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PRIMER NOTE

Eleven polymorphic microsatellite loci for the salmonid trematode *Plagioporus shawi*

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Abstract

We isolated and characterized 11 microsatellite loci from *Plagioporus shawi*, a parasitic trematode of salmonid fishes (*Oncorhynchus* spp.). Extensive polymorphism (expected heterozygosities ranging from 0.476 to 0.981 and number of alleles from 10 to 55) was found in a sample of 94 trematodes from 24 infected cutthroat trout (*Oncorhynchus clarki*) individuals. Nine of the 11 loci will be useful for future genetic studies on within population dynamics of *P. shawi*.

Keywords: microsatellites, *Oncorhynchus*, *Plagioporus shawi*, salmon, trematode

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Plagioporus shawi is a digenean trematode that matures in the intestines of salmonid fishes (*Oncorhynchus* spp.). This trematode has a distribution that ranges west of the Cascade Mountains from northern California to northern Washington, with reports in eastern Washington and western Idaho, USA (Hoffman 1999). The life cycle of *P. shawi* occurs in freshwater streams and involves a snail (first intermediate host) and an aquatic arthropod (second intermediate host) (Schell 1975). Fish becomes infected upon ingestion of an infected second intermediate host. Mitochondrial data indicate that this parasite is highly subdivided among freshwater drainages (Criscione & Blouin 2004). Microsatellites will be useful for elucidating fine scale structure and transmission dynamics within streams.

DNA was extracted using a pooled sample of seven adult trematodes obtained from a cutthroat trout (*Oncorhynchus clarki*). We used a standard phenol–chloroform procedure (Sambrook *et al*. 1989). Microsatellite isolation followed the protocol of Cabe & Marshall (2001) with minor modifications. Briefly, a degenerate oligonucleotide-primed-polymerase chain reaction (DOP-PCR) was used to produce random genomic fragments of suitable size (200–2000 bp) for cloning. See Cabe & Marshall (2001) for cycling and reaction conditions. Fragments from the DOP-PCR were enriched with either a $(GATA)_8$ probe that was biotinylated on the 3′-end or a 5′-biotinylated $(CA)_{12}$ probe that had a 3′-inverted dT. Hybridization of the probe to the genomic fragments followed that of Cabe & Marshall (2001) except the temperature was reduced to 60 °C. Streptavidin-coated magnetic particles (Promega) were used to capture the biotinylated probes. Magnetic particles (0.3 mg) were first washed twice, each in 1 mL of 1× SSC and then 6× SSC. The hybridization reaction and 35 μL of 6× SSC were added to the beads and moderately agitated for 20 min. Washes were the same as Cabe & Marshall (2001), but the final two washes were at 60 °C. Repeat-enriched DNA was eluted and made double-stranded following the methods of Ardren *et al*. (2002). Two enrichments were done for the $(GATA)_8$ probe, whereas one enrichment was used for $(CA)_{12}$. PCR fragments were cloned using Invitrogen's TOPO TA Cloning Kit. Colonies were screened for repeats using the methods of Cabe & Marshall (2001).

Several sequences of clones from the $(GATA)_8$ enrichment had flanking sequences that matched on the one end of the repeat motif, but did not match on the other end. These clones may have originated as amplification artefacts by recombination of different PCR fragments (for a proposed mechanism see Koblížková *et al*. 1998). Thus, we were not able to determine cloning efficiency. However, we were able to design primers that worked successfully for five $(GATA)_n$ loci (Table 1). To avoid amplification artefacts in the $(CA)_{12}$ enrichment, we added a 3′-inverted dT to the probe (Koblížková *et al*. 1998). We sequenced 46 positive $(CA)_n$ clones (out of 94 screened) and observed no PCR

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Table 1 Microsatellite primer pairs for *Plagioporus shawi* ($n = 94$)

Locus	Primer sequence (5′–3′)	T_a (°C)	Clone repeat	Clone size (bp)	No. alleles	H_O	H_E
PLSHM02	GATCCAACCGACCAATGT ^{F}CTATATTGTGTCTGTCGATC	56	$(GATA)_{14}$GTTAGATG GATAGTTA$(GATA)_{14}$	200	55	0.862*	0.977
PLSHM12	GATCGAACATCCCTCAAG ^{H}CGTTCGTATGACTAAAATCG	60	$(GATA)_2(GACA)_2(GATA)_{44}$	249	54	0.872*	0.981
PLSHM26	GAACTTTTACAATAGATAGTC ^{H}CTCATATACTTTTCCAAGC	54	$(AGTT)_4(AGAT)_5$TGAT $(AGAT)_4$GGAT$(AGAT)_2$	129	22	0.809	0.84
PLSHM41	^{F}ATGATGAATTCCCAAAAGG TCGGAATTTCGCACGTAC	48	$(TAGA)_8$TAAA$(TAGA)_2$TAGG $(ATAG)_4$ATTG$(ATAG)_8$	181	21	0.489	0.476
PLSHM48	GCTTACATACAGATACAATTC ^{N}CGGATAAACATCAATCTTGC	48	$(GATA)_{12}$	123	35	0.968	0.961
PLSHD04	^{F}ACATCGTTGGTCAGAAGC GACAACTGATGAGATGCG	58	$(CA)_{11}$	172	10	0.777	0.813
PLSHD09	TTTTGTTCTGTCTTTGGATG ^{H}ATGACCTTGTAGTGAATCG	52	$(CA)_{13}$	154	30	0.915	0.954
PLSHD13	GTTTTGTTGGTGCTAGTG ^{F}TATCTTGCGGTATTTGTT	54	$(AC)_{12}$	297	14	0.809	0.855
PLSHD36	^{H}CATTAAAATTAAACCCTACCTG GGTCCAAAGTCCAATCCT	54	$(CA)_{17}$TACT$(CA)_3$	163	28	0.926	0.902
PLSHD43	^{N}AAGCTAAGCGTTGAAGTC GTAACATTGGTAACTTGCT	52	$(CA)_{11}$	203	25	0.894	0.913
PLSHD47	TCATGGGCATACAATAATCC ^{N}CTATGAATACGTTCCTTGG	52	$(CA)_{17}$	203	17	0.787	0.828

T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity. ^{F,H,N}refer to the 5′ fluorescent-labelled primer (6-FAM, HEX, NED). *Statistically significant deviation from Hardy–Weinberg equilibrium ($P < 0.01$). GenBank Accession nos: AY894895–AY894905.

artefacts. All sequences had $(CA)_n$ motifs and six were duplicates. We designed primers for 15 clones and found six $(CA)_n$ loci that worked consistently (Table 1).

We screened 94 individuals from Cascade Creek, Oregon, USA for polymorphism at the 11 loci shown in Table 1. Because *P. shawi* is hermaphroditic, there is a potential for allelic contamination from outcrossed matings if whole adult worms (2 mm long) are used. Fertilized eggs in the uterus and stored sperm in the seminal receptacle lie anterior to the two tandem testes. Thus, worms were cut between the two testes and only the posterior end was used for extractions. Worm tissue of individuals was placed in 200 µL of 5% Chelex containing 0.2 mg/mL of proteinase K, incubated for 2 h at 56 °C, and boiled at 100 °C for 8 min. PCR amplifications were performed with 25 µL reactions [4 µL of extraction supernatant, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 µM each primer, 0.5 U *Taq* DNA polymerase (Promega), 95 °C for 3 min; 35 × (94 °C for 45 s, annealing temperature for 30 s, 72 °C for 45 s), 72 °C for 7 min]. *PLSHM12* and *PLSHM41* had 38 cycles. We also ran control reactions using extracted DNA from cutthroat trout, steelhead trout (*Oncorhynchus mykiss*), and coho salmon (*Oncorhynchus kisutch*). Fish controls were negative. PCR products were run on an ABI 3100 with GENESCAN software and scored using GENOTYPER (Applied Biosystems). ROX500 size standard was used for all loci except for *PLSHM02* and *PLSHM12*, which used Mapmarker 1000.

We tested for Hardy–Weinberg equilibrium (HWE) and genotypic disequilibrium for pairs of loci using GENEPOP version 3.4 (Raymond & Rousset 1995), raising the default Markov chain parameters fivefold. Significance was determined at $P = 0.05$. A sequential Bonferroni method was used to correct for multiple tests (Rice 1989). Genotypic linkage was not detected. Only *PLSHM02* and *PLSHM12* showed significant deviations from HWE (Table 1). Low frequency null alleles may be responsible for these deviations. Alternatively, we suspect that allelic dropout or partial null amplification was a problem with these two highly polymorphic loci as indicated by large variations in peak intensities for several alleles within and among individuals. Thus, ambiguous scoring may have resulted in homozygous excess. All other loci showed substantial polymorphism and could be scored reliably (Table 1). These loci provide the first set of microsatellite markers isolated for *P. shawi*.

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