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A consecutive self-proliferate silver carp (*Hypophthalmichthys molitrix*) variety created through artificial meiotic gynogenesis

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ABSTRACT

In this research, meiotic gynogenesis (meio-gynogenesis) was used in our silver carp (*Hypophthalmichthys molitrix*) breeding program. Three natural sex-reversed male individuals were found in a second generation meio-gynogenetic strain, and these were used to construct self-proliferate strain. Through morphological trait selection and growth rate comparison, an excellent variety was established, which was named the "Changfeng silver carp" (CF variety). In this study, we compare the genetic, morphological traits and growth rate between the CF variety and the common silver carp species. The average genetic identity of the CF variety is 0.98, and the ratios of full length/body height and body length/body height are 5.87% and 7.21%, respectively, which are significantly higher than those of the common silver carp. These data indicate that the caudal fin of the CF variety is shorter than that of the common silver carp. Principal component analysis (PCA) showed that the morphology between CF variety and the common species is diverged at principal component 1 (PC1), and stepwise discriminate analysis showed that the CF variety and common species can be discriminated by the combined usage of full length, body height and head length, the respective total discrimination accuracies of which are 90.9% (original) and 90.7% (cross-validation). Growth rate comparison tests in two consecutive years proved that there is no significant difference ($P > 0.05$) at age of one year, while the growth rate of the CF variety is 12.27–17.88% faster than that of the common species at the age of two years. These results demonstrate that the combined usage of meio-gynogenesis and morphological traits selection is a feasible and effective method to create an excellent and creditable variety, especially for fish with a relatively long sexual maturity cycle.

1. Introduction

The silver carp (*Hypophthalmichthys molitrix*) is a freshwater cyprinid fish species, native to north and northeast Asia. The silver carp typically feeds on phytoplankton, without the need for extra feed, which is important in reducing production costs and increasing profit. Furthermore, the culture of silver carp may control the burst of algal bloom and improve water quality (Starling, 1993; Chen et al., 2006). The world production capacity has reached 5.54 million tonnes in 2012 (FAO year book Fishery and Aquaculture Statistics, 2012), among which most of the cultured fish are unselected, and these fish lead to lower production and weaker resistance to disease. The development of silver carp culturing is further restrained by inferior fish fries; thus the breeding of outstanding variety is beneficial to the culture of silver carp and protection of water quality. Silver carp have a relatively long sexual maturity cycle; in central China the initial sexual maturity occurs after more than four years, and therefore a period of several decades is usually required for the stabilization of selected traits with conventional breeding methods, such as full sib-mating. As a result, the utilization of a suitable and reliable method to shorten the breeding period is urgently needed.

Gynogenesis is a mode of reproduction in which offspring are formed exclusively from maternal genetic information (Thorgaard, 1983; Gui et al., 1992; Donaldson and Devlin, 1996; Arai, 2001; Komen and Thorgaard, 2007; Gui and Zhou, 2010). Two types of gynogenesis exist, involving either meiotic or mitotic chromosomes (Ihssen et al., 1990; Alsaqufi et al., 2014). Meiotic gynogenesis (meio-gynogenesis) is formed by the second polar body after the second meiotic division, and the polar body is retained within the egg where it fuses with the maternal pronucleus to form a diploid zygotic nucleus. The polar body contains a maternal chromosome set which is produced during the nonreductional division of the meiosis, but is not genetically identical to the haploid egg nucleus, due to the recombination events which exchange genetic information during chromosome pairs (Purdom, 1983). Consequently, meio-gynogens are not homozygous for all loci in genomes. Alternatively, in mitotic gynogenesis (mit-gynogenesis), the maternal nucleus normally duplicates during the first embryonic cell cycle to generate two identical haploid chromosome sets. Due to the fact that the two chromosome sets in a mit-gynogen are derived from a duplicated

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haploid maternal nucleus, these cells are completely homozygous for all loci. Compared to meio-gynogenesis, mit-gynogenesis does not produce as many viable fish as it is completely homozygous for all loci, and every harmful recessive allele will be able to produce its phenotype (Zhang et al., 2013). Different from this, meio-gynogens have a higher survival rate than mit-gynogens, because they have less inbreeding. For practical purposes, meio-gynogens manipulation is easier to perform than mit-gynogenesis (Tave, 1999).

Theoretically, the sexual phenotype of the gynogenetic embryo relies on the gamete via meiosis only; thus the sexual phenotype is varied among different sex determination fishes. In contrast to higher mammals, the sex determination of fish is usually determined genetically, but this is a delicate process, which in many cases can be influenced by environmental factors (Pandian and Sheela, 1995; von Hofsten and Olsson, 2005; Hulak et al., 2008; Senior et al., 2012). Even in a single species, there are different sex determination models, such as tilapia (XX♀/XY♂ & ZW♀/ZZ♂) (Graves and Peichel, 2010; Lee et al., 2011; Piferrer et al., 2012). Previous studies have demonstrated two types of sex determination existing in fishes, namely heterosome and autosome (Devlin and Nagahama, 2002; Kitano and Peichel, 2012; Gui and Zhu, 2012). Four typical heterochromosome determination types exist in fish, which are (1) XX♀/XY♂, (2) ZW♀/ZZ♂, (3) ZO♀/ZZ♂ and XX♀/XO♂, (4) $X_1X_1X_2X_2$♀/X_1X_2Y♂. By artificial gynogenesis, some super male (YY) and super female (WW) individuals have been created respectively in yellow catfish (Wang et al., 2009; Liu et al., 2013) and flatfish (Chen et al., 2014), and all male variety has been produced in yellow catfish (Liu et al., 2013; Dan et al., 2013). In theory, for the autosome type fishes (NN♀/NN♂), all the gynogenetic offspring are female (NN♀), and the consecutive self-proliferation of the gynogenetic population seems unrealistic without male individuals. Current knowledge of sex determination in the silver carp is largely unknown, and no morphological differences in the chromosomes of the two sexes have been identified by classical karyotype.

We performed artificial meio-gynogenesis in our silver carp breeding program in 1987 (Pan, 1988), and three natural sex-reversed male individuals were found in the second generation of the meio-gynogenetic strain in 2002, in which self-proliferate strains were constructed by having three male individuals mate with other female individuals. After performing systematic and continuous comparisons of the morphology and growth rates between common species, the offspring of strain II were certified by the Aquatic Raw Variety Approval Committee (ARVAC) of China in 2010 with the formal name "Changfeng silver carp" (CF variety) (certification number: GS-01-001-2010). Here we present the results of the comparative studies on the genetic traits, morphological traits and growth rate characterizing the meio-gynogenetic variety. As the first consecutive self-proliferate of the meio-gynogenetic sliver carp variety, the selection process provided an ideal example for the breeding of other fishes with relatively long sexual cycles.

2. Material and methods

2.1. Selection process of CF variety

We performed artificial meio-gynogenesis in the silver carp breeding program in 1987, using inactivation by UV irradiation of the sperms of common carp (*Cyprinus carpio*), fertilized with eggs from a single excellent female silver carp from the Yangtze River, from which more than 5000 individuals of meio-gynogenetic diploid fries were obtained (Pan, 1988), and all these meio-gynogenetic offspring were cultured in separated ponds to avoid intermingling with common sliver carp. In 1996, two female meio-gynogenetic parents with outstanding growth traits were selected for the second round of meio-gynogenesis to construct a family, and 3000 offspring from each parent were kept for culture in separated ponds. In 2002, three naturally sex-reversed male individuals in family-II were found in the second meio-gynogenetic strain, which were confirmed to have isozymes (supplemental material). Self-proliferate strains were produced after these three male individuals mating with other female individuals (Fig. 1). Strain II shows the greatest economic traits among these strains. The excellent male and female individuals in Strain II were selected for self-proliferation (Pan et al., 2004). The growth rates were continually compared between the offspring of the Strain II and the common species, and the growth rate of the Strain II was shown to be 13.3–20.47% faster than that of the common species. The offspring of Strain II were certified by ARVAC of China in 2010.

2.2. Measurement of morphological traits

Conventional methods were used to measure the morphological traits, including body weight, full length, body length, head length, and body height. CF variety and common species of the fish in each growth stage from 1 to 3 years old were individually sampled and measured. The effective sample size of the CF variety is 390 (weight: 50.3–4150.2 g, body length 13.7–60.7 cm), and the common species is 386 (weight: 50.2–4400.4 g, body length: 13.8–61.6 cm).

2.3. Comparison of the growth rate in two consecutive years between CF variety and common species

Fish in the first growth circle period since hatching are named 0^+ (from early larva to end of growth in winter); correspondingly, fish in the second growth circle after 0^+ are named 1^+.

For the 0^+ fish, individuals with the same size (2.5 ± 0.1 g) were marked by coded wire tag, the CF variety (1300 individuals) and common species (1300 individuals) were cultured in the same pond (1000 m^2), and a total of three ponds were used as replicates. The growth cycle of 0^+ was between June 15 and December 15, under natural conditions.

Individuals of the 0^+ tests were then collected and randomly selected, and further marked by cutting off the pectoral fin (either left or right) for 1^+ fish testing. Fish of the CF variety (200 individuals) and common species (200 individuals) with the same size were cultured in a single pond (1000 m^2), and three ponds were used as replicates. The growth cycle of 1^+ was from May 1 to December 1, under natural conditions.

2.4. SSR analysis

The method for DNA extraction was described elsewhere (Sambrook et al., 1989), and the SSR protocol was described previously (Zhang et al., 2010). The SSR premiers are listed in Table 1.

2.5. Data processing

The data were analyzed using the following statistical method: POPGENE 3.2 software (Yeh et al., 1999) was used for genetic diversity analysis. The MEGA 5.0 software package (Tamura et al., 2011) was used for cluster analysis. ANOVA (Mean $\pm$ SD) was used for the statistical analysis of the morphological traits of the CF variety and the common species. The least squares method was used for the regression analysis of morphological traits. The maximum variance cluster algorithm was used for principal component analysis (PCA). Stepwise discriminate analysis (Wilks' Lambda) was used to differentiate the CF and common species.

3. Results

3.1. Self-proliferate strain construction and genetic diversity of CF variety

3.1.1. Self-proliferate strain construction by meio-gynogenesis

In our selection procedure, all of the meio-gynogenetic offspring were cultured in separated ponds so as to avoid intermingling with the common sliver carp. Three naturally sex-reversed male individuals were found in the second meio-gynogenetic strain, which were previously identified with isozymes (supplemental material). To further