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PRIMER NOTE

Trinucleotide microsatellite loci for the zebra mussel *Dreissena polymorpha*, an invasive species in Europe and North America

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Abstract

The high mutation rate at microsatellite loci can supply important demographic information on founder events and range expansion in an invasive species such as the zebra mussel *Dreissena polymorpha*, following its initial introduction. In order to facilitate studies into the colonization patterns of this species in new habitats in Europe and North America, five trinucleotide microsatellite loci were isolated from a partial DNA library. Allelic diversity at all described loci was high, ranging from 20 to 35 alleles per locus. Homologous loci were not amplified in a second related invasive species, *Dreisenna bugensis*, using the primers developed here.

Keywords: *Dreissena polymorpha*, invasive species, primer note, trinucleotide microsatellites, zebra mussel

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Maruyama & Fuerst (1985) pointed out that it was possible to detect if a population had experienced a recent reduction in effective population size by comparing the allele numbers at a locus with the heterozygosity within a population. The former decreases faster than the latter after a bottleneck. However, measuring population expansion and bottlenecks in a population's history has been difficult to test until the advent of microsatellite markers. The large number of alleles at a microsatellite locus allows the statistically robust computation of heterozygosity expectations at a locus at mutation-drift equilibrium (Cornuet & Luikart 1996; Luikart *et al*. 1997). It is now possible to practically examine the dynamics of invasion in a population undergoing range expansion or contraction.

The zebra mussel *Dreissena polymorpha* is a successful invader of freshwater bodies in Europe and, more recently, in North America. Certain life history traits, such as byssal thread attachment and a planktonic veliger stage, have facilitated its spread by shipping through these water bodies. The species has been undergoing range expansion in Europe for approximately 300 years (Kinzelbach 1992) and in North America since 1986 (Hebert *et al*. 1989). Using microsatellite markers, it is now possible to study the demographics of this invasion. This paper describes the isolation of such markers, which may be used for this purpose.

Microsatellite isolation followed the approach of Strassman *et al*. (1996); with modifications. Zebra mussel DNA was extracted from 10 mg of frozen gill tissue following the methods of Winnepenninckx *et al*. (1993) and Claxton *et al*. (1997). DNA from 10 individuals was pooled and restricted overnight with *Rsa*I and *Hae*III. Restricted DNA in the 300–1000 size range was isolated from an agarose gel using a silica-bead based method (Pharmacia) and ligated to the *Eco*RV site of a plasmid vector, PZeRo 2.2 (Invitrogen) following manufacturer's recommendations. The ligated plasmids were then electrotransformed into an *Escherichia coli* host, Top10F′ (Invitrogen), using a Biorad Genepulser.

Approximately 50 000 colonies were screened for microsatellite-containing sequences. The colonies (fixed on nylon membrane; Zetaprobe, Biorad) were first probed overnight with AAT_{10} and $AAAT_6$, end-labelled with [γ^{33}P]-ATP (ICN), at a hybridization temperature of 50 °C. Following autoradiography, these same membranes were then probed with a second group of end-labelled

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Table 1 Description of primer sequences used to amplify trinucleotide microsatellite loci in the zebra mussel *Dreissena polymorpha*. Reaction conditions are described in the text. Numbers of alleles and observed heterozygosity values were derived from a survey of 12 populations (n = 648) from the North American Great Lakes

Locus name	Primer sequence 5′–3′	Core Sequence of cloned DNA	Size of cloned allele	Size range (bp)	Denaturing Temp.	No. of cycles	No. of alleles	$H_O/*H_E$	GenBank Accession no.
Dpol A6	TGCCGGTCTAATAATAGAGTTAAC GTGATTGTGTATCTGCTATAAACC	$[ATT]_3ACT[ATT]_2ATC$ $[ATT]_8[ACT]_3[ATT]_3$	301	301–358	58 °C	27	20	0.658/ 0.893	AF317427
Dpol B6	CGTTGTTCAAGCAATAAGAAAGAC CGTGTGCTCATGTTTCCTCC	$[AAT]_{28}$	305	256–352	61 °C	25	32	0.611/ 0.935	AF317426
Dpol B8	TTTGAACATTAAACATTTGTCCAC GTCTAGTGCTACAGTTG	$[AAT]_{20}$	170	127–244	50 °C	30	35	0.619/ 0.931	AF317428
Dpol B9	TTGACAATATCCTGTCTAATGGTT CGTCTACAAGTTTATGTG	$[AAT]_{21}$	240	217–292	57 °C	30	20	0.741/ 0.909	AF317429
Dpol C5	GCACTGTCAACGTCACACTTTTGC CTTGCTAACAGCTCGGTTGTATC	$[AAT]_{16}$	213	187–247	63 °C	27	20	0.819/ 0.891	AF317430

*H_O/H_E, observed and expected heterozygosity, respectively.

oligonucleotides, AAC_9, AAG_8 and $GATA_7$ at a hybridization temperature of 55 °C.

Plasmid DNA from positive colonies was isolated, purified (plasmid extraction columns; Qiagen) and their DNA sequences were determined using a cycle-sequencing reaction using dye-terminated primers. The sequencing reactions were read using an ABI 377 automated sequencer and the resultant chromatograms were used to identify microsatellite loci with a suitable flanking sequence on each side of the tandem repeats. Flanking polymerase chain reaction (PCR) primers were designed for 16 microsatellites using GENERUNNNER 3.0 (Hastings Software Inc). Putative loci were amplified in 10 µL volumes with the following components; 20–100 ng DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.0 mM $MgCl_2$, 0.02 mM each dNTP (Promega), 0.2 pmoles of primer and 0.5 U of *Taq* polymerase (Promega). Amplification was performed in a Stratagene robocycler; one cycle of denaturation at 95 °C for 5 min, followed by 25–30 cycles of 95 °C for 30 s, an annealing temperature range of 48–65 °C for 30 s and extension at 72 °C for 1 min.

Following primer extension, 2 µL of 6× sucrose loading buffer (Sambrook *et al*. 1989) was added to the reaction mixture; 3 µL of this mixture was assayed by electrophoresis in 5% nondenaturing polyacrylamide (19:1 acrylamide:bis-acrylamide) run with 1× TBE buffer (Sambrook *et al*. 1989; 7.8 V/cm). Gel dimensions were 20 cm wide, 16 cm long and 1 mm thick. Microsatellite alleles were visualized by silver staining (Naish & Skibinski 1998).

A total of 65 positive colonies were sequenced. Of those, five were $(AAAT)_n$ repeat microsatellites, 25 were $(AAT)_n$, 15 were $(AAG)_n$, 15 were $(AAC)_n$ and two were $(GATA)_n$. The remaining were false positives or second copies of certain loci. Of the 16 primer pairs designed, five amplified polymorphic repeats, four had null alleles at the target loci (verified by sequencing) and seven amplified monomorphic loci.

The primer sequences, PCR product sizes, average heterozygosities and annealing temperatures for the five polymorphic loci are given in Table 1. Loci derived for *D. polymorpha* did not amplify homologous loci in *D. bugensis* (annealing range: 45–65 °C), a second invasive species in the Great Lakes. Six sets of PCR primers have been derived for microsatellites occurring in the latter species (Wilson *et al*. 1999).

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