

Bowdoin College

## Bowdoin Digital Commons

---

Biology Faculty Publications

Faculty Scholarship and Creative Work

---

3-1-2006

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

Vladimir Douhovnikoff  
*Simmons University*

Caterina Nerney  
*Department of Environmental Science, Policy, and Management*

George K. Roderick  
*Department of Environmental Science, Policy, and Management*

Craig H. Newton  
*CANTEST Ltd.*

Stephen C. Welter  
*Department of Environmental Science, Policy, and Management*

Follow this and additional works at: <https://digitalcommons.bowdoin.edu/biology-faculty-publications>

---

#### Recommended Citation

Douhovnikoff, Vladimir; Nerney, Caterina; Roderick, George K.; Newton, Craig H.; and Welter, Stephen C., "Isolation of nine microsatellite loci in *Dolichogenidea homoeosomae* (Hymenoptera) a parasitoid of the sunflower moth *Homoeosoma electellum* (Lepidoptera)" (2006). *Biology Faculty Publications*. 48.  
<https://digitalcommons.bowdoin.edu/biology-faculty-publications/48>

This Article is brought to you for free and open access by the Faculty Scholarship and Creative Work at Bowdoin Digital Commons. It has been accepted for inclusion in Biology Faculty Publications by an authorized administrator of Bowdoin Digital Commons. For more information, please contact [mduoye@bowdoin.edu](mailto:mduoye@bowdoin.edu), [a.sauer@bowdoin.edu](mailto:a.sauer@bowdoin.edu).

## PRIMER NOTE

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

VLADIMIR DOUHOVNIKOFF,\* CATERINA NERNEY,† GEORGE K. RODERICK,† CRAIG H. NEWTON‡ and STEPHEN C. WELTER†

\*Department of Biology, Simmons College, 300 The Fenway, Boston, MA 02115, USA, †Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720, USA, ‡BC Research Inc., 3650 Wesbrook Mall, Vancouver, BC V6S 2L2, Canada

## Abstract

Nine microsatellite loci were isolated from the insect *Dolichogenidea homoeosomae* (Hymenoptera: Braconidae), an important parasitoid of the sunflower moth *Homoeosoma 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. homoeosomae* and will be useful for studies of population dynamics and connectivity.

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

Received 16 June 2005; revision accepted 25 July 2005

*Homoeosoma 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. homoeosomae* microsatellite clones were obtained and screened from biotin-enriched genomic DNA as described by Khasa *et al.* (2000) using oligonucleotides TG<sub>12</sub>, GA<sub>12</sub>, AAG<sub>8</sub>, AAC<sub>8</sub> and GATA<sub>6</sub>. 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 µL polymerase chain reaction (PCR) contained 100–130 ng genomic DNA, 1× buffer (Invitrogen), 2 mM MgCl<sub>2</sub>, 200 µM of each dNTPs, 20 ng BSA, 0.5 M Betaine, 1.6 µM unlabelled primer, 0.8 µM 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. homoeosomae*. Genetic variability and linkage was analysed using GENEPOP 3.3 (Raymond & Rousset 1995).

**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<br>Accession no. | Primer sequences (5'-3')                                    | Temperature<br>range (°C) | Size<br>range (bp) | No of<br>alleles | $H_O$ | $H_E$ |
|--------|----------------------|---------------------------|-------------------------------------------------------------|---------------------------|--------------------|------------------|-------|-------|
| Dh-27a | (CAA) <sub>14</sub>  | DQ003261                  | F: TTGTCTGAGGATCTCATACACA<br>R: TGCTTTTATTCCTGGACAATCA      | 55–60°                    | 149–173            | 7                | 0.59  | 0.71  |
| Dh-2a  | (AG) <sub>12</sub>   | DQ003268                  | F: CTTGTGAAAAAGAATGACC<br>R: TTAACCAGCAGGCGCTCTTCAGC        | 55–60°                    | 149–159            | 5                | 0.29  | 0.73  |
| Dh-11a | (CA) <sub>50</sub>   | DQ003268                  | F: GGTCATAGGAAGCTTCCCTGAC<br>R: GTGCGTAACATTCTGAAAGGC       | 55–60°                    | 136–182            | 9                | 0.72  | 0.86  |
| Dh-8a  | (CTAT) <sub>11</sub> | DQ003262                  | F: GTTGTTTTGCCATTGGGCCAACAG<br>R: TAATAGCAGTTGATCGTAAGAAATG | 55–60°                    | 114–138            | 7                | 0.69  | 0.76  |
| Dh-3a  | (GATA) <sub>7</sub>  | DQ003263                  | F: GAGAAGGACCAGAGAAACCAAG<br>R: CATTTTCGGTCGCTAATGCACG      | 55–60°                    | 145–149            | 2                | 0.25  | 0.39  |
| Dh-20a | (GTT) <sub>10</sub>  | DQ003264                  | F: TAGAGTGTGGTTTGTAGCCAGCAC<br>R: GGTTCGCGCGGAAGCCACATTC    | 55–60°                    | 208–229            | 8                | 0.53  | 0.78  |
| Dh-19a | (TTG) <sub>16</sub>  | DQ003265                  | F: GCAACTGCTGTTGTGTGCTGCTG<br>R: CCTTCGAAAGCCCAATATTGCTG    | 55–60°                    | 127–175            | 14               | 0.61  | 0.89  |
| Dh-14a | (CAA) <sub>12</sub>  | DQ003266                  | F: GGTCAGAAATGTATGATACGC<br>R: TATCCAGGATGACACGTGAC         | 55–60°                    | 121–139            | 6                | 0.61  | 0.71  |
| Dh-26a | (GTT) <sub>20</sub>  | DQ003267                  | F: CCTTGTTTTTTGCTGTTGTGCC<br>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 ( $P-11a \times P-8a$ ,  $P < 0.05$ ) indicated possible linkage. In conclusion, these first microsatellite primers developed for *D. homeosomae* will be useful for studies of population dynamics and connectivity.

### Acknowledgements

The authors thank Richard S. Dodd for advice and laboratory support, Adelaide M. Cheng for laboratory assistance, and Per Palsboll for discussions and assistance. Access to sampling sites was generously granted by The Nature Conservancy, Pioneer International, Pureseed Inc., California Department of Fish and

Game, and the US Department of Fish and Game. Partial funding was provided by The Land Institute.

### References

- Chen YH, Welter SC (2002) Abundance of a native moth, *Homoeosoma electellum* (Lepidoptera: Pyralidae), and activity of indigenous parasitoids in native and agricultural sunflower habitats. *Environmental Entomology*, **31**, 626–636.
- Chen YH, Welter SC (2003) Confused by domestication: incongruent behavioral responses of the sunflower moth, *Homoeosoma electellum* (Lepidoptera: Pyralidae) and its parasitoid, *Dolichogenidea homoeosomae* (Hymenoptera: Braconidae), towards wild and domesticated sunflowers. *Biological Control*, **28**, 180–190.
- Khasa PD, Newton CH, Rahman MH, Jaquish B, Dancik BP (2000) Isolation, characterization and inheritance of microsatellite loci in alpine larch and western larch. *Genome*, **43**, 439–448.
- Moysés CB, Mockford S, Almeida-Toledo LF, Wright JM (2005) Nine polymorphic microsatellite loci in the Neotropical electric eel *Eigenmannia* (Teleostei: Gymnotiformes). *Molecular Ecology Notes*, **5**, 7–9.
- Raymond M, Rousset F (1995) GENEPOP: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.