主論文の要約

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学 位 論 文 題 目

Studies on genetic and reproductive characteristics of natural polyploid silver crucian carp

Carassius auratus langsdorfii

（自然倍数体ギンブナの遺伝および生殖特性に関する研究）

Silver crucian carp *Carassius auratus langsdorfii* that is most widely distributed in the wild populations of Japan comprises a diploid-polyploid complex. Bisexually reproducing diploids (2n = 100) are sympatrically distributed with gynogenetically developing triploids (3n = 156) and tetraploids (4n = 206) (Kobayasi et al., 1970). Genetic diversity of natural gynogenetic triploid Japanese silver crucian carp has been studied using different types of genetic markers such as isozyme (Shimizu et al., 1993), random amplified polymorphic DNA assay (RAPD-PCR) (Murakami et al., 2001), mitochondrial DNA (mtDNA) sequencing (Murakami and Fujitani, 1997), restriction fragment length polymorphism (RFLP) and microsatellite loci (Ohara et al., 1999, 2003). However, the genetic and reproductive characteristics of triploids and tetraploids are still required to be studied due to the presence of relatively fewer tetraploid female and very rare polyploid male in wild population.

In this thesis, silver crucian carp samples were collected from rearing population in the Gunma Prefectural Fisheries Station. This population is originated from natural population in Jounuma Lake, Gunma Prefecture and rearing population has been maintained by artificial fertilization. Firstly, ploidy status was determined. Then, genetic structure was studied based on the microsatellite DNA markers. Finally, reproductive performances of triploid and tetraploid *C. a. langsdorfii* were tested under laboratory conditions.

In chapter 1, many triploids and a few tetraploids appeared in Jounuma origin population. Additionally, genetic variations were also found in triploid and tetraploid *C. a. langsdorfii* by six microsatellite loci as in previous studies (Ohara et al., 1999, 2000; Bai et al., 2011). All the triploid and tetraploid individuals were heterozygotes, suggesting complicated genetic structures of polyploids. A major clone (3nC1) and several minor clones (3nC2–C6) were detected.

Because of few reports on bisexual reproduction and very rare appearances of polyploid males in wild population in Japan, polyploid silver crucian carp should possess an exclusive gynogenetic reproductive mode. Additionally, the hexaploid *C. a. langsdorfii* has not been reported in nature. Therefore, the author speculated that triploid *C. a. langsdorfii* reproducing unisexually might be produced from bisexual diploids. However, the origin of tetraploid *C. a. langsdorfii* was not still clear. Here, phylogenetic relationships among the 14 triploid and 4 tetraploid were investigated and the results indicated that Jounuma origin tetraploids (4nC1, 4nC2, 4nC4) might have arisen from triploid. However, one tetraploid but 4nC3 (Nanae, no record of the origin) showed a distant relationship from Jounuma-Group.

In chapter 2, reproductive performance of triploid female from a major clonal line 3nC1 was tested by artificial insemination with sperm of diploid goldfish *C. a. auratus*. Rates of fertilization and hatching were examined. Ploidy status of normal larvae was assessed by nuclear DNA content flow cytometry analysis. Then, using selected samples, isogenic characteristics of these progeny were verified by random amplified polymorphic (RAPD) DNA-PCR fingerprints.

Ploidy analysis and RAPD-PCR finger prints demonstrated that gynogenesis was initiated by fertilization with sperm from goldfish in eggs of triploid, because all the progeny were triploids and had no paternally derived DNA fragments. Thus, clonal triploid eggs may be formed by apomixis, i.e., ameiotic division of oocytes by skipping the first meiotic division, in triploid crucian carp (Yamashita et al., 1993). Interestingly, two hypertriploids (3.6C) surviving for a long time were found in normal progeny. This result suggests that silver crucian carp can tolerate hyper-ploidy

comprising extra or supernumerary chromosomes. Here, gynogenesis of silver crucian carps in major clone (3nC1) was also verified by reproductive experiment. But the gynogenesis of minor tentative clone individuals has not been confirmed due to the poor egg qualities. Further experiments are still required to be conducted.

In chapter 3, the author examined genetic characteristics of progeny of a pair of tetraploid crucian carp which were reproduced in a tank by natural spawning. Here, 120 individuals were randomly selected from the large population of progeny arising from a pair of the tetraploids and were assessed for their ploidy status by flow cytometry analysis. Then, using selected samples, isogenic characteristics of these progeny were verified by random amplified polymorphic (RAPD) DNA-PCR fingerprints and microsatellite DNA analysis. Chromosomes of these progeny were also prepared and assessed by conventional karyotyping and rDNA detection using fluorescence in situ hybridization (FISH).

All the progeny examined revealed tetraploid-range DNA contents, RAPD and microsatellite genotypes which were identical to the mother. These results indicated that these progeny occurred by gynogenetic development in tetraploid eggs spawned by a tetraploid female after fertilization by a tetraploid male sperm. Thus, sperm from the tetraploid male had triggered gynogenesis in unreduced tetraploid eggs. As shown in the present study, eggs from a tetraploid female initiated gynogenetic development even after fertilization with homospecific sperm from a tetralploid male. Among 120 individuals examined, no hexaploid individuals arose due to the incorporation of a sperm nucleus from the tetraploid male. At the five tested microsatellite loci, mother (TetFA1) and all 14 progeny had the same allelic type, while father (TetMA1) showed different type. In the present study, the father-specific allele was not found in all progeny tested. The microsatellite results strongly indicated the occurrence of gynogenetic development in tetraploid eggs even after fertilization by a tetraploid male sperm.

The karyotypes of the goldfish comprised 20 M + 40 SM + 40 ST/T chromosomes, which is similar to those previously reported in diploid *C. auratus subsp*. (2n = 100) and diploid Miyazaki race silver crucian carp (2n = 100) (Kobayasi et al., 1970). The karyotype of a tetraploid silver crucian carp gave 4n = 206 chromosomes which were

categorized into 44 M + 82 SM + 80 T. The presence of six microchromosomes found in the present study suggested that a gynogenetic tetraploid may have arisen from a triploid with 3n = 156 chromosomes by incorporation of haploid chromosomes. Ribosomal rDNA signals were detected in four chromosomes of diploid goldfish with 2n = 100 when hybridized with 5.8S + 28S rDNA probes. Whereas eight signals were detected in chromosomes of tetraploid silver crucian carp with 4n = 206. These FISH results indicated that the progeny of tetraploid silver crucian carp had four sets of chromosomes. However, the exact morphologies of rDNA-bearing chromosomes have not been determined and additional molecular cytogenetic studies will be required in the near future.

In this thesis, the author clarified the genetic and reproductive characteristics of natural polyploid silver crucian carp *C. a. langsdorfii* in Japan. The molecular and cytogenetic results show that Japanese triploid and tetraploid possesses an exclusive unisexual reproductive mode. Such a reproductive system is different from that in Chinese silver crucian carp *C. a. gibelio*, which reproduces both unisexually and bisexually.