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PRIMER NOTE

Isolation and characterization of DNA microsatellite from cotton bollworm (*Helicoverpa armigera*, Hübner)

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Abstract

Five microsatellite loci of *Helicoverpa armigera* were isolated from a partial genomic library screened by oligonucleotide probes. Primers were designed to detect allelic variability and heterozygosity in 60 individuals collected from different host species. All loci were found to be polymorphic, have 8–11 alleles with expected heterozygosity ranging from 0.81 to 0.88. Our results indicate that the five microsatellite loci could provide valuable markers for population genetic and ecological studies of the cotton bollworm.

Keywords: genetic structure, *Helicoverpa armigera*, microsatellite, PCR, primer

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The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepitoptera: Noctuinae) is one of the most serious agricultural insect pests throughout Asia, Europe, Africa and Australia. It has adapted to many different host species and caused huge damage. Some researches suggest that *H. armigera* is a facultative migratory insect because of its ability to fly over long distances (Wu & Guo 1995; Guo 1997). Population genetic analysis offers a convenient approach to understanding the process of local adaptation and migration in this pest, and can provide useful data for pest control. Studies using random amplified polymorphic DNA (RAPD) markers have suggested little deviation among populations and low genetic variation within populations (Lei *et al*. 1997; Zhou *et al*. 2000). However, the relationship between RAPD marker evolution and population differentiation is poorly known. Consequently, we turned to the development of microsatellite markers. In this paper we describe the isolation of five microsatellite loci from the genome of *H. armigera*.

Genomic DNA was extracted from eight individuals according to Chen *et al*. (2000), and digested with two restriction enzymes *Hae*III and *Rsa*I and electrophoresed in a 0.8% agarose gel. DNA fragments in a range of 200–800 bp were excised and purified. A partial genomic library was constructed by ligating the DNA fragments into the vector pGEM-7Zf(+) at the *Sma*I site. Approximately 600 transformed clones were screened using a mixture of digoxygenin 3′ end labelled $(CA)_n$, $(GT)_n$, $(ATT)_n$ and $(GATA)_n$ oligonucleotide probes. Twenty-four positive clones were purified and sequenced on an ABI PRISM 377 automated sequencer. Five microsatellite loci were detected and primer sets were designed with the help of program OLIGO (National Bioscience Inc. Version 4.0). These five pairs of primers produced unambiguous and reproducible polymorphic bands. Allelic variability and heterozygosity of the loci were determined with DNA extracted from individuals obtained from different host species (cotton, maize, soybean, peanut, sesame and Monsanto's *Bt* cotton 33B). Ten individuals were used for each population. Polymerase chain reaction (PCR) amplification was carried out in a Perkin-Elmer 9600 Cetus thermal cycler in 25 μL of reaction mixture including approximately 20–40 ng of template DNA, 0.2 mM dNTP, 0.5 μM of each primer, ddH_2O, 1 × PCR buffer (Promega Co.; 50 mM KCl, 2 mM MgCl, 10 mM Tris-HCl), and 1 unit of *Taq* DNA Polymerase (Promega Co.). After initially denaturation at 94 °C for 5 min, the reaction underwent 35 cycles with 94 °C for 1 min, specific annealing temperature (Table 1) for 30 s, 72 °C for 40 s and a final extension at 72 °C for 5 min. PCR products were electrophoresed on 8% nondenaturating polyacrylamide gel and visualized after silver staining (Cairns & Murray 1994).

The variability of the five loci within 60 individuals is summarized in Table 1. All loci were found to be polymorphic,

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Table 1 Microsatellite markers for cotton bollworm *Helicoverpa armigera*. Size range refers to the PCR product sizes of each locus. Number of alleles was determined from 60 individuals from six kinds of host crops in the field. (Acc., GenBank accession number; T_a, annealing temperature; N, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity)

Locus Acc.	Primer sequence (5′–3′)	Size range (bp)	Repeat region	T_a (°C)	N	H_O	H_E
Ham2 AF330634	F: cataggaagtggtgaagggt R: cacattcgtctttcatcgac	227–275	$(ttttga)_9$	49	9	0.43	0.87
Ham3 AF330629	F: acgtcgatgaaagacgaatgtga R: aagctggtctgtgctgccat	171–241	$(taaa)_2(taaat)_4$	53	8	0.45	0.81
Ham4 AF330630	F: gccgtaatgccctcaattctt R: ttccctcggagagccgt	207–277	$(tctg)_6tctt(tctg)_6$	55	11	0.58	0.87
Ham5 AF330631	F: tagtctgggaattttgtctggtgt R: cgtgccattgaaatagtaagccat	248–296	(t)n(g)n	52	8	0.47	0.88
Ham6 AF330632	F: taagtatgccctcgactgtcgt R: cactttccaattagcctcgatgct	202–252	$(gat)_2tt(gat)_2tt$ … $(aata)_5$	53	10	0.65	0.85

with 8–11 alleles and expected heterozygosity ranging from 0.81 to 0.88. These markers show excellent properties and have been used to examine the genetic differentiation of populations on different host crops. Previous studies showed that isolation of microsatellite loci from Lepitoptera is unusually difficult, and very few studies have successfully used microsatellite markers in this insect group (Saccheri *et al*. 1998; Keyghobadi *et al*. 1999). Our results indicate that this set of five microsatellite loci would provide valuable markers for population genetic and ecological studies in the cotton bollworm. It might also be useful for studies on other insect.

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References

Cairns MJ & Murray V (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques*, **17**, 915–919.

Chen X, Tan S, Liu R, Wang Y, Li D (2000) Study on the genetic variation of the cotton bollworm (*Helicoverpa armigera*) populations in China. *Entomologia Sinica*, **7**, 243–249.

Guo YY (1997) Progress in the researches on migration regularity of cotton bollworm and relationship between the pest and its host plants. *Acta Entomologica Sinica*, **40** (Suppl.), 1–6.

Keyghobadi N, Roland J, Strobech CE (1999) Influence of landscape on the population structure of the alpine butterfly *Parnassius smintheus* (Papiliomidae). *Molecular Ecology*, **8**, 1482–1495.

Lei ZR, Li L, Guo YY (1997) RAPD analysis of DNA polymorphism in cotton bollworm (*Helicoverpa armigera*) from different regions of China. *Acta Entomologica Sinica*, **40** (Suppl.), 13–19.

Saccheri IJ, Koussaari M, Kankare M *et al.* (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Wu KM & Guo YY (1995) Intra-variation of disperse in population of cotton bollworm *Helicoverpa armigera*. *Sinozoologia*, **12**(Suppl), 44–48.

Zhou X, Fartor O, Applebaum SW, Coll M (2000) Population structure of the pestiferous month *Helicoverpa armigera* in the Eastern Mediterranean using RAPD analysis. *Heredity*, **85**, 251–256.