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PRIMER NOTE

Isolation of nine microsatellite loci in *Dolichogenidea homoeosomae* (Hymenoptera) a parasitoid of the sunflower moth *Homoeosoma electellum* (Lepidoptera)

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Abstract

Nine microsatellite loci were isolated from the insect *Dolichogenidea homoeosomae* (Hymenoptera: Braconidae), an important parasitoid of the sunflower moth *Homosoeosoma electellum* (Lepidoptera: Pyralidae), and assayed for polymorphism. All nine loci were polymorphic within the five populations tested, with two to 14 alleles per locus. Expected and observed heterozygosities ranged from 0.39 to 0.90 and 0.25 to 0.72 respectively. These are the first microsatellite primers developed for *D. homeosomae* and will be useful for studies of population dynamics and connectivity.

Keywords: *Homosoeosoma*, Hymenoptera microsatellites, parasitoid, population structure

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Homosoeosoma electellum (Lepidoptera: Pyralidae) is recognized as the pre-eminent pest in sunflower populations across North America, and *Dolichogenidea homoeosomae* (Hymenoptera: Braconidae) is its most important parasitoid in the San Joaquin Valley in California (Chen & Welter 2003). Previous work has shown that *H. electellum* is parasitized more in wild populations than in agricultural settings which suggests that *D. homoeosomae* may have great potential as a tool in the integrative pest management of this species (Chen & Welter 2002). Microsatellite markers were developed in order to better understand the spatial genetic structure and metapopulation dynamics of *D. homoeosomae*.

Genomic DNA was extracted and pooled from five *Dolichogenidea homoeosomae* wasps using the QIAmp DNA Micro Kit (QIAGEN). *D. homeosomae* microsatellite clones were obtained and screened from biotin-enriched genomic DNA as described by Khasa *et al*. (2000) using oligonucleotides TG_{12}, GA_{12}, AAG_8, AAC_8 and $GATA_6$. Of the 36 clones sequenced 34 were positive for the presence of a dinucleotide, trinucleotide, or tetranucleotide repeat. From these, 19 primer pairs were designed and tested. One primer from each pair of primers was end-labelled with fluorescent dye (6-FAM). Each 25 L polymerase chain reaction (PCR) contained 100–130 ng genomic DNA, 1× buffer (Invitrogen), 2 mM $MgCl_2$, 200 M of each dNTPs, 20 ng BSA, 0.5 M Betaine, 1.6 M unlabelled primer, 0.8 M labelled primer and 0.5 U *Taq* DNA polymerase (Invitrogen). Amplification was performed in Techne Genius thermocycler using an initial denaturation at 94 C for 5 min; 19 cycles were performed, each consisting of 45 s at 94 C, 45 s of annealing starting at 45 C and decreasing by 0.5 C per cycle, and 45 s at 70 C. An additional 20 cycles were run consisting of 30 s at 94 C, 30 s at 50 C and 60 s at 70 C. A final extension was performed at 70 C for 5 min. Amplified fragments were analysed on an ABI PRISM 3100 automated sequencer using GeneScan-500 [ROX] size standard (Applied Biosystems), and allele sizes were called using ABI GENEMAPPER 3.0.

Polymorphism at nine loci was assayed in 51 samples of *D. homoeosomae* collected from five sunflower populations located within the California Central Valley. Only female samples were used in the analysis of heterozygosity due to the haplodiploid nature of *D. homeosomae*. Genetic variability and linkage was analysed using GENEPOP 3.3 (Raymond & Rousset 1995).

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Table 1 Characterization of nine *Dolichogenidea homoeosomae* microsatellite loci based on 51 individuals collected from five sunflower populations located within the California Central Valley. (H_O, observed heterozygosity; H_E, expected heterozygosity)

Locus	Repeat	GeneBank Accession no.	Primer sequences (5′–3′)	Temperature range (C)	Size range (bp)	No of alleles	H_O	H_E
Dh-27a	$(CAA)_{14}$	DQ003261	F: TTGTCTGAGGATCTCATACACA R: TGCTTTATTCTCCTGGACAATCA	55–60	149–173	7	0.59	0.71
Dh-2a	$(AG)_{12}$	DQ003268	F: CTTGTGAAAAAAGAATGACC R: TTAACCAGCAGGCGCTCTTCAGC	55–60	149–159	5	0.29	0.73
Dh-11a	$(CA)_{50}$	DQ003268	F: GGTCATAGGAAGCTTCCCTGAC R: GTGCGTAACATTCTGAAAGGC	55–60	136–182	9	0.72	0.86
Dh-8a	$(CTAT)_{11}$	DQ003262	F: GTTGTTTGCCATTGGGCCAACAG R: TAATAGCAGTTGATCGTAAGAAATG	55–60	114–138	7	0.69	0.76
Dh-3a	$(GATA)_7$	DQ003263	F: GAGAAGGACCAGAGAAACCAAAG R: CATTTCGGTCGCTAATGCACG	55–60	145–149	2	0.25	0.39
Dh-20a	$(GTT)_{10}$	DQ003264	F: TAGAGTGTGGTTTTAGCCAGCAC R: GGTTTCGGCGGAAGCCACATTC	55–60	208–229	8	0.53	0.78
Dh-19a	$(TTG)_{16}$	DQ003265	F: GCAACTGCTGTTGTTGCTGCTG R: CCTTCGAAAGCCCAATATTGCTG	55–60	127–175	14	0.61	0.89
Dh-14a	$(CAA)_{12}$	DQ003266	F: GGTCAGAATGTATGATACGC R: TATCCAGGATGACACGTGAC	55–60	121–139	6	0.61	0.71
Dh-26a	$(GTT)_{20}$	DQ003267	F: CCTTGTTTTTGCTGTTGTCCC R: GCATCCACAAACACCGTCATCAC	55–60	159–192	12	0.59	0.82

All loci tested were polymorphic with two to 14 alleles per locus (Table 1). Expected and observed heterozygosities ranged from 0.39 to 0.90 and 0.25 to 0.72, respectively. With the exception of locus *P-8a*, all loci had a significant heterozygote deficiency ($P < 0.05$). One explanation is the presence of null alleles, however, this explanation is not satisfactory as nearly all loci show a deficiency. Alternative explanations are small sample size or population fragmentation and genetic drift (Moysés *et al*. 2005). Only one of the 36 pairwise tests of genotypic disequilibrium (P-11a × P-8a, $P < 0.05$) indicated possible linkage. In conclusion, these first microsatellite primers developed for *D. homeosomae* will be useful for studies of population dynamics and connectivity.

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