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Rapid development of polymorphic microsatellite markers for the Amur sturgeon (*Acipenser schrenckii*) using next-generation sequencing technology

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ABSTRACT. Anthropogenic activities have seriously impacted wild resources of the Amur sturgeon, *Acipenser schrenckii*, and more information on local and regional population genetic structure is required to aid the conservation of this species. In this study, we report the development of 12 novel polymorphic microsatellite loci using next-generation sequencing technology, and the genotyping of 24 individuals collected from a sturgeon farm. The results show that the mean number of observed alleles per locus is 6.6 (ranging from 2 to 17). Observed and expected heterozygosity values ranged from 0 to 0.958 and from 0.508 to 0.940, respectively. Not a single locus showed significant departure

from Hardy-Weinberg equilibrium and no linkage disequilibrium was observed among any pairwise loci. These highly informative microsatellite markers will be useful for genetic diversity and population structure analyses of *A. schrenckii* and other species of this genus.

Key words: Microsatellite; Polymorphism; Genetic diversity; *Acipenser schrenckii*; Next-generation sequencing

INTRODUCTION

Amur sturgeon (*Acipenser schrenckii*) is one of the most ancient fish in the world. In recent decades, anthropogenic impacts, such as water pollution, overfishing, and habitat deterioration, have caused widespread population declines of wild resources (Shmigirilov et al., 2007), and this species was listed in the Appendices to CITES at the 10th meeting of the Conference of the Parties in 1997 (Zhang et al., 2013a). However, artificial rearing of *A. schrenckii* is rapidly developing due to its high commercial value. A previous study of *A. schrenckii* mainly centered on its cultivation, breeding, and reproductive traits (Zhang et al., 2013b). The population genetic diversity and genetic structure of the species has also been investigated using the mitochondrial DNA cytochrome oxidase subunit I and microsatellite DNA markers (Zhang et al., 2013a). However, lack of sufficient polymorphic molecular markers has limited development of molecular phylogeny, population structure, and molecule-assisted selective breeding in this species. Therefore, isolation and development of additional novel polymorphic microsatellite or other molecular markers are necessary for analyzing genetic information in *A. schrenckii*. In this study, we isolated and developed polymorphic microsatellite loci for *A. schrenckii* using next-generation sequencing technology.

MATERIAL AND METHODS

Total RNA was extracted from liver tissue of *A. schrenckii* using Trizol (Invitrogen, USA), following the manufacturer protocol. Then, the cDNA library was constructed and sequenced using high-throughput Illumina sequencing technology. Simple sequence repeat (SSR) reads were identified from assembled sequences using the SSR Hunter software (Version 1.3) (Li and Wan, 2005). Primer pairs for selected microsatellites were designed using the Primer 5.0 software (Premier Biosoft International, USA). Genomic DNA of eight individuals of *A. schrenckii* was used to optimize amplification conditions and to screen for polymorphic SSRs.

To test the level of genetic polymorphism for these loci, fin tissue from 24 individuals of *A. schrenckii* from the Engineering and Technology Center of Sturgeon Breeding and Cultivation of the Chinese Academy of Fishery Science (Beijing, China) were sampled. Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen, Germany). PCR amplifications were performed in a 15-μL reaction volume containing approximately 1 μL template DNA, 1 μL of each primer, 4.5 μL H_2O, and 7.5 μL premix Taq DNA polymerase (Takara, Japan). The amplification conditions were as follows: 95°C for 5 min, 35 cycles at 94°C for 35 s, annealing temperature (Table 1) for 35 s, 72°C for 40 s, and a final extension at 72°C for 10 min, ending with a 4°C hold. Electrophoresis was conducted on non-denaturing 10% polyacrylamide gels (100 V, 12 h) with a 50-bp ladder molecular maker (Tiangen, China) and a known allele size sample as a control, and gels were visualized using silver staining. Allele sizes were analyzed and digitalized by Gelpro 3.2. Observed

heterozygosity, expected heterozygosity, and tests for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed using the GENEPOP 4.0 software (Rousset, 2008) and FSTAT 2.9.3.2 for windows (Goudet, 1995), respectively.

RESULTS

Using the Illumina transcriptome sequencing method, a total of 50,142,328 clean reads and 214.3 Mbp were obtained from *A. schrenckii* cDNA (GenBank accession No. SRR1131121), which were assembled into 203,507 contigs with a mean length of 1053 bp. Of these contigs, 40,048 (19.7% of all contigs) were identified that contained microsatellite loci, but only 24,135 contigs (60.3% of all microsatellite-containing contigs) contained potentially amplifiable loci. Within the 24,135 contigs, dinucleotides were the most dominant [18,174 (75.3%)]. The longest motif class (hexanucleotides) was present at very low frequency [5 (0.02%)] and the numbers of the other motif classes were 5,612 (23.3%), 327 (1.35%), and 17 (0.07%) for trinucleotides, tetranucleotides, and pentanucleotides, respectively. From these potential microsatellite locus contigs, 27 (6, 11, and 10 contigs of dinucleotides, trinucleotides, and tetranucleotides, respectively) were selected for primer design. Of the 27 potential microsatellite markers, 8 pairs were not easily amplified, 7 pairs were monomorphic, and the remaining 12 pairs of primers were polymorphic in the 24 individuals of *A. schrenckii* (Table 1). The observed numbers of alleles per locus ranged from 2 to 17 (mean 6.6). Observed heterozygosity ranged from 0 to 0.958 and expected heterozygosity ranged from 0.508 to 0.940 (Table 1). However, of the 12 microsatellite loci, 2 marker pairs (AS1266 and AS2189) showed homozygotes with more than 2 alleles. No one locus deviated significantly from HWE after Bonferroni's correction and no significant LD was observed in pairwise loci (Table 1).

Table 1. Characterization of 12 novel polymorphic microsatellite markers in *Acipenser schrenckii*.

Locus	Primer sequence (5'-3')	GenBank accession No.	Repeat motif	Ta (°C)	N_A	Size rang (bp)	H_O/H_E	P
AS352	F: AATGTCGTCCCATCTCAA R: ATCATACCTCGTCTCACC	KJ623720	$(GT)_6$	53	9	213-237	0.250/0.770	1.000
AS426	F: AACCGACGGCTATGTAAT R: TTCCTTCTCACGCTGTAT	KJ623721	$(GT)_7$	53	6	293-303	0.125/0.653	1.000
AS633	F: CCTGAACGGGAACCAAGT R: AGGACACCTCACAGCACC	KJ623722	$(GT)_6$	57	17	286-328	0.583/0.940	1.000
AS1266	F: GCAGATTGATAACCACCAG R: TTTGAATAAAGCGGAAGC	KJ623723	$(AC)_6$	53	2	181-183	0.000/0.508	1.000
AS2189	F: GGCACTTTGTTAGGATTG R: AAGACAGAGCCATTGAGG	KJ623724	$(TA)_5$	53	3	303-307	0.000/0.524	0.608
AS2725	F: CTGTCCGTTTATCCGTCC R: CCTCTGCTGCCAACTCAA	KJ623725	$(CA)_6$	57	5	161-185	0.130/0.557	1.000
AS3148	F: GCCTGGTGTTCACCTACCC R: ATCCATTCGTCCACTCATTCT	KJ623728	$(GCA)_5$	53	5	263-281	0.167/0.660	1.000
AS6682	F: CGCCTGGATTCTGTGAGT R: AGAGTGTTGAGGTCGTAC	KJ623729	$(TAT)_5$	53	5	184-208	0.042/0.660	1.000
AS10282	F: AAATCTGCTTTGGGACACCC R: CCGCTGACAACCAGTCTGAG	KJ623727	$(TGTT)_5$	55	5	227-247	0.739/0.741	1.000
AS13185	F: CGAGTTGCCCAAGTCATT R: GCGTCTCGCATCCATTTA	KJ623730	$(CAT)_6$	54	10	189-216	0.958/0.899	1.000
AS16977	F: ATCCACGTATCCTTGCTT R: AGTGCATGTGCCTTGTG	KJ623726	$(CAGA)_5$	55	4	250-258	0.167/0.712	1.000
AS74678	F: TTGGCCCATTTATGCTTGTC R: GGACAAAATCAGGGGCATCA	KJ623726	$(CAGA)_5$	55	4	250-258	0.167/0.712	1.000

Ta, annealing temperature of primer pairs; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

DISCUSSION

This study was the first report on microsatellite loci development in *A. schrenckii* based on transcriptome using the next-generation sequencing technology (Illumina transcriptome sequencing method). Although relatively high proportion of monomorphic and not easy amplified loci were found, we have successfully detected and designed enough polymorphic microsatellite loci. Compared to traditional library-based approach such as magnetic bead enrichment (Hua et al., 2014), we could isolated and developed polymorphic microsatellite loci more faster and cheaper. This result is in agreement with recent studies (Perry and Rowe, 2011; Castoe et al., 2012; Nowak et al., 2014). Therefore, we could predict that the next-generation sequencing technology brings us powerful molecular tools for population genetic investigation in non-model organism.

Conflicts of interest

The authors declare no conflict of interest.

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