SHORT COMMUNICATIONS

Rapid establishment of pure lines of silver carp (*Hypophthalmichthys molirix*) and bighead carp (*Aristichthys nobilis*)*

WANG Zhongwei, YE Yuzhen, ZHOU Jianfeng and WU Qingjiang**

(State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China)

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Abstract The diversity of gynogenetic, artificial sex reversal and natural silver carp and bighead carp is examined using randomly amplified polymorphic DNA (RAPD) method. All of the 187 bands are obtained and 19 (10.16%) of them are polymorphic in gynogenetic silver carp. Meanwhile 32 (15.61%) out of 205 bands are polymorphic in control group. In gynogenetic bighead carp a total of 232 bands are identified and 11 (4.74%) out of them are polymorphic, while 25 (10.37%) out of 241 bands are polymorphic in control group. The genetic distance of four populations is calculated and it is 0.102 and 0.023 for gynogenetic silver carp and gynogenetic bighead carp respectively. The values of natural silver carp and bighead carp are 0.161 and 0.104. From the UPGMA trees constructed based on genetic distance, the sex reversal individuals that match with the gynogenetic female individuals are picked out. A new breeding process of establishing a pure line is developed.

Keywords: *Hypophthalmichthys molirix*, *Aristichthys nobilis*, RAPD, genetic diversity, pure line, gynogenesis.

Silver carp (*Hypophthalmichthys molirix*) and bighead carp (*Aristichthys nobilis*) are semi-migration fish in the Yangtze River, Zhujiang River and Heilongjiang River and they are also the most important cultured objects in China. They play important roles in the food chain of freshwater because they mainly feed on plankton. It was demonstrated that the intense stocking of the filter-feeding fish, silver carp and bighead carp, plays a decisive role in the elimination of water bloom from East Lake in Wuhan City, Hubei Province[1]. At present water resource decreases rapidly because of dam construction, overfishing and water pollution. And the qualities of the two kinds of fish degenerate for lack of scientific management. Therefore, to breed new varieties with a high output and fast growth is an urgent task.

A pure line in common carp (*Cyprinus carpio*)[2] was first established using artificial gynogenesis and artificial sex reversal, and a pure line of zebrafish[3] was established by the same method. But two consecutive gynogeneses have to be carried out and more rapid methods should be established with respect to the fish with a long generation time. Liu attempted to establish a pure line through artificial gynogenesis and nuclear transplantation[4]. This paper is the result of establishing pure lines of silver carp and bighead carp through artificial gynogenesis, sex reversal and RAPD marker selection. In this process only one gynogenesis should be performed.

1 Materials and methods

1.1 Materials

The gynogenetic and sex reversal silver carp and bighead carp were collected from the Institute of Hydrobiology, CAS. The natural populations as controls were obtained from the Yangtze River. The gynogenetic and sex reversal carps were from the same mother. The functional sex reversal was obtained from gynogenetic offspring of 15 days old fry fed on diet containing methyltestosterone (30mg/kg) for 90 days.

1.2 Methods

1.2.1 DNA extraction. Fin tissues were digested over night in 750μL extracting buffer (10mmol/L

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** To whom correspondence should be addressed. E-mail: qjwu@ihb.ac.cn

Tris, 0.1 mol/L EDTA, 0.5% SDS, pH8.0) and proteinase K at the final concentration of 200 mg/mL. The standard method of phenol-chloroform extraction was performed to extract DNA.

1.2.2 PCR amplification and electrophoresis. Forty random primers (OPO and OPJ, Operon product) were used (Table 1). Amplifications were performed in a 25 μL reaction mixture, which contained 2.5 μL 10× reaction buffer, 1 μL dNTP (2.5 mmol/L), 1 μL primer, 0.5 μL Taq polymerase (2 U/μL), 2 μL template DNA and 18 μL sterile water. PCR profile consisted of an initial denaturation for 5 min at 94 ℃ followed by 40 cycles of denaturation at 94 ℃ for 45 s, annealing at 37 ℃ for 45 s and extending at 72 ℃ for 90 s, and a final extension at 72 ℃ for 5 min. PCR products were separated by 1.4% agarose gel electrophoresis and were visualized under UV light.

1.2.3 Data analysis. The clear bands on the gel were analyzed and the existence or absence of the bands were presented by 1 or 0 respectively. Genetic distance was calculated using the RAPD104 software only from the bands amplified by polymorphic primers and every band was regarded as one locus. UPGMA trees were constructed according to the genetic distance using Mega 2.1 software[5].

2 Results

2.1 PCR amplification

Different degrees of polymorphism were revealed by the PCR amplification (Figs. 1 and 2). In gynogenetic silver carp 187 bands were produced by 32 random primers and 19 polymorphic bands were produced by 11 polymorphic primers (10.16%). In contrast, 205 bands and 13 polymorphic bands were amplified by 32 random primers in control silver carp, the rate of polymorphism was 15.61%. In gynogenetic bighead carp 35 random primers produced 232 bands and out of them 11 (4.74%) polymorphic bands were obtained by 7 polymorphic primers. In control bighead carp a total of 241 bands were amplified and 25 bands were identified by 15 polymorphic primers. The rate of polymorphic bands occupied 10.37%. The polymorphism found in control groups was higher than that of gynogenetic groups.

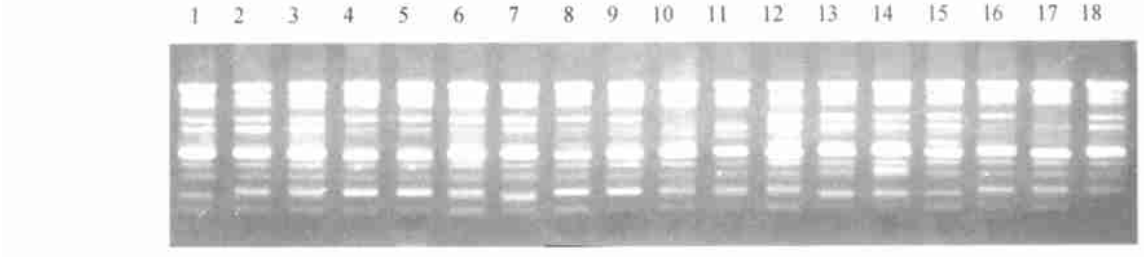

Fig. 1. RAPD pattern of silver carp amplified by primer OPO13. 1 and 2, Sex reversal silver carp; 3~18, gynogenetic silver carp.

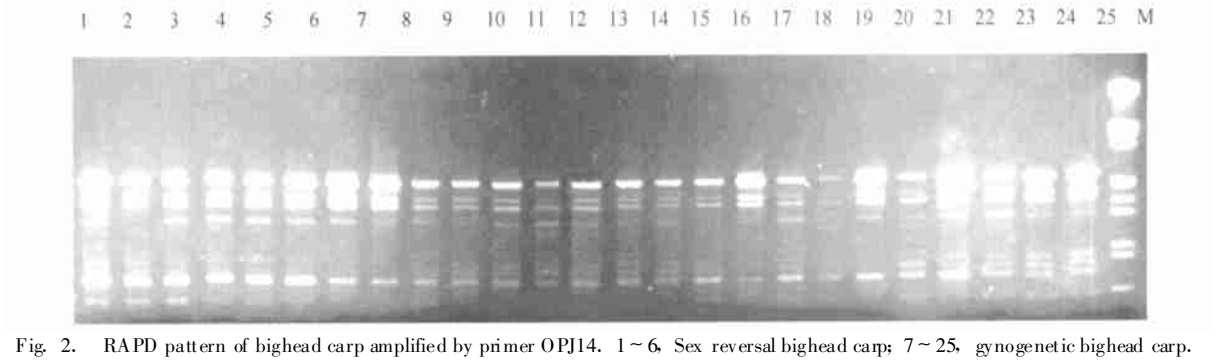

Fig. 2. RAPD pattern of bighead carp amplified by primer OPJ14. 1~6, Sex reversal bighead carp; 7~25, gynogenetic bighead carp.

Table 1. Polymorphic primers and sequences

Primers	Sequence	Populations			Primers	Sequence	Populations			
OPJ1	CCCGGCATAA			GS S	OPJ20	AAGCGGCCTC		B		
OPJ4	CCGAACACGG	GB		GS S	OPO1	GGCACGTAAG		B	GS	S
OPJ5	CTCCATGGGG		B	GS S	OPO2	ACGTAGCGTC		B		
OPJ6	TCGTTCCGCA		B		OPO4	AAGTCCGCTC	GB	B	GS	S

To be continued

Continued

Primers	Sequence	Populations				Primers	Sequence	Populations			
OPJ7	CCTCTCGACA	GB	B			OPO6	CCACGGGAAG	GB			
OPJ9	TGAGCCTCAC		B			OPO7	CAGCACTGAC			GS	S
OPJ10	AAGGCCGAGG			GS	S	OPO9	TCCCACGCAA	GB	B		
OPJ11	ACTCCTGCGA			GS	S	OPO10	TCAGAGCGCC		B		
OPJ12	GTCCCGTGGT			GS	S	OPO11	GACAGGAGGT		B		
OPJ13	CCACACTACC		B			OPO13	GTCAGAGTCC		B	GS	S
OPJ14	CACCCGGATG	GB				OPO14	AGCATGGCTC			GS	S
OPJ16	CTGCTTAGGG		B			OPO15	TGGCGTCCTT	GB			S
OPJ18	TGGTCGCAGA		B			OPO17	GGCTTATGCC				S

GB, Gynogenetic bighead carp; B, control bighead carp; GS, gynogenetic silver carp; S, control silver carp

2.2 Genetic diversity

The genetic diversity of four populations was assessed by RAPD analysis software. The genetic distance of gynogenetic silver carp ranged from 0 to 0.42 and the mean distance was 0.102. It was from 0 to 0.355 and the mean value was 0.161 for natural silver carp. The genetic distance of gynogenetic bighead carp ranged from 0 to 0.042 and the mean distance was 0.023. It was from 0.011 to 0.194 and the mean value was 0.104 for the control population. The genetic distances of gynogenetic populations were higher than those of control populations.

2.3 Screening of individuals with same genetic loci

The UPGMA trees of gynogenetic silver carp and bighead carp were constructed (Figs. 3 and 4). It can be seen from Fig. 3 that the same genetic loci were shared by sex reversal silver carp individuals 1 and 2 and gynogenetic silver carp individuals 6, 8, 9, 16, and sex reversal bighead carp individual 1 shares the same loci with gynogenetic bighead carp individual 15 and sex reversal bighead carp individual 3 with gynogenetic bighead carp individuals 9, 19, 22 (Fig. 4). In gynogenetic bighead carp and the rest of sex reversal bighead carp individuals 2, 4, 5, and 6, no common loci was identified among them.

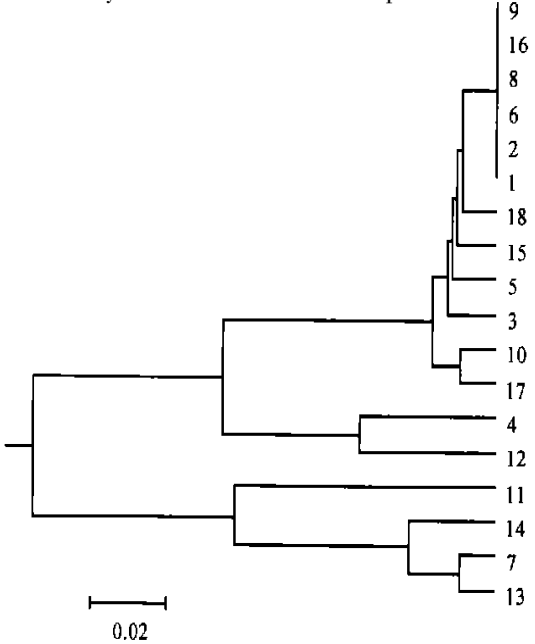

Fig. 3. UPGMA tree of gynogenetic and sex reversal silver carp. 1 and 2, Sex reversal silver carp; 3~18, gynogenetic silver carp.

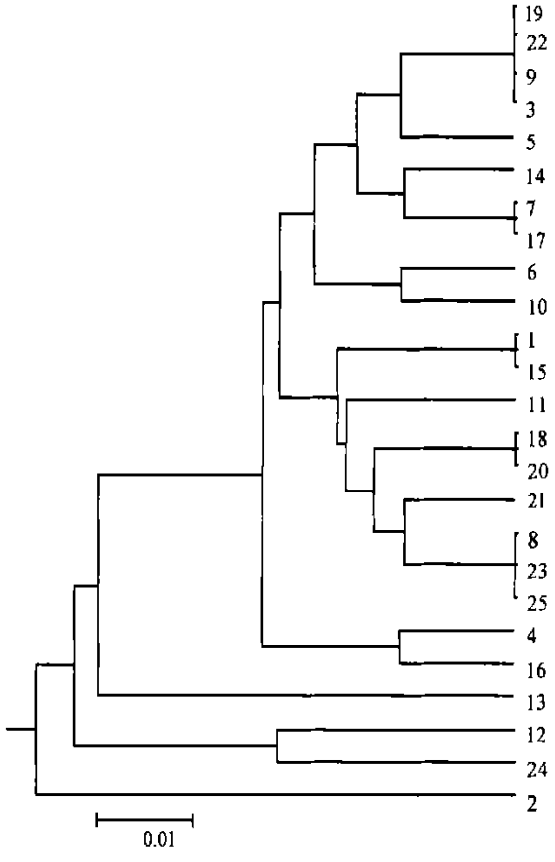

Fig. 4. UPGMA tree of gynogenetic and sex reversal bighead carp. 1~6, Sex reversal bighead carp; 7~25, gynogenetic bighead carp.

3 Discussion

3.1 Genetic diversities of silver carp and bighead carp

Genetic diversity of fish is the result of adaptation to environment and evolution. Some studies on the fish diversity have been carried out using different genetic markers. Wu et al. demonstrated the diversity and genetic structure of the bighead carp from lower reaches of the Yangtze River and the silver carp from middle reaches of the Yangtze River using isozyme markers[6,7]. Li et al. examined the genetic diversity of the silver carp and bighead carp from the mid-down reaches of the Yangtze River by PCR-RFLP of mtDNA[8]. The natural and artificial cultured populations were also analysed with RAPD method and it disclosed that artificial breeding decreased the genetic diversities[9,10]. Moreover, the genetic differentiation of gynogenetic silver carp was assessed using RAPD and protein markers by Deng et al.[11]. Using different markers in identification of the genetic polymorphism resulted in different conclusions, and among the methods RAPD showed a much higher polymorphism.

The cause of genetic diversity in gynogenetic individuals is the crossing over of chromosomes in meiosis[12]. It was known from this study that natural populations were higher than gynogenetic populations in either polymorphic loci rate or genetic distance.

3.2 Feasibility of establishing pure lines

Artificial gynogenesis has been widely used in genetics and breeding for increasing homozygosity and establishing a pure line. The traditional method of establishing a pure line has to carry out sib mating of 8 to 10 consecutive generations. Therefore, it takes a long time to establish pure lines of silver carp and bighead carp. In general the gynogenesis increases homozygosity and decreases heterozygosity because paternal chromosomes are rejected. The coefficient of inbreeding of one generation of gynogenesis is about 0.6 and it is higher than those of sibling (0.25) and self-fertilization (0.5). And it is about 0.9 for the two consecutive gynogeneses, and the individuals obtained from their offspring may be considered as pure lines. Wu et al. developed a new method of establishing a pure line through combining the artificial gynogenesis with artificial sex reversal in common carp[2]. This study developed a new strategy of rapidly establishing pure lines of silver carp and bighead carp through artificial gynogenesis, functional sex reversal and RAPD marker selection. In the process only one gynogenesis was carried out. RAPD markers were dominant markers and the sensitivity of this method was higher than that of other genetic markers used for analysis, such as isozyme, protein and RFLP. Moreover, stability and speciality of RAPD was achieved by optimization of the PCR conditions. The gynogenetic female individuals sharing the same loci of sex relversal male were picked out, so this method was reliable. The homozygosity will reach the expected degree of two consecutive gynogeneses. However, the random primers used in this study do not cover the whole genome, so more primers or other genetic markers should be adopted to further analyze the established pure lines in the future.

References

1 Liu, J. K. et al. Unraveling the enigma of the disappearance of water bloom from the East Lake (Lake Donghu) of Wuhan. Resources and Environment in the Yangtze Basin (in Chinese), 1998, 8(3): 312.

2 Wu, Q. J. et al. Investigation on the carp gynogenesis with reference to establishing a pure line. Acta Genetica Sinica (in Chinese), 1981, 8 (1): 50.

3 Streisinger, G. et al. Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). Nature, 1981, 291: 293.

4 Liu, T. M. et al. Factors affecting the efficiency of somatic cell nuclear transplantation in the fish embryo. Journal of Experimental Zoology, 2002, 293: 719.

5 Kumar, S. et al. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics, 2001, 17(12): 1244.

6 Wu, L. Z. et al. Biochemical genetic structure and variation in a natural population of silver carp from the middle reaches of the Yangtze River. Acta Hydrobiologica Sinica (in Chinese). 1997, 21 (2): 157.

7 Wu, L. Z. et al. Biochemical genetic structure and variation in a natural population of bighead carp from the lower reaches of the Yangtze River. Acta Hydrobiologica Sinica (in Chinese), 1991, 15 (1): 94.

8 Li, S. F. et al. Diversity of mitochondrial DNA in the populations of silver carp, bighead carp, grass carp and black carp in the middle-and lower reaches of the Yangtze River. Acta Zoologica Sinica (in Chinese), 1998, 44(1): 82.

9 Zhang, D. C. et al. Studies on genetic diversity of bighead carp (*Aristichthys nobilis*) in the Yangtze River. Journal of Wuhan University (*Natural Science Edition*) (In Chinese), 1999, 45(6): 857.

10 Zhang, X. Y. et al. Analysis on genetic diversity of *Hypophthalmichthys molitrix* in Changjiang River. Journal of Fisheries of China (suppl.) (in Chinese), 1999, 23: 7.

11 Deng, H. et al. Genetic analysis of gynogenetic silver carp (*Hypophthalmichthys molitrix*) by means of RAPD and protein electrophoresis. Freshwater Fishery (in Chinese), 1998, 28(6): 10.

12 Lou, Y. D. et al. Artificial gnogenesis and its application in genetics and aquaculture. Journal of Fisheries of China (in Chinese), 1986, 10 (1): 111.