



## Microsatellite Markers for the Paddlefish (*Polyodon spathula*)

Edward J. Heist<sup>1,2\*</sup>, Erica H. Nicholson<sup>2</sup>, Justin T. Sipiorski<sup>2</sup> & Devon B. Keeney<sup>1,2</sup>

<sup>1</sup>Fisheries and Illinois Aquaculture Center; <sup>2</sup>Department of Zoology, Southern Illinois University Carbondale, Carbondale, IL 62901-6511; Fax: (618) 536-7761; E-mail: edheist@siu.edu (\*Author for Correspondence: E-mail: edheist@siu.edu)

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The American paddlefish (*Polyodon spathula*) is a large (up to 80 kg) planktivorous fish that inhabits large rivers of central North America. While formerly much more abundant, American paddlefish have significantly declined in number because of habitat destruction (pollution and dams) and also due to over-harvest initially for their meat and later for their roe, which is used to make caviar (Graham 1997; Jennings and Zigler 2000). While the American paddlefish is not currently listed under the US Endangered Species Act it is listed on Appendix II of the Convention on International Trade of Endangered Species (CITES) and the Committee on the Status of Endangered Wildlife in Canada lists it as extirpated from Canada. There are only two extant species of paddlefishes: *P. spathula* and *Psephurus gladius* (Chinese paddlefish), both of which face similar threats (Bemis et al. 1997). These two species diverged from the sturgeons (their closest living relatives) during the Mesozoic (Birstein and Desalle 1998). Because these “living fossils” exhibit such novel morphologies and ancient divergences from other vertebrates, their conservation value is extremely high.

Current conservation strategies call for a re-establishment of extirpated American paddlefish populations using hatchery stocks (Graham 1997). The use of fish from hatcheries presents challenges for conservation, including the avoidance of inbreeding depression, domestication, and the breakup of locally adapted gene complexes by mixing genetically distinct stocks. There is an urgent need for the development of genetic markers that can be used to guide management of these ancient fish so that the genetic diversity that has survived to this day is not eroded in an attempt to increase numbers of fish. The purpose of this project is to develop a suite of polymorphic genetic markers

(microsatellites) in American paddlefish for use in conservation and research of the American paddlefish.

Tissue samples (posterior tips of opercula) were collected from 28 individual American paddlefish. Nineteen American paddlefish were collected during May and June of 2000 from commercial fishers at the confluence of the Kaskaskia and Mississippi Rivers in Randolph County, Illinois. Nine American paddlefish were collected in April 1999 by recreational fishers from the Big Muddy River near its confluence with the Mississippi River in Union County, Illinois. Genomic DNA was isolated using the Quiagen DNeasy tissue kit (Quiagen Inc.) and stored at  $-20^{\circ}\text{C}$ . Construction and screening of a subgenomic library followed Heist and Gold (2000). Total genomic DNA from a single American paddlefish was digested with the restriction enzyme *Mbo* I. Fragments spanning approximately 300–800 base pairs were excised from a 1.4% agarose TAE gel, ligated into a pUC 18 cloning vector and used to transform *Dh5 $\alpha$*  competent cells. Individual colonies were transferred to nylon membranes (Hybond, Inc.) and probed with  $^{32}\text{P}$  radiolabeled (GT)<sub>10</sub> and (GA)<sub>10</sub>. Positive colonies were sequenced manually. PCR primers were designed using the MacVector software package (Oxford Molecular).

PCR reactions contained approximately 1–10 ng genomic DNA, 0.1 units Taq DNA polymerase, 0.5  $\mu\text{M}$  each primer, 200  $\mu\text{M}$  each dNTP, 2 mM MgCl<sub>2</sub>, and 1X Taq buffer (50 mM KCl, 10 mM Tris, 0.1% Triton X-100, pH 9.0). One primer was radiolabeled with  $\gamma$ - $^{32}\text{P}$  using T4 polynucleotide kinase prior to amplification. Amplification consisted of a two-minute denaturation step at  $94^{\circ}\text{C}$ , 25 cycles of  $94^{\circ}\text{C}$  for 30 s,  $56$ – $60^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 30 s, followed by a single five-minute extension step at  $72^{\circ}\text{C}$ . Alleles at individual loci were sepa-

Table 1. Characteristics of American Paddlefish (*Polyodon spathula*) microsatellite loci. Locus Psp-32 contained an imperfect repeat with numerous short (GT)<sub>n</sub> motifs. The sequences from which primers were developed have been submitted to Genbank (Accession numbers AF406733–AF406740)

Locus	Inheritance	Longest Repeat	Primers (5' → 3')	Ta (°C)	Length of PCR Products (bp)	Alleles	H <sub>e</sub>	H <sub>o</sub>
Psp-12	Disomic	(GA) <sub>15</sub>	F: ATCTGATACAATCTTCACAGTCC R: GAGTTCAGCTCGCTCTCC	56	218–228	6	0.613	0.571
Psp-18	Tetrasomic	(GA) <sub>20</sub>	F: CTATGTGAGGAATGCCACGAC R: TGACATCACCATGACCTATTGG	56	164–174	6	–	–
Psp-20	Disomic	(GA) <sub>13</sub>	F: GCATAGTTTTTGGGGGATGGC R: ACAACTGCTTCACCGCATTCC	60	202–208	4	0.562	0.714
Psp-21	Disomic	(GA) <sub>25</sub>	F: TTCAGCAGGTAGTGAGACAGGCAG R: TCAAGTCCCATCCACTCTTCGC	56	142–170	7	0.721	0.714
Psp-26	Disomic	(GT) <sub>25</sub>	F: TCGGTGTTTGTGTGTGTGTATGC R: TGGTTCAGTTTCGCTCATCC	56	130–160	11	0.822	0.821
Psp-28	Disomic	(GA) <sub>37</sub>	F: CAAAGGCATCCCCTACCAC R: GCTGGACAAAAAGTATGGAGTGC	56	224–260	14	0.889	0.929
Psp-29	Tetrasomic	(GCAC) <sub>6</sub>	F: GGGGTCTAATAAAATCCACCGTTC R: TTGCCTTGTGCTCTGTGTTCC	56	195–215	4	–	–
Psp-32	Disomic	imp.	F: AATGACTCAGTTGTGTGCTGC R: AAGTGTAGGGGAATCTACCAG	60	173–177	3	0.198	0.214

rated on denaturing polyacrylamide gels and visualized via autoradiography using the cloned fragment at each locus as a size standard. We also tried to amplify polymorphic microsatellite loci in nine genomic DNAs from each of shovelnose sturgeon (*Scaphirhynchus platyrhynchus*) and green sturgeon (*Acipenser medirostris*) at an annealing temperature of 56 °.

Twenty-six of 1536 colonies strongly hybridized to the probe and were sequenced. Several loci were rejected because they had either no apparent repeat, short repeats, not enough sequence between the vector and the insert, or self-complementary flanking sequence that prevented the design of PCR primers. One locus (Psp-29) contained a tetrameric repeat flanked by a short dinucleotide repeat. Ultimately primers were designed for fourteen loci, eight of which reliably amplified polymorphic microsatellite loci. Six primer sets amplify polymorphic microsatellite loci exhibiting disomic inheritance, with three to fourteen alleles per locus and observed heterozygosities ranging from 0.214 to 0.929 (Table 1). Two loci exhibited up to four alleles per individual (tetrasomic inheritance) similar to the tetrasomic loci described in sturgeons by May et al. (1997). All members of the Acipenseriformes (sturgeons and paddlefishes) are

presumed to have polyploid ancestors (Blackledge and Bidwell 1993). Because of the uncertain homology of bands and the difficulty of determining dosage when less than four unique alleles are present, these loci are less useful than loci exhibiting disomic inheritance. Nevertheless they may have some utility (e.g., for assessing relatedness to avoid inbreeding in captive stocks) and therefore their primer sequences are included in this study (Table 1). No primer set amplified polymorphic microsatellite DNA in either of the sturgeon species. Monomorphic products of approximately the correct size were observed in both sturgeons for loci Psp-28 and Psp-32. The remaining five primer sets failed to amplify any products of the expected size range.

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