análisis genético de los gusanos sureños de la raíz del maíz, recolectados en los Estados de Dakota del Sur, Nebraska, y Arkansas usando secuencias del ADN de la primera región espaciadora (spacer region) interna tránscrita del ribosoma nuclear (ITS1), y una porción de los genes del citócromo oxidase I y II del ADN mitocondrial (mtADN). Entre los 22 escarabajos sujetados al análisis de secuencia del AND, no se obervaron sitios de nucleótidos polimórficos para el marcador ITS1 y se observó un sitio variable de nucleótido para el marcador de mtADN. La falta de la distinción genética observada en las poblaciones del gusano de la raíz del maíz, sugíere un nivel alto de dispersión o una recién expansión geográfica de una base relatívamente pequeña.

Corn rootworms, *Diabrotica* spp., are part of the large group of chysomelid beetles, many of which attack agricultural crops (Levine & Oloumi-Sadeghi 1991). Southern corn rootworm (SCR) *Diabrotica undecimpunctata howardi* Barber, or as adults known as spotted cucumber beetle, is widely distributed in North America, occurring in most areas east of the Rocky Mountains, in southern Canada, and in Mexico. It is most abundant and destructive in the southern United States. This insect is multivoltine and overwinters as adults in the southern parts of its range (Branson & Krysan 1981). Southern corn rootworms infest the roots of many grass crops and weeds, as well as those of peanuts, alfalfa, and occasionally cucurbits. They are most damaging to corn and peanuts. Annually 20 to 25 million acres of corn are treated with soil insecticides to protect the crop from corn rootworm larval feeding damage (Fuller et al. 1997). Soil insecticides applied for the corn rootworm represent one of the major uses of insecticide in the United States. Costs associated with insecticides applied to control larval damage to corn roots and adult damage to corn silks, along with crop losses can approach $1 billion annually (Metcalf 1986).

Despite the possible benefits that molecular genetic analysis of SCR may provide towards diagnostics, dispersal, insecticide resistance, and the implementation of area wide control programs, very little research in this area has been conducted. Previous population genetic analysis of SCR is limited to a single study by Krafsur (1999) on allozyme diversity in leaf beetles. An assessment of 56 southern corn rootworms collected from Iowa corn fields using allozymes revealed an average heterozygosity of 11.3% among the 39 loci studied. Although this study did find genetic variation in SCR, the study was directed towards detecting genetic variation in the species and not studying the extent of variation within and among populations.

The goal of our study was to survey population diversity and to determine if molecular marker systems can be used for identifying strains and emerging phenotypes that might affect area wide

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control programs. We surveyed two molecular markers, the first internal transcribed spacer region (ITS1), located between the repeating array of nuclear 18S and 5.8S ribosomal genes, and portions of the mitochondrial DNA cytochrome oxidase I and II genes. These markers have been successfully used for monitoring population genetics of western corn rootworm, *D. virgifera virgifera* LeConte (Szalanski et al. 1999) and northern corn rootworm, *D. barberi* Smith and Lawrence (Roehrdanz et al. 2003) and for differentiating *Diabrotica* species (Szalanski & Powers 1996; Szalanski et al. 2000).

Materials and Methods

Southern corn rootworm adults were obtained from the field locations as listed in Table 1. Adult rootworms were preserved by immersion in 95% alcohol in the field or transported live to the laboratory and frozen at -80°C. Voucher specimens are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

Alcohol preserved specimens were allowed to dry on filter paper, and DNA was extracted from individual thoraces using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 µl of Tris: EDTA and stored at -20°C. Polymerase chain reaction for the nuclear DNA marker was conducted using the primers rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') described by Vrain et al. (1992), and primer rDNA1.58s (5'-GCCACCTAGTGAGCCGAGCA-3') by Cherry et al. (1997). These primers amplify a 3' portion of the 18S gene, the entire ITS1 region and a 5' section of the 5.8S gene. The 3' portion of the mtDNA cytochrome oxidase (CO) I gene, tRNA leucine, and a 5' portion of the CO II gene was amplified using the primers C1-J-2797 (5'-CCTCGACGTTATTCAGATTACC-3') (Simon et al. 1994) and c2-N-3400 (5'-TCAATATCATTGATGACCAAT-3') (Taylor et al. 1997). PCR reactions were conducted using 1 µl of the extracted DNA Szalanski et al. (1997). The ITS1 PCR protocol was 40 cycles of 94°C for 45 s, 54°C for 45 s and 72°C for 60 s. The mtDNA PCR protocol consisted of 40 cycles of 94°C for 45 s, 42°C for 45 s, and 72°C for 60 s. Amplified DNA from individual beetles was purified, and concentrated using Microcon-PCR Filter Units (Millipore, Bedford, MA). Samples were sent to the University of Arkansas DNA Resource Center (Fayetteville, AR) for direct sequencing in both directions using an ABI Prism 377 DNA sequencer. Consensus sequences were obtained from the sequences using GCG Wisconsin Package software (Accelrys, San Diego, CA). GenBank accession numbers for the beetles subjected to DNA sequencing in this study are AY191606 to AY191608. The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model (Kimura 1980) of sequence evolution.

Table 1. Sampling locations and dates for collections of Southern corn rootworm.

City	State	n	Year
Dannebrog	NE	5	2001
Lincoln	NE	4	1999
North Platte	NE	1	1996
Brookings	SD	3	1996
Fayetteville	AR	5	2002
Kaiser	AR	4	2002

Results and Discussion

DNA sequencing of the ITS1 PCR-amplified product revealed a 762 bp amplicon for all of the SCR subjected to DNA sequencing (Fig. 1). The average base frequencies were A = 0.37, C = 0.14, G = 0.10, and T = 0.39. Of the 21 SCR individuals sequenced (Table 1), no ITS1 sequences were variable. For the mtDNA COI/COII amplicon, a total of 580 bp were sequenced. The average base frequencies were A = 0.30, C = 0.17, G = 0.20, and T = 0.33. One nucleotide site, 304, was variable among the 21 southern corn rootworm DNA sequences (Fig. 2). One of the SCR sampled from Brookings, SD had a C at this site, while the remaining DNA sequences had a T at this site. To verify this nucleotide polymorphism, the Brookings, SD sample was resequenced in both directions, and all four individual sequences had the polymorphism at site 304. Based on a study by Kobayashi et al. (1999) on PCR error, almost all of the *Epilachna* ladybird beetle mtDNA COI PCR errors, 19 out of 20, were transitions, while the substitution observed in SCR is a transition. In addition, based on the PCR error rate of 7.3×10^{-5} substitutions per site per cycle from Kobayashi et al. (1999), we calculate that the chance of a PCR error at site 304 for two independent PCR amplications is 8.5×10^{-6}. Based on this, we believe that this nucleotide difference represents an actual polymorphism in the population.

The low level of molecular genetic differentiation within and among SCR populations has been observed in western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and Mexican corn rootworm, *D. v. zeae* Krysan and Smith (Szalanski et al. 1999). No ITS or mtDNA markers were found that were diagnostic for the subspecies despite the fact that there are obvious color differences. What little genetic polymorphism that was detected had no geographical component. Low levels of genetic diversity in the ITS1 and mtDNA regions among widely dispersed populations has been reported for other insect species besides

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1    TTGATTACGT CCCTGCCCTT TGTACACACC GCCCGTCGCT ACTACCGTAT

51   TGAATGATTT ACTGAGGTTC TTCGGACTGA GCGCGGTGGC GTTTCGGCGT

101  CGTCGATGTT TCGGAAAGAT GACCAAACTT GATCATTTAG AGGAAGTAAA

151  AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT CATTACAGTG

201  TTGTCAATAA ACGACAAGTC TGTATTAATT ATTTCGAACT GTTAAAATAA

251  CTGACGTGTG TCTTATCTTC GTATACTGCA AGGAAGAAAA ATTAATAGTA

301  ATTAATTATG CTTTATTGTG GAAAATACGA AAAAATAGAG ACACATACAC

351  GTGTATTGTT ATTATTATTG TGATCGTGTA GATGCAAAAC GTTTTAATGT

401  AATAAATATC TCGATAATAT ATCGGGTACC TAGAGAACGA AGTCTTTCGG

451  GATTAGTTCT TCGATGGTGA TAAAGATTTT CGCCCGATTA TCAAGAGATG

501  TTACACTGAA ACGTAATTTC TTGATCGCAT TAAGAGAGGA AATTCGTGTA

551  CCGTTTTTTT GCACGCCTCG TTTGTTTAAC ATATGTGCTG TGCTTAAAAA

601  AAAAACGTAA TAAACGATTT CTTTTCTGAT TCGTATAAAT ATACTCGCCA

651  TTTTCTATAA ATGACGTTGT GTTTCGTACT TAAATAAACT TTTGGGTTAT

701  AATTTTCGAA CGGGACGCGT CTAATTAAAC GATTACCCTG AACGGTGGAT

751  CACTCGGCTC GT
```

Fig. 1. Nucleotide sequence of the amplified ITS1 region of SCR including portions of the 18S gene (nucleotides 1-177) and 5.8S gene (716-762). Ribosomal gene sequences are in italics, and primer recognition sequences are underlined.

SCR, WCR, and MCR including European corn borer, *Ostrinia nubialis* (Hübner) (Marcon et al. 1998), stable fly, *Stomoxys calcitrans* L. (Szalanski et al. 1996), and alfalfa weevil, *Hypera postica* (Gyllenhal) (Erney et al. 1996). These species have high mobility or recent expansion from a genetic bottleneck in common. These factors may contribute to the lack of genetic variation among SCR populations.

This is not the case with northern corn rootworm, *Diabrotica barberi* Smith and Lawrence (Roehrdanz et al. 2003). ITS1 DNA sequencing and PCR-RFLP analysis of ITS1 and mtDNA revealed genetic variation within and genetic differentiation among northern corn rootworm populations. With both ITS1 and mtDNA markers there appeared to be a phylogeographic pattern of genotype frequencies. They observed that populations east of Illinois were homogeneous for one ITS1 genotype, two ITS1 genotypes were observed in Illinois, Wisconsin and North Dakota, while South Dakota, Nebraska, Iowa, and Kansas populations were composed of all three ITS1 genotypes. The mtDNA had 58 haplotypes that displayed a strong east-west geographical partition. The region of overlap occurred in a few counties of east-central Illinois. Possible reasons for this population structure include expansion from different glacial relicts, historic host plant differences, and *Wolbachia* driven reproductive incompatibilities (Roehrdanz et al. 2003). The lack of genetic distinction observed in southern corn rootworm

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1    CCTCGACGTT ATTCAGATTA CCCCGATATT TTCATATTAT GAAATATTGT

51   ATCTTCTATT GGATCTCTAA TTTCATTAAT TAGAGTAATC TTCTTAATTT

101  ATATTTTTTG AGAAGCATTA TCTATAAAAC GTAAAAGATT AAGACCATTA

151  AGATTAACAT CATCAATTGA ATGATTACAA TTTAATCCAC CTGCTGAACA

201  TAGATATTCT GAATTACCAA TACTATCTTC AAATTTCTAA TATGGCAGAT

251  TAGTGCACTG GATTTAAACC CCAAATATAA AGTTTAAACT TTTTTTAGAA

301  ATTTCAACTT GAAAAAATTT CATATTACAA GATAGATCCT CTCCACTAAT

351  AGAACAATTA TCTTACTTTC ATGACCATGC ATTAATAATT CTAGTAATTA

401  TTACAGTATT AGTTGGTCAA TTAATATTTT TTTTATTTTT TAATAAATTT

451  TTACATCGAA ATTTACTTGA AGGACAATTA ATTGAAATTA TTTGAACTAT

501  CCTCCCTACA ATTACATTAA TTTTCATTGC AATTCCTTCA TTACGTTTAA

551  TTTATATTTT AGATGAAGTT AATAACCCAT CTATTACTAT TAAAACTATT

601  GGTCATCAAT GATATTGA
```

Fig. 2. Nucleotide sequence of the amplified mtDNA COI/COII region of SCR including portions of the COI gene (nucleotides 1-240), tRNA-leu (245-318), and COII gene (322-618). The polymorphic nucleotide site 342 is in bold, and primer recognition sequences are underlined.

populations suggests either high levels of dispersal or a recent geographical expansion from a relatively small base.

The lack of genetic polymorphism in the ribosomal spacer and mitochondrial COI/COII regions diminishes the prospect of using these tools to quickly find molecular markers to track insecticide resistance, or other behavioral modifications in SCR. This is especially relevant given the impending release of genetically modified corn with the *Bacillus thuringiensis* var. *tenebrionis* gene which encodes for an endotoxin specific for Coleoptera, and is targeted towards corn rootworms. Techniques detecting higher levels of polymorphism such as AFLP or microsatellites may reveal markers more suitable for population level analyses of gene flow in SCR. Assays for genes or gene products directly involved may also prove useful, especially for resistance management.

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