Discovery of Two Different Reproductive Development Modes of the Eggs of Artificial Multiple Tetraploid Allogynogenetic Silver Crucian Carp

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Introduction

Artificial multiple tetraploid allogynogenetic silver crucian carp possesses the reproductive function of gynogenesis in spite of incorporating one genome of common carp, because all chromosomes of silver crucian carp are maintained[1]. This was first confirmed in the artificial propagation experiments in which the eggs of the multiple tetraploid fish were respectively inseminated with normal and UV-irradiated sperms of red common carp[2]. Recently, our laboratory has found in studying the gynogenetic mechanism of silver crucian carp that there exist two different fertilized cytological behaviors as the sperm of homologous silver crucian carp and the sperm of heterologous red common carp or general crucian carp enter the eggs, i. e. the heterologous sperm cannot be promoted to develop, and sperm nucleus is kept in the state of condensation, representing the typical gynogenetic process; whereas the homologous sperm can be decondensed and associated with female nucleus, but cannot be further developed to transform male pronucleus[3, 4]. The newly discovered multiple tetraploid fish incorporates one foreign genome and elevates ploidy. Will the ability of the eggs responding to the homologous and heterologous sperms be influenced by the incident? In order to answer this question, we performed artificial propagation experiments with the sperm of paternal species red common carp and the sperm of maternal species silver crucian carp, and carried out cytogenetic analysis for the embryos. 4 multiple tetraploids were selected in the experiments. The results reveal that the responses of the eggs to insemination of paternal and maternal species sperms are obviously different, especially the response to homologous sperm of the maternal species changes a lot, representing two completely different reproductive results. Chromosome analysis of embryo cells preliminarily suggests that they result from two different reproductive development modes, i. e. allogynogenesis and pseudoamphimixis.

1 Materials and Methods

1.1 *Origin of Experimental Fishes and Artificial Propagation Groups*

Artificial multiple tetraploids were discovered from artificially propagated populations of allogynogenetic silver crucian carp[1]. Red common carp (*Cyprinus carpio* red variety) and transparent colored crucian carp (*Carassius auratus* transparent colored variety) were taken from Guanqiao Experimental Station of the Institute of Hydrobiology. Considering each multiple tetraploid fish as the mother of one "clone", the propagated 4 multiple tetraploid fishes were respectively termed 9102, 9103, 9104 and 9105 (9101 had been used in the preliminary confirmation of gynogenetic reproduction[2]) according to the order of ovulation. In the first 3 fishes (9102, 9103 and 9104), the eggs were respectively inseminated with the sperm of paternal species red common carp and the sperm of maternal species silver crucian carp, and the artificial gynogenetic control experiments, in which the eggs of the tetraploid fishes and the eggs of bisexual diploid transparent colored crucian carp or red common carp were respectively inseminated with UV-irradiated (by the method reported by Jiang *et al.* [5]) sperm of red common carp, were also performed. As for 9105, only two propagation groups inseminated with the paternal and maternal species sperms were carried out because of insufficient eggs.

1.2 *Statistics of Survival Rates of Embryos and Fingerlings*

The inseminated eggs in every experimental group were even sprinkled in the culture dishes of 15 cm diameter (about 300—500 eggs per dish). Each group had 2—8 dishes according to the number of eggs. Before gastrula stage, the number of eggs per dish was counted, dead embryos were picked out and counted respectively at gastrula, tail-bud, somitation and pigmentation stages. In this way, the number of survival embryos and survival rates were calculated at each stage. Hatching rates included all larvae with movable ability. The survival rates at first-feeding and after one month of culture were the proportion of the survival larva number cultured in fishpond and the survival fingerling number after one month of culture respectively to the number of inseminated eggs.

1.3 *Chromosome Analysis of Embryonic Cells*

Chromosome preparations and analysis on embryos at gastrula and somitation stages were made according to the method reported by Gui *et al.* (1990)[6].

2 Results

The results (Fig. 1) of artificial propagations indicated that the eggs of the 4 multiple tetraploid fishes displayed two absolutely different development fates as the eggs were inseminated with paternal red common carp sperm and maternal silver crucian carp sperm. In the groups inseminated with red common carp sperm (including normal sperm and UV-irradiated sperm), embryos, larvae, fingerlings and adults developed and grew normally. However, in the groups inseminated with silver crucian carp sperm, the majority of embryos displayed deformation before and after hatching stage. Most of the embryos were capable of hatching, and the hatched larvae were movable, obviously different from the deformed larvae without moving ability in gynogenetic haploid controls of bisexual fish, but the majority of them had no ability of normal swimming, only turned circle unceasingly in water until twisting to crescent moon pattern and died successively. The rates of deformed larvae after one day of hatching were somewhat different among the 4 fishes, but were all over 50%, i. e. 53.6%, 66.1%, 70.3% and 65.8% respectively. Although some larvae could survive after culturing in fishpond, the vast majority of them could not escape from the fate of death. After one month of culture, only a few individuals could survive. The rates of these survivors relative to the inseminated eggs were respectively 0.85% (30 fishes from 3549 eggs), 0.12% (3 fishes from 2506 eggs), 0.33% (4 fishes from 1199 eggs) and 0.52% (3 fishes from 581 eggs), and basically neared the survival rates of spontaneously doubling gynogenetic diploids in haploid gynogenetic controls of bisexual fishes, because the proportions of survivors from the 3 haploid gynogenetic control groups relative to the inseminated eggs were respectively 0.22%, 0.63% and 0.27% after one month of the same culture.

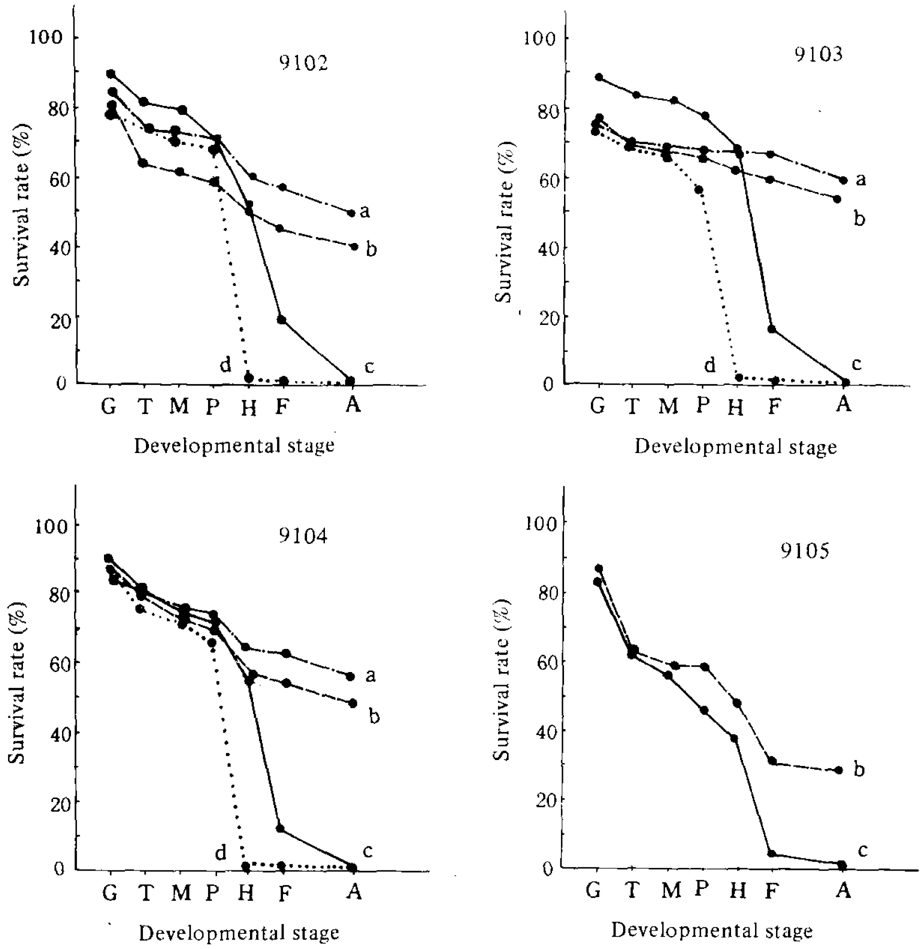

Fig. 1. Survival rates at different developmental stages (gastrula, G; tail-bud, T; myocomma appearance, M; pigmentation, P; hatching, H; first-feeding, F; after one month of culture, A) in the experimental groups of the eggs of 9102, 9103, 9104 and 9105 multiple tetraploid fishes that were respectively inseminated with UV-irradiated sperm (a) and normal sperm (b) of red common carp as well as silver crucian carp sperm (c), and in the control groups of the eggs from transparent colored crucian carp or red common carp that were inseminated with the same UV-irradiated sperm (d, gynogenetic haploid control).

The number of eggs per group: 9102: a(4058), b(1123), c(3549), d(1364) (eggs of transparent colored crucian carp); 9103: a(446), b(472), c(2506), d(478) (eggs of transparent colored crucian carp); 9104: a(1522), b(652), c(1199), d(1115) (eggs of red common carp); 9105: b(641), c(581).

Another characteristic of artificial propagations was that the survival rates in the groups inseminated with UV-irradiated sperm were higher than those in the groups with normal sperm. This phenomenon had been observed in our previous study[2]. The reasons leading to the difference might be related to the mechanism of oogenesis.

Chromosome analysis of embryonic cells revealed the reproductive mechanism of the two different development fates about the eggs of multiple tetraploid fishes. As the same reported by us[2], there are about 212 chromosomes in the majority of metaphases prepared from the embryos developing from the inseminated eggs of multiple tetraploid fishes with red common carp sperm, no matter what the sperms were, i.e. the embryos were developed by gynogenetic mode so that they maintain all chromosomes of the female parent. Whereas in the specimens prepared from the embryos developing from the inseminated eggs with silver crucian carp sperm, the majority of metaphases contain more than 212 chromosomes, most of them are hypertetraploid cells with 230—240 chromosomes (Fig. 2b). Some metaphases with 293 chromosomes (Fig. 2a) were also observed, in which the number was just equal to the sum of chromosome number (212) of multiple tetraploid ameiotic egg and chromosome number (81) of silver crucian carp meiotic sperm. This suggests that the female nucleus of the egg and male nucleus of the sperm might undergo fusion in some degree after the multiple tetraploid egg accepts the silver crucian carp sperm, which is obviously different from the development mode responding to the red common carp sperm.

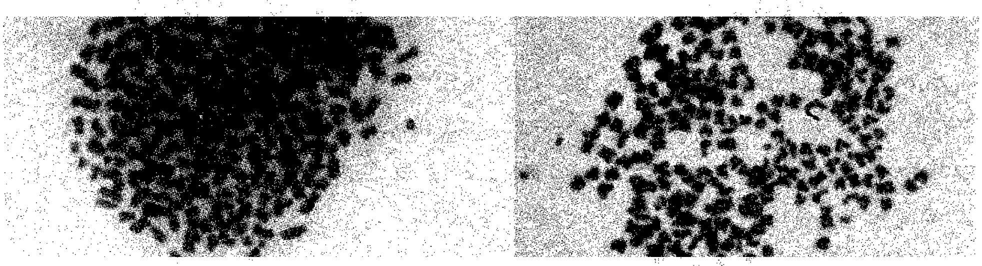

Fig. 2. The metaphases observed from the specimens that were prepared from the embryos developing from the eggs of the multiple tetraploid fish inseminated with silver crucian carp sperm. a, CN=293; b, CN=236.

3 Discussion

The above results suggest that the multiple tetraploids possess two different development modes. When the egg is inseminated by the sperm of paternal species, the egg nucleus will be activated to develop and hatch normal multiple tetraploid offsprings. The responding reproductive mode is a typical gynogenesis. When the egg is inseminated with the sperm of the maternal species silver crucian carp, the responding mechanism is quite different. Judging from cytogenetic analysis, the sperm nucleus and egg nucleus seem to have undergone fusion, but as the chromosome number (212) of egg nucleus is much larger than that (81) of sperm nucleus, the reactive consequences might result in partial chromosome elimination and loss, and therefore lead to the formation of aneuploid embryos without hatching. Since the lost chromosomes and chromosome number are various among aneuploid embryos, and the degrees of the influence on embryos are also various, thereby, deformation and death occur early or late among these embryos. The responding result is somewhat similar to that of distant hybridization between two bisexual species with various chromosome numbers, but the genetic bases of the eggs are different, i.e. the egg of multiple tetraploid fish does not undergo conventional meiosis, and the fertilization process and mechanism remain to be further studied. So, we call the second responding development mode the pseudoamphimixis.

The two different reproductive development modes of multiple tetraploid fish eggs might be the expansion of differences that silver crucian carp eggs react to heterologous sperm and homologous sperm, but the propagation consequences between them are quite different. As concerns silver crucian carp, although there exist different cytological behaviors[3, 4] for the two types of sperms, both the inseminated eggs can hatch normal offsprings[7]. As we performed the above experiments, the control experiments, in which silver crucian carp eggs were inseminated with the same red common carp sperm and silver crucian carp sperm, were also carried out. The results show that both of them can hatch normal larvae with more than 60% hatching rate, and the larvae can develop and grow normally. There exist two different reproductive development modes for multiple tetraploids, but only when the eggs are stimulated by the heterologous sperm of paternal species red common carp can a lot of normal offsprings be reproduced by the gynogenetic mode; whereas homologous sperm of maternal species silver crucian carp does not have the capability. We also discovered that multiple tetraploid fish eggs inseminated with silver carp (*Hypophthalmichthys molitrix*) sperm also hatched normal gynogenetic offsprings. It means that the extensivity of responding heterologous sperms to gynogenesis is similar to silver crucian carp. Of course, this requires to be evaluated by propagations with various species sperms. Additionally, the pseudoamphimixis mode of multiple tetraploids might possess the obvious genetic breeding potential, because it can be preliminarily observed from some biological character such as body color and scale (unpublished data) that the surviving individuals might contain the additive chromosome set or chromosomes.

The responding mechanism of two different reproductive development modes might be the first discovery in vertebrates so far. It seems that the mechanism exists in the eggs of silver crucian carp, which merely form a delicate control system in evolution. The control system can not only coordinate development of sperm nucleus, but also impel sperm nucleus to bring into play (for example, the appearance of males, etc.). Multiple tetraploid individuals might further extend the differences that silver crucian carp eggs respond heterologous and homologous sperms owing to incorporating one genome of red common carp. Therefore, the distinctness between the two responding reproductive modes will explore a new breakthrough point for revealing the control system and gynogenetic mechanism of silver crucian carp eggs, and convince us that the gynogenetic silver crucian carp and the newly discovered multiple tetraploid individuals are of special implication in evolutionary genetics research of unisexual and polyploid vertebrates[8—11].

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