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Genetic similarity among pheromone and voltinism races of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae)

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Abstract

The genetic variability of seven European corn borer populations, *Ostrinia nubilalis*, from North America and Europe was assessed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis and DNA sequencing. The nuclear ribosomal internal transcribed spacer 1 (ITS-1) region (\approx 500 base pair [bp]) and four mitochondrial (mtDNA) regions (1550 bp total) were examined. The smartweed borer, *Ostrinia obumbratalis*, and south-Western corn borer, *Diatraea grandiosella*, were used for comparisons. Of 106 restriction sites identified (80 in mtDNA and 26 in ITS-1), none differentiated geographical populations, pheromone races, or voltine ecotypes of the European corn borer. The lack of variation in the ITS-1 of European corn borer was confirmed by DNA sequence analysis. The genetic similarity of European corn borer populations, despite their wide geographical range and physiological differences, may be explained by a relatively recent origin for the voltinism and pheromone races, gene flow among races, and/or expansion from genetic bottlenecks.

Keywords: internal transcribed spacer, mitochondrial DNA, *Ostrinia nubilalis*, pheromone races, voltinism ecotypes.

Introduction

The European corn borer, *Ostrinia nubilalis* (Hübner), is of agricultural significance in much of the Northern hemisphere, including Europe, Asia, Northern Africa, North America, the Philippines, Guam and Japan (Beck, 1987). It affects the production of maize, as well as other crops including sorghum, cotton, potatoes and many vegetables. Overall, yield losses and control expenditures associated with the European corn borer are estimated to exceed US\$1bn annually (Mason *et al.*, 1996). The European corn borer is native to Southern Europe (Beck, 1987) and is believed to have been introduced into North America between 1909 and 1914, probably on broom corn imported from Hungary or Italy (Vinal, 1917). However, based on differences in voltinism and sex pheromone composition among European corn borer ecotypes and races, it is likely that multiple introductions occurred (Showers, 1993). In the 80 years since its introduction, the European corn borer has spread rapidly northward into Canada, westward to the Rocky Mountains, and southward to Florida and Mexico (Mason *et al.*, 1996).

Two pheromone races of the European corn borer have been identified in North America. Both use 11-tetradecenyl acetate isomers (*E* and *Z*) as sex pheromones; females of the *Z* strain produce a blend with an *E*:*Z* ratio of 3:97, whereas the ratio in females of the *E* strain ranges from 97:3 (DuRant *et al.*, 1995) to 99:1 (Roelofs *et al.*, 1987). Liebherr & Roelofs (1975) demonstrated the selective mating of European corn borer pheromone races, based not only upon their isomeric blends, but also on mating periodicity. Nevertheless, hybrid moths have been produced in the laboratory and are found in nature in areas where the races occur sympatrically (Roelofs *et al.*, 1987). The *Z* race predominates over most of the range in Europe and North America, whereas the *E* strain is found in Switzerland, Italy and Eastern North America, from Massachusetts

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Table 1. European corn borer populations studied.

| Collection | Voltine ecotype | Pheromone race | Pheromone gland analysis ^a | | | Collection | | |
|-------------------------------|-------------------------------|----------------|---------------------------------------|-----|--------------------|------------|------------|----------------|
| | | | Generation tested | N | Frequency Z allele | Stage | Host plant | Date collected |
| Burleigh Co., ND ^d | Univoltine | Z ^b | — | — | — | Larva | Corn | Jul 1995 |
| Hooper, NE | Biovoltine ^c | Z ^b | — | — | — | Adult | Corn | Jun 1995 |
| Selingsgrove, PA ^e | Uni- & Bivoltine ^c | Z and E | F ₂ | 20 | 0.90 | Egg | Corn | Jun 1995 |
| Bouckville, NY ^f | Univoltine | Z | F ₂ and F ₄ | 100 | 1.00 | Larva | Corn | Feb 1989 |
| Geneva, NY ^f | Bivoltine | E | F ₂ and F ₄ | 100 | 0.00 | Larva | Corn | Dec 1988 |
| Plymouth, NC ^g | Multivoltine ^c | E and Z | F ₃ | 68 | 0.18 | Larva | Potato | Jun 1995 |
| Lombardia, Italy ^h | Bivoltine ^c | E and Z | F ₃ | 27 | 0.44 | Larva | Corn | Jul 1995 |

^a Female pheromone gland analyses for PA, NC and Europe by CEM; pheromone gland analyses for NY colonies by W. Roelofs (Roelofs *et al.*, 1985).

^b Pheromone race based on geographical origin (Mason *et al.*, 1996).

^c Voltine ecotype based on geographical origin (Mason *et al.*, 1996).

^d Collected by M. Weiss (North Dakota State University). Diapausing larvae maintained in dark at 10 °C for 120 days and then 30 °C, 16:8 (L:D) photophase, with 1% agar solution for moisture, to break diapause.

^e Collected by S. Inch (Selingsgrove, Pennsylvania).

^f Laboratory colonies from C. Linn (NY State Ag. Exptl Sta., Cornell University). Colony started from 10 mated pairs and maintained on artificial diet (Roelofs *et al.*, 1985).

^g Collected by J. VanDyann (North Carolina State University).

^h Collected by K. Steffey (University of Illinois).

to South Carolina (Klun & Huettel, 1988; Mason *et al.*, 1996).

Showers (1993) recognized three European corn borer voltine ecotypes in the USA; northern univoltine, central bivoltine and southern multivoltine. The E pheromone race has bi- and multivoltine ecotypes, whereas the Z race occurs as uni-, bi- and multivoltine ecotypes (Glover *et al.*, 1991). Differences in pheromone blend discrimination between bivoltine Z and univoltine Z males have also been reported (Glover *et al.*, 1987). In New York state, three distinct races occur sympatrically: bivoltine Z, univoltine Z and bivoltine E (Roelofs *et al.*, 1985).

Despite the world-wide economic importance of the European corn borer, the population genetics and population structure of this insect species remain largely unknown. The detection and characterization of genetic differentiation among populations is particularly relevant to an understanding of the evolution of a species and to improving pest management practices (Baverstock & Moritz, 1990). The ability to distinguish amongst geographically distinct populations of European corn borer would allow us to estimate intraspecific levels of gene flow, a critical piece of information for any appropriate management of the development of resistance to insecticides and insecticidal crops (Roush & Daly, 1990; Tabashnik, 1991; Caprio & Tabashnik, 1992). Data on the baseline susceptibility of the European corn borer to *Bacillus thuringiensis* (Bt) across the species range in the USA (Marçon, 1997) show no distinct differences among pheromone races, voltinism types, or geographical location. These data suggest little genetic differentiation among populations, at least in terms of susceptibility to Bt. The

limited number of allozyme polymorphisms reported for the European corn borer suggest that genetic differentiation among races and ecotypes is limited (Harrison & Vawter, 1977; Cardé *et al.*, 1978; Cianchi *et al.*, 1980; Glover *et al.*, 1990).

The purpose of this study was to evaluate levels of genetic divergence among European corn borer races, ecotypes and geographical populations using mitochondrial and nuclear ribosomal ITS regions. Two other crambid species, the smartweed borer, *Ostrinia obumbratalis* (Lederer) and the south-western corn borer, *Diatraea grandiosella* Dyar, were included for comparisons of intra- and interspecific variation.

Results

Pheromone analysis

The pheromone composition of European corn borer populations with relevant information regarding sample collections is presented in Table 1.

Amplicons

mtDNA amplicons I, II, III and IV (Table 2) were estimated to be ≈ 333, 347, 261 and 624 bp in size, respectively, for *O. nubilalis* as well as for *O. obumbratalis* and *D. grandiosella*. These values are consistent with those predicted by the *D. yakuba* mtDNA map (Clary & Wolstenholme, 1985). The rDNA amplicon V varied in length among the three species, with estimated sizes of 507 bp for the European corn borer, 521 bp for the smartweed borer and 624 bp for the south-western corn borer.

Table 2. Polymerase chain reaction primers.

| Amplicon | Forward primer ^a | Sequence 5'-3' | Reverse primer ^a | Sequence 5'-3' |
|--------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| I ^b | N1-J-12585 | GGTCCCTTACGAATTTGAATATATCCT | LR-N-12854 | GAGTTCAAACCGGCGTAAGCCAGGT |
| II ^b | CB-J-11545 | ACATGAATTGGAGCTCGACCACT | N1-N-11841 | GGTACATTACCTCGGTTTCGTTATGAT |
| III ^c | N4-J-8502 | GTAGGAGGAGCTGCTATATTAG | N4-N-8718 | GCTTATTCATCGGTTGCTCA |
| IV ^d | C1-J-2792 | ATACCTCGACGTTATTGAGA | C2-N-3380 | TCAATATCATTGATGACCAAT |
| ITS-1 ^e | rDNA ₂ | TTGATTACGTCCTGCCCTTT | rDNA _{1,58S} | ACGAGCCGAGTGATCCACCG |

^a Mitochondrial primer names follow nomenclature of Simon *et al.* (1994).

^b Pruess *et al.* (1992); 30 cycles: 95 °C–30 s, 50 °C–30 s, 70 °C–1 min.

^c Pruess *et al.* (1992); 30 cycles: 95 °C–30 s, 45 °C–30 s, 70 °C–1 min.

^d Bogdanowicz *et al.* (1993) (C1-J-2792), Erney *et al.* (1996) (C2-N-3380); 30 cycles: 94 °C–1 min, 50 °C–1 min, 72 °C–2 min.

^e Vrain *et al.* (1992) (rDNA₂), Cherry *et al.* (1997) (rDNA_{1,58S}); 35 cycles: 95 °C–45 s, 52 °C–1 min, 72 °C–2 min.

Restriction fragment length patterns

BanII, *BsrI* and *MspI* did not cut any of the amplicons. Restriction fragment patterns of the four mitochondrial amplicons revealed a total of fifty-three restriction sites in European corn borer and eighty scoreable restric-

tion sites among the three species, eighteen in amplicon I, twenty-four in amplicon II, seventeen in amplicon III and twenty-one in amplicon IV. Twenty-six restriction sites were identified in the ITS-1 amplicon V (Table 3). Mitochondrial and ITS-1 restriction fragment pat-

Table 3. Restriction fragment lengths (bp) estimated on 2.5% MetaPhor agarose gels for European corn borer (ECB), smartweed borer (SWB), and south-Western corn borer (SWCB).

| R.E. | ECB | SWB | SWCB |
|---------------------------|---|---|--------------------------|
| Amplicon I | (333 bp) | (333 bp) | (333 bp) |
| <i>AluI</i> | 350 | 202, 130 | 350 |
| <i>ApoI</i> | 202, 93, 18 | 221, 93 | 249, 93 |
| <i>Asel</i> | 118, 110 ^a | 226, 110 | 273, 72 |
| <i>DraI</i> | 208, 41 ^b | 208, 49, 41 ^a | 231, 82, 41 |
| <i>MseI</i> | 103, 55, 40, 30, 17 | 103, 55, 40 ^a , 17 | 110, 84, 55, 48 |
| Amplicon II | (347 bp) | (347 bp) | (347 bp) |
| <i>AluI</i> | 167, 128, 45 | 155, 128, 45, 7 | 192, 142 |
| <i>ApoI</i> | 154, 110, 57, 23, 18 | 268, 57, 18 | 154, 110, 99 |
| <i>Asel</i> | 122, 94, 63, 37, 33, 8 | 92, 63, 52, 45, 33 ^a , 21, 8 | 122, 92, 65, 33, 30, 14 |
| <i>DraI</i> | 290, 73 | 268, 73 | 247, 73, 22 ^a |
| <i>MseI</i> ^c | 59, 39, 26 | 39, 26 | 91, 39, 26, 15 |
| <i>SspI</i> | 181, 83, 59, 17 | 181, 83, 46, 17 | 350 |
| Amplicon III | (261 bp) | (261 bp) | (261 bp) |
| <i>AluI</i> | 138, 121 | 138, 121 | 255 |
| <i>ApoI</i> | 194, 75 | 194, 75 | 262 |
| <i>Asel</i> | 141, 88, 46 | 141, 88, 46 | 141, 70, 46, 14 |
| <i>DraI</i> | 154, 112 | 154, 112 | 262 |
| <i>MseI</i> | 89, 66, 54, 15, 10 | 89, 66, 54, 15, 10 | 89, 54, 35, 32, 25, 10 |
| <i>SspI</i> | 130 ^a | 140, 130 | 140, 62, 35, 29 |
| Amplicon IV | (624 bp) | (624 bp) | (624 bp) |
| <i>AluI</i> | 551, 114 | 551, 352, 174, 114 | 385, 198 |
| <i>ApoI</i> | 396, 156, 78 | 396, 156, 78 | 203, 187, 156, 78 |
| <i>AseI</i> ^c | 225, 98, 72, 64, 34, 26 | 225, 142, 126, 98, 64 | 362, 101, 89, 68 |
| <i>DraI</i> | 357, 268 | 357, 330, 268 | 330, 268 |
| <i>MseI</i> ^c | 98, 84, 71, 51, 46, 24 | 101, 90, 73, 50 | 98, 88, 73, 58 |
| <i>SspI</i> | 592, 55 | 537, 37 | 338, 199, 96 |
| Amplicon V | (507 bp) | (521 bp) | (624 bp) |
| <i>ApoI</i> | 397, 116 | 397, 81, 45 | 447, 116, 65 |
| <i>BfaI</i> | 517 | 298, 228 | 410, 210 |
| <i>DraI</i> | 507 | 541 | 491, 171 |
| <i>DpnII</i> ^c | 131 ^a , 64 ^a , 56, 37, 27 | 131 ^a , 64 ^a | 433, 131, 62 |
| <i>HaeIII</i> | 422, 99 | 433, 99 | 531, 99 |
| <i>HinfI</i> | 504 | 531 | 412, 219 |
| <i>MseI</i> | 212, 192, 104 | 261, 192, 35, 15, 14 | 235, 192, 163 |
| <i>RsaI</i> | 173, 138, 122, 35, 16 | 304, 173 | 350, 254 |
| <i>SspI</i> | 481 | 506 | 562, 78 |
| <i>TaqI</i> | 281, 209, 30 | 323, 209 | 323, 146, 80, 59 |

Total length of amplicon indicated parenthetically.

^a Interpreted as double band.

^b Interpreted as triple band.

^c Pattern difficult to interpret due to the large number of fragments generated and/or loss of small fragments.

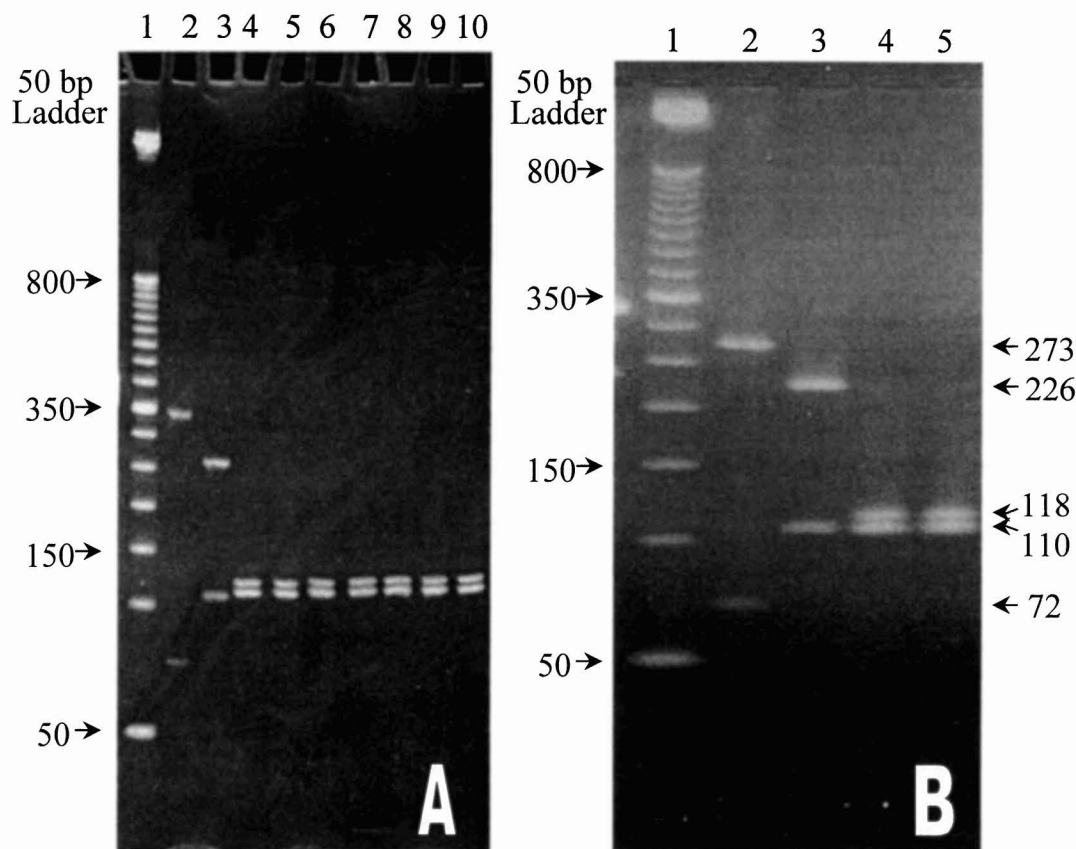


Figure 1. *Asel* digestion of mitochondrial amplicon I. (A) PAGE (10%); Lane 1, molecular standard (50 bp ladder); lane 2, *D. grandiosella*; lane 3, *O. obumbratalis*; lanes 4–10, *Ostrinia nubilalis* (lane 4, Italy; lane 5, North Carolina; lane 6, New York bivoltine E race; lane 7, New York univoltine Z race; lane 8, Pennsylvania; lane 9, North Dakota; lane 10, Nebraska). (B) MetaPhor agarose (2.5%). Lane 1, molecular standard (50 bp ladder); lane 2, *D. grandiosella*; lane 3, *O. obumbratalis*; lane 4–5, *Ostrinia nubilalis* (lane 4, Italy; lane 5, Nebraska).

terns were identical for all European corn borer samples (Figs 1 and 2). The estimated number of nucleotide substitutions per nucleotide, d , calculated from mitochondrial DNA restriction site data (Nei & Tajima, 1981) was 0.043 between the European corn borer and smartweed borer, 0.112 between the European corn borer and south-western corn borer, and 0.106 between the smartweed borer and the south-western corn borer. Estimates of d from ITS-1 restriction site data were 0.122 between the European corn borer and smartweed borer, 0.160 between European corn borer and south-western corn borer, and 0.158 between smartweed borer and south-western corn borer.

DNA sequence analysis

ITS-1 sequences were obtained from four European corn borers, two from Nebraska and two from Italy, and two smartweed borers. Sequence data indicate that amplicon V is 505 bp in length for the European corn borer and 497 bp for the smartweed borer (Fig. 3). The size estimate for the intact European corn borer ampli-

con, as indicated by the RFLP data (507 bp) is consistent with the corresponding sequence, while the smartweed borer sequence is slightly less than that predicted by RFLP analyses. ITS-1 sequences were identical for the European corn borers from Nebraska and Italy. For the ITS-1 amplicon, the pair-wise absolute distance (Felsenstein, 1993) between the European corn borer and the smartweed borer was 0.17. All of the restriction patterns observed in the European corn borer and smartweed borer ITS-1 RFLP analyses were supported by RE sites in the corresponding sequences (Fig. 3; Table 3).

Discussion

The existence of different pheromone races, voltine ecotypes and the geographical distance between European corn borer populations suggests the potential for reproductive isolation and concomitant genetic divergence of populations. However, no genetic divergence was observed among the European corn borer populations we examined. PCR-RFLP patterns for the

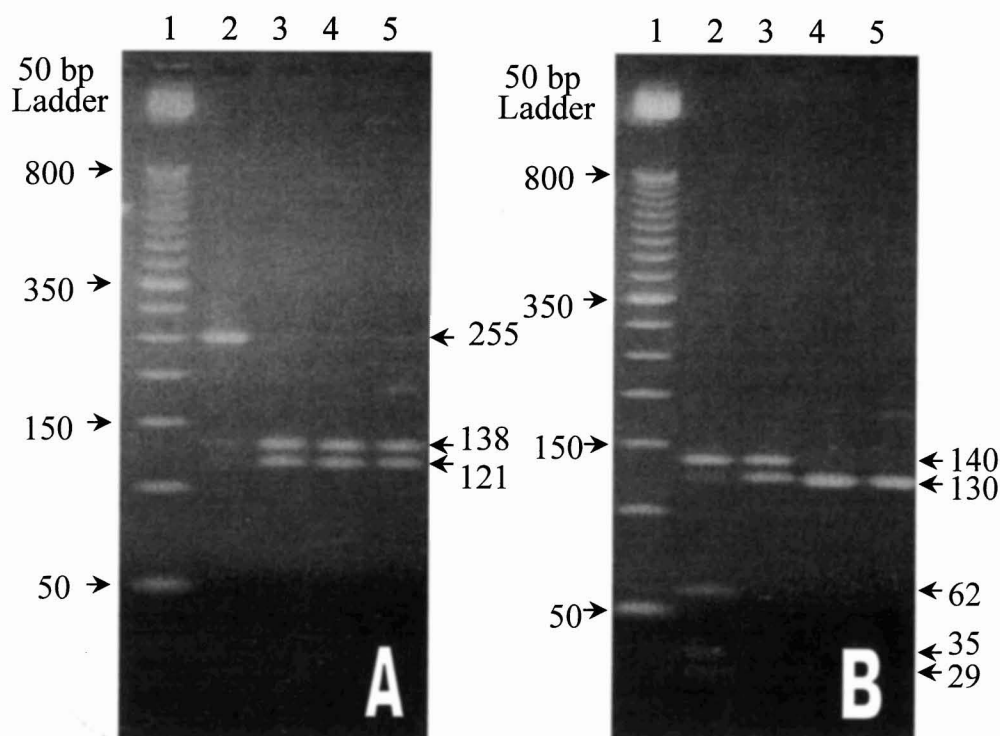


Figure 2. *AluI* (A) and *SspI* (B) digests of mitochondrial amplicon III on a 2.5% MetaPhor agarose gel. Lane 1, molecular standard (50 bp ladder); lane 2, *D. grandiosella*; lane 3, *O. obumbratalis*; lanes 4–5, *Ostrinia nubilalis* (lane 4, Italy; lane 5, Nebraska).

nuclear and mitochondrial regions were identical and a sequencing of the ITS-1 region confirmed the lack of differentiation (Table 3; Fig. 3). These results support previous allozyme studies which indicate a minimal genetic divergence among European corn borer races and ecotypes (Harrison & Vawter, 1977; Cianchi *et al.*, 1980; Glover *et al.*, 1990). However, genetic variation in the form of pheromone races (Zhu *et al.*, 1996) and voltine ecotypes (Showers, 1993) has previously been documented. The lack of differentiation among pheromone races and voltine ecotypes from North America and Europe indicates that the evolution of these traits may be a fairly recent event, on an evolutionary time scale. A more extensive survey of European corn borers from various locations in Europe may provide information on the genetic variation in the founding population and additional insight as to the historical basis of the present-day population structure of the European corn borer in North America.

Mitochondrial and ITS sequences are especially useful for detecting genetic divergence in the early stages of speciation and population structure within species. Mitochondrial tree-trimming (Avise *et al.*, 1987) and the concerted evolution of repetitive sequences (Elder & Turner, 1995) push these regions towards homogeneity in interbreeding populations. However, with the onset of reproductive isolation, both

regions have the potential to differentiate relatively rapidly on an evolutionary timescale. Because mitochondrial DNA is primarily inherited from the mother and does not recombine, it can also serve as an excellent marker for identifying populations which may have been isolated historically, but are currently interbreeding (Avise *et al.*, 1987; Taylor *et al.*, 1996b). The homogeneity of the mitochondrial and ITS-1 sequences observed in European corn borer indicates that the pheromone races and voltinism ecotypes have evolved relatively recently (< 10 000–100 000 years ago). A generally accepted estimate for mtDNA divergence is 2% per million years (Powell *et al.*, 1986). Based upon our ITS-1 and mitochondrial RFLP data for the European corn borer and smart weed borer, the ITS-1 amplicon has diverged 3–4-fold faster than the mitochondrial amplicons. Given a divergence rate of 7% per million years, we expect one fixed nucleotide difference approximately every 30 000 years in a 500-bp ITS-1 sequence.

In a few of our RFLP patterns, the sum of the fragment sizes do not equal the size of the amplified region. This is not uncommon in RFLP analyses, particularly for REs with a large number of restriction sites (Roehrdanz *et al.*, 1994). Such REs can generate very small, undetectable, DNA fragments as well as co-migrating fragments of similar sizes. This is particu-

| | | | | | | |
|--------------------------|-----|--------------------------|--------------------|---------------------|---------------------|----------------------|
| ECB | 1 | <u>TTGATTACGT</u> | <u>CCCTGCCCTT</u> | <u>TTACACACCG</u> | <u>CCCGTCGCT</u> | <u>ACTACCGATT</u> |
| SWB | 1 | <u>TTGATTACGT</u> | <u>CCCTGCCCTT</u> | <u>TTACACACCG</u> | <u>CCCGTCGCT</u> | <u>ACTACCGATT</u> |
| Hae III | | | | | | |
| ECB | 51 | GGAATGATTT | AGTGAGGTCT | TCGGACCGAC | AGCCGGTGGC | TTACCGGCGG |
| SWB | 51 | GAAATGATTT | AGTGAGGTCT | TCGGACCGAC | ACGCGGTGGC | TTACCGGCGG |
| Dpn II | | | | | | |
| ECB | 101 | TCGGCGTTGC | TGGGAAGTTG | ACCAAACCTG | ATCATT TAGA | GGAAGTAAAA |
| SWB | 101 | TCGGCGTTGC | TGGGAAGTTG | ACCAAACCTG | ATCATT TAGA | GGAAGTAAAA |
| Dpn II Mse I | | | | | | |
| ECB | 151 | GTCGTAACAA | GGTTTCCGTA | GGGGAACCTG | CGGAAG GATC | ATTAACGTGT |
| SWB | 151 | GTCGTAACAA | GGTTTCCGTA | GGGGAACCTG | CGGAAG GATC | ATTAAGGGGG |
| Rsa I Dpn II | | | | | | |
| ECB | 201 | ACGTT CACAT | GTGATACACA | AGTGTCATGT | GGGAAT GATC | ATACAATAAT |
| SWB | 201 | GTGTT00000 | 00000ATTAG | GATGGGGGAG | AATAATCATO | AAAAAATNNN |
| Dpn II/Taq I Bfa I Mse I | | | | | | |
| ECB | 251 | CCAGACACAO | AAACAAAAGT | TCT GATCGAA | GGAGGTCTC | GT TTAATT TAT |
| SWB | 251 | CCAAACACAA | CAACAACATC | TCTGAGCGGA | GGACGGT CTA | GT TT CATTAC |
| Dpn II Taq I Rsa I Rsa I | | | | | | |
| ECB | 301 | GAG ATCGTTC | GACGATT GGC | GTC GATC GAT | ATTCTACGTG | TACAGATTGA |
| SWB | 301 | GAG ATCGTTC | GACGGT CGGC | GTC GATC GAT | ATTCTACGTG | TACAGATTGA |
| Dpn II Rsa I Apo I Apo I | | | | | | |
| ECB | 351 | AAATCCG CGA | TCTCAGT AGT | TTTC GATC GT | TCGTAA AA TO | GAC AAATT CA |
| SWB | 351 | AAATCCG CGA | TCTCAGT AGT | TTTCGTATGT | TCGTAA AAATT | CAC AAATT CA |
| Mse I | | | | | | |
| ECB | 401 | TGCGGTTGAA | AAOTTGTAOT | ATOTTTTCAG | ACATTTATAA | AATTATTATT |
| SWB | 401 | TGCGGTTGAA | AATGTATATT | ATCTTTTCAG | OOOATAAATC | TATTATTAAA |
| Mse I Apo I Mse I | | | | | | |
| ECB | 451 | ATTOATTGTT | TATTATAAC | CACTATAAAC | CATTACCCTG | <u>GACGAGCCGA</u> |
| SWB | 451 | ACTATTTCCT | TAATAAATT | ATTATTAAAC | CATCACCTG | <u>GACGAGCCGA</u> |
| Dpn II | | | | | | |
| ECB | 501 | <u>GTGATCCAAC</u> | <u>G</u> | | | |
| SWB | 501 | <u>GTGATCCAAC</u> | <u>G</u> | | | |

Figure 3. Alignment of European corn borer (ECB) and smartweed borer (SWB) nuclear rDNA sequences (amplicon V). Restriction enzyme recognition sites are emboldened, with the name of the enzyme above. Deletions are represented by zeros. Ambiguous nucleotides are represented by an 'N'. Primer sequences are underlined.

larly evident for *Mse*I in amplicons II and IV and *Asel*I in amplicon IV. Heteroplasmy, the co-existence of more than one mitochondrial haplotype within an individual, can explain the fragment pattern observed for *Alu*I and *Dra*I in amplicon IV from the smart weed borer. A polymorphic *Dra*I site about 30 bp from one end of the 357 bp fragment, which is absent in the European corn borer, but present in south-western corn borer, would result in the observed *Dra*I pattern. Likewise, a polymorphic *Alu*I site in the 551 bp fragment could cut it into the 352 and 174 bp fragments. Although uncommon, this phenomenon has been reported for other insect species (Boyce *et al.*, 1989; Azeredo-Espin *et al.*, 1991; Valle & Azeredo-Espin, 1995).

PCR-RFLP and sequencing analyses of mitochondrial and nuclear amplicons proved useful for differentiating the European corn borer not only from the south-western corn borer (which belongs to a different genus in the same family as European corn borer), but more importantly, from the smartweed borer, a closely related congeneric species which is morphologically very similar to the European corn borer (Munroe, 1976). The genetic distance between the two species was only 0.043 for mitochondrial PCR-RFLPs. This suggests that genetic differentiation may be limited,

even among different species within the genus *Ostrinia*. The genetic distance calculated from nuclear PCR-RFLPs of the ITS-1 region was threefold greater than that calculated from PCR-RFLPs of mitochondrial amplicons. This faster rate of divergence was confirmed by sequencing data (Fig. 3), suggesting that the nuclear ITS-1 marker should provide a greater power to detect differences among European corn borer populations. Information from ITS-1 PCR-RFLPs and DNA sequences yielded essentially the same results. Therefore, PCR-RFLP may be preferable for population genetics studies, because many more individuals can be surveyed quickly and at less cost (Simon *et al.*, 1993; Taylor *et al.*, 1996a).

Low levels of mtDNA variation among widely dispersed populations has been reported among populations of other insect species, such as gypsy moth, *Lymantria dispar* (L.) (Harrison *et al.*, 1983), horn fly, *Haematobia irritans* (L.) (McDonald *et al.*, 1987), stable fly, *Stomoxys calcitrans* (L.) (Szalanski *et al.*, 1996), secondary screwworm, *Cochliomyia macellaria* (Taylor *et al.*, 1996b), monarch butterfly, *Danaus plexippus* L. (Brower & Boyce, 1991), and tobacco budworm, *Heliothis virescens* (F) (Roehrdanz *et al.*, 1994). These species all share high gene flow or a

recent expansion from a genetic bottleneck. These factors may also contribute to the lack of genetic variation observed among European corn borer populations. The sporadic movement of the European corn borer on surface airflow currents has been documented (Showers *et al.*, 1995), and it is possible that gene flow is contributing to the maintenance of genetic similarity among widely dispersed European corn borer populations. From a resistance management standpoint, an important consequence would be the potential for the rapid spread of insecticide resistance genes from one location throughout the species' geographical range (Caprio & Tabashnik, 1992).

Experimental procedures

Insects

European corn borer adults used for the genetic analyses were obtained from six locations across the USA and one location in northern Italy, representing different voltinism and pheromone strains (Table 1). For most of the locations, European corn borer were field-collected as larvae and shipped to our laboratory by overnight mail. Larvae were reared to adults, frozen and stored at -80°C . The European corn borer samples from New York were obtained from laboratory colonies as pupae (C. Linn, New York State Agricultural Experiment Station, Cornell University, Ithaca, NY). Pupae were reared to adults, frozen, and stored at -80°C .

Two other crambid species, the smart weed borer, *O. obumbratalis* (Lederer) and the south-western corn borer, *D. grandiosella* Dyar were analysed for comparison. Smart weed borers were field-collected as adults from weedy patches in Polk County, Iowa and delivered as live moths to the University of Nebraska. South-western corn borers were obtained as pupae from a laboratory colony maintained by the USDA-ARS Cotton Insects Research Unit, Starkville, Mississippi. This colony was initiated from insects collected in corn fields throughout Mississippi and is annually infused with wild insects (Frank M. Davis, personal communication).

Pheromone analysis

Fifth instar larvae were sent to the University of Delaware for pheromone gland analysis to determine the percentage of E and Z pheromone alleles. Larvae were reared to adults and pheromone glands were excised from adult females during the 7th hour of scotophase on the second day after eclosion. Each gland was extracted for at least 30 min in 5 μL heptane containing Z-7-tetradecenyl (0.9 ng/ μL) acetate as an internal standard. Samples (3 μL) were analysed by gas chromatography on a 15 m \times 0.25 mm i.d. fused silica capillary column containing a 0.5 μm film of Stabilwax (Restek Corporation). A Varian 3500 gas chromatograph (Varian Associates) equipped with a split/splitless injector, autosampler and flame ionization detector was used for all analyses. The column oven was programmed at 80°C for 1.5 min, 80 – 130°C at $20^{\circ}/\text{min}$, 130 – 210°C at $7^{\circ}/\text{min}$, 210 – 245°C at $20^{\circ}/\text{min}$ and 245°C for 5 min. The injector temperature was 200°C , detector temperature was 250°C , and nitrogen flow was 19 cm/s. The E and Z isomers were identified based on co-elution with known standards. The internal

standard and two pheromone isomers eluted at ≈ 15 min, with peaks of interest being separated by 0.2 min.

Female moths were assigned to pheromone strains by the ratio of the two pheromone isomers. Percentages were determined by a comparison of isomer peak heights at appropriate retention times. Samples with $> 85\%$ E isomer were classified as E strain, those with 50–80% E isomer were classified as hybrids, and those with the E isomer $< 15\%$ were classified as Z strain.

PCR-RFLP

Procedures were carried out according to the methods of Taylor *et al.* (1996a), with minor modifications. Adult thoraces were individually homogenized in 100 μL of lysis buffer. Proteinase K and RNAase were increased to 20 and 10 μg , respectively. DNA was isolated by phenol/chloroform extraction using quantities one half those of Taylor *et al.* (1996a). DNA was resuspended in 50–100 μL of TE buffer. After the extractions, DNA presence and quality was confirmed with 1% agarose gels. Samples were stored at 4°C for subsequent analysis.

Because mtDNA sequence information was not available for the European corn borer, an initial screen of potential mitochondrial primers was performed and four primer pairs were chosen which produced reliable and consistent DNA amplification products (amplicons) (Table 2). Based upon the *Drosophila yakuba* mitochondrial genome (Clary & Wolstenholme, 1985), the expected length and content of the amplicons were: amplicon I, 320 base pairs (bp) containing the 3' end of the NADH dehydrogenase (NADHDH) 1 gene, the entire tRNA_{Leu} gene, and the 5' end of the 16S rRNA gene; amplicon II, 345 bp containing the 3' end of the cytochrome B gene, the entire tRNA_{Ser} gene and the 5' end of the NADHDH 1 gene; amplicon III, 257 bp containing part of the NADHDH 4 gene; amplicon IV, 628 bp containing the 3' end of the cytochrome oxidase (CO) I gene, the entire tRNA_{Leu} gene and the 5' end of the CO II gene. Primers rDNA₂ and rDNA_{1,58S} (Table 2) were used to amplify a region of the nuclear ribosomal DNA (rDNA) which includes a portion of the 18S rDNA gene, the internally transcribed spacer 1 (ITS-1), and a portion of the 5.8S rDNA gene (Vrain *et al.*, 1992; Cherry *et al.*, 1997).

Fifteen restriction enzymes (*AluI*, *ApoI*, *Asel*, *BanII*, *BfaI*, *BsrI*, *DpnII*, *DraI*, *HaeIII*, *HintI*, *MseI*, *MspI*, *RsaI*, *SspI* and *TaqI* (New England Biolabs) were screened on five adult *O. nubilalis* from each of the seven populations—five adult *O. obumbratalis*, and five adult *D. grandiosella*. Restriction enzyme digests followed the manufacturer's recommended procedures. Reaction mixtures contained 1 μL of PCR product, 0.2 μL of restriction enzyme, 0.5 μL of 10X buffer, and autoclaved double-distilled water to a total volume of 5 μL . Samples were incubated at 37°C for 18–24 h. Restriction fragments were separated by polyacrylamide (10%) and Metaphor (FMC Bio-products) agarose (2.5%) gel electrophoresis. A 50 bp ladder was included on each gel. Fragment sizes were estimated with GEL-JLM (LaCroix, 1994). A matrix of restriction site presence/absence was derived manually from the restriction fragment patterns without explicitly mapping RE sites. Restriction fragment patterns which could not be explained by the gain or loss of individual restriction sites were considered not scoreable and were not included in the among species restriction site analysis.

DNA sequencing

The ITS-1 amplicon was sequenced from four European corn borers (two from Nebraska and two from Italy) and two smartweed borers. Amplified DNA was purified using the Prep-A-Gene DNA purification kit (Bio-Rad) and resuspended in double-distilled water to a final concentration of 30 ng/μL. Sequencing was performed via the dideoxy chain termination method (Sanger *et al.*, 1977) by the Iowa State University DNA Sequencing Laboratory (Ames, Iowa). Two primers, rDNA₂ and rDNA_{1.58S} (5 pmol/μL) (Table 2), were used to sequence in both directions. Sequences were aligned manually. A computer program, DIGEST (Ramin Nakisa, unpublished data), was used to scan the DNA sequences for restriction sites. Nucleotide sequences for European corn borer and smartweed borer have been deposited in GENBANK with accession numbers AF077013 and AF077014.

Statistical analysis

Nucleotide sequence divergence (*d*) was calculated from the restriction site data using the Restriction Enzyme Analysis Package (REAP) (McElroy *et al.*, 1992) following the procedures of Nei & Tajima (1981) and Nei & Miller (1990). Pair-wise absolute distances (*d*) between species were calculated from DNA sequence data using the Dnadist module of PHYLIP 3.5 (Felsenstein, 1993).

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