

PRIMER NOTE

# Isolation and characterization of microsatellites in *Aphidius ervi* (Hymenoptera: Braconidae) and their applicability to related species

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## Abstract

*Aphidius ervi* (Hymenoptera: Braconidae) is an important biological control agent of aphids, and a model organism for the study of host–parasitoid ecology and evolution. We isolated microsatellite loci from *A. ervi* to examine the genetic effects of its introduction from Europe to North America. We present primers for 11 microsatellites from *A. ervi*. We have assayed six of these loci for variability and found from two to 37 alleles. These six primer pairs were tested on 17 related species and appear broadly applicable. These microsatellite loci provide a new tool for research on *A. ervi* and its relatives.

**Keywords:** *Aphidius*, Braconidae, introduction, microsatellites, parasitoid

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In classical biological control, predators, parasitoids and diseases of invasive alien pests are imported from the native range of the invader in an attempt regulate its population size. Although introductions and founder effects are thought to play an important role in population differentiation and even speciation (e.g. Barton & Charlesworth 1984), the genetic effects of biological control introductions on biological control agents have received little attention. *Aphidius ervi* is an important biological control agent of aphids, and has been introduced from its native range in Eurasia to North and South America and Australia (Angalet & Fuester 1977). Additionally, *A. ervi* is a model for the study of parasitoid ecology, behaviour, and physiology, and host–parasitoid interactions (e.g. Sequeira & Mackauer 1992; Henter 1995; Du *et al.* 1996; Losey *et al.* 1997; Hufbauer & Via 1999; Ives *et al.* 1999; Falabella *et al.* 2000). We developed a suite of microsatellite loci for *A. ervi* to: (i) explore the genetic effects of the introduction to North America; and (ii) facilitate detailed studies of this model species' population structure, dispersal, and host use.

*A. ervi* were obtained from three sources: the Koppert Biological Systems laboratory colony in the Netherlands, and clover and alfalfa fields in Tompkins Co., NY. Wasps were killed by freezing at –80 °C. DNA was extracted using the DNeasy™ Tissue Kit (Qiagen) with the following modifications. A total of 0.065 g of *A. ervi* were used as starting material. The first four steps of the protocol for animal tissues (DNeasy™ Tissue Kit Handbook, April 1999, Qiagen) was scaled up 1.5 times. Genomic DNA was eluted with 200 µL buffer AE after incubating for 5 min at room temperature.

*A. ervi* genomic DNA was digested with *ApoI* and *BstYI*. The genomic library was constructed by ligating size-selected fragments (700–1600 bp) into pUC 19 that had been digested with *Bam*HI and *Eco*RI and gel-purified. Vectors were transformed into competent *Escherichia coli* cells (Life Technologies Max Efficiency DH5α), and plated on LB/ampicillin agar. Colonies were lifted with Magna Graph membranes (Osmonics, Inc.) and screened with a <sup>32</sup>P-labelled probe of pooled oligonucleotides (AC and AG dimers and all possible trimers excluding homopolymers). This screening yielded 168 clones with positive signals. A total of 38 were amplified with M13 forward or reverse primers and sequenced with a BigDye Terminator Cycle Sequencing Kit and an ABI 377 automated sequencer (PE

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**Table 1** Characteristics of 11 microsatellite loci isolated from *Aphidius ervi*, including locus name (clone no.) and GenBank Accession no., primer sequences, PCR annealing temperature, repeat motif in cloned allele, and size of the sequenced allele. The number of alleles found in 240 diploid (female) individuals, the size range of the amplified alleles, observed heterozygosity and expected heterozygosity are reported for six loci. The top primer was dye-labelled for visualization

Locus (Accession)	Primer Sequences (5'–3')	$T_a$ (°C)	Repeat of cloned allele	Size (bp)	No. of alleles	Size range (bp)	$H_O$	$H_E$
4 (AF336990)	CGAGACGAAGACAGACTGGTG TGCCCGCAATTTCTCATAAC	50	(AAC) <sub>26</sub>	279	37	204–342	0.64	0.82
11 (AF336991)	ATGCGTCGCGGGATAAC TGAACATAAAACACGAGGGAAATC	50	(TC) <sub>12</sub>	138	2	132–138	0.11	0.10
47a (AF336992)	GATGCAAAACACTATAACCACTT TGCCACAATATTCACAACATCA	51	(GTT) <sub>7</sub>	292	9	286–316	0.53	0.55
47b (AF336992)	TGGATGCGACGGATGTGGT TGCAATGTATCAAGCAACAAGTGG	54	(GTT) <sub>8</sub>	166	—	—	—	—
51 (AF336993)	CGTAAAATAACACGTCCTGGTA TTTTTGTGTGAATTATGTTGTTGA	50	(GTG) <sub>6</sub>	299	6	287–302	0.47	0.64
74 (AF336994)	TCACGATCTCTTCTTGCAACAC TGTCACCACGAATAGCATCACC	52	ACCACG(ACC) <sub>5</sub> ACG(ACC) <sub>2</sub>	127	6	124–139	0.46	0.56
78a (AF336995)	ACGTGCTGTATCACCAGTTGC CACCCAGTGCCATACGAATAC	51	(CAA) <sub>7</sub>	125	6	119–134	0.54	0.55
78b (AF336995)	GTATGGCACTGGGTGGGTCTC ACCGATAGTATTTTGTCCAACACC	52	(CAA) <sub>7</sub>	193	—	—	—	—
96 (AF336996)	TTCATCTCGGTTTATTCGTTTTG CAGATCGTTAAAGCTCACTGTGT	50	(TA) <sub>7</sub>	149	—	—	—	—
107 (AF336997)	ACTCTAGAGGATCCTTTGTGTCG AATAAAAATAAAATACCTGGTGACG	52	(GA) <sub>10</sub>	231	—	—	—	—
129 (AF336998)	GCATCATCGTCCACGTTCTACAA ACTCTGGGATGTTGGGGTTTGA	53	(CAA) <sub>55</sub>	295	—	—	—	—

Applied Biosystems; GenBank accession numbers AF336990–AF337032). Primers were designed for 14 loci, 11 of which produced reliable amplification products from nine clones (Table 1). Dye-labelled primers were ordered for six of these to assay levels of variability (Table 1). These six primer pairs were also tested for utility on 17 other species of *Aphidius*, but not assayed for variability (Table 2).

Microsatellite loci were amplified using polymerase chain reaction (PCR) Express thermocyclers (Hybaid) in 10 µL reactions containing 1 µL genomic DNA, 1× PCR Buffer (20 mM Tris-HCL, pH 8.4, 50 mM KCl), 2 mM MgCl<sub>2</sub> 0.2 mM each dNTP, 2 pmol of each primer, 0.5 units *Taq* polymerase (Life Technologies), and 0.1 µL TaqStart antibody (Clonetech). Amplification cycle conditions consisted of approximately 1 min at 90 °C, and then 35 cycles of 50 s at 95 °C, 1 min at annealing temperature (Table 1), 1 min 30 s at 72 °C, and then a final extension step for 45 min at 72 °C. Reactions were held at 0–4 °C before separation in an ABI 377 automated sequencer.

Allele sizes were scored by comparison with internal size markers using GENOTYPER software (PE Applied Biosystems). Expected heterozygosities ranged from 0.10 to 0.82 (Table 1). Only two of the six loci (11 and 47) conform to Hardy–Weinberg expectations when tested with the

**Table 2** Utility of six microsatellite primer pairs on other relatives of *Aphidius ervi*. + indicates strong products of correct size unless otherwise noted, w indicates weak products, – indicates reaction failed. Products were not sequenced

Species	Locus					
	4	11	47a	51	74	78a
<i>A. absinthii</i>	+	+	+	w	+	+
<i>A. cingulatus</i>	w	+	w	+	+	+
<i>A. colemani</i>	+	–	+	–	+	w
<i>A. eadyi</i>	+	+*	+	–	+	w
<i>A. matricariae</i>	w	+†	+	–	+	w
<i>A. microlophii</i>	+	+	+	+	+	+
<i>A. nr smithi</i>	+	–	+	+	w	+
<i>A. picipes</i>	–	+*	+	+	–	+
<i>A. pisivorus</i>	+	+	+	+	+	w
<i>A. rhopalosiphii</i>	+	+	+	+	+	w
<i>A. rosae</i>	+	+*	+	+	+	+
<i>A. rubi</i>	+	w	+	+	+	+
<i>A. setiger</i>	w	+†	w	+	w	+
<i>A. smithi</i>	+	+	+	+	+	+
<i>A. sonchi</i>	+	–	+	w	+	+
<i>A. urticae</i>	+	+	+	+	+	+
<i>Diaeretiella rapae</i>	+	+	+	+	w	w

\*PCR product too large; †two bands produced.

probability test of GENEPOP (<http://wbiomed.curtin.edu.au/genepop/index.html>; Raymond & Rousset 1995). The deficit of heterozygotes could be due to the presence of null alleles. Alternatively, the parasitoid's association with temporary agricultural habitats might lead to violations of the assumptions inherent to Hardy–Weinberg (e.g. no migration). Additionally, the deviations could be an artefact of our sampling strategy. That is, we could have created an apparent heterozygote deficit by sampling structured subpopulations across which allele frequencies at these loci are differentiated.

The primer pairs for these six loci worked quite well on closely related species (Table 2), suggesting they may prove useful for ecological and population genetic studies in species other than *A. ervi*.

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