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PRIMER NOTE

Characterization of polymorphic microsatellite markers in the Indian meal moth, *Plodia interpunctella* (Hübner)

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Abstract

The Indian meal moth, *Plodia interpunctella* (Hübner), is a serious and widespread pest of stored food commodities worldwide. Studies on the population genetics and breeding structure of *P. interpunctella* are lacking. Understanding the breeding structure of this pest has implications for its management. Fifteen microsatellite loci were isolated, cloned and characterized using an enrichment method. Forty individuals from six subpopulations were tested for polymorphism. Nine loci were found to be polymorphic. The number of alleles varied from three to six per locus.

Keywords: genetic markers, Indian meal moth, microsatellites, *Plodia interpunctella*

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Microsatellites are simple sequence repeats (SSRs) of two to five nucleotide motifs that occur ubiquitously in eukaryotic genomes. Because of their high degree of polymorphism and abundance in eukaryotic genomes, microsatellites have found many genetic applications in insects. However limited success has been achieved in their isolation from lepidopteran species (Ji *et al*. 2003, Williams *et al*. 2002). The Indian meal moth (IMM), *Plodia interpunctella* (Hübner) (Pyralidae: Lepidoptera) is a destructive pest of raw and processed foods worldwide including the United States. *P. interpunctella* is resistant to most of the insecticides used to control it (Subramanyam & Hagstrum 2003). Studies on the population genetics and breeding structure of IMM are lacking. Population genetic studies can aid in estimating rates of gene flow and dispersal patterns and these data are likely to be useful in fine-tuning existing management programs.

High molecular weight genomic DNA was extracted from laboratory reared *P. interpunctella* using the DNAeasy Kit (QIAGEN). Microsatellites were isolated using an enrichment procedure (Hamilton *et al*. 1999). The microsatellite enrichment protocol involved the digestion of DNA into approximately 500 bp fragments with restriction enzymes *Rsa*1 and *Hae*111 (New England BioLabs). This was followed by ligation of double stranded *Snx* linker primers (*SNXF*: 5′-CTAAGGCCTTGCTAGCAGAAGC-3′ and *SNXR*: 5′-GCTTCTGCTAGCAAGGCCTTAGAAAA-3′) to each end of the DNA fragment. Enrichment was carried out by hybridizing DNA with biotinylated microsatellite probes containing di-, tri- and tetranucleotide repeats. Fragments were then PCR-(polymerase chain reaction) amplified using *Snx* primers and cloned into a T-vector (Invitrogen), followed by transformation. Positive clones were picked at random, and the inserts were PCR-amplified using *M13* primers. (forward: 5′-CATTTTGCTGCCGCTG-3′; reverse: 5′-GTCCTTTGTCGATACTG-3′) Amplifications were carried out in a PTC200 thermal cycler (MJ Research) in 25 μL volume. The reaction mixture contained 19.5 μL of water, 10X *Taq* buffer (500 mM KCl, 100 mM Tris-HCl pH 9.2, 1% Triton X–100), 2.5 mM $MgCl_2$, 0.86 mM dNTPs, 5 μM each primer, 0.6 U *Taq* DNA polymerase and 50–100 ng DNA. Cycling conditions were: denaturation step at 94 °C for 3 min followed by 35 cycles at 95 °C for 30 s, 50 °C for 45 s and 72 °C for 30 s and a final extension at 72 °C for 10 min. The PCR product was electrophoresed on a 1% agarose gel and the fragments larger than 400 bp were excised and purified using QIAquick Gel Extraction Kit (QIAGEN), following the manufacturer's instructions. Sequencing reactions were carried out in 5-μL volumes using dRhodamine dye kit (Applied Biosystems) as per the recommended protocol. Fragments were then sequenced on an ABI 371 automated sequencer at the DNA sequencing laboratory at the University of Florida. Oligonucleotide primers flanking the microsatellite repeat were designed for 20 loci using OLIGO Analyser version 3.0, primer analysis

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Table 1 Primer sequence and characteristics of microsatellite loci in *Plodia interpunctella*

Locus name/Genbank accession no.	Repeat motif	Primer sequence (5′–3′)	Annealing temperature (°C)	Size of alleles (bp)	Number of alleles	H_O*	H_E*
IMM211 AY743332	GT(9)	F: TCACTCGATATCGCCATGGAGT R: AAACACGGACCGTGTACACAAG	58	133–151	6	0.759	0.649
IMM214 AY743333	GA(16)	F: GCTCTTATTACCGCTGCGTCTAG R: GAAGAGGACAAACTTACTTTGGCA	59	270–288	3	0.227	0.358
IMM216 AY743334	TA(6)	F: GAATTTCAAGAAGTGGGCCCTCAG R: GGAAAGGGAACGGTTGTTGATC	59	192–262	6	0.583	0.618
IMM218 AY743335	TG(7)	F: CCGGTCTCAATTTTCGTTCGA R: CCAACATCGTGCTGATCAGAAG	59	250–268	4	0.840†	0.549†
IMM220 AY743336	TC(10)	F: GACGAAGCCCAAGGTCAGAATAG R: GAAAACATTTTGCGGTGTCTCCG	60	225–243	3	0.409	0.516
IMM38 AY743337	TCTA(6)	F: CATCATCGTTGAGCCATGACC R: GACCATAAGATACTTGTTATGCTC	58	274–310	5	0.329	0.445
IMM313 AY743338	CATT(14)	F: CATCAAGGGCACGCACAAC R: GTCTTTGGTCTTTTCCATCCG	58	312–368	4	0.150	0.155
IMM314 AY743339	TGAC(5)	F: CGAGTCGCATTCACGTATGTG R: GAAGTCACTAACTTCAACGGTGTAG	60	271	4	0.075	0.210
IMM319 AY743340	TCTG(10)	F: CAGATGAGACAATAACGAAACTTC R: GAACACCGCTACAAATTACATC	58	202–226	5	0.292	0.323

*H_O, observed heterozygosity and H_E, heterozygosity expected under Hardy–Weinberg equilibrium; †, observed and expected heterozygosities are significantly different from each other.

software (Integrated DNA Technologies) (period). A gradient PCR to optimize the annealing temperature for each primer was set up as previously described in 25-μL reaction volume. The annealing temperatures for the gradient cycle ranged from 47 to 60 °C. Optimum temperature was determined for each primer after visualizing the bands on a 1% agarose gel. Fifteen primers amplified the fragment of interest.

These loci were tested for polymorphism on 40 individuals from six field-collected IMM subpopulations [Abilene, Salina, Oswego and Hays from Kansas, Bet Dagan (Israel) and Montreal (Canada)]. The PCR products were electrophoresed on a 0.4 mm, 6% polyacrylamide gel in a Hoefer SE600 vertical gel apparatus (GE Healthcare). Alleles were scored by comparing the migration of the fragments to a standard 100-bp ladder (Promega).

Nine loci showed polymorphism (.95 criterion, Hartl & Clark 1997) across the subpopulations tested (Table 1). The number of alleles varied from three to six among all loci with a mean (± SD) of 4.4 ± 0.369. Deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were analysed by means of exact tests using the program FSTAT version 2.9.3.2 (Goudet 2001). Tests for deviation from HWE showed significant differences in observed heterozygosity at only one of the nine loci. No LD between loci was detected.

Extensive spatial and temporal sampling are in progress and the analysis of these populations using the markers described in this study will provide important information on the population genetics of this pest. These microsatellite markers may also be used to develop a DNA fingerprinting system to discriminate among populations and infestations originating from multiple areas.

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