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Short Communication

Genetic characterization of 18 novel microsatellite loci in northern pike (*Esox lucius* L.)

Jun Wang[1], Chenghui Wang[1], Long Qian[2], Yuqing Ma[1], Xinxin Yang[1], Zsigmond Jeney[3] and Sifa Li[1]

[1]*Key Laboratory of Aquatic Genetic Resources and Utilization, Ministry of Agriculture, Shanghai Ocean University, Shanghai, China.*
[2]*Fisheries Technology Extension Station, Xinjiang Production and Construction Corps, Urumqi, Xinjiang, China.*
[3]*Research Institute for Fisheries, Aquaculture and Irrigation, Szarvas, Hungary.*

Abstract

The northern pike (*Esox lucius* L.), an important predatory freshwater species, is undergoing significant population decline. In this study, 18 novel polymorphic microsatellite loci were isolated and used for assessing genetic variation in the Chinese Ulungur and Hungarian Balaton populations of the species. The number of alleles ranged from 2 to 13, observed heterozygosity from 0.154 to 0.920 and expected heterozygosity from 0.145 to 0.921, thereby indicating the specific usefulness of these suites of markers for investigating genetic variability.

Key words: northern pike, microsatellite loci, genetic variability.

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The northern pike (*Esox lucius* L.) is a predatory freshwater fish with circumpolar distribution in the Northern Hemisphere above latitude 40 and up to the Arctic zone (Jacobsen *et al.*, 2005; Lucentini *et al.*, 2006). In China, this fish is encountered only in an upstream region of the Irtse (Eltrixhe) River drainage area, in the northern part of Xinjiang Province, where the Ulungur and Jili lakes, the two major habitats (Li, 1981; Ren *et al.*, 2002), are located. In the 1960's, more than 120 tons of this species were harvested in China, this accounting for 20% of the total fish production in this watershed. However, since the end of the 1960's, there has been a sharp decline, as a consequence of commercial overexploitation and environmental changes. In 1999, the total harvest was only 15 tons (Ren *et al.*, 2002), finally dropping to 7.5 tons in 2006 (Huo *et al.*, 2009). A similar drop-off has also been reported in other continental populations (Lorenzoni *et al.*, 2002; Launey *et al.*, 2003). Thus, it is urgently necessary to conduct genetic conservation and management programs on this species.

The investigation of genetic diversity, a crucial step in the implementation of conservation strategies for endangered species, is however, dependent on the availability of appropriate genetic molecular markers. So far about 25 *E. lucius*-specific microsatellite markers have been developed (Miller and Kapuscinski, 1996; Miller and Kapuscinski, 1997; Launey *et al.*, 2003). Nevertheless, due to limitations in the numbers of alleles and the low degree of polymorphism for these markers, the study of genetic variability in the northern pike will require the use of a larger number of loci (Launey *et al.*, 2003). Therefore, the aims were to (1) isolate further novel polymorphic microsatellite loci, and (2) evaluate the applicability of these loci in the assessment of genetic variability in this fish.

In 2004, specimens of three wild populations of the northern pike in China were collected from the lakes Ulungur ($n = 26$), Jili ($n = 21$) and Beitun-183 ($n = 21$). Simultaneously, 25 individuals, sampled from Lake Balaton in Hungary in 2003, were used as reference population. A small piece of the caudal fin in each sample was removed and then stored in 95% ethanol.

Genomic DNA was extracted using a standard phenol-chloroform method (Sambrook and Russell, 2001). The pooled genomic DNAs of 30 individuals from China and 10 from Hungary were digested with the restriction enzyme *Sau*3AI. Fragments of 400-1,000 bp were isolated and purified, and then ligated to short linkers (Micr-A: 5'-GAT CGT CGA CGG TAC CGA ATT CT-3', Micr-B: 5'-GTC AAG AAT TCG GTA CCG TCG AC-3'). Microsatellite-containing fragments were selectively coupled with a biotin-labeled $(CA)_{15}$ probe. These fragments were then ligated into a *pMD*19-T vector and used for transforming competent *Escherichia coli* DH5α cells. Positive clones were subsequently tested by polymerase chain reaction (PCR) using M13 universal primers and sequenced using

Send correspondence to Chenghui Wang. Key Laboratory of Aquatic Genetic Resources and Utilization, Ministry of Agriculture, Shanghai Ocean University, 201306 Shanghai, China. E-mail: wangch@shou.edu.cn.

Table 1 - Characteristics of 18 polymorphic microsatellite loci for northern pike.

Locus	Primer sequences(5'-3')	Repeat motif	Allele range	T_a (°C)	Total number of alleles*	N_A (Ulungur/Balaton)	H_O (Ulungur/Balaton)	H_E (Ulungur/Balaton)	P_{HWE} (Ulungur/Balaton)	GenBank accession no.
Eluc001	F:ACCCATACTCTGTCCCCAAT R:TGAAAGTGCTCCGTTGCT	$(CA)_{10}$	188-204	56	5	2/5	0.154/0.640	0.145/0.796	0.617/0.000	GQ358204
Eluc002	F:TGATGACACTGTCCGTGTGT R:AGCCATCTGTTCCTGCAA	$(GT)_7N_2(TG)_{17}N_2(TG)_8$	229-286	56	12	6/12	0.539/0.920	0.680/0.921	0.052/0.078	GQ358205
Eluc004	F:TGATTGTAACTGCACAGCGG R:TAGCGCAGACACACTACTGGA	$(TG)_{12}N_2(GT)_9$	242-312	56	15	5/13	0.269/0.600	0.561/0.915	0.000/0.400	GQ358206
Eluc005	F:AGACACCACTGCACAACCTT R:GGCACACAGACTAGGATGAT	$(CA)_{17}CG(CA)_7$	166-198	62	6	3/6	0.808/0.400	0.604/0.764	0.053/0.060	GQ358207
Eluc014	F:GCAAAGTAGGGCATTGAAGC R:ATGTGCGTGTAACTGGCGAA	$(CA)_{26}$	168-214	60	9	5/9	0.577/0.440	0.768/0.817	0.118/0.001	GQ358208
Eluc018	F:ATTTGACCACTACAGCTGCG R:TGTGTAGGAACGGGTTCACT	$(TC)_7N_2(TC)_{10}$	198-220	60	5	3/3	0.308/0.040	0.570/0.631	0.000/0.000	GQ358209
Eluc019	F:GCAGAACCTTAGTGAACCCGT R:TGTCCTGAGGGAGAAAGGAA	$(CA)_9$	154-170	60	5	5/5	0.385/0.520	0.658/0.693	0.000/0.126	GQ358209
Eluc021	F:CATTCTTCTCATCAGCACCC R:TGAAGGTGCACTGTAAGACG	$(GT)_{28}$	212-242	60	3	2/3	0.308/0.480	0.483/0.656	0.059/0.076	GQ358210
Eluc025	F:TGTGTGTGTGTGCATTCGTG R:GCTTACATTTCAGGCCGTCT	$(GT)_{12}$	248-316	62	4	3/4	0.154/0.480	0.4800.675	0.000/0.074	GQ358211
Eluc027	F:TCTCTGTCTAACACGAGCGA R:GTGTGTGTGCAGGTTCACAT	$(CA)_{10}$	140-178	60	10	6/6	0.423/0.960	0.753/0.823	0.000/0.403	GQ358212
Eluc030	F:CAGACTGACGGGGGTATTTT R:TAGACAGTTTGGGGCTCGTA	$(GT)_{16}$	182-208	56	3	2/2	0.500/0.360	0.419/0.350	0.284/0.883	GQ358213
Eluc033	F:CCAGCTCAGGTGTACTGAAA R:ATGGCAACAGCAGCCTCCT	$(CA)_{14}$	340-402	56	8	6/2	0.615/0.320	0.756/0.509	0.062/0.055	GQ358215
Eluc037	F:CAACACCTGGTTCCTCTCAT R:CTGGTTGGTTGACTAAGCTG	$(CA)_5N_2(CA)_{14}$	301-321	60	5	5/4	0.731/0.520	0.742/0.634	0.058/0.342	GQ358217
Eluc040	F:CAGGATGAGAAGCAAGTGTG R:TGTTCTCCAGAACCATGGTG	$(CA)_{16}$	242-270	60	8	3/6	0.269/0.200	0.242/0.710	0.816/0.000	GQ358218
Eluc041	F:GTGTGTAGACTTTGGCTCGAT R:ACCCAGACAGAAACAAAGACC	$(GT)_{20}$	192-228	62	12	7/8	0.846/0.680	0.775/0.805	0.069/0.172	GQ358219
Eluc042	F:TGGCACAGGAAGAACAACAG R:CGGACCAAGGCAAGACAATT	$(TG)_{21}N_2(AG)_5$	218-242	62	7	3/7	0.423/0.760	0.601/0.801	0.052/0.099	GQ358220
Eluc045	F:AGCATCAGGGAGTAGTTGCA R:CAGGTAAGCGTCCAGGTAAA	$(CA)_{19}$	140-180	62	13	6/10	0.385/0.640	0.634/0.886	0.089/0.124	GQ358221
Eluc046	F:TGTGTCAGTAGCATCGCAAG R:ATGTACAGAGCCTGTTCCACC	$(CA)_{23}$	188-228	62	8	6/2	0.615/0.280	0.817/0.429	0.133/0.082	GQ358222

F, forward primer; R, reverse primer; T_a, annealing temperature; N_A, No.of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HWE}. *P* value for Hardy-Weinberg equilibrium. The total number of alleles* comprising all the four populations (Ulungur, Jili, Beitun-183, and Balaton Lakes).

an ABI 3730 automatic sequencer (Applied Biosystems, USA). Specific PCR primers for the microsatellite loci were then designed, using the online software Web Primer.

PCR conditions were optimized by means of the AG-6321 gradient thermal cycler (Eppendorf, Hanburg, Germany), with an annealing step of 52-62 °C for selecting the optimum annealing temperature. The volume of each reaction mixture (10 μL) was composed of 1 μL of genomic DNA (20 ng/μL), 5 μL of buffer (0.2 μM dNTPs, 1.5 μM MgCl2, 0.5 μM Taq DNA polymerase), 1 μL of primers (0.5 μM each) and 3 μL of distilled water. The PCR conditions were as follows: initial denaturation for 5 min at 94 °C, followed by 35 cycles for 30 s at 94 °C, 30 s at optimal annealing temperature (Table 1), and 30 s at 72 °C, followed by a terminal extension step of 10 min at 72 °C. The PCR products were visualized by means of the QIAxcel multicapillary gel electrophoresis system (Qiagen, Hilden, Germany).

The observed heterozygosity (H_O), and expected heterozygosity (H_E) according to Nei's unbiased estimate (Nei, 1978) were calculated using POPGENE software (Yeh *et al.*, 1999). FSTAT 2.9.4 software (Goudet, 1995) was used to test the linkage disequilibrium for all loci. Deviation from Hardy-Weinberg equilibrium (HWE) in each locus was estimated by using GENEPOP 3.4 software (Raymond and Rousset, 1995), and null alleles were detected with Micro-Checker (Van Oosterhout *et al.*, 2004).

A total of 97 positive clones were selected for sequencing, of which 76 (78.4%) contained microsatellite sequences (motifs repeated more than five times). From amongst these 76 clones, 48 microsatellite loci with adequate flanking regions were chosen to design primer pairs using the online software, which successfully generated PCR products. 18 primer pairs (Table 1) with a high degree of demonstrated polymorphism were used for population analysis. These 18 microsatellite loci were deposited in GenBank (accession no. GQ358204-GQ358222). Significant departure from HWE was evident in several loci in both populations (Table 1).

A total of 138 alleles were amplified by the 18 primer pairs in four populations. The mean number of alleles across loci and populations was 7.67. The values of observed and expected heterozygosities for each locus in the Ulungur and Balaton populations are presented in Table 1. In the Ulungur population, H_O ranged from 0.154 to 0.846, and H_E from 0.145 to 0.817, whereas in the Balaton population H_O ranged from 0.040 to 0.920 and H_E from 0.350 to 0.921 (Table 1). By means of the Micro-Checker software, six loci (Eluc004, Eluc014, Eluc018, Eluc019, Eluc027 and Eluc045) were detected to have a null allele in the two populations. There was no evidence of large allele dropout in any of the loci.

Investigating and detecting genetic diversity in northern pike hinges on both the availability of molecular markers and their implication. In the present study, 18 loci were detected as polymorphic, with relatively high numbers of alleles in the two populations tested. Some loci were found to have deviated from HWE in the two populations, which could have resulted from the presence of a null allele or the dramatic population decline in China (*e.g.*, the total harvest in 2006 was only 6.25% of that in the 1960s). There was no significant linkage disequilibrium in loci combinations following sequential Bonferroni correction for multiple tests. In conclusion, the results on genetic variability in the two populations analyzed herein indicate that these suites of markers are useful for investigating genetic diversity in the northern pike.

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