Molecular Ecology Notes (2007) **7**, 1132–1134 doi: 10.1111/j.1471-8286.2007.01806.x

PRIMER NOTE

# Characteristics of 13 polymorphic microsatellite markers in the corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae)

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**Abstract**

**Thirteen polymorphic microsatellite loci suitable for population genetic studies of *Helicoverpa zea* were discovered by screening partial genomic libraries enriched for microsatellite sequences. Insects collected (*N* = 96) in Stoneville, Mississippi were used to characterize these markers. The observed and effective number of alleles per locus ranged from two to nine (average 4.46) and from 1.07 to 2.45 (average 1.81), respectively. Fisher exact tests detected significant deviations from Hardy–Weinberg equilibrium at three loci, probably due to inbreeding, null alleles, or Wahlund's effect. Significant genotypic disequilibrium was not detected between any pair of loci.**

*Keywords*: bollworm, genetic markers, Helicoverpa zea, Lepidoptera, microsatellite, SSR

*Received 2 February 2007; revision accepted 20 March 2007*

The bollworm or corn earworm, *Helicoverpa zea* (Boddie), is a major pest of cotton and corn, and causes damage to at least 30 other crop species in the USA (Metcalf & Flint 1962; Martin *et al*. 1976). Greater knowledge of the genetics of *H. zea* would improve the development and implementation of integrated pest management programs and the design of insecticide resistance management strategies. Population genetic and mapping studies of this pest have been difficult to conduct due to scarcity of genetic markers.

Although microsatellite markers have not been developed for *H. zea*, those isolated in *Helicoverpa armigera* (Tan *et al*. 2001; Ji *et al*. 2003; Scott *et al*. 2004) have been tested in specimens of *H. zea* from laboratory colonies (Grasela & McIntosh 2005). Only four loci out of the 14 tested showed consistent results. Suitability of *H. armigera* primer pairs for field studies of *H. zea* was not evaluated. In addition, each of these primer pairs required a different annealing temperature which makes large-scale genotyping difficult. In the present communication, we report the isolation and characterization of 13 polymorphic microsatellite loci in *H. zea* that we developed by testing a field population.

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Corn earworms collected from a field location in Stoneville, Mississippi (*N* = 96) were used to evaluate loci suitable for genetic mapping and population studies. DNA extractions were performed using the MasterPure™ reagent system (Epicentre Technologies) following manufacturer's instructions. Genomic DNA extracted from 20 pupae obtained from a laboratory colony was used in developing microsatellite libraries. Partial genomic libraries enriched for di-, tri-, and tetranucleotide microsatellite sequences of *H. zea*, were constructed following Hamilton *et al*. (1999), with modifications by Perera *et al*. (2007). Enrichment of microsatellite sequences was carried out by magnetically capturing genomic DNA fragments hybridized to 24 biotinylated oligonucleotides (IDT DNA, Inc). Biotinylated oligonucleotides were pooled into four groups based on hybridization temperatures and the absence of complementary sequences. The hybridization temperatures of oligonucleotide pools of group 1 ($[AC]_{13}$, $[AACC]_5$, $[AACG]_5$, $[AAGC]_5$, $[AAGG]_5$, $[ATCC]_5$), group 2 ($[AG]_{12}$, $[AAC]_6$, $[AAG]_8$, $[ACT]_{12}$, $[ATC]_8$), group 3 ($[AAAC]_6$, $[AAAG]_6$, $[AATC]_6$, $[AATG]_6$, $[ACAG]_6$, $[ACCT]_8$, $[ACTC]_6$, $[ACTG]_6$), and group 4 ($[AAAT]_8$, $[AACT]_8$, $[AAGT]_8$, $[ACAT]_8$, $[AGAT]_8$) were 58, 52, 48, and 42 °C, respectively. Washing, amplification, and cloning enriched DNA fragments into pGemT-Easy vector (Promega) were carried out as described in Perera *et al*. (2007).

**Table 1** Characteristics of 13 microsatellite loci isolated from *Helicoverpa zea*. Locus names, GenBank Accession nos, forward(F) and reverse (R) primer sequences, repeat sequence and length of the cloned allele, size range of observed alleles, observed and effective allele numbers ($n_o$ and $n_e$, respectively), and observed and expected heterozygosities ($H_O$ and $H_E$, respectively) for each locus are given. Sample size column indicate the number of insects successfully genotyped from the 96 insects used in the analysis

| Locus (Accession no.) | Primer sequence (5′–3′) | Cloned allele: Repeat Sequence | Cloned allele: Size | Observed size range (bp) | Sample size | $n_o$ | $n_e$ | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|---|
| HzMS1-4 (EF152205) | F: CAAGTGATAAAAGACGCCGAAGAT R: GTTGATCGTCAAGGAAGTGGCTAT | $(TGA)_6$ | 118 | 111–144 | 86 | 5.0 | 1.46 | 0.326 | 0.315 |
| HzMS1-6 (EF152206) | F: GTTTTGTCATTTGTCAAGCCGAA R: AGCTCCCATACAACAAACGTGATT | $(TGA)_7$ | 237 | 208–245 | 91 | 7.0 | 1.66 | 0.099 | 0.401* |
| HzMS3-1 (EF152207) | F: CAGTAGTTCCTGAGATTAGCGCGT R: ATCACGTTCTCGAAAAACATTGCT | $(CAAA)_4$ | 113 | 106–110 | 96 | 3.0 | 1.61 | 0.354 | 0.379 |
| HzMS3-4 (EF152208) | F: GGTCAAGATTCGTGCCGATAACTA R: TTTTCGGTTCAGTGGCTTGTAGTAG | $(TCTG)_4$ | 118 | 115–117 | 77 | 2.0 | 1.07 | 0.042 | 0.068 |
| HzMS3-11 (EF152209) | F: ACTTCAAAGTTCGATTCTTGGGAT R: GCTCAAAGAGGACTACGTAGCTGA | $(AGCT)_4$ | 106 | 93–106 | 96 | 6.0 | 2.01 | 0.469 | 0.505 |
| HzMS3-41 (EF152210) | F: AAATTTCAACCAAATCGGTCTAGC R: TGGCCGAACTATAATATCTTACTTCCTA | $(ACAT)_4$ | 121 | 121–135 | 96 | 5.0 | 1.68 | 0.542 | 0.408 |
| HzMS3-48 (EF152211) | F: GGTGAAATGGAAATTGTTATCTATCCC R: TCAGTCCAGTGGTTTAGACGTGAA | $(TCTG)_4$ | 101 | 94–102 | 96 | 3.0 | 2.08 | 0.406 | 0.522 |
| HzMS3-86 (EF152212) | F: GGGGAAAAGAGGAAACAAATCATC R: GAAACACGTTTGAGGAGGTCAGAT | $(CAT)_4$ | 140 | 136–151 | 82 | 4.0 | 1.13 | 0.122 | 0.1175 |
| HzMS4-3 (EF152213) | F: ACTTTCCGCATCCGATTAAAATGT R: CAAATCGGACCAGTAGTTCCTGAG | $(GTTT)_4$ | 122 | 122–126 | 96 | 2.0 | 1.43 | 0.313 | 0.306 |
| HzMS4-10 (EF152214) | F: CTAGAACGGGCTTCATGGTGAG R: AAAAATAAAATGTATTCCGGGCGT | $(ATT)_4$ | 113 | 110–113 | 62 | 2.0 | 1.08 | 0.081 | 0.078 |
| HzMS4-14 (EF152215) | F: CAACATACAACATTCAGCCTGTCC R: TCAGTCGTCAGTTTTTGTCTTTGC | $(AC)_7$ | 132 | 110–135 | 95 | 9.0 | 1.60 | 0.168 | 0.376* |
| HzMS4-16 (EF152216) | F: AGTGTATACGGAGCAAGAATTGGA R: TTTTGCAAATCAAACTATTGAAAAGTAA | $(ACAT)_6$ | 147 | 134–149 | 59 | 6.0 | 2.43 | 0.288 | 0.771* |
| HzMS4-23 (EF152217) | F: GTTCAGCGGTTTAGATGTGAAAGG R: TAAGGGTTCGTGTAGAAGTTCCCA | $(GACA)_4$ | 135 | 130–139 | 96 | 4.0 | 2.45 | 0.500 | 0.595 |
| | | | | Mean | 86.77 | 4.46 | 1.81 | 0.285 | 0.372 |
| | | | | S.E. | 13.23 | 2.14 | 0.83 | 0.169 | 0.205 |

*Loci with significant deviations from Hardy–Weinberg equilibrium ($P < 0.01$).

All DNA sequence analyses were carried out at the USDA-ARS Mid-South Area Genomics Laboratory using an ABI 3730xl instrument and BigDye version 3.1 (Applied Biosystems). Microsatellite detection and primer design were performed using SSRFINDER (Sharopova *et al*. 2002) software using the criteria described in Perera *et al*. (2007). Forward and reverse primers were modified for use under universal PCR condition by adding 'tail' sequences 5′-CAGTTTTCCCAGTCACGAC-3′ and 5′-GTTT-3′, respectively, and a universal 6-carboxyfluorescein (FAM)-labelled primer containing the tail sequence of the forward primer was used to label the amplicons (Taliercio *et al*. 2006).

Genotyping reactions were run on an ABI 3730xl instrument using ROX 500 dye marker (Applied Biosystems) as the standard. Alleles were scored using GENEMAPPER® software (Applied Biosystems) and manually confirmed. Fisher exact tests for Hardy–Weinberg equilibrium (HWE) and genotype disequilibria were performed using a Markov Chain method (1000 dememorization steps, 100 batches, and 1000 iterations per batch), implemented using the GENEPOP web version 3.4 software (Raymond & Rousset 1995).

DNA sequences from 144 clones yielded 50 independent microsatellite sequences. Only 34 primer pairs meeting the design criteria were selected for screening. Any primer pair that produced stutter peaks were eliminated from further evaluation. Characteristics of 13 polymorphic microsatellite loci, consisting of eight, four, and one tetra-, tri-, and dinucleotide repeats, respectively, are summarized in Table 1. The observed and effective number of alleles per locus ranged from two to nine (average 4.46 ± 2.14) and from 1.07 to 2.45 (average of 1.81 ± 0.83). Exact tests for (HWE) indicated significant deviations at the loci, HzMS1-6, HzMS4-14, and HzMS4-16. These three loci were significantly deficient in heterozygotes [$F_{IS}$ estimates of 0.777, 0.589, and 0.559,

respectively ($P < 0.0001$)], probably due to inbreeding, Wahlund's effect, or the presence of null alleles. No significant genotypic disequilibrium was detected between any pair of loci. A pair of loci was considered in linkage disequilibrium if the *P*-value associated with the test was ≤ 0.01.

We isolated and characterized 13 polymorphic microsatellite marker loci in *H. zea*. These are the first set of microsatellite markers characterized in this species. Only primers with optimal annealing temperatures around 60 °C were selected. This enabled the use of uniform PCR conditions across all loci and facilitated high throughput sample processing.

## Disclaimer

Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

## Acknowledgements

We thank Linda Ballard, Mary Duke, Xiaofen Liu, Sheron A. Simpson, and Torey Looft for their technical support and Drs Gary Fritz (Eastern Illinois University) and Steven Valles (USDA-ARS, Gainesville, Florida) for critically reviewing the manuscript. Parts of this research were supported by CRIS 6402-22000-047-00 and 6402-21310-001-00.

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