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RAPD and microsatellite analysis of diploid gynogens from allotetraploid hybrids of red crucian carp (*Carassius auratus*)×common carp (*Cyprinus carpio*)

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Abstract

Genetic variation was comparatively analyzed between the artificially induced diploid gynogen population from F_{10} allotetraploid hybrids of red crucian carp (♀) (*Carassius auratus* red var., 2n=100)×common carp (♂) (*Cyprinus carpio* L., 2n=100) and the normal F_{10} allotetraploid hybrid population used as the control, using random amplified polymorphic DNA (RAPD) assay and microsatellite analysis. The specific 600-bp fragment for diploid gynogen population was detected by S45 and the specific 900-bp fragment for allotetraploid F_{10} hybrid population was detected by S134. The results from RAPD assay and microsatellite analysis were in agreement with each other, that is to say, the diploid gynogens presented lower level of polymorphism than allotetraploid F_{10} hybrids. Furthermore, as expected, microsatellite analysis revealed more detailed information on genetic diversity than RAPD assay. The mean percentage of polymorphic loci (12.71%) and Shannon's index of phenotypic diversity (0.25) from RAPD data for diploid gynogen population were significantly lower than those (30.69% and 0.63, respectively) for F_{10} allotetraploid hybrid population. The mean number of alleles per microsatellite locus (1.73) in diploid gynogen population was considerably lower than that (2.55) in F_{10} allotetraploid hybrid population. The average observed (0.36) and expected heterozygosity (0.26) in diploid gynogen population were lower than those (0.58 and 0.40, respectively) in F_{10} allotetraploid hybrid population, indicating that the diploid gynogens presented lower genetic diversity than the allotetraploids. In addition, the mean effective number of alleles at 11 microsatellite loci (1.60) in diploid gynogen population was lower than that (1.88) in F_{10} allotetraploid hybrid population. The significant differences between two populations in the average observed and expected heterozygosity, mean number of alleles and effective number of alleles, suggested that the effect of gynogenesis resulted in rather higher genetic homogeneity in diploid gynogens. The comparative RAPD analysis of diploid gynogens and their parents was performed with 34 primers. The identical RAPD pattern was detected between diploid gynogens and their female parent, however, some clear specific RAPD bands were detected between diploid gynogens and their male parents, but not

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detected in their female parent. The result indicated that heterologous genetic material had incorporated into diploid gynogenetic fish (G_1).

Keywords: Gynogenesis; Random amplified polymorphic DNA; Genetic similarity; Genetic diversity; Microsatellite; Allotetraploid; *Cyprinus carpio*; *Carassius auratus*

1. Introduction

In our previous studies (Liu and Zhou, 1986; Liu et al., 2001), the F_1–F_2 hybrids of red crucian carp (♀) (*Carassius auratus* red var., 2n=100)×common carp (♂) (*Cyprinus carpio* L., 2n=100) were found to be diploid. In the F_1 hybrids, 4.67% males and 44.3% females were fertile, which is an exception to the general case of sterility in interspecific hybrids. The fertile males and females mated each other, producing F_2. It was confirmed that the females and males of diploid F_2 hybrid were able to produce diploid eggs and diploid spermatozoa, respectively, which fertilized each other to form the bisexual fertile allotetraploid fish in F_3. The tetraploidy was maintained from one generation to another generation. So far, the F_{13} hybrids were formed. Based on examining the numbers and karyotypes of chromosomes, the F_3–F_{11} hybrids were proved to be allotetraploids with 200 chromosomes containing two chromosome sets of red crucian carp and two chromosome sets of common carp (Liu et al., 2001; Sun et al., 2003). Similar to F_3–F_{11}, F_{12}–F_{13} hybrids were also confirmed as allotetraploids (data not published). It is the first case of creation a bisexual fertile allotetraploid population in fish (maybe in vertebrates). The main practical interest in tetraploids is for producing triploids. The large-scale allotriploid crucian carp and allotriploid common carp were produced in China through mating the F_3–F_{13} hybrids (♂) with Japanese crucian carp (♀) (*C. auratus* cuvieri) and common carp (♀), respectively, which showed some enhanced performances such as high anti-disease ability, faster growth rate, sterility and good flesh quality (Zhou et al., 1999; Liu et al., 2000a,b).

The allotetraploids possessed four sets of chromosomes and produced the diploid gametes. So it is worth making the gynogenetic experiment to elucidate their sex determining system and the diploid eggs' trait. The gynogenetic experimental results (Liu et al., 2004) indicated that following activation by UV-irradiated sperm, the diploid eggs of allotetraploid hybrids developed into normal live first-generation diploid gynogens (abbreviated G_1). Cold-shocking these activated diploid eggs failed to induce the expected tetraploidy in them. All these gynogenetic progenies, generated with or without the cold shock, were female diploids with 100 chromosomes, indicating the XXXX genotype of allotetraploid females. The gynogens were not morphologically distinguishable from the F_2 hybrids. Like the diploid F_2 hybrids, the diploid G_1 also produced diploid eggs, for these eggs developed into triploids with 150 chromosomes when crossed with diploid red crucian carp and developed into tetraploids with 200 chromosomes when crossed with tetraploids. Furthermore, without the treatment for doubling the chromosome number, the eggs, produced by the G_1, developed into second-generation diploid gynogens (abbreviated G_2) following activation by UV-irradiated sperm. Recently, we found that both G_1 and G_2 grew 30% faster than the allotetraploids (data not shown). Production of these diploid eggs from the diploid gynogens may have resulted from the pre-meiotic endoreduplication of chromosomes. For aquaculture, these diploid eggs provided an important gamete source for the production of tetraploids and triploids, and establishment of diploid gynogenetic hybrid line.

DNA polymorphisms were extensively employed as a means of assessing genetic diversity in aquatic organisms. A relatively small sample size was informative from the DNA polymorphisms. The polymerase chain reaction (PCR) provided a simple, fast and inexpensive means for genome analysis. With the advent of recombinant DNA and PCR techniques, a single, short oligonucleotide primer was used to amplify specific sequences of genomic DNA. Random oligonucleotide primers produced random amplified polymorphic DNA (RAPD) that were extensively used as molecular markers (Kikuchi et al., 1997; Koh