Mitochondrial DNA variation in wild and hatchery populations of northern pike, *Esox lucius* L.

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Abstract

Esox lucius is an economically important freshwater species. Mitochondrial cytb, 12SrRNA, and 16SrRNA gene sequences were used in order to clarify the genetic variation and population structure in three *E. Lucius* populations, i.e., one Wild population (W) and two hatchery populations (Hatchery Population I-HPI and Hatchery Population II-HPII). A total of 55 individuals, with 19 from wild and 18 from each hatchery population, were sequenced. The results are as follows: 1) cytb. Eleven variable sites defined eight distinct haplotypes, with 1137 base pairs (bp) complete cytb sequences. The nucleotide diversity (π) values were W 0.009%, HPI 0.075% and HPII 0.068%, respectively. Genetic distances within and between populations were both between 0 and 0.1%. Analysis of molecular variance (AMOVA) analysis revealed that variation within populations accounts for 98.49% of total variation. 2) 12SrRNA. We obtained 380 bp consensus 12SrRNA sequence and found little variation among the 55 sequences. 3) 16SrRNA. Twelve variable sites defined seven haplotypes, with 519 bp consensus 16SrRNA sequence. The π values were W 0.041%, HPI 0.310% and HPII 0.021%, respectively. Genetic distances were between 0 and 0.3% within populations and from 0 to 0.2% between populations. AMOVA disclosed that variation within populations accounts for 91.79% of total variation. Variation within populations is the main source of total variation. The results suggest that low genetic variation can be found in both wild and hatchery populations of *E. lucius*, and the effect of genetic drift and adaptation to culture conditions gradually emerged under only two generations' artificial cultivation.

Keywords: *Esox lucius*, mitochondrial DNA, genetic diversity, genetic variability, hatchery

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Introduction

Northern pike, *Esox lucius* L. (Family Esocidae), exhibits a circumpolar geographical distribution in the Northern Hemisphere (Grande et al., 2004; Larsen et al., 2005). It is a large sized fish, and is one of the most economically important freshwater species because it supports both commercial and recreational fisheries (Westers and Stickney, 1993; Grande et al., 2004). In China, the distribution of *E. lucius* is very restricted in the Irtysh River basin of northern Xinjiang Province (Zhu, 1995). It is one of the most popular aquatic products in China for its less bone, very delicious, and high nutrition value. However, in the recent years, several factors such as overfishing and environmental pollutions play an important role in the drastic decline of natural populations of northern pike.

Enhancing the numbers of fish in wild populations through the release of cultured fish has recently become widespread (Iguchi et al., 1999). To protect and sustainably utilization the natural resource of *E. lucius*, it is necessary to enhance the artificial culture product of *E. lucius*. Hatchery population released into natural waters might have genetic effects on wild populations through interbreeding and competitive exclusion, which usually appear to be negative compared to unaffected populations (Hindar et al., 1991; Waples, 1991). It has been shown that reduced mitochondrial DNA variation appeared in hatchery populations cultured for multiple generations (Iguchi et al., 1999). Thus, the genetic variation of hatchery populations should be quantified and considerable effort should be made to minimize the genetic impact of releasing hatchery-reared fish into wild populations (Bergan et al., 1991).

The rapid rate of mutation, low level of recombination and exclusive maternal inheritance of extra nuclear mitochondrial DNA makes it useful for the study of evolutionary genetics and molecular taxonomy (Avise et al., 1987). Mitochondrial DNA variation may also be useful as a tool for identifying and managing stocks of fish species (Grewe and Hebert, 1988; Billington and Hebert, 1991). In this study, complete cytochrome *b*, partial 12SrRNA and 16SrRNA sequences were employed as molecular markers to assess the levels of genetic variation within, and genetic differentiation between wild and hatchery populations of *E. lucius*. This study is the first on mtDNA variation of wild and hatchery populations of *E. lucius* from China, and may benefit the sustainable utilization of *E. lucius*.

Materials and methods

Nineteen individuals of wild *E. lucius* population were randomly collected from Irtysh River (N47°22', E87°39'), Xinjiang, China. Thirty-six individuals of two hatchery *E. lucius* populations, with 18 individual of each, were both randomly obtained from Jiuquan (N39°44', E98°31'), Gansu, China. All wild and hatchery individuals were transported to the laboratory

on dry ice and stored at -80°C until used. For the sake of convenience, these three populations are respectively abbreviated as W (Wild Population), HPI (Hatchery Population I), and HPII (Hatchery Population II).

Firstly, thousands of wild individuals (W individuals) were collected from Irtysh River, Xinjiang and about one hundred matured wild individuals were used as parents for reproducing HPI individuals. Then, thousands of HPI Individuals were transferred to Jiuquan. After two years' cultivation, about 100 matured individuals of HPI were also used as parents and perform artificial propagation to generate HPII individuals. Finally, thousand of HPII individuals were cultured at Haimaquan Aquaculture Base. Thus, we regarded the W population as the 1st generation, and the HPI and HPII populations as the 2nd and 3rd generation, respectively.

Total genomic DNA was extracted from muscle tissues. Muscle tissues were dissected, digested in proteinase K, and extracted in phenol-chloroform and precipitated in 100% ethanol (Sambrook et al., 1989). Extracted DNA was checked using 0.8% agarose gel electrophoresis, then diluted to appropriate concentration (about 100 ng/ul) for PCR amplification.

Amplification reactions of the mitochondrial cytochrome *b*, 12SrRNA, and 16S rRNA genes were in 50 µl volumes, and amplification reaction mixtures consisted of 100 ng DNA template, 0.2 mmol/l dNTPs, 0.2 µmol/l primers each, 2.0 mmol/l $MgCl_2$, 5.0 µl 10× reaction buffer, 2 U Taq plus polymerase (Takara), with sterilized water added to make up the final volume to 50µl. The PCR profile consisted of an initial denaturation step (5 min at 94°C), followed by 36 (cytb), or 30 (12SrRNA and 16SrRNA) cycles of denaturation (45 s at 94°C for cytb, and 30 s at 94°C for 12SrRNA and 16SrRNA), annealing (45 s at 52°C for cytb, and 30 s at 55°C for 12SrRNA and 16SrRNA), and extension (at 72°C for 1 min), and a final extension step (7 min at 72°C). Primer sequences used for cytochrome *b* gene amplification were L14724 and H15915 (Xiao et al. 2001), for 12SrRNA gene amplification were L1091 and H1478 (Kocher et al., 1989), and for 16SrRNA gene amplification were L2510 and H3059 (Bouchon et al., 1994).

All six primers were used for sequencing corresponding gene segments. PCR products were purified in 1.5-2.0% agarose (Biowest) and sequenced using BigDye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems) with an automated DNA sequencer (Applied Biosystems 3130) following the manufacturer's instructions.

The complete DNA sequences of cytochrome *b* and the partial of 12SrRNA and 16S rRNA sequences were compiled with BioEdit 5.0.9 (Hall, 1999) and aligned with ClustalX (Thompson et al., 1997) using default gap penalties. Mitochondrial DNA diversity was evaluated using haplotype diversity (h) and

nucleotide diversity (π) for each population using Arlequin 3.1 (Excoffier et al., 2005). The genetic distances among haplotypes were estimated by using p-distance.

In order to evaluate hypothesized patterns of spatial genetic structure, a hierarchical analysis of molecular variance (AMOVA) was performed using Arlequin 3.1 (Excoffier et al., 2005) to partition variance components attributable to (1) variance among populations; and (2) variance among individuals within populations.

Results

The cytb, 12SrRNA, and 16SrRNA gene can all be amplified clearly in these three *E. lucius* populations. The sizes of the cytb, 12SrRNA, and 16SrRNA were respectively 1300, 500, and 600 bp.

The cytb sequences were corrected and aligned, and 1137 bp complete sequences were obtained. Among the 55 sequences of these three populations, eight distinct haplotypes were detected (including 2 haplotypes in W, 3 in HPI, and 5 in HPII). One haplotype was shared by the three populations, and the other seven haplotypes were unique to one population. All eight haplotypes sequences were submitted to Genbank databases (the accession numbers were between FJ425091 and FJ425097, plus HM177470). Eleven variable sites were found in these sequences, accounting for 0.97% of total sites, and three were parsimony informative sites.

The 12SrRNA gene sequences were corrected and aligned, and 380 bp consensus sequences were obtained. Among the 55 sequences of these three populations, little variation was found and only one haplotype (the Genbank accession number was FJ425098) was detected in two hatchery populations. Due to little variation, 12SrRNA sequences were not used for following analyses.

The 16SrRNA gene sequences were corrected and aligned, and 519bp consensus sequences were obtained. Among the 55 sequences of these three populations, seven distinct haplotypes were detected (including 3 haplotypes in W, 4 in HPI, and 2 in HPII). One shared haplotype was found. All seven haplotypes sequences were submitted to Genbank databases (the accession numbers were between FJ425099 and FJ425103, plus HM177477 and HM177478). Twelve variable sites were found in these sequences, accounting for 2.31% of total sites, and one was parsimony informative sites.

The genetic diversity parameters of these three populations based on cytb and 16SrRNA gene sequences are listed in Table 1. Among 55 individuals studied, haplotype diversity (h) was 0.105 (W), 0.216 (HPI) and 0.405 (HPII) for cytb, and 0.205 (W), 0.529 (HPI) to 0.111 (HPII) for 16SrRNA. For cytb, the nucleotide diversity (π) values of W, HPI and HPII populations are 0.009%, 0.075% and 0.068%, respectively. For 16SrRNA, the respective π values for these three populations were 0.041%,

0.310% and 0.021%. The HPI population had the highest nucleotide diversity values for both cytb and 16SrRNA markers (Table 1), about 8 times than that of W population.

Genetic distances within and between populations based on cytb and 16SrRNA are shown in Table 2. For cytb, the results indicate that genetic distances within and between populations were both between 0 and 0.1%. For 16SrRNA, genetic distances were between 0 and 0.3% within populations and from 0 to 0.2% between populations.

For cytb, genetic distances among different haplotypes of *E. lucius* were between 0.09% and 0.62%. For 16SrRNA, genetic distances among haplotypes of *E. lucius* were between 0.19% and 1.73% (Table 3). The variable sites and frequency of haplotypes are listed in Table 4.

Results of AMOVA analysis are shown in Table 5. For cytb, the genetic variation among populations accounts for 1.51% of total variation, whereas within population variation accounts for 98.49% of total variation. For 16SrRNA, the genetic variation among populations accounts for 8.21% of total variation, whereas within population variation accounts for 91.79% of total variation. Thus, within-population variation is the main source of total variation for both genetic markers.

Table 1: Genetic diversity of wild and hatchery populations of *Esox lucius* based on mitochondrial cytb and 16SrRNA gene sequences

Marker	**cytb**			**16SrRNA**		
Population	W	HPI	HPII	W	HPI	HPII
Number of individuals	19	18	18	19	18	18
Number of sites(bp)	1137	1137	1137	519	519	519
Number of polymorphic sites (s)	1	5	6	2	9	1
Number of haplotypes	2	3	5	3	4	2
Haplotype diversity (h)	0.105	0.216	0.405	0.205	0.529	0.111
Nucleotide diversity (π)	0.00009	0.00075	0.00068	0.00041	0.00310	0.00021
Standard deviation of π	0.00008	0.00045	0.00027	0.00024	0.00129	0.00019

Table 2: Genetic distances within and between populations of *Esox lucius* based on mitochondrial cytb and 16SrRNA gene sequences

Marker		**cytb**			**16SrRNA**	
Population	W	HPI	HPII	W	HPI	HPII
W	0.000			0.000		
HPI	0.000	0.001		0.002	0.003	
HPII	0.000	0.001	0.001	0.000	0.002	0.000

Table 3: Pairwise p-distances (%) matrix among haplotypes of *Esox lucius* based on mitochondrial cytb and 16SrRNA sequences

gene		**p-distance**						
cytb		C-Hap2	C-Hap3	C-Hap4	C-Hap5	C-Hap6	C-Hap7	C-Hap8
	C-Hap1	0.44	0.26	0.18	0.18	0.18	0.09	0.09
	C-Hap2		0.26	0.53	0.44	0.62	0.53	0.53
	C-Hap3			0.35	0.35	0.44	0.35	0.35
	C-Hap4				0.26	0.35	0.26	0.26
	C-Hap5					0.35	0.26	0.26
	C-Hap6						0.26	0.26
	C-Hap7							0.18
16SrRNA		R-Hap2	R-Hap3	R-Hap4	R-Hap5	R-Hap6	R-Hap7	
	R-Hap1	1.35	0.39	0.19	0.19	0.19	0.19	
	R-Hap2		1.73	1.16	1.54	1.54	1.54	
	R-Hap3			0.58	0.58	0.58	0.58	
	R-Hap4				0.39	0.39	0.39	
	R-Hap5					0.39	0.39	
	R-Hap6						0.39	

Table 4: Variable sites and frequencies of haplotypes of *Esox lucius* based on mitochondrial cytb and 16SrRNA sequences (., identical nucleotides to haplotype C-Hap1 for cytb and haplotype R-Hap1 for 16SrRNA)

gene	haplotype	Variable sites												Number (Frequency)		
														W	HPI	HPII
cytb													2			
				1	1	1	1	1	2	2	6	8				
		5	6	3	4	5	6	7	3	9	0	2				
	C-Hap1	C	C	C	G	A	A	A	A	T	T	T		18（94.7%）	16（88.9%）	14（77.8%）
	C-Hap2	.	.	T	T	C	C	C	.	.	.	.		0(0%)	1(5.56%)	0(0%)
	C-Hap3	.	.	.	C	C	C	.	.	.	.	.		0(0%)	1(5.56%)	0(0%)
	C-Hap4	.	.	.	A	.	.	.	C	.	.	.		0(0%)	0(0%)	1(5.56%)
	C-Hap5	.	.	.	T	.	.	.	.	A	.	.		0(0%)	0(0%)	1(5.56%)
	C-Hap6	A	A	.	.	.	.	.	.	.	.	.		0(0%)	0(0%)	1(5.56%)
	C-Hap7	.	.	.	.	.	.	.	.	.	C	.		0(0%)	0(0%)	1(5.56%)
	C-Hap8	.	.	.	.	.	.	.	.	.	.	C		1(5.26%)	0(0%)	0(0%)
16SrRNA									1	1	4	5	5			
				4	5	8	8	8	4	7	9	0	1			
		7	9	9	7	1	4	9	3	5	4	0	7			
	R-Hap1	T	C	T	T	T	A	T	T	A	C	G	G	17(89.5%)	12(66.7%)	17(94.4%)
	R-Hap2	.	.	.	.	G	G	G	G	.	T	A	T	0(0%)	1(5.56%)	0(0%)
	R-Hap3	A	T	.	.	.	.	.	.	.	.	.	.	0(0%)	4(22.2%)	0(0%)
	R-Hap4	.	.	.	.	.	.	.	.	.	.	.	T	0(0%)	1(5.56%)	0(0%)
	R-Hap5	.	.	G	.	.	.	.	.	.	.	.	.	0(0%)	0(0%)	1(5.56%)
	R-Hap6	.	.	.	.	.	.	.	.	T	.	.	.	1(5.26%)	0(0%)	0(0%)
	R-Hap7	.	.	.	A	.	.	.	.	.	.	.	.	1(5.26%)	0(0%)	0(0%)

Table 5: AMOVA analyses of wild and hatchery populations of *Esox lucius* based on mitochondrial cytb and 16SrRNA gene sequences

Source of variation	Cytb (F_{ST}=0.0151)				16SrRNA (F_{ST}=0.0821)			
	df	Sum of squares	Variance components	Percentage of variation(%)	df	Sum of squares	Variance components	Percentage of variation (%)
Among populations	2	0.728	0.00436Va	1.51	2	1.676	0.02840Va	8.21
Within populations	52	14.781	0.28424Vb	98.49	52	16.506	0.31742Vb	91.79
Total	54	15.509	0.28861		54	18.182	0.34582	

Discussion

The one wild and two hatchery populations of *E. lucius* share only one cytb haplotype and one 16SrRNA haplotype, with highest frequency (For cytb, haplotype C-Hap1 accounting for 94.7% in W, 88.9% in HPI and 77.8% in HPII; For 16SrRNA, haplotype R-Hap1 accounting for 89.5% in W, 66.7% in HPI and 94.4% in HPII)(Table 4). This result means that C-Hap1 and R-Hap1 are the most common haplotypes of *E. lucius* in the three generations being investigated. Only one haplotype appear in both HPI and HPII population for 12SrRNA, may reflect the different rates of evolution of the three molecular markers. The 12SrRNA gene is a relatively conservative mitochondrial gene marker with a low evolutionary rate (Meyer 1993). This result suggests that 12SrRNA evolving slower than cytb and 16SrRNA, and it is not a good marker for population genetics of *E. lucius*.

The three *E. lucius* populations exhibited a low level of polymorphism and divergence at the mtDNA genome. This result is comparable patterns of genetic variation observed for European and North American pike populations (Maes et al., 2003; Nicod et al., 2004). The low genetic variation within populations conforms to the small population sizes of pike (Maes et al., 2003).

Nucleotide diversity indicates the mean number of differences between all pairs of haplotypes in each population, and is, therefore, a better genetic diversity index of a population than haplotype diversity. For both cytb and 16SrRNA markers in *E. lucius*, the highest π value was observed within the HPI population (Table 1). The genetic distances within HPI (0.1% for cytb and 0.3% for 16SrRNA, see Table 2) are also higher than W and HPII populations. It means that HPI, the 2nd generation of wild individuals, maintain higher genetic variation than HPII, the 3rd generation of wild individuals. Low π value often observed in hatchery population because interhaplotype nucleotide substitution in the hatchery population was within the range of that in the wild population (Iguchi et al., 1999). However, in this study, the π value of HPI was about 8 times than that of W population. This phenomenon may cause by random genetic drift and sampling bias.

The results of AMOVA analysis for both cytb and 16SrRNA markers show that within population's variation accounts for significantly more of the total variation than that of among populations (cytb: 98.49% vs 1.51%; 16SrRNA: 91.79% vs 8.21%). This suggests that these three populations have not isolated genetic structure.

Usually, haplotype and nucleotide diversities both decreased with successive hatchery generations (Iguchi et al., 1999). Genetic drift and adaptation to culture conditions may cause a loss of genetic variation (Allendorf, 1993; Ferguson, 1995). In this study,

the nucleotide diversity decreased from HPI to HPII for both cytb and 16SrRNA markers, whereas the haplotype diversity decreased from HPI to HPII for 16SrRNA and increased from HPI to HPII for cytb. It suggests that the effect of genetic drift and adaptation to culture conditions gradually emerged under only two generations' artificial cultivation. The genetic variation of hatchery populations for multiple generations and the impact of stocking population on wild population should be traced and evaluated in the future.

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