Reproduction and Breeding 2 (2022) 22–29

Contents lists available at ScienceDirect

Reproduction and Breeding

journal homepage: www.keaipublishing.com/en/journals/reproduction-and-breeding

The formation and biological characterization of two allotriploid fish derived from interploid crosses

Jialin Cui [a,b,c,1], Mengxue Luo [a,b,c,1], Xin Gao [a,b,c], Hong Zhang [a,b,c], Xueyin Zhang [a,b,c], Li Ren [a,b,c,**], Shaojun Liu [a,b,c,*]

[a] State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha, 410081, Hunan, PR China
[b] Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry, Changsha, 410081, Hunan, PR China
[c] College of Life Sciences, Hunan Normal University, Changsha, 410081, Hunan, PR China

ARTICLE INFO

Keywords:
Interploid cross
Allotriploid
Sterility
Mitochondrial genome

ABSTRACT

Interploid cross is regularly observed in plants and often results in phenotypic changes, including reduced fertility and heterosis. Yet few triploids have been observed in animals due to the rare coexistence of diploid and allotetraploid populations in species. However, heterosis in growth traits and innate immunity have been detected in the allotriploids obtained from interploid crossing of the male allotetraploids of red crucian carp and common carp (4nAT) with the females of their inbred parents. Here, we obtained two new types of allotriploids ($3nR_2C$ and $3nRC_2$) by the interploid crossing of female 4nAT with the inbred parents (red crucian carp and common carp, respectively). The embryos of the two allotriploids developed normally through the zygote, cleavage, blastula, gastrula, segmentation and hatching stages, and high fertilization rates ($3nR_2C$: 91.31%, $3nRC_2$: 84.09%) and hatching rates ($3nR_2C$: 72.96%, $3nRC_2$: 71.15%) were observed. The measured DNA content and chromosome numbers suggested that both $3nR_2C$ and $3nRC_2$ were allotriploids with 150 chromosomes. Intermediate characteristics of some morphological traits, such as the body length to body height ratio (BL/BH), were observed in $3nR_2C$ and $3nRC_2$. In contrast to the stage II oocytes in the ovaries of the inbred parents, cystic structures formed by small, underdeveloped oogonia were observed in the two allotriploids. In contrast to the observation of mature spermatids in the testes of the inbred parents, spermatids with irregular shapes that began to degrade and disintegrate were observed in the two allotriploids. These results revealed abnormal gonad development in the allotriploids. Taken together, our results indicate that the two new allotriploids will be significant in aquaculture.

1. Introduction

Hybridization is a genetic breeding technology for effectively obtaining varieties with hybrid vigour in aquaculture [1]. Examples of heterosis achieved via hybridization include the reduction of intermuscular bone counts in a *Culter* hybrid compared with *Culter alburnus* and strong innate immunity detected in hybrid populations [2,3]. All of these improved varieties not only meet consumer demands but are also beneficial to economic efficiency [4]. Many investigators have explored the genetic basis of heterosis at the genomic, epigenomic, and transcriptional levels [5–7]. Many relevant experiments have also been conducted to obtain improved varieties, such as reciprocal intergeneric hybrid lineages of *Megalobrama amblycephala* and *Culter alburnus* and the hybrid progenies of *Ctenopharyngodon idellus* (female) × *Erythroculter ilishaeformis* (male) [8,9]. Diploid and tetraploid fish populations have been obtained via hybridization, providing valuable germplasm resources for genetic breeding [10].

Heterosis has often been studied in triploid plants but has rarely been studied in fish [11,12]. Sterile fish in natural waters do not damage the ecological environment. One study on the ovaries and testes of allotriploids showed that they could not produce mature eggs and sperm [13]. Other studies using cytogenetic and molecular methods confirmed that pachytene arrest occurred in the oocytes of allotriploid fish [14]. The growth rate of the triploids was faster than that of their parents [15,16].

* Corresponding author. State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha, 410081, Hunan, PR China.
** Corresponding author. State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha, 410081, Hunan, PR China.
E-mail addresses: cuijialin8023@163.com (J. Cui), lmxmxl123@163.com (M. Luo), gao634401733@126.com (X. Gao), hongzhang0609@qq.com (H. Zhang), XueyinZhang0219@qq.com (X. Zhang), renli_333@163.com (L. Ren), lsj@hunnu.edu.cn (S. Liu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.repbre.2022.02.004
Received 18 November 2021; Received in revised form 6 January 2022; Accepted 22 February 2022
Available online 16 March 2022

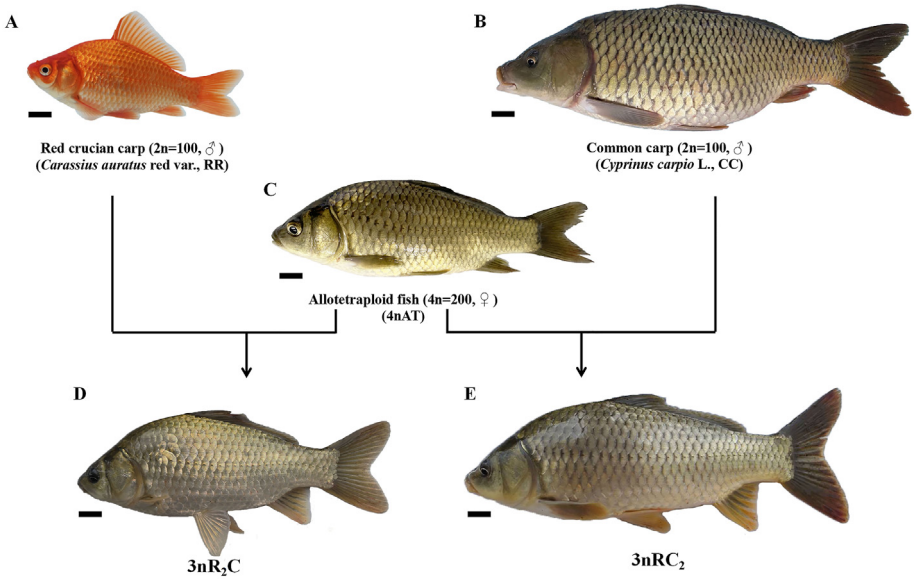

Fig. 1. Crossing procedure and appearance of two allotriploids. (A)–(C) Parents of the two allotriploids. (D) Hybrid of 4nAT (female) × RR (male). (E) Hybrid of 4nAT (female) × CC (male). Bar = 3 cm.

However, the slow gonadal development of the triploids suggests that a portion of the energy originally allocated to gonadal development may be transferred to the growth system in these individuals, leading to a faster growth rate [17]. Under induction by physical and chemical treatments, only low survival of triploids can be achieved because of damage to fertilized eggs [18]. However, hybridization is an efficient method for obtaining triploids through the interploid crossing of tetraploids and diploids [1,19].

Both red crucian carp (RR) and common carp (CC) [20,21], belonging to the Cyprinidae family, are important common aquaculture species in China. In a previous study, an allotetraploid population derived from the intergeneric crossing of RR (female) with CC (male) was proven to show bisexual fertility [22]. There are some types of triploid fish generated by crossing the male allotetraploid with female diploids, including white crucian carp [17], CC [23], and RR [24]. In this study, we obtained two allotriploids derived from the interploid crossing of allotetraploid hybrids (4nAT, female) with the inbred parents (RR and CC). We investigated some biological characteristics of the two allotriploids, including their embryonic development, DNA content, chromosome numbers, morphological traits, gonadal development and mitochondrial genome. The two allotriploids may become new varieties used in aquaculture and could provide models for research on the mechanism of heterosis.

2. Material and methods

2.1. Ethics statement

All experiments conducted in this study were approved by the Animal Care Committee of Hunan Normal University and followed the stated guidelines of the Administration of Affairs Concerning Animal Experimentation of China and ARRIVE. Fish collection and crossing were approved by the Animal Care Committee and Protection Station of Polyploidy Fish of Hunan Normal University (approval ID: 03/2018). The fish were deeply anaesthetized with 100 mg/L MS-222 (Sigma-Aldrich, St.LOUIS, MO, USA) prior to dissection.

2.2. Animals and cross breeding

All the RR, CC, and 4nAT individuals referenced in this study were raised in the Engineering Research Center of Polyploid Fish Breeding and Reproduction of the State Education Ministry at Hunan Normal University. Mature parent fish were selected during the breeding season (from March to May). The interploid crosses were performed in two groups. In one group ($3nR_2C$), 4nAT and RR were used as the female and male parents, respectively. In the other group ($3nRC_2$), the male parent was changed to CC. Embryos were produced by artificial fertilization and developed in culture dishes at a water temperature of 18–20 °C. Six culture dishes for each group were selected at random for continuous observation of embryonic development and photographed with a Pixera Pro 600 ES digital camera (Nikon, Japan). In each group, approximately 2000 embryos were randomly chosen to examine the fertilization rate (number of embryos at the blastula stage/total number of eggs × 100%) and hatching rate (number of hatched fry/number of eggs × 100%). The hatched fry were transferred to a pond for further culture.

2.3. Measurement of DNA content and preparation of chromosome spreads

Peripheral blood samples of RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$ were collected to measure the DNA content using a flow cytometer (Cell Counter Analyser, Partec, Germany). All blood samples were processed according to the method described in a published paper [25]. Each sample was measured under the same conditions. To examine the deviation in the ratios of the DNA content of the hybrids to the sum of contents of the parents, we employed the χ^2 test with Yate's correction.

Kidney tissues were used to detect chromosome number in RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$. The applied method followed that found in a published paper [26] with minor modifications. After culturing for two to three days at 20–22 °C, the samples were injected one to three times with phytohemagglutinin (PHA) at a dose of 6–15 μg/g body weight at an interval of 12–24 h. Two to three hours prior to dissection, each sample was injected with colchicine at a dose of 4–6 μg/g body weight. The kidney tissue was ground in 0.9% NaCl and subjected to hypotonic treatment with 0.075 M KCl at 37 °C for 40–60 min and then fixed three times in 3:1 methanol-acetic acid. The cells were added dropwise to cold, wet microslides and stained with 4% Giemsa stain for 40–60 min. Chromosome shapes and numbers were assessed under a light microscope. One hundred metaphase spreads (10 spreads per sample) in $3nR_2C$ and $3nRC_2$ were examined. Thirty metaphase spreads in the parents were examined.

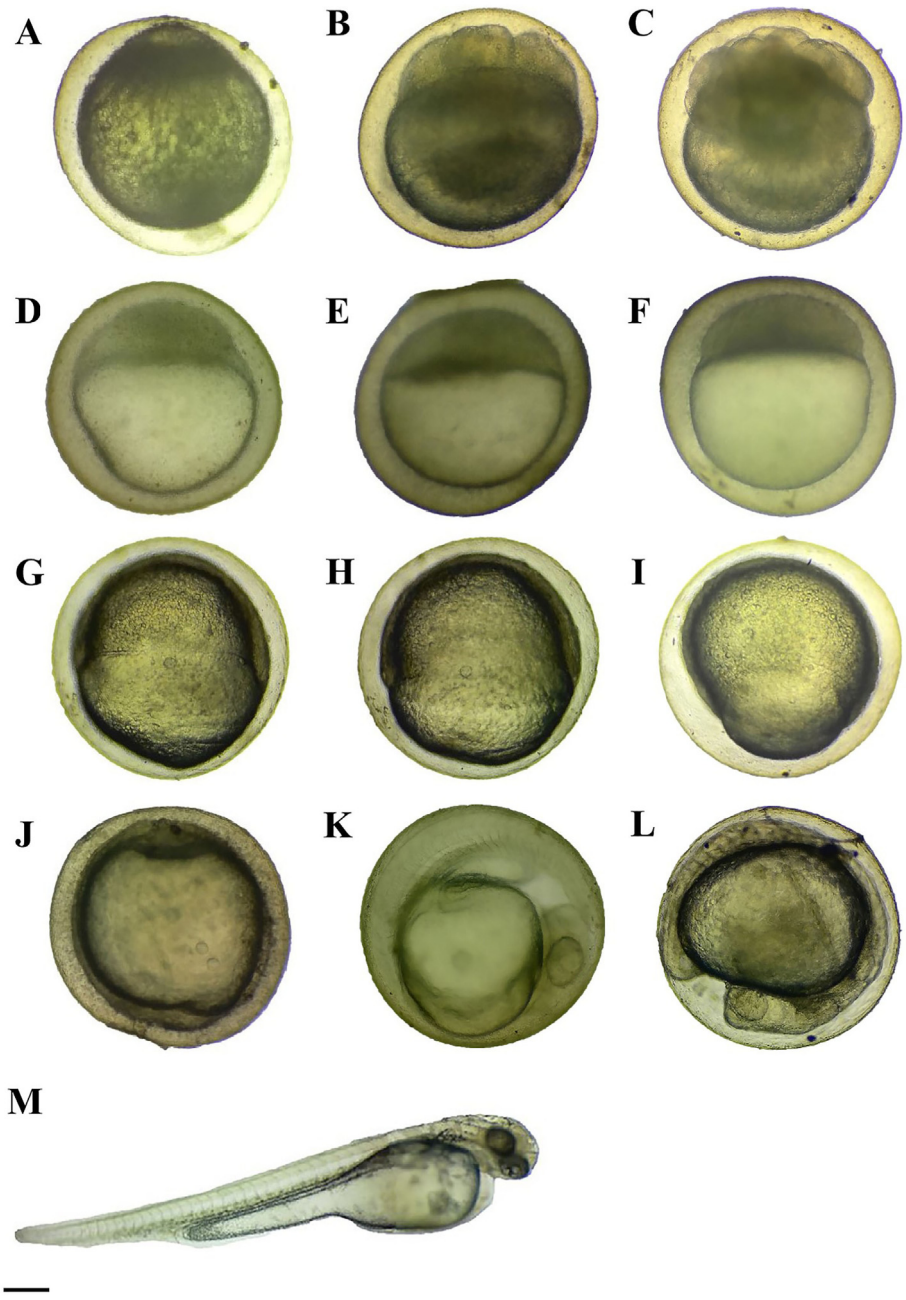

Fig. 2. Early embryonic development of $3nRC_2$. (A) Zygote of $3nRC_2$. (B)–(C) Cleavage stage. (D)–(F) Blastula stage. (G)–(I) Gastrula stage. (J) Segmentation stage. (K)–(L) Pharyngula stage. (M) Fry of $3nRC_2$. Bar = 0.1 mm.

Table 1
Fertilization rate and hatching rate.

Hybridization groups	Fertilization rate (%)	Hatch rate (%)
$3nR_2C$	91.31%	72.96%
$3nRC_2$	84.09%	71.15%

2.4. Measurement of morphological traits and body weight

Thirty $3nR_2C$ and thirty $3nRC_2$ individuals at one year of age were randomly selected for the study of morphological traits. Quantitative characteristics of the scales and fins, including the numbers of lateral scales, upper lateral scales, lower lateral scales, dorsal fins, abdominal fins and anal fins of each fish, were recorded. The recorded quantitative characteristics of appearance included whole length (WL), body length (BL), body height (BH), head length (HL), head height (HH), caudal peduncle length (CPL) and caudal peduncle height (CPH). Moreover, we translated the measurable data into proportional data to more clearly represent the morphological traits. SPSS Statistics 17.0 (IBM Corp., NY, USA) was used to perform ANOVA and pairwise comparisons of the data. After feeding for eight months, the body weights of the two allotriploids and their inbreed parents (RR and CC) were measured (n = 30).

2.5. Observation of gonadal structure

Twenty individuals of $3nR_2C$ and $3nRC_2$ were randomly sampled at the age of eight months for the examination of gonad development by histological sectioning. The gonad samples, including three ovarian and testis samples of RR and CC, were selected as controls. The gonads were fixed in Bouin's solution, embedded in paraffin, sectioned and stained

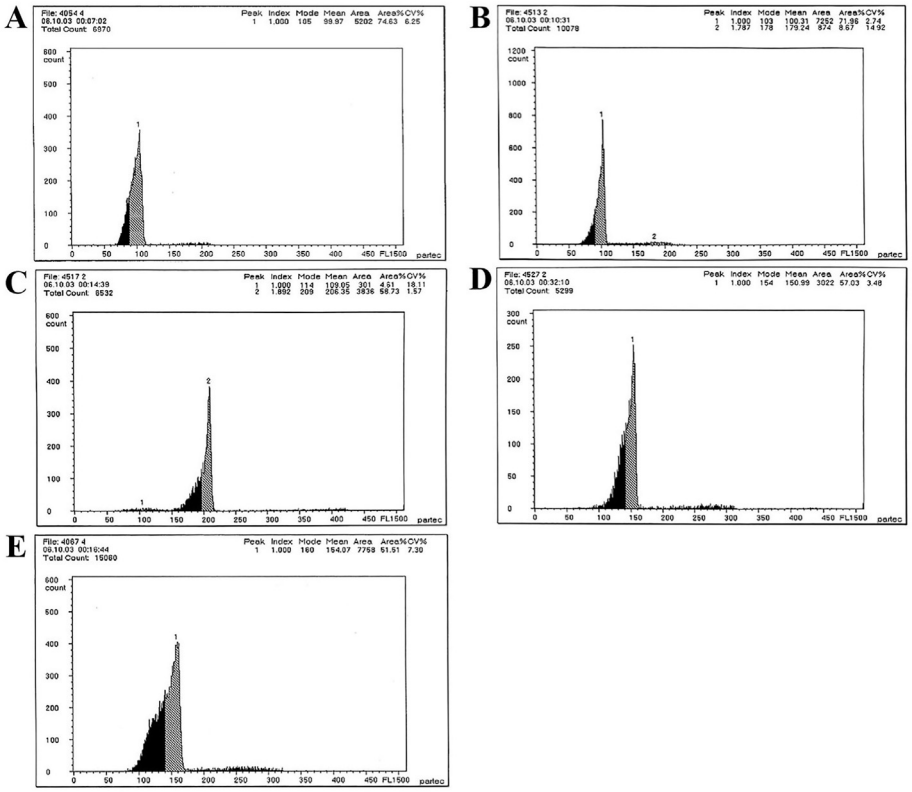

Fig. 3. Cytometric histogram of DNA fluorescence for RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$. (A) Mean DNA content of RR (peak 1: 99.97). (B) Mean DNA content of CC (peak 1: 100.31). (C) Mean DNA content of 4nAT (peak 1: 206.35). (D) Mean DNA content of $3nR_2C$ (peak 1: 150.99). (E) Mean DNA content of $3nRC_2$ (peak 1: 154.07).

Table 2
Mean DNA content of RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$.

Fish type	DNA content	Ratio	
		Observed	Expected
RR	99.97		
CC	100.31		
4nAT	206.35		
$3nR_2C$	150.99	$(4nAT + RR)/3nR_2C = 2.02$[a]	2
$3nRC_2$	154.07	$(4nAT + CC)/3nRC_2 = 1.99$[a]	2

[a] The observed ratio is not significantly different ($p > 0.05$) from the expected ratio.

Table 3
Chromosome numbers of RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$.

Fish type	Distribution of chromosome numbers						
	No. of metaphase	<100[a]	100	<150[a]	150	<200[a]	200
RR	30	4	26				
CC	30	7	23				
4nAT	30					6	24
$3nR_2C$	100			16	84		
$3nRC_2$	100			11	89		

[a] The chromosome number is less than what they should be, owning to the loss of chromosomes in the procedure of chromosome preparation.

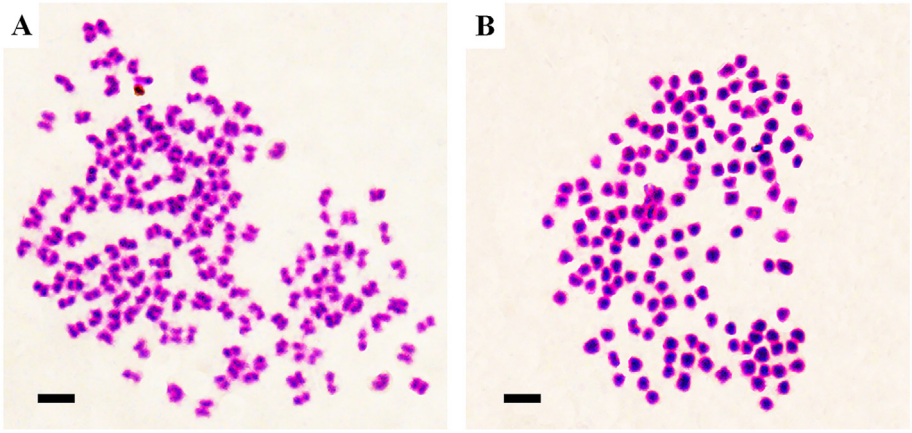

Fig. 4. Metaphase chromosome spreads of $3nR_2C$ and $3nRC_2$. (A) Metaphase chromosome spreads of $3nR_2C$ ($3n = 150$). (B) Metaphase chromosome spreads of $3nRC_2$ ($3n = 150$). Bar = 3 μm.

Table 4
Quantitative characters of the scales and fins of RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$.

Fish type	Lateral scales	Upper lateral scales	Lower lateral scales	Dorsal fins	Abdominal fins	Anal fins
RR	28–30	6	6	III+18-20	8–9	III+7
CC	35–38	5–6	5–6	III+17-19	8–9	III+6-7
4nAT	30–32	5–6	6–7	III+18-19	8–9	III+6-7
$3nR_2C$	28–32	6	6	III+16-18	9–10	III+7
$3nRC_2$	28–34	6	6	III+17-21	8–9	III+7

Table 5
Quantitative characters of the appearance of RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$.

Fish type	WL/BL	BL/BH	BL/HL	HL/HH	CPL/CPH	HH/CPH
RR	1.23 ± 0.02	2.20 ± 0.16	3.72 ± 0.27	1.18 ± 0.04	0.79 ± 0.02	1.60 ± 0.04
CC	1.22 ± 0.07	2.94 ± 0.01	4.10 ± 0.02	1.23 ± 0.07	1.15 ± 0.10	1.86 ± 0.02
4nAT	1.15 ± 0.01	2.77 ± 0.01	4.20 ± 0.01	0.93 ± 0.03	1.05 ± 0.02	1.79 ± 0.03
$3nR_2C$	1.24 ± 0.09	2.41 ± 0.17	3.34 ± 0.08	1.23 ± 0.06	1.11 ± 0.09	1.65 ± 0.07
$3nRC_2$	1.29 ± 0.03	2.54 ± 0.08	3.43 ± 0.21	1.16 ± 0.14	0.99 ± 0.18	1.85 ± 0.29

Whole length to body length ratio (WL/BL), body length to body height ratio (BL/BH), body length to head length ratio (BL/HL), head length to head height ratio (HL/HH), caudal peduncle length to caudal peduncle height ratio (CPL/CPH), and head height to caudal peduncle height ratio (HH/CPH).

with haematoxylin and eosin. Gonadal structures were observed and photographed with a Pixera Pro 600 ES digital camera (Nikon, Japan).

2.6. Genomic DNA extraction, PCR and sequencing

Total genomic DNA was extracted from the peripheral blood cells of $3nR_2C$ and $3nRC_2$ fish using a Universal Genomic Blood DNA Extraction kit (TaKaRa, Dalian, China). Twenty-two pairs of primers were synthesized to amplify the mitochondrial DNA (mtDNA) by polymerase chain reaction (PCR) [27,28]. The PCR products were separated on a 1.2% agarose gel using TBE buffer. The targeted fragments were purified using a gel extraction kit (Sangon, Shanghai, China) and ligated into the pMD18-T vector (TaKaRa, Dalian, China). The plasmids were transformed into *E.coli* DH5α cells and purified. The targeted fragments inserted into the pMD18-T vector were sequenced with an automated DNA sequencer (ABI PRISM 3730). All sequences were analysed using the BLAST, Clustal W (http://www.ebi.ac.uk/) and MEGA 4.0 programs to determine identity.

3. Results

3.1. Embryonic development, fertilization rate and hatching rate

The two allotriploids ($3nR_2C$ and $3nRC_2$) were obtained via the interploid crossing of 4nAT females with their inbred parents (RR and CC) (Fig. 1D and E). The embryonic development of the two allotriploids was observed from the zygote to hatching stages (Fig. 2 and Supplementary Fig. S1) [29]. We observed that each stage of embryonic development took more time in the two allotriploids than in RR (Supplementary Table S1). Higher fertilization and hatching rates were detected in $3nR_2C$ than in $3nRC_2$ (Table 1).

3.2. Detection DNA content and chromosome numbers

To determine the ploidy level of the two allotriploids, the measurement of erythrocyte DNA content and chromosome spread preparation were performed in RR, CC, 4nAT, $3nR_2C$ and $3nRC_2$ (one year old) (Fig. 3). The DNA content of $3nR_2C$ was equal to the sum of that of RR and half of that of 4nAT, while the DNA content of $3nRC_2$ was equal to the sum of that of CC and half of that of 4nAT ($p > 0.05$) (Table 2). Additionally, a chromosome number of 150 was observed in both $3nR_2C$ and $3nRC_2$ based on the results of the analysis of metaphase spreads (Fig. 4 and Table 3).

3.3. Detection of morphological traits and growth traits

The appearances of $3nR_2C$ and $3nRC_2$ are shown in Fig. 1. Red or steel grey coloration was observed on the bodies of RR and CC, respectively. However, the two allotriploids both showed steel grey coloration. Additionally, RR and CC showed no barbels or two pairs of barbels, respectively. Interestingly, $3nR_2C$ and $3nRC_2$ also showed no barbels or two pairs of barbels, respectively. We observed hybrid phenotypes (intermediate types between RR and CC) of some quantitative traits, including scales, fins, and body length to body height ratio, in $3nR_2C$ and $3nRC_2$ (Table 4 and Table 5). Mean body weights of four types of fish are shown in Fig. S2. The mean body weight of the two hybrids is bigger than their inbred parents.

3.4. Fertility detection of the two triploids

The observation of histological sections (obtained at eight months of age) showed that the ovaries of RR and CC developed normally and were mainly composed of oocytes at stage II (Fig. 5A and B). However, the ovaries of $3nR_2C$ and $3nRC_2$ were occupied by small volume of underdeveloped oogonias, which were usually grouped together to form cystic structures. Among these small cells, there were some primary oocytes in which no mature eggs were observed (Fig. 5C–F). The observation of histological sections (eight months of age) further showed that the testes of RR and CC were full of mature spermatids (Fig. 6A–B, 6E-F). The testes of $3nR_2C$ and $3nRC_2$ were mainly composed of seminiferous tubules, some of which contained spermatids, but the spermatids showed irregular shapes. They were not clear and began to degrade and disintegrate. Mature spermatids could not be observed (Fig. 6C–D, 6G-H). These results revealed that the gonads of $3nR_2C$ and $3nRC_2$ fish developed abnormally.

3.5. Sequence analysis of mitochondrial genome

The complete mtDNA sequences of the two allotriploids were obtained through Sanger sequencing. The mtDNA sequences of $3nR_2C$ and $3nRC_2$ were both circular molecules of 16,580 bp and contained one noncoding control region (D-loop), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes and 13 protein-coding genes (Supplementary Table S2). The nucleotide sequence alignment of the mitochondrial genes were compared between the inbred parents and the two allotriploids (Supplementary Table S3). Similarity rate of mtDNA sequences was higher in the comparsion of $3nRC_2$ *vs.* maternal 4nAT (99.5%) than $3nRC_2$ *vs.* paternal CC comparsion (89.8%). Similarity rate of mtDNA sequences was similar between $3nR_2C$ and its parents (99.4% and 99.6%). Moreover, mitochondrial protein-coding genes were analysed between the hybrids and the inbred parents (Table 6). These results suggest that maternal inheritance was detectable in the mtDNA of the two allotriploids.

4. Discussion

An intergeneric cross between RR (female) and CC (male) was performed to establish an allotetraploid population [22]. The two previously

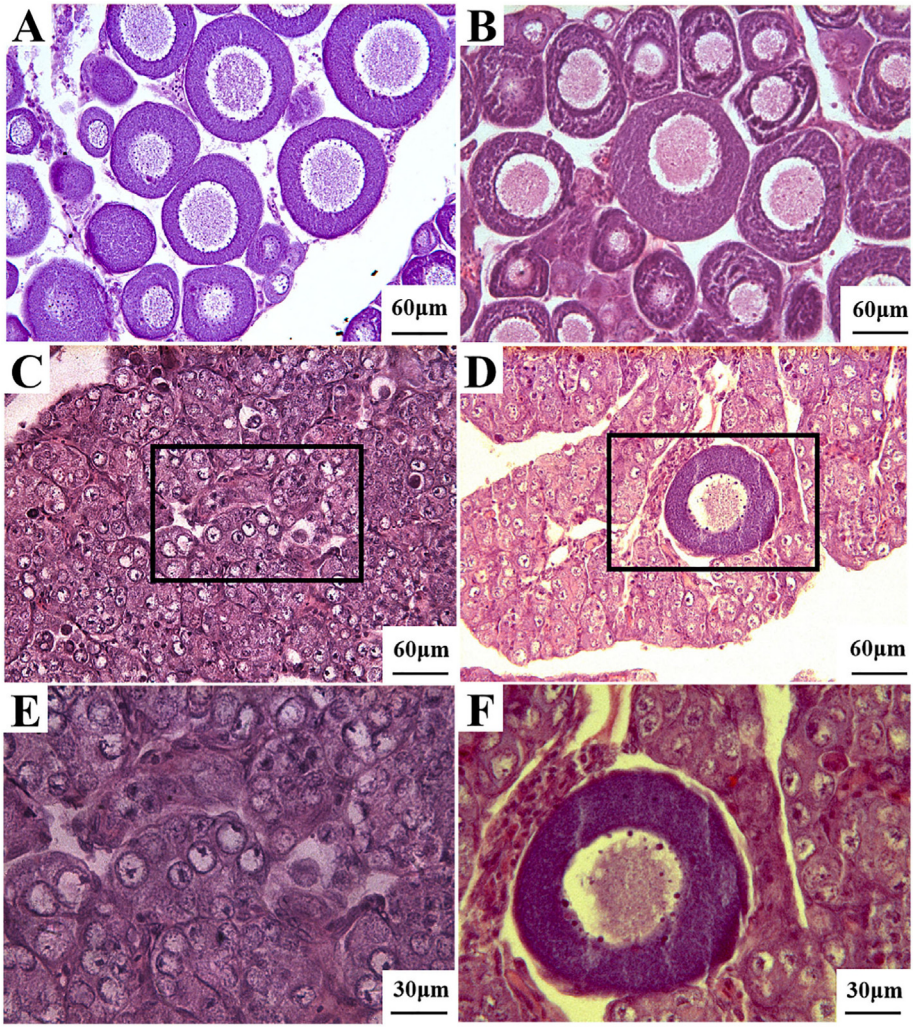

Fig. 5. Ovary microstructure of RR, CC, $3nR_2C$ and $3nRC_2$. (A) Histological section of the ovary of RR. Bar = 60 μm. (B) Histological section of the ovary of CC. Bar = 60 μm. (C) Histological section of the ovary of $3nR_2C$. Bar = 60 μm. (D) Histological section of the ovary of $3nRC_2$. Bar = 60 μm. (E) Further magnified view of (C). Bar = 30 μm. (F) Further magnified view of (D). Bar = 30 μm.

existing types of allotriploids were obtained from two crosses: 1) RR (female) x 4nAT (male) and 2) CC (female) x 4nAT (male) [30]. The characteristics of fast growth and strong innate immunity have been beneficial in commercial fish breeding for many years. In our studies, we obtained two new types of allotriploids: 1) 4nAT (female) x RR (male) and 2) 4nAT (female) x CC (male). Our goal is to obtain commercial fish with heterosis. Our next work will focus on the growth traits and innate immunity of the two allotriploids.

The distinct types of mitochondrial and nuclear genomes present in the four types of allotriploids provide us with an excellent model for investigating interactions between the mitochondrial and nuclear genomes, which are conserved from yeast to mammalian cells [31]. Additionally, the R (originally from RR) and C (originally from CC) subgenomes were detected in 4nAT. Our most recent studies identified genomic variations (*e.g.*, homoeologous recombination) in these subgeneomes [22]. One set of the R and C subgenomes found in 4nAT was inherited in all four types of allotriploids, while the R or C subgenome with no homoeologous recombination was obtained from RR or CC, respectively. The various genotypes and phenotypes obtained from the above breeding strategies will be useful for further studies on interactions between the mitochondrial and nuclear genomes.

Nucleo-cytoplasmic incompatibility is an interesting phenomenon [32]. Different traits of embryonic development, including the timing of embryonic development, and fertilization and hatching rates, were observed in the allotriploids and their inbred parents. Comparative analyses of expression regulation in these fish also provided us with an excellent basis for future work.

The two subgenomes originating from two species (100 chromosomes originating from RR and 50 ones originating from CC in $3nR_2C$, 50 chromosomes originating from RR and 100 ones originating from CC in $3nRC_2$) were the key causes of sterility for the two allotriploids (Figs. 5 and 6). A recent study reported that abnormal meiosis at metaphase I was the potential cause of abnormal testicular development for an allotriploid of *Carassius auratus* × *Cyprinus carpio* [33]. However, a fertile triploid *Carassius auratus* (chromosome number: 150) in natural water exhibited sexual and gynogenetic reproduction modes [34].

A reduced expression of fertility-related genes (*e.g. cyp11c1*, *esr2b*, *krt18b*, *napsa*, *prkacba*) and increased expression of activin β_A and β_B in allotriploid of *Carassius auratus* × *Cyprinus carpio* may exert a key role in aberrant ovarian development and lead to female sterility [35,36]. The hub miRNAs (*e.g.* let-7a, miR-100, miR-122) play roles in endocrine secretion and gamete maturation and may lead to the sterility of the allotriploid of *Carassius auratus* × *Cyprinus carpio* [37]. These results provide valuable evidences to investigate gonad development and sterility in allotriploids.

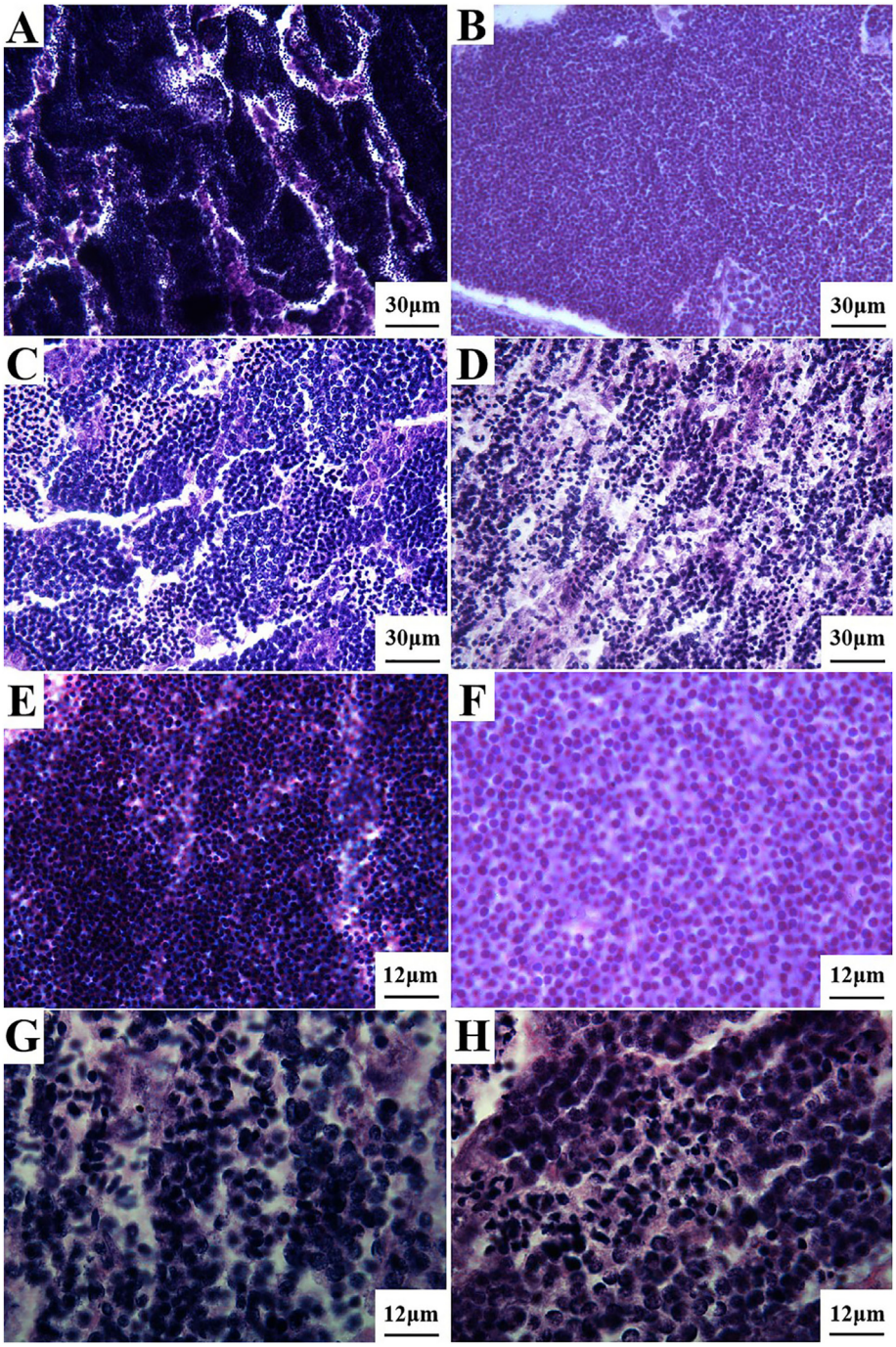

Fig. 6. Testis microstructure of RR, CC, $3nR_2C$ and $3nRC_2$. (A) Histological section of the testis of RR. Bar = 30 μm. (B) Histological section of the testis of CC. Bar = 30 μm. (C) Histological section of the testis of $3nR_2C$. Bar = 30 μm. (D) Histological section of the testis of $3nRC_2$. Bar = 30 μm. (E) Further magnified view of (A). Bar = 12 μm. (F) Further magnified view of (B). Bar = 12 μm. (G) Further magnified view of (C). Bar = 12 μm. (H) Further magnified view of (D). Bar = 12 μm.

Table 6
Amino acid sequence alignment of 13 mitochondrial protein-coding genes among 4nAT, $3nR_2C$ and $3nRC_2$.

Region	$3nR_2C$ *vs.* 4nAT (%)	$3nRC_2$ vs. 4nAT (%)	$3nR_2C$ vs. $3nRC_2$ (%)
Complete genes	99.2	99.5	99.7
ND1	100	100	100
ND2	98.5	98.5	100
COX I	99.8	100	99.8
COX II	100	100	100
ATP8	100	100	100
ATP6	100	100	100
COX III	100	100	100
ND3	100	100	100
ND4L	100	100	100
ND4	100	100	100
ND5	99.4	99.8	99.6
ND6	95.5	95.5	100
Cytb	99.4	100	99.4

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (U19A200122, 31730098, U19A2040, 31702334), 111 Project (D20007), the Earmarked Fund for China Agriculture Research System (CARS-45), Natural Science Foundation of Hunan Province (Grant No. 2020JJ5355), High-Level Talent Agglomeration Program of Hunan, China (2019RS1044), Huxiang Young Talent Project (Grant No. 2021RC3093), Laboratory of Lingnan Modern Agriculture Project (NT2021008), Resources Sharing Service Platform of Science and Technology in Hunan Province of China (2020TP3001).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.repbre.2022.02.004.

References

[1] S. Wang, C. Tang, M. Tao, Q. Qin, C. Zhang, K. Luo, R. Zhao, J. Wang, L. Ren, Establishment and application of distant hybridization technology in fish, Sci. China Life Sci. 62 (1) (2019) 22–45.
[2] C. Wu, X. Huang, Q. Chen, F. Hu, L. Zhou, K. Gong, W. Fu, D. Gong, R. Zhao, The formation of a new type of hybrid culter derived from a hybrid lineage of *Megalobrama amblycephala* (♀) × *Culter alburnus* (♂), Aquaculture 525 (2020), 735328.
[3] L. Ren, X. Zhang, J. Li, X. Yan, X. Gao, J. Cui, C. Tang, S. Liu, Diverse transcriptional patterns of homoeologous recombinant transcripts in triploid fish (*Cyprinidae*), Sci. China Life Sci. (2021) 1–11.
[4] R.D. Houston, T.P. Bean, D.J. Macqueen, M.K. Gundappa, Y.H. Jin, T.L. Jenkins, S.L. Selly, S.A. Martin, J.R. Stevens, Harnessing genomics to fast-track genetic improvement in aquaculture, Nature 21 (7) (2020) 389–409.
[5] D.L. Powell, M. García-Olazábal, M. Keegan, P. Reilly, K. Du, A.P. Díaz-Loyo, S. Banerjee, D. Blakkan, D. Reich, Natural hybridization reveals incompatible alleles that cause melanoma in swordtail fish, Science 368 (6492) (2020) 731–736.
[6] Q. Qin, C. Wang, Y. Zhou, H. Qin, C. Zhao, L. Yang, T. Yu, S. Liu, Rapid genomic and epigenetic alterations in gynogenetic *Carassius auratus* red var. Derived from distant hybridization, Mar. Biotechnol. 22 (3) (2020) 433–442.
[7] D. Gong, L. Xu, W. Li, R. Shang, J. Chen, F. Hu, S. Wang, Q. Liu, C. Wu, Comparative analysis of liver transcriptomes associated with hypoxia tolerance in the gynogenetic blunt snout bream, Aquaculture 523 (2020), 735163.
[8] L. Ren, W. Li, Q. Qin, H. Dai, F. Han, J. Xiao, X. Gao, J. Cui, C. Wu, The subgenomes show asymmetric expression of alleles in hybrid lineages of *Megalobrama amblycephala* × *Culter alburnus*, Genome Res. 29 (11) (2019) 1805–1815.
[9] C. Wu, X. Huang, F. Hu, Y. Ouyang, L. Zhao, S. Wang, W. Li, J. Fan, C. Zhang, Production of diploid gynogenetic grass carp and triploid hybrids derived from the distant hybridization of female grass carp and male topmouth culter, Aquaculture 504 (2019) 462–470.
[10] Q. Liu, J. Liu, L. Yuan, L. Li, M. Tao, C. Zhang, Q. Qin, B. Chen, M. Ma, The establishment of the fertile fish lineages derived from distant hybridization by overcoming the reproductive barriers, Reproduction 159 (6) (2020) R237–R249.
[11] C. Liu, T. Liu, E.W. Ohlson, L. Wang, D. Wu, Q. Guo, M.P. Timko, G. Liang, Loquat (*Eriobotrya japonica* (Thunb.) circadian clock gene cloning and heterosis studies of artificial triploid loquat, Sci Hortic-Amsterdam 246 (2019) 328–337.
[12] M.J. Serapiglia, F.E. Gouker, L.B. Smart, Early selection of novel triploid hybrids of shrub willow with improved biomass yield relative to diploids, BMC Plant Biol. 14 (1) (2014) 1–12.
[13] F. Hu, C. Wu, Y. Zhou, L. Cao, J. Xiao, S. Wang, Y. Wu, L. Ren, Q. Liu, Production of androgenetic, triploid and tetraploid hybrids from the interspecific hybridization of female Japanese crucian carp and male blunt snout bream, Aquaculture 491 (2018) 50–58.
[14] R. Zhou, R. Shang, D. Gong, X. Xu, S. Liu, Characterization of H3 methylation in regulating oocyte development in *cyprinid* fish, Sci. China Life Sci. 62 (6) (2019) 829–837.
[15] W. Huang, Q. Liu, J. Xie, W. Wang, J. Xiao, S. Li, H. Zhang, Y. Zhang, S. Liu, Characterization of triploid hybrid groupers from interspecies hybridization (*Epinephelus coioides* ♀ × *Epinephelus lanceolatus* ♂), Aquacult. Res. 47 (7) (2016) 2195–2204.
[16] J. Shen, S. Liu, Y. Sun, C. Zhang, K. Luo, M. Tao, C. Zeng, Y. Liu, A new type of triploid crucian crap-red crucian carp (♀) × allotetraploid (♂), Prog. Nat. Sci. 16 (12) (2006) 1348–1352.
[17] M. Tao, S. Liu, Y. Long, C. Zeng, J. Liu, L. Liu, C. Zhang, W. Duan, Y. Liu, The cloning of Dmc1 cDNAs and a comparative study of its expression in different ploidy cyprinid fishes, Sci. China, Ser. A C 51 (1) (2008) 38–46.
[18] J. Xu, F. You, X. Wu, P. Zhang, Y. Lin, H. Jiang, C. Zheng, Induction of triploidy in large yellow crocker *Pseudosciaena* crocea (Richardson, 1846): effects of pressure shocks and growth performance in the first rearing year, Aquacult. Res. 39 (13) (2008) 1369–1376.
[19] S. Liu, Distant hybridization leads to different ploidy fishes, Sci. China Life Sci. 53 (4) (2010) 416–425.
[20] Z. Chen, Y. Omori, S. Koren, T. Shirokiya, T. Kuroda, A. Miyamoto, H. Wada, A. Fujiyama, A. Toyoda, De novo assembly of the goldfish (*Carassius auratus*) genome and the evolution of genes after whole-genome duplication, Sci. Adv. 5 (6) (2019) eaav0547.
[21] P. Xu, J. Xu, G. Liu, L. Chen, Z. Zhou, W. Peng, Y. Jiang, Z. Zhao, Z. Jia, The allotetraploid origin and asymmetrical genome evolution of the common carp *Cyprinus carpio*, Nat. Commun. 10 (1) (2019) 1–11.
[22] S. Liu, J. Luo, J. Chai, L. Ren, Y. Zhou, F. Huang, X. Liu, Y. Chen, C. Zhang, Genomic incompatibilities in the diploid and tetraploid offspring of the goldfish × common carp cross, Proc. Natl. Acad. Sci. Unit. States Am. 113 (5) (2016) 1327–1332.
[23] L. Cai, F. Tong, T. Tang, Z. Ao, Z. Wei, F. Yang, Y. Shu, S. Liu, K. Mai, Comparative evaluation of nutritional value and flavor quality of muscle in triploid and diploid common carp: application of genetic improvement in fish quality, Aquaculture 541 (2021), 736780.
[24] F. Yu, H. Zhong, G. Liu, S. Liu, Z. Zhang, Y. Zhou, M. Tao, Y. Liu, Characterization of *vasa* in the gonads of different ploidy fish, Gene 574 (2) (2015) 337–344.
[25] S. Liu, Y. Liu, G. Zhou, X. Zhang, C. Luo, H. Feng, X. He, G. Zhu, H. Yang, The formation of tetraploid stocks of red crucian carp ×common carp hybrids as an effect of interspecic hybridization, Aquaculture 192 (2–4) (2001) 171–186.
[26] J. Xiao, X. Kang, L. Xie, Q. Qin, Z. He, F. Hu, C. Zhang, R. Zhao, J. Wang, The fertility of the hybrid lineage derived from female *Megalobrama amblycephala* × male *Culter alburnus*, Anim. Reprod. Sci. 151 (1–2) (2014) 61–70.
[27] X. Guo, S. Liu, Y. Liu, Evidence for maternal inheritance of mitochondrial DNA in allotetraploid, DNA Sequence 18 (4) (2007) 247–256.
[28] C. You, R. Zhao, J. Hu, S. Liu, M. Tao, C. Zhang, Y. Chen, Q. Qin, J. Xiao, Inheritance of the complete mitochondrial genomes in three different ploidy fishes, Curr. Mol. Med. 14 (10) (2014) 1322–1330.
[29] C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullmann, T.F. Schilling, Stages of embryonic development of the zebrafish, Dev. Dynam. 203 (3) (1995) 253–310.
[30] L. Ren, X. Yan, L. Cao, J. Li, X. Zhang, X. Gao, J. Liu, J. Cui, S. Liu, Combined effects of dosage compensation and incomplete dominance on gene expression in triploid cyprinids, DNA Res. 26 (6) (2019) 485–494.
[31] M.D. Doynova, A. Berretta, M.B. Jones, C.L. Jasoni, M.H. Vickers, J.M. O'Sullivan, Interactions between mitochondrial and nuclear DNA in mammalian cells are non-random, Mitochondrion 30 (2016) 187–196.
[32] K.J. Dunham-Snary, M.W. Sandel, M.J. Sammy, D.G. Westbrook, R. Xiao, R.J. McMonigle, W.F. Ratcliffe, A. Penn, Mitochondrial-nuclear genetic interaction modulates whole body metabolism, adiposity and gene expression in vivo, EBioMedicine 36 (2018) 316–328.
[33] C. Zhang, Q. Li, L. Zhu, W. He, C. Yang, H. Zhang, Y. Sun, L. Zhou, Y. Sun, Abnormal meiosis in fertile and sterile triploid cyprinid fish, Sci. China Life Sci. (2021) 1–12.
[34] L. Zhou, Y. Wang, J. Gui, Genetic evidence for gonochoristic reproduction in gynogenetic silver crucian carp (Carassius auratus gibelio bloch) as revealed by RAPD assays, J. Mol. Evol. 51 (5) (2000) 498–506.
[35] S. Li, Y. Zhou, C. Yang, S. Fan, L. Huang, T. Zhou, Q. Wang, R. Zhao, C. Tang, Comparative analyses of hypothalamus transcriptomes reveal fertility-, growth-, and immune-related genes and signal pathways in different ploidy cyprinid fish, Genomics 113 (2) (2021) 595–605.
[36] M. Tao, H. Hu, L. Huang, S. Li, L. Yuan, T. Zhou, C. Song, R. Zhao, S. Liu, Differential expression of activin β_A and β_B genes in female allotriploid and diploid red crucian carp *Carassius auratus* red var, J. Fish. Biol. 95 (6) (2019) 1523–1529.
[37] Y. Zhou, S. Li, S. Fan, J. Wang, Z. Guo, Q. Wang, W. Qin, T. Li, M. Tao, Integration of miRNA-mRNA co-expression network reveals potential regulation of miRNAs in hypothalamus from sterile triploid crucian carp, Reproduction and Breeding 1 (2) (2021) 114–121.