Conserv Genet (2008) 9:973–979
DOI 10.1007/s10592-007-9417-9

TECHNICAL NOTE

Development of new microsatellite primers for green and white sturgeon

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Received: 9 August 2007 / Accepted: 20 August 2007 / Published online: 5 September 2007
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Abstract Sixty-eight primer sets for microsatellite loci were developed from microsatellite motif enriched genomic libraries of pooled DNA from the polyploid green and white sturgeon (*Acipenser medirostris* and *A. transmontanus*). Four individuals from each species were screened for polymorphism at these loci. Forty-eight loci amplified in both species, and some exhibited species-specific amplification for white or green sturgeon (8 and 12 loci, respectively). The number of alleles per locus ranged from one to 12. At least 68% of the green and 65% of the white sturgeon loci we developed are polysomic.

Keywords Microsatellite · Sturgeon · *Acipenser* · Primer

White sturgeon (*Acipenser transmontanus*) and green sturgeon (*Acipenser medirostris*) are long-lived anadromous fishes inhabiting estuaries and bays along the western coast of North America. Like most sturgeon worldwide, white and green sturgeon populations are in decline in many portions of their distribution due to overharvest, habitat degradation, and barriers blocking access to spawning areas (Duke et al. 1999).

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The US Fish and Wildlife Service (USFWS) listed the Kootenai River white sturgeon population as endangered under the Endangered Species Act in 1994 (USFWS 1994), and the US National Marine Fisheries Service (NMFS) listed the Southern green sturgeon distinct population segment as threatened in 2006 (NMFS 2006).

Small population size and decreased gene flow have reduced genetic diversity in the Kootenai River white sturgeon populations (Rodzen et al. 2004) and perhaps in the Sacramento River green sturgeon populations (Adams et al. 2006). Protection of genetic diversity plays a prominent role in the recovery plan for the Kootenai River white sturgeon (USFWS 1999) and will likely be significant in green sturgeon recovery. However, microsatellite development has been complicated in these species due to their polysomic genome (Ludwig et al. 1998, Rodzen and May 2002; Israel et al. 2004; but see McQuown et al. 2000; Welsh et al. 2003). We describe the development of additional microsatellite markers necessary to monitor and protect the genetic diversity among and between populations of these fish (Wirgin et al. 1997, Israel et al. 2004).

Microsatellite loci were isolated per Jones et al. (2000) by Genetic Identification Services Inc. (GIS). High molecular weight DNA was extracted from muscle tissue of an equal mix of both species using chloroform/phenol/isopropanol extraction (Sambrook et al. 1989). The DNA was digested with the blunt-end restriction enzymes *Rsa*I, *Hae*III, Bsr B1, *Pvu*II, *Stu*I, *Sca*I, and Eco RV. A *Hin*dIII site was attached to the 5 ′ end of DNA fragments ranging from 300 to 750 bp. The fragments were subjected to magnetic bead capture using 5 ′-biotinylated oligonucleotides, Biotin-AAC(12), Biotin-CAG(10), Biotin-CATC(8) and Biotin-TAGA(8), per the manufacturer's protocol (CPG, Inc). Captured molecules were amplified, digested with *Hin*dIII to remove the adaptor sequences, and ligated

Table 1 Characterization of 66 microsatellite loci in green and white sturgeon (*Acipenser* spp.): library species, primer sequences, repeat motif, GenBank Accession numbers, number of alleles and allele size range in *A. medirostris* and *A. transmontanus*, and library clone size in base pairs.

Identifier	*Primer Sequence (5′-3′)*	*Motif*	GenBank accession no.	Total alleles in *A. medirostris* (maximum per individual)	Allele size range (bp)	Total alleles in *A. transmontanus* (max. per individual)	Allele size range (bp)	Sequenced clone size (bp)
AciG2	TCGTGCATCAATAAGAGTCCA CTGCGAGACGGAGTGTCATA	AAAC	EF639104	NS[a]		5 (3)	250–300	280
AciG 4	GAGGGCCAAACAAAAGGTCT GATAAAAGCCAGGGGAGAGG	AAAC	EF639105	3 (3)	280–320	3 (3)	280–320	191
AciG 9	TCAATGACGCCTTTGTTCAG AGACCAGGACACAGGGATTG	CATC	EF639106	4 (3)	280–320	3 (3)	280–320	308
AciG 13	CTCGCATCACCTCAAACTCA TTCCGGGAATTTTCTTTTCC	TAGA	EF639107	4 (3)	360–400	2 (3)	380–400	313
AciG 17	AGACTGCAGTGCTGCTCAAA GCTGAGCTTCTGCAATAGGG	TAGA	EF639108	3 (3)	120–160	5 (5)	100–160	185
AciG 18	AGGGAGGCTTTCACTTTTCC ATTTGGAATTTGGGCAGATG	TAGA	EF639109	4 (4)	380– >400	2 (2)	320–360	325
AciG 22	TACCTTTGGGCTGCTCATTT GGCAGTGAGGAATCTGTCGT	AAAC	EF639110	2 (1)	360–400	1 (1)	380–400	395
AciG 24	GGTGATCACGGGGTTGATAC CAGGAAATGCAGGACACAGA	AAAC	EF639111	4 (4)	200–300	3 (3)	240–230	235
AciG 27	TCCTGCCACAATACCACAGA CCCCGCTCTTGAAAATAACA	AAAC	EF639112	2 (1)	320–340	3 (2)	320–340	359
AciG28	CTTCCCAAAACGCAAGTTGT AACGGTGCACAAGAAAGGTC	AAAC	EF639113	4 (4)	360–400	NA[b]		407
AciG 29	TGCATGTTCAGATGAATAGAGTGA CTGCCGTGTGAAGAACAAGA	AAAC	EF639114	1 (1)	300	NA		281
AciG 30	CAGCGAGTAAATGCATGGAA GAGAGTACGAGGAGCGGAGA	AAAC	EF639115	7 (7)	320–380	6 (6)	300–380	381
AciG 32	CCATCCCTGCATCATCTTCT TCCAGGACTGTGTTTCAGCA	AAAC	EF639116	5 (3)	140–160	NS		158
AciG 33	GCCAAGAATCGGAGGATACA CGCGGCTGTCAGTATCAGTA	AAAC	EF639117	2 (1)	380–400	NS		396
AciG 34	AAGGCAGATTTGAACACCAGA AGAGACACAGAGCGCAGTCA	AAAC	EF639118	3 (3)	280	NS		206
AciG 35	CAACAACAGTGGCAGGGTTA ACCCTTCCAAAGCAGGAAAT	TAGA	EF639119	11 (9)	230–400	9 (7)	230–300	239

Table 1 continued

Identifier	*Primer Sequence (5′-3′)*	*Motif*	GenBank accession no.	Total alleles in *A. medirostris* (maximum per individual)	Allele size range (bp)	Total alleles in *A. transmontanus* (max. per individual)	Allele size range (bp)	Sequenced clone size (bp)
AciG 36	CGCTGTGCTACGTTTGTTGT ACAGCGGCAATAAGTCAAGC	TAGA	EF639120	4 (4)	340–400	4 (3)	340–380	350
AciG 38	AATCGAGGTGAGCGAGAGAG TGAAACACCACGTGTTAGAGACA	TAGA	EF639121	9 (9)	180–320	12 (12)	180–320	201
AciG 39	CACCCAGTCCAGGATTCACT CTGATTTTATAGAGAAGACAGCTACA	TAGA	EF639122	4 (4)	180–220	NS		200
AciG 40	TCCGGTCAAGAGTCCAGAAG TTCGTGCTCACAACCATCTC	TAGA	EF639123	2 (2)	340–360	NA		224
AciG 41	GCTGCAGTCCAGTTTTCTCC ATCTGCTGGAGCGTGAATTT	TAGA	EF639124	NA		4 (3)	160–180	168
AciG 42	ACAATTTAAGTGCCGGTTGC ACTCGACCTTTCGGTTCTCA	TAGA	EF639125	1 (1)	>400	NA		396
AciG 44	CAGGGGCGCTATAAGAGGAT ATGATGGGGAATGCTACTGC	TAGA	EF639126	5 (4)	340–400	3 (3)	340–400	405
AciG 46	AAGTTTTGCCCACTGAATGC GGTTCCATATGGGTGGCAAT	TAGA	EF639127	5 (5)	380–400	3 (3)	340–380	380
AciG 47	GGGGAAGCAATGTTTTCAGA TGCTGATGGCTCACATTAGG	TAGA	EF639128	1 (1)	390–410	2 (2)	390–410	422
AciG 48	TTGCCTTCAAGATGAACAGG AGCTGAATCTGCACGGAAGT	TAGA	EF639129	6 (6)	280–420	7 (4)	320–400	411
AciG 49	CGGAGACCTCCTGGTTACAA TTCTTGTGTTCCCATCCACA	TAGA	EF639130	13 (9)	220–360	NA		292
AciG 51	GAGCTTACAGTCCGGAGCAC GGGTATTGGAAACACAAGCAA	TAGA	EF639131	NS		5 (5)	220–270	244
AciG 52	TTATCCCGGTCCTGAAACAC TGAGGAGGAGACACTTGAAGC	TAGA	EF639132	11 (6)	160–220	9 (7)	160–220	187
AciG 53	TTAAACAGCACAGGCAGTGG GCTTTGTTGCCTCAAAGGAG	TAGA	EF639133	5 (4)	200–280	3 (3)	200–260	241
AciG 54	CGTTGTAAGGCGAAACACAA GGTGGCTCTGTCGTTACCAT	TAGA	EF639134	2 (2)	>400	NA		425
AciG 56	TGTTGCACTGTGTGCTGTGT ACCCAAATCCTGCAGACAAG	TAGA	EF639135	3 (2)	280–300	2 (2)	280–320	294

Table 1 continued

Identifier	*Primer Sequence (5′-3′)*	*Motif*	GenBank accession no.	Total alleles in *A. medirostris* (maximum per individual)	Allele size range (bp)	Total alleles in *A. transmontanus* (max. per individual)	Allele size range (bp)	Sequenced clone size (bp)
AciG 57	CTGGATAAGGGCGTCTGCTA AGATTTGCCAGAATCGCTTG	TAGA	EF639136	NS		2 (2)	160–180	314
AciG 60	ACCGAAACTGGAGACACACC GGATGAGAGGCTTTCTTTGC	TAGA	EF639137	4 (2)	400–440	2 (2)	400–440	395
AciG 61	TGCCGTTGGATAAATGGACT GCAGTGGAACCATAACAGCA	TAGA	EF639138	5 (2)	360–420	7 (4)	320–420	376
AciG 63	TTTGCCAAGGACTGGGTATC GCTGTTGACTTCCCCACACT	AAAG	EF639139	4 (4)	220–300	4 (4)	220–300	250
AciG 71	GCCATTTCAAATTGGGAGAA TGAATCAGGGGTGAGAAAGG	AAAT	EF639140	1 (1)	360	NS		371
AciG 74	AGGGAGAGTGCCAGTGTTTG TAGGGACATGTTCCACCACA	AAAG	EF639141	5 (3)	180–200	NS		187
AciG 75	AGCTCTGGTGGACATTCCTG GCCATGTGTTTTGCAAAGGT	AAAG	EF639142	NS		4 (3)	260–300	279
AciG 76	CAGCAAGGTAAACCGCTCTC GCGGCGATGTCTCTCATATT	CAGA	EF639143	4 (4)	320–360	3 (3)	320–360	330
AciG 88	CCCTCTTTGTCTTGCTACGC TGCTGATTGTGTGGTGGATT	AAAC	EF639144	3 (3)	140–160	1 (1)	140–160	157
AciG 92	CAGCCTTCTGCACACTTCAA GGTCGTGTTTGTGATGTTCG	AAAC	EF639145	2 (2)	220–260	2 (2)	220–260	268
AciG 93	CCAAATTGGGGAGAATGAGA GGGGAACGCATGTAGAAAGA	AAAC	EF639146	2 (2)	360–380	1 (1)	370–300	386
AciG 95	GGACAGCAGTGGCTTCCTAA TCTGGCAATTCACTGAAAACA	TAGA	EF639147	5 (5)	220–240	3 (3)	180–200	194
AciG 97	ATTCTGGTTTGATTCTTAGCC CGGGTTCTGAGATGGACTGT	TAGA	EF639148	3 (3)	320–360	3 (3)	320–360	333
AciG 105	CCATTGGGAAGCAAAAGTGT CGCAGGGGCATCTATCTATC	TAGA	EF639149	6 (5)	340–360	NS		357
AciG 107	TGCGTGTCACACTAATTTCCA ACCTCTCCCTCTTCCAGCTC	AAAC	EF639150	2 (2)	200–210	2 (2)	200–210	199
AciG 110	GATGCTTGCTCTTGCTTATCG GCAGGTGCACTGCAAGTCTA	TAGA	EF639151	9 (4)	280–340	5 (4)	280–340	276

Table 1 continued

Identifier	*Primer Sequence (5′-3′)*	*Motif*	GenBank accession no.	Total alleles in *A. medirostris* (maximum per individual)	Allele size range (bp)	Total alleles in *A. transmontanus* (max. per individual)	Allele size range (bp)	Sequenced clone size (bp)
AciG 113	AGCATTGCAAAAGCCGATAC ATGGGGATAACTGCATGGAA	AAAC	EF639152	2 (2)	300	1 (1)	300	310
AciG 114	TCCAACCCCAAGGTATTCTG GAACGGGCCAAAATGAAATA	TAGA	EF639153	NA		4 (3)	240–260	249
AciG 120	CGCTGTGCTGGTCTTTACAC CAGCAGTTGTGGAAGCAAAA	TAGA	EF639154	4 (4)	320–360	1 (1)	320–360	356
AciG 121	CGCTATCCCAGGACGATTTA TCTCAGTGAAAGCAGGGAAA	AAAC	EF639155	4 (3)	180–200	2 (2)	180–220	193
AciG 124	GCCAAGCTTACACTAACACATACA TAACGATGCACGGATAGCAG	CAGA	EF639156	NS		3 (3)	250–300	260
AciG 137	ACGAGCGGGTTAAAGAATGA CCTGCTTCCTAAACCACCAA	CAGA	EF639157	3 (3)	190–240	3 (3)	320–360	197
AciG 140	TTCAGACCTCCGTCCTTTTG TCTTGCCGTCACACTTTGAG	CAGA	EF639158	6 (5)	160–180	6 (6)	160–190	177
AciG 142	TGCAGTAAAATAGGGAGCAGA CCCATCCTGGCAGTAACACT	CAGA	EF639159	8 (7)	260–320	5 (5)	260–320	305
AciG 153	GTTTCCACCTGCAGATCCAT TTCACAGCCCTCACACTCTG	CAGA	EF639160	9 (8)	160–240	6 (4)	180–260	251
AciG 157	GATCCACTCCCGGTTCACTA CCAAAGAGGTGCTTTTCCTG	CAGA	EF639161	2 (2)	300–320	2 (2)	300–320	326
AciG 159	CGCTGATTTTAGCCCTGGT TCGCTCTCAATGCTGATGTC	CAGA	EF639162	1 (1)	120	3 (3)	120–190	193
AciG 198	ACCATCCCCTCCCATATCTC TGGTTCTTAGCGGAGGAAGA	AAAT	EF639163	3 (3)	170–200	2 (2)	160–180	198
AciG 200	CAATCAGTTTGGACAAAAGTTCA GCCCTTTGAATCACGAAAAA	AAAT	EF639164	3 (3)	190–220	3 (2)	190–220	120
AciG 203	ACAATAGGGTGAGGCACCAG ATGCCATCGCAGTTTTGTCT	AAAT	EF639165	2 (2)	180–200	8 (3)	180–200	141
AciG 204	TGACATGCTATACAAATGTATTGTGG CCAGGGTTGAATACCGAAGA	AAAT	EF639166	4 (3)	140–170	4 (3)	150–170	188
AciG 205	CCGAGCTTTCAGATGCTGTT CCTTAAAGCCACTTTGGGTTC	AAAT	EF639167	3 (3)	180–220	3 (3)	180–220	145

Table 1 continued

Identifier	*Primer Sequence (5′-3′)*	*Motif*	GenBank accession no.	Total alleles in *A. medirostris* (maximum per individual)	Allele size range (bp)	Total alleles in *A. transmontanus* (max. per individual)	Allele size range (bp)	Sequenced clone size (bp)
AciG 207	GTCCTGTCCCATACCCACTG AGCAGGAGTTGACAGGGAGA	AAAT	EF639168	3 (3)	120–160	1 (1)	120	163
AciG 209	TTAGATTGCCGTGCTTTTCC CTGACTCGGTTTGAAGCACA	AAAT	EF639169	3 (3)	200–220	3 (3)	200–220	178
AciG 213	CTCCACCACAGCAGATCGTA CCTGTACTGCCCTGTAACACC	AAAT	EF639170	3 (2)	180–200	2 (2)	200–240	169
AciG 219	AAAACTCCCTGCTGCCCTAT CTGGGGTTGTTACTGGAGA	AAAT	EF639171	NA		3 (3)	140–200	117

[a] NS indicates non-specific amplification

[b] NA indicates no amplification

into the *Hin*dIII site of pUC19. The plasmids were then electroporated into *Escherichia coli* DH5α. Recombinant clones were identified by blue–white selection and were sequenced on an ABI 377 using ABI Prism Taq dye terminator cycle sequencing methodology. Before sequencing, regions of interest were amplified with 1 × BiolaseC Buffer, $MgCl_2$ (2.0 mM), dNTPs (0.2 mM each), 6 mM each primer (forward primer fluorescent-labeled), BiolaseC Taq polymerase (0.25 U), and 1.0 µl template DNA (0.2 ng/µl) in a 50 µl reaction. Cycling parameters were: 94°C for 3 min, 35 cycles of (94°C, 40 s; 55°C, 40 s; 72°C, 30 s) and a final 4 min extension at 72°C.

The DNA sequences were analyzed for tandem repeats using the program *mreps* (http://bioinfo.lifl.fr/mreps/). In total, 291 clones were sequenced, yielding 188 potentially useful sequences with microsatellite motifs and sufficient flanking sequences. After eliminating duplicates, 154 sequences remained for analysis. Primers flanking the microsatellites were designed with *Primer3* (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and synthesized by Integrated DNA Technologies Inc.

The 154 primer pairs were screened for consistent amplification with four Kootenai River broodstock (*A. transmontanus*) and four wild *A. medirostris* (two from Sacramento River and two from Klamath River). DNA was extracted using the Promega Wizard SV 96 Genomic DNA Purification System. The amplification mix consisted of 1.0µl template DNA (~5 ng/ul), 1X $MgCl_2$-free buffer (Promega), $MgCl_2$ (2.5 mM), dNTPs (0.2 mM each), 1 µl each of forward/reverse primers at 5 µM, Promega Taq DNA polymerase (0.075 U), and dH_2O to a 10 µl volume. PCR was conducted on a PTC 220 Dyad thermoycler (MJ Research, Inc.) at: 95°C for 90 s, 35 cycles of (95°C, 60 s; 56°C, 45 s; 72°C, 2 min) and a final 5 min extension at 72°C. PCR products were diluted with 5µl 98% formamide buffer, denatured at 95°C for 3 min, and chilled on ice for 2 min. Products were separated on a 5% denaturing polyacrylamide gel at 65 W for 45 min–90 min. The products were dyed with a Sybr-Green™ (Invitrogen)–agarose overlay (Rodzen et al. 1998), and then visualized with a FluorImager 595 (Molecular Dynamics).

This screening resulted in 66 primer pairs that produced consistently scorable alleles for one or both species. Primers were named *AciG* (*Aci* for *Acipenser* and G for the UC Davis Genomic Variation Lab), followed by a numerical designation. Repeat motifs and clone sizes were derived from the single clone sequenced by GIS for each microsatellite and could be from either *A. transmontanus* or *A. medirostris*.

Forty-eight loci amplified consistently in both species, while 12 amplified only in *A. medirostris* and 8 only in *A. transmontanus* (Table 1). The number of alleles observed at each locus ranged from one to 12. 19 (31.7%) of the loci

in *A. medirostris* and 19 (34.5%) of the loci in *A. transmontanus* showed one to two alleles; the remaining loci were polysomic. Some of those loci showing one to two alleles may show more alleles when tested against additional individuals. Due to the polysomic nature of these loci, we were unable to test for conformance to Hardy-Weinberg equilibrium, and the small sample size prevented estimates of heterozygosity. More extensive study of these loci will be necessary to estimate these parameters.

The markers described in Table 1 should provide ample resolution for detailed analysis of genetic diversity within and among *A. transmontanus* and *A. medirostris* populations. The high correlation of marker utility between these species suggests these markers may also prove useful in other sturgeon species. Although the polysomic nature of many of these loci precludes traditional population genetic analysis, conservation genetic studies can be completed using an alternative method of data analysis (Rodzen et al. 2004a, b; Israel, unpub. data).

Acknowledgements We thank the Kootenai Tribe of Idaho (Award #01633; subcontract from a grant to them from the Bonneville Power Authority) and CALFED (Award # ERP-02D-P57) for funding this work.

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