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Characterization of allodiploid and allotriploid fish derived from hybridization between *Cyprinus carpio haematopterus* (♀) and *Gobiocypris rarus* (♂)

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ABSTRACT

The production of hybrid progeny through distant hybridization holds great significance in enriching germplasm resources for fish breeding. In this study, a hybridization experiment was conducted between female KOC (*Cyprinus carpio haematopterus*) and male GR (*Gobiocypris rarus*), resulting in the production of two distinct types of hybrid offspring. These progenies were classified as allodiploid and allotriploid based on their DNA content and chromosome numbers, hereafter referred to as CG and CCG. Subsequently, a comprehensive comparative analysis was performed between the CG and CCG hybrids and their parents, focusing on countable traits, measurable traits, erythrocyte morphology, as well as karyogene and mitochondrial gene composition. The majority of the examined countable and measurable traits in both CG and CCG exhibited similarities predominantly with GR, except for the ratios of body length (BL) to body height (BH) and head length (HL). Moreover, observing erythrocytes revealed the presence of dumbbell-shaped nuclei in CCG, a characteristic not observed in CG hybrids or the parents. Sequencing alignment revealed that the *homeobox* (Hox) genes and 5S RNA in CG and CCG were inherited from both KOC and GR, signifying their status as allodiploid and allotriploid organisms. The mitochondrial genes in CG and CCG showed substantial similarity to KOC, albeit with a few sites displaying paternal leakage inheritance from GR. In comparison to CG, the growth rate of CCG was found to be significantly faster, which could be attributed to the upregulation of *growth hormone 1* (*gh1*) and the downregulation of *myostatin b* (*mstn*). This study successfully produced two hybrid offspring with distinct growth characteristics but similar genetic backgrounds, making them ideal subjects for future investigations into growth traits. The findings of this research established a fundamental basis for investigating the growth mechanism of fish and provided significant implications for the advancement of fish breeding through hybridization.

1. Introduction

Natural hybridization is a widespread phenomenon observed in various organisms, including bacteria, fungi, plants, and animals, playing a significant role in speciation and evolutionary innovations [1–4].

Artificial hybridization, involving carefully selected parents with distinct genetic conditions and specific traits, has played a significant role in plant and animal breeding practices. In the realm of rice cultivation, hybrid rice has emerged as the predominant form in China since the 1970s and has gained global popularity. This advancement has greatly contributed to improved yield potentials and enhanced food security on a global scale [5]. Within the domain of animal breeding, artificial hybridization is predominantly employed in fish breeding, which benefits from external fertilization. Notably, by 2017, hybrid fish accounted for nearly half of the approved improved fish varieties by the Chinese government, underscoring the prevalence of hybridization as a widely adopted breeding technology in the field of fish breeding [6], which indicates that hybridization is the most commonly used breeding

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technology in fish. The crossbreeding of fish can be categorized into two main types based on the genetic relationship between the parent organisms: close hybridization and distant hybridization. Close hybridization refers to crosses between different strains or varieties within the same species, also known as intraspecific hybridization. On the other hand, distant hybridization involves crosses between different species within the same or different genera and families. Distant hybridization can lead to genome-level alterations and the production of diploid, triploid, and tetraploid hybrid individuals [7–9].

The common carp (*Cyprinus carpio* L) belongs to *Cyprininae* subfamily and holds the position as the third most extensively cultivated freshwater species globally, owing to its favorable growth characteristics and significant economic value [10]. Previous studies have revealed that the common carp possesses a complex tetraploid genome, which has historically undergone additional whole-genome duplication, resulting in a total of 100 chromosomes [11,12]. KOC is an ornamental variant of common carp, selectively bred for its distinctive coloration and scale patterns. It exhibits an extended lifespan in captivity, reaching up to 40 years, and has gained widespread popularity across various countries. KOC possess the potential for significant economic value and can develop a remarkable level of tameness, thus often holding a deep emotional value for their owners [13]. Extensive research on carp populations worldwide has revealed significant variability in growth rates in temperate latitudes. Within the first year, carp size can vary from 105 to 190 mm fork length (FL), with further growth during the second year reaching 195–300 mm FL. By the age of 7–11 years, carp may reach a length of up to 500 mm FL [14]. The age at which both male and female carp reach sexual maturity can vary between 1 and 5 years. Moreover, there exists a correlation between water temperature and sexual maturity [15–18]. KOC has a rather high fecundity, with mean fecundity being about 299,000 oocytes and relative fecundity ranged from 19,300 to 216,000 oocytes/kg [14].

The rare minnow (GR) is a small freshwater fish species that is native to the upper reaches of the Yangtze River. This species is found in a limited area consisting of various rivulets in Sichuan, Southwest China [19]. According to recent molecular phylogenetic studies, GR belongs to the subfamily *Gobioninae* [20] and exhibits a diploid status with 50 chromosomes [21]. This species boasts numerous appealing attributes, such as its sensitivity to chemicals, small adult size (2–8 cm), wide temperature tolerance (0–35 °C), ease of laboratory culturing, high egg production with an average of 266 eggs per hatch and continuous batch spawning, short embryonic development period (72 h at 26 °C), and a brief life cycle of about 4 months [22]. Due to these characteristics, GR is extensively utilized as a native laboratory fish for chemical testing and research in the fields of disease, toxicology, behavior, and genetics [23–29].

The aforementioned two species pertain to distinct subfamilies within the Cyprinidae family, displaying significant disparities in terms of body size, reproductive cycle, and spawning behavior. Therefore, it is of great interest to study the relevant characteristics of their hybrids. In this study, hybridization between female KOC (2n = 100) and male GR (2n = 50) was conducted and resulted in the formation of allodiploid and allotriploid hybrids. The biological characteristics, chromosome number, karyotype, blood cell size, gene expression, and genetic composition were investigated.

2. Materials and methods

2.1. Ethics statement

All animal experiments in this were approved by the Institutional Animal Care and Use Committee of Hunan Normal University (Permit Number: 633).

2.2. Hybridization

Both the KOC and GR fish used as parents in the hybridization were obtained from the Polyploid Fish Breeding and Propagation Engineering Center, Hunan Normal University. During the breeding season, three to five mature female KOC exhibiting visible pregnancy and about fifty robust 8-month-old male GR were selected. Then the distant hybridization was performed using dry artificial insemination. Approximately 7 days after fertilization, the F1 hybrid larvae were capable of horizontal swimming and were subsequently transferred to an outdoor concrete pool with running water for rearing. After six months, the F1 hybrid offspring were collected and utilized for measurements of morphological traits, analysis of DNA content, observation of erythrocytes, preparation of chromosome spreads, amplification of karyogenes and mitochondrial genes, as well as extraction of RNA from several tissues.

2.3. Morphological traits

Following a feeding period of six months, the DNA content of the F1 offspring was assessed using flow cytometry as previously reported [30,31]. Based on these measurements, the F1 hybrids were initially classified into two groups, referred to as CG and CCG. Subsequently, 10 samples from each of the KOC, GR, CG, and CCG groups were selected for measurement and analysis of morphological traits. Measurable traits mainly included body weight (WT), whole length (WL), head length (HL), head height (HH), body length (BL), body height (BH), caudal peduncle length (CPL), and caudal peduncle height (CPH). Countable characters mainly included lateral-line scales, upper lateral scales, lower lateral scales, and counts of pectoral fin-ray, dorsal fin-ray, ventral fin-ray, and anal fin-ray. To eliminate the influence of size differences, ratio analysis was conducted for measurable traits, including WL/BL, BL/HL, BL/BH, HL/HH, BL/HH, and CPL/CPH. T-tests [32,33] were performed for statistical analysis between two groups, while ANOVA was used for comparisons among multiple groups.

2.4. Determination of DNA content

The DNA contents of KOC, GR, CG, and CCG were determined through flow cytometry detection. The following procedure was employed: Approximately 0.2 mL blood sample was collected with a disposable syringe that had been rinsed with acid-citrate dextrose (ACD). The blood sample was then squeezed into a 1.5 ml centrifuge tube containing pre-added ACD. Subsequently, the blood was diluted to a faint yellow color, and 300 μL of the diluted blood was mixed with 300 μL of DAPI staining solution (05–5002, Sysmex, USA). After incubating at room temperature in the dark for 10 min, the samples were filtered through a 30 μm-filter. The filtrate was collected and analyzed using a flow cytometer (Partec, Germany) according to the instructions. The ploidy of the sample was normalized with the DNA content of diploid red crucian carp.

2.5. Chromosome spreads preparation

Approximately 0.3 mL of blood was drawn from each fish using a syringe rinsed with 0.2 mL of 0.1 % sodium heparin. The collected blood was placed in a refrigerator at 4 °C for 1–2 h, allowing the red blood cells to precipitate. The supernatant was removed, and the remaining supernatant was added to a 50 mL EP tube containing 10 mL of culture medium. The culture medium consisted of 1.5 % fetal bovine serum, 0.001 % sodium heparin, 0.3 mg/mL phytohaemagglutinin, and 100 IU/mL penicillin and streptomycin in RPMI-1640 (C11875500BT, ThermoFisher Scientific, USA). The blood cells were then incubated at 25 °C for 72 h, with gentle mixing every 8 h. After 68 h of incubation, 50–60 μL of colchicine at a concentration of 10 μg/mL was added to reach a final concentration of 0.05–0.07 μg/mL. The cultured cells were subsequently collected by centrifugation and immersed in 10 mL of hypotonic solution

(0.075 mol/L KCl) in a water bath at 24 °C for 30 min. Following this, the cultured cells were mixed with 1 mL of ready-made fixative (methanol: glacial acetic acid = 3:1) and gently collected again through centrifugation. This fixation process was repeated twice, followed by an overnight third fixation at 4 °C. The fixative cells were once again collected by centrifugation, mixed with 1 mL of fixative, and the resulting cell suspension was dropped onto frozen slides from a height of approximately 40 cm. The slides were quickly heated on an alcohol lamp until dry. After drying, the slides were stained with Giemsa staining solution for 30 min, washed with running water from the backside of the slide, and observed and photographed under a Leica DM2500 microscope (Leica, Germany) at 100× magnification.

2.6. Determination of erythrocyte morphology and nuclear volume

Blood samples were drawn separately from the tail veins of KOC, GR, CG, and CCG fish. The samples were diluted with phosphate buffer solution and used to prepare blood smears. These smears were then dried and stained with Wright's staining solution for 5 min. After staining, the slides were washed with PBS and underwent a second staining for 5 min. Once staining was complete, the slides were washed with running water from the backside until no obvious staining remained. For measurement purposes, at least 50 cells from each group (KOC, GR, CG, and CCG) were selected.

2.7. Whole genomic DNA extraction and DNA amplification

Whole genomic DNA was extracted from caudal fin cells of KOC, GR, CG, and CCG fish using HOM buffer (80 mM EDTA, 100 mM Tris-HCl, 0.5 % SDS, pH = 8.0). Caudal tissue was appropriately digested at 55 °C for at least 3 h. After complete digestion, NaCl and chloroform were added, and the mixture was vigorously vortexed for 20 min. The mixture was then centrifuged to collect the supernatant, which was subsequently mixed with isopropanol and centrifuged for 20 min. The resulting precipitate was washed with 75 % ethanol one or two times. Finally, the white precipitate was dried and dissolved in 100 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH = 8.0). The obtained genomic DNA was used as a template for the amplification of mitochondrial genes, 5S rDNA, and Hox family genes. Thedegenerate PCR primers used for mitochondrial genes [34,35], 5S rDNA [36], and Hox genes [37,38] were based on previous studies and were shown in Tables 5 and 6

2.8. RNA extraction and q-PCR

Total RNA was extracted from the liver, pituitary, brain, and muscle tissues of KOC, GR, CG, and CCG fish using the Trizol reagent (15596026, Invitrogen, USA). Tissues were fully homogenized with a homogenizer to release RNA. The homogenate was then mixed with chloroform, and the aqueous phase was separated after centrifugation. Subsequently, isopropyl alcohol was added to the aqueous phase, and after precipitation and centrifugation, the white RNA precipitate was obtained. The RNA precipitate was washed with 75 % ethanol to remove residual liquid and was then dried. Finally, the RNA was dissolved in DEPC water and treated with DNase to eliminate DNA contamination. The purified RNA was extracted using phenol-chloroform, and the resulting RNA precipitate was dissolved in DEPC water.

To obtain cDNA, the RNA was subjected to reverse transcription using the Revert-Aid First Strand cDNA Synthesis Kit (K16215, ThermoFisher Scientific, USA). The synthesized cDNA was used as a template for quantitative PCR (q-PCR) using the primers listed in Table 7. The qPCR primers were designed utilizing the 'primer blast' tool available at NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). The qPCR reaction was prepared with SYBR Green Master Mix (low Rox) (MQ10501S, Monad, China) and performed on ABI real-time system (QuantStudio™ 5, USA). The results obtained were subjected to statistical analysis using the t-test method and graphs were generated using GraphPad Prism 8.

3. Results

3.1. Formation of allodiploid and allotriploid hybrids

Two types of hybrid offspring were obtained through the hybridization between female KOC and male GR. The figures (Fig. 1A–D) illustrate the appearance of the female parent KOC, the male parent GR, the allodiploid hybrid CG, and the allotriploid hybrid CCG. It was observed that the body color of CG and CCG resembled that of their paternal parent GR, while their body shape and size were closer to their maternal parent KOC. Flow cytometry was employed to detect the relative DNA content of the parents and hybrid progenies with the red crucian carp serving as the reference, given its established diploid status with 100 chromosomes [39]. To enhance the visibility of ploidy, the gain value was adjusted to establish a fluorescence value of 100 for the DNA content in diploid red crucian carp. Subsequently, the results (Fig. 1E–H) revealed that the DNA content per cell in KOC and GR was approximately 100 and 50, respectively, whereas it was around 75 in CG and 125 in CCG. Accordingly, the chromosome numbers of KOC, GR, CG, and CCG were 100, 50, 75, and 125, respectively, consistent with their respective DNA content (Fig. 1I-L). The average DNA content of CG was approximately the sum of half of the maternal DNA content and half of the paternal DNA content (Table 1), indicating that CG was an allodiploid hybrid containing a set of chromosomes from KOC and a set of chromosomes from GR. The average DNA content of CCG was nearly the sum of the maternal DNA content and half of the paternal DNA content, indicating that CCG was an allotriploid hybrid containing two sets of chromosomes from KOC and one set of chromosomes from GR.

3.2. Morphological traits

Countable traits were compared and analyzed between the parental fish and the two types of hybrids (Table 2). The number of lateral line scales, upper lateral scales, and lower lateral scales in GR was significantly lower than in KOC, while CG and CCG exhibited the same number as GR rather than KOC. Likewise, the fin ray numbers in the dorsal fin, abdominal fin, and anal fins of GR were significantly lower than those of KOC. The fin ray composition of CG and CCG was essentially the same, with their abdominal fin ray counts matching GR, but their dorsal fin ray counts were between those of their parents, and their anal fin ray counts were significantly lower than those of the parents. Overall, the countable traits of CG and CCG resembled their maternal parent. Comparative analysis was also conducted on measurable traits, including WL/BL, BL/BH, BL/HL, HL/HH, and CPL/CPH, among KOC, GR, CG, and CCG (Table 3). It revealed no significant differences in the values of WL/BL, HL/HH, and CPL/CPH among KOC, GR, CG, and CCG, but both the BL/BH and BL/HL in CG and CCG were significantly lower than those of their parents and closer to KOC, indicating that the body shape of CG and CCG resembled that of the maternal parent, consistent with the observations in appearance.

3.3. Erythrocyte morphological analysis

The morphology of erythrocytes in KOC, GR, CG, and CCG was observed by preparing blood smears (Fig. 2), and the average nuclear volume of erythrocytes in hybrids and their parents was measured (Table 4). The erythrocytes of the parents and hybrid offspring were oval-shaped, with the nucleus stained blue-purple in the center. Macroscopically, the erythrocyte nuclear volume had a positive correlation with their DNA content, with CCG having the largest nuclear volume, followed by KOC, CG, and GR (Fig. 2), consistent with the measurements of erythrocyte nuclear volume in these four types of fish (Table 4). The measured value of erythrocyte nuclear volume in CG was the sum of half of KOC and half of GR, while the measured value in CCG

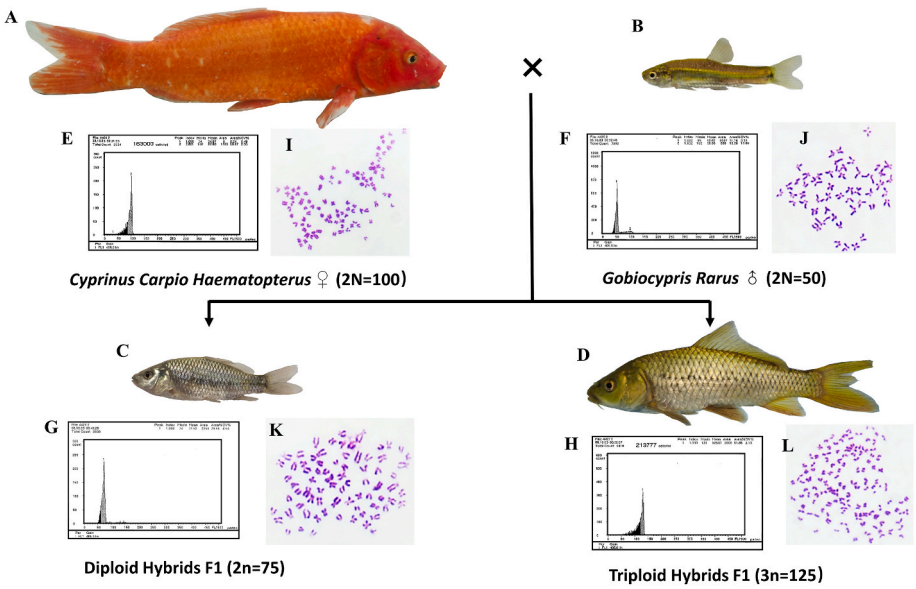

Fig. 1. Generation of diploid and triploid hybrids by crossing female KOC with male GR. (A–D) The representative appearance of KOC, GR, CG, and CCG respectively. (E–H) Cytometric histograms of DNA fluorescence for KOC, GR, CG, and CCG respectively. (I–L) The metaphase chromosome spreads of KOC, GR, CG, and CCG respectively. Three individuals were used for KOC, CG and CCG. For GR, a total of 24 fish were separated into three groups for preparing chromosome spread.

Table 1
Statistical analysis of average DNA content among 2nKOC, 2nGR, 2nCG and 3nCCG

Fish	DNA content	Ratio		
		Observed	Expected	P value
2nKOC	97.80 ± 3.23			
2nGR	52.26 ± 0.98			
2nCG	77.49 ± 4.32	CG/(0.5KOC+0.5 GR) = 1.03 ± 0.11	1	0.195
3nCCG	129.07 ± 1.35	CCG/(KOC+0.5 GR) = 1.04 ± 0.03	1	0.113

Note: T-test was used for the analysis of significant differences difference between the observed value and the expected value. Three individuals were used for each group.

Table 2
The countable traits of 2nKOC, 2nGR, 2nCG, and 3nCCG

Fish	No. of lateral scales	No. of upper lateral scales	No. of lower lateral scales	No. of dorsal fins	No. of abdominal fins	No. of anal fins
2nKOC	36^a	8^a	6^a	III+20-21^a	12^a	III+10^a
2nGR	34–35^b	4–5^b	5^b	III+8^b	8–9^b	III+6^b
2nCG	30–34^b	4–5^b	4–5^b	III+13^c	8^b	III+4^c
3nCCG	34–35^b	5–6^b	5^b	III+13-14^c	8^b	III+4^c

Note: Different letters indicate significant difference between different groups ($P < 0.05$).

was the sum of KOC and half of GR (Table 4), consistent with their genome composition. Additionally, erythrocytes with dumbbell-shaped nuclei were observed only in CCG, but not in KOC, GR, and CG, which is a phenomenon generally observed in polyploids without clear explanations [40].

Table 3
The measurable traits of 2nKOC, 2nGR, 2nCG, and 3nCCG

Fish	WL/BL	BL/BH	BL/HL	HL/HH	CPL/CPH
2nKOC	1.17 ± 0.01^a	3.35 ± 0.06^a	4.06 ± 0.05^a	1.15 ± 0.02^a	1.30 ± 0.03^a
2nGR	1.20 ± 0.02^a	3.95 ± 0.09^b	4.56 ± 0.03^a	1.21 ± 0.03^a	1.67 ± 0.06^a
2nCG	1.25 ± 0.05^a	3.15 ± 0.08^c	3.39 ± 0.12^b	1.29 ± 0.06^a	1.61 ± 0.02^a
3nCCG	1.27 ± 0.04^a	2.94 ± 0.07^c	3.21 ± 0.14^b	1.40 ± 0.05^a	1.53 ± 0.05^a

Note: WL, whole length; BL, body length; HL, head length; BH, body height; HH, head height; CPL, caudal peduncle length; CPH, caudal peduncle height Different letters indicate significant difference between different groups ($P < 0.05$).

3.4. Genetic composition of nuclear genes

The hybrid progenies theoretically contain genomes from both parents. Therefore, nuclear genes, including 5S rRNA and the Hox family, were amplified to analyze their composition (Fig. 3). Despite the high sequence similarity observed between the sequences amplified from KOC and GR, there were specific sites with intraspecific conserved bases that could help distinguish the sequences from the maternal or paternal parent. In this study, five Hox genes, Hox-A2b, Hox-A4a, Hox-B5b, Hox-C4a, and Hox-D10a, were amplified from KOC, GR, CG, and CCG, and the sequence alignment revealed that there was only one type of sequence for Hox genes in KOC and GR, while both CG and CCG had two sets of Hox gene sequences from both parents. Amplification and alignment were also performed to analyze the genetic composition of 5S rRNA in CG and CCG, and the results were consistent with the findings in the Hox genes, as CG and CCG had two sets of 5S rRNA from the maternal and paternal parents, respectively. However, the ratios of maternal sequence to paternal sequence in CG and CCG did not correspond to their genetic composition completely. Additionally, some mutations in the sequence of Hox-A2b in CG and CCG were found to be identical to one of the parents, while the entire sequence shared higher similarities with the other parent, which has been reported as chimeric gene formation in previous studies [41,42]. These results, to some extent, demonstrate the hybrid origin of CG and CCG.

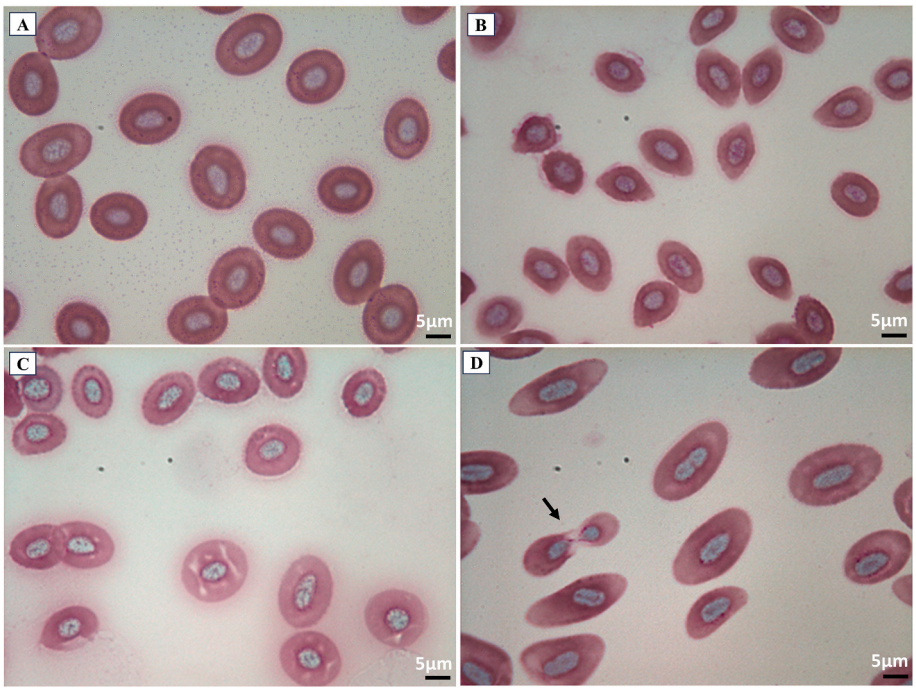

Fig. 2. Observation of erythrocytes in peripheral blood. (A) Mature erythrocytes of KOC. (B) Mature erythrocytes of GR. (C) Mature erythrocytes of CG. (D) Mature erythrocytes of CCG. The black arrow indicates the heteromorphic red blood cell in CCG. Scale bar, 5 μm.

Table 4
Mean erythrocyte nuclear volume measurements for 2nKOC, 2nGR, 2nCG and 3nCCG

Fish	Major axis (μm)	Minor axis (μm)	Volume (mm^3)	Volume ratio		
				Observed	Expected	P value
2nKOC	5.12 ± 1.08	3.2 ± 0.32	28.81 ± 11.41			
2nGR	4.91 ± 0.63	2.79 ± 0.41	20.01 ± 9.70			
2nCG	4.85 ± 0.92	2.93 ± 0.29	21.83 ± 9.30	CG/(0.5KOC+0.5 GR) = 0.89 ± 0.03	1	0.184
3nCCG	6.92 ± 1.10	3.08 ± 0.27	34.29 ± 12.83	CCG/(KOC+0.5 GR) = 0.88 ± 0.05	1	0.223

Note: T-test was used for the analysis of significant differences difference between the observed value and the expected value. Three individuals were used for each group.

3.5. Genetic composition of mitochondrial genes

The mitochondrial genome is known to be maternally inherited, but paternal leakage also occurs [35,43,44]. In this study, 12 fragments from mitochondrial genes, including 12S rRNA, 16S rRNA, ND1 (*NADH dehydrogenase subunit 1*), ND2 (*NADH dehydrogenase subunit 2*), ATPase 8 (*ATP synthase 8*), ND4 (*NADH dehydrogenase subunit 4*), ND5 (*NADH dehydrogenase subunit 5*), Cytb (*cytochrome b*), and CR (control region), were amplified and sequenced. The alignment results showed that the mitochondrial sequences of CG and CCG were highly homologous to KOC, with identities greater than 99 % (Fig. 4). The mitochondrial sequences of GR were significantly different from those of KOC, CG, and CCG, displaying a portion of intraspecific-conserved sites. Importantly, several sites in ND4 and ND5 of CG and CCG were identical to GR rather than KOC, indicating paternal leakage in these hybrid progenies. Additionally, a few mutations in ND1 and Cytb of CG and CCG were found that differed from both parents. These results suggest that the mitochondrial genome of CG and CCG is primarily inherited from the maternal parent, with a minor influence from paternal leakage and genetic mutations.

3.6. Growth performance

It was observed that CG and CCG raised in the same pool exhibited obvious differences in body size. Therefore, their average body weights at 6 months old were measured, and the statistical results showed a significantly higher weight in CCG (Fig. 5A). While the growth difference was expected, as CCG possessed an additional set of chromosomes from KOC, the expression levels of growth-related genes were analyzed. The genes analyzed included *gh1*, *insulin-like growth factor 1* (*igf1*), *phosphoinositide-3-kinase, regulatory subunit 1* (*pik3r1*), and *mstn* in the pituitary, liver, brain, and muscle of CG and CCG, respectively (Fig. 5B–E). It was revealed that there was no significant difference in the expression of *igf1* ($P = 0.178$) and *pik3r1* ($P = 0.086$) between CG and CCG (Fig. 5D and E), but CCG exhibited significantly higher expression of *gh1* ($P = 0.045$) in the pituitary (Fig. 5B), which is known as the major gene involved in promoting growth through endocrine activities [45,46]. Accordingly, the expression of *mstn* ($P = 0.042$), a major inhibiting gene for muscle growth [47–49], was dramatically downregulated in CCG compared to CG (Fig. 5C). These results indicate that the expression patterns of *gh* and *mstn* in CG and CCG are consistent with their growth performance.

4. Discussion

Distant hybridization has been proven as an effective method for generating hybrid fish with varying ploidies. The research conducted in our laboratory has revealed that first-generation hybrid progenies exhibit a range of ploidies, from diploid to tetraploid [7]. Generally, if the parent fish have the same number of chromosomes, their first-generation hybrid progenies are typically allodiploid [50–52]. However, when the female parent has more chromosomes than the male parent, the first hybrid generation can produce polyploid progenies,

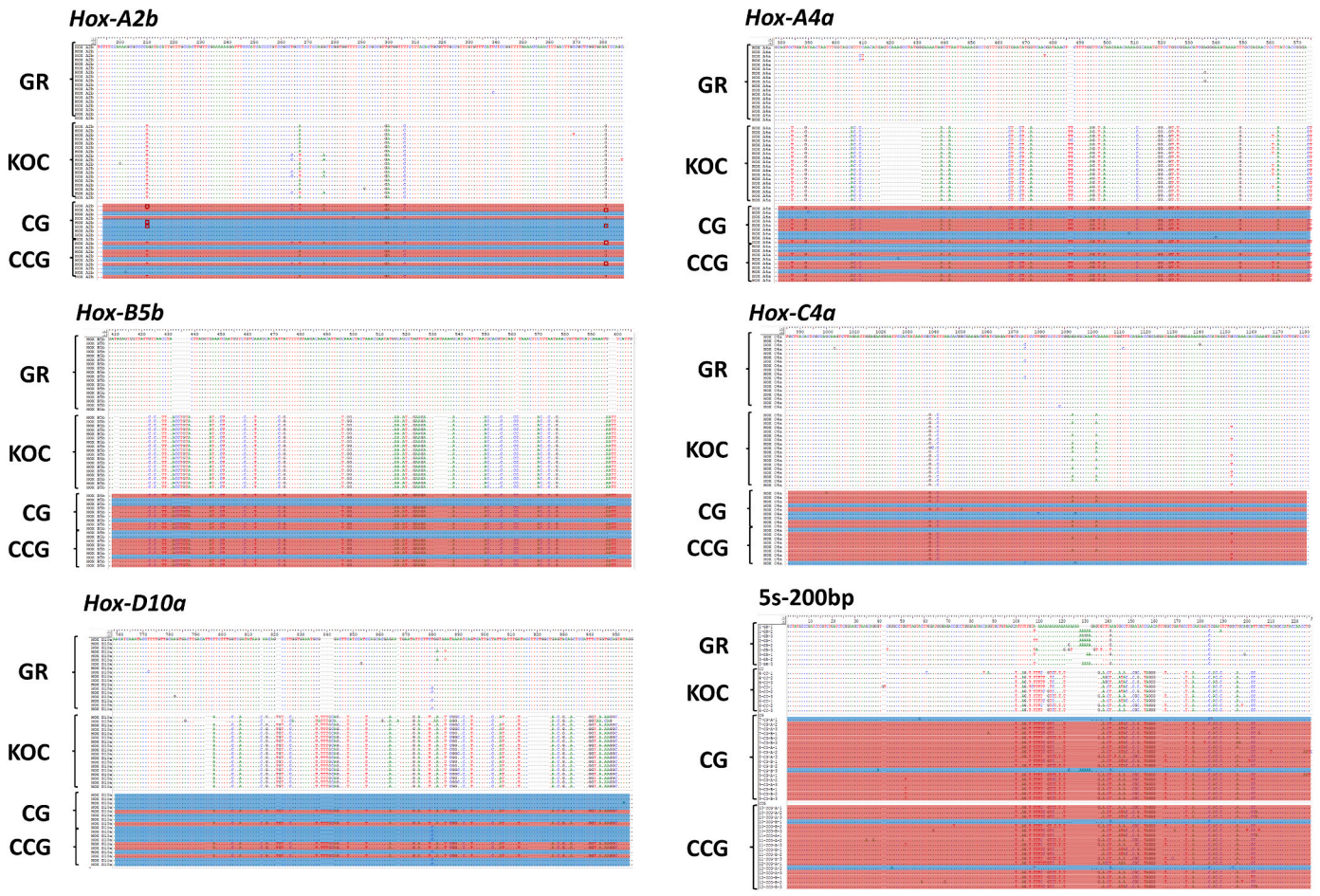

Fig. 3. Nucleotide sequence alignment of Hox family and 5s RNA among KOC, GR, CG, and CCG. The sequence in red or blue indicates the sequence is basically identical to the female or male parent. The red box indicates the mutation that differed from the particular parent.

including triploid and tetraploid individuals [6,53]. In this study, we performed distant hybridization between subfamilies of *Cyprininae* and *Gobioninae*, resulting in two different types of hybrid progenies through the crossing of female KOC and male GR. The F1 hybrids were found to be allodiploid and allotriploid with DNA content values and chromosome numbers of 75 and 125 respectively (Fig. 1). Hybrid progenies typically exhibit characteristics inherited from both parents due to the combination of their genomes [54–56]. Herein, the alloploid (CG) and allotriploid (CCG) offspring in this study displayed similar body shapes to the female parent, primarily determined by the ratio of body length to body height or head length (Fig. 1 and Table 3). However, they exhibited the cyan coloration from the paternal parent, and their scale distribution and fin composition were more similar to the paternal parent (Fig. 1 and Table 2). Compared to the allodiploid hybrids, the allotriploid hybrids resembled the female parent more closely in appearance, with larger body size and a pair of thick barbels (Fig. 1). This phenomenon can be explained by the fact that allotriploid possess two sets of maternal genomes, while allodiploid only have one set of genomes from the female parent.

Previous studies have reported the presence of red blood cells with divided nuclei in polyploid fish but not in diploid fish, and the exact reason behind this remains unclear [56–58]. Most researchers believe that the divided nuclei in red blood cells are formed through cell division, while others suggest that it may result from apoptosis induced by cell senescence or heteromorphosis to increase the surface area of the red blood cell for better exchange of material and information [59,60]. In this study, nuclear-divided red blood cells were observed only in the allotriploid hybrids, but not in the autodiploid parents or allodiploid hybrids (Fig. 2). Similar findings have been reported in other studies on autotriploid rainbow trout [61], suggesting that nuclear division in red blood cells is primarily attributed to polyploidy or high DNA content rather than hybridization or artificial induction, thus supporting the heteromorphosis hypothesis.

Hox genes and 5S RNA are usually used to investigate the composition of subgenomes in hybrids [62–64]. Therefore, we amplified several karyogenes, including Hox family and 5S RNA using degenerate primers and analyzed their sequences in hybrids and parents. The results showed high similarity in the examined gene sequences between parents and hybrids. Both diploid and triploid hybrids contained sequences identical to those of both parents, further confirming their hybrid nature (Fig. 3). However, the ratios of sequences from the maternal and male parents in diploid and triploid hybrids were not consistent with their ploidies, potentially due to primer bias. Additionally, the amplified Hox-A2b from hybrids exhibited base pairs specific to both parents simultaneously, indicating the presence of chimeric genes resulting from genome recombination induced by hybridization [41,42]. Furthermore, chimeric genomes also were described in natural hybrids of *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. This natural hybrid exhibited the formation of chimeric chromosomes resulting from non-reciprocal recombination events involving homeologous chromosomes. Interestingly, these nonreciprocal recombinations between homeologous chromosomes occurred in highly conserved regions [65]. This phenomenon is consistent with our observation herein (Fig. 3).

Despite the maternal inheritance characteristic of animal mitochondrial DNA (mtDNA), studies have shown occurrences of mtDNA recombination and paternal leakage [43,66–68]. In this study, we

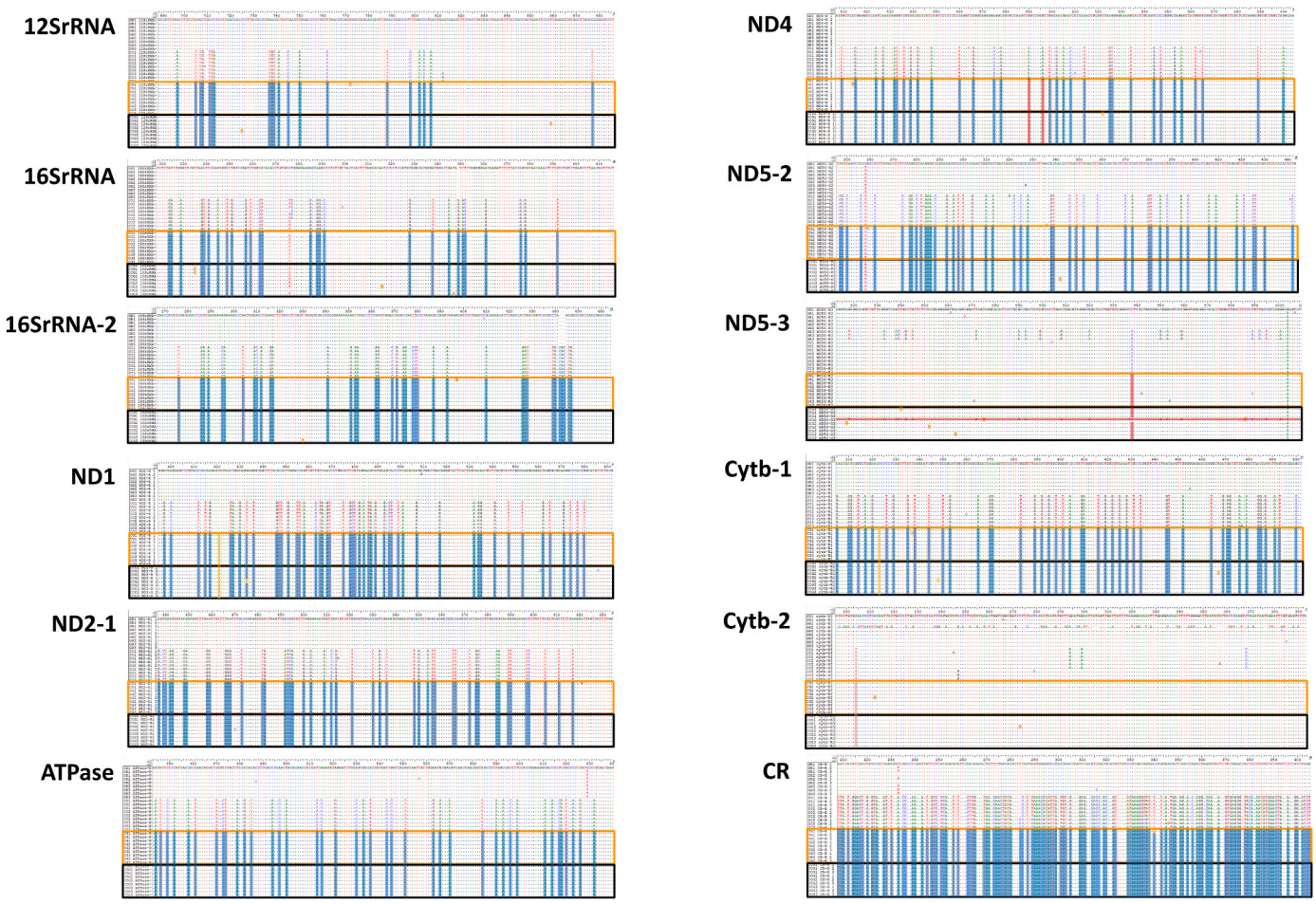

Fig. 4. Nucleotide sequence alignment of mitochondrial structural regions (genes) among KOC, GR, CG, and CCG. The blue or red box indicates the specific site with maternal or paternal inheritance. The orange and black box indicates the sequence belongs to CG and CCG respectively.

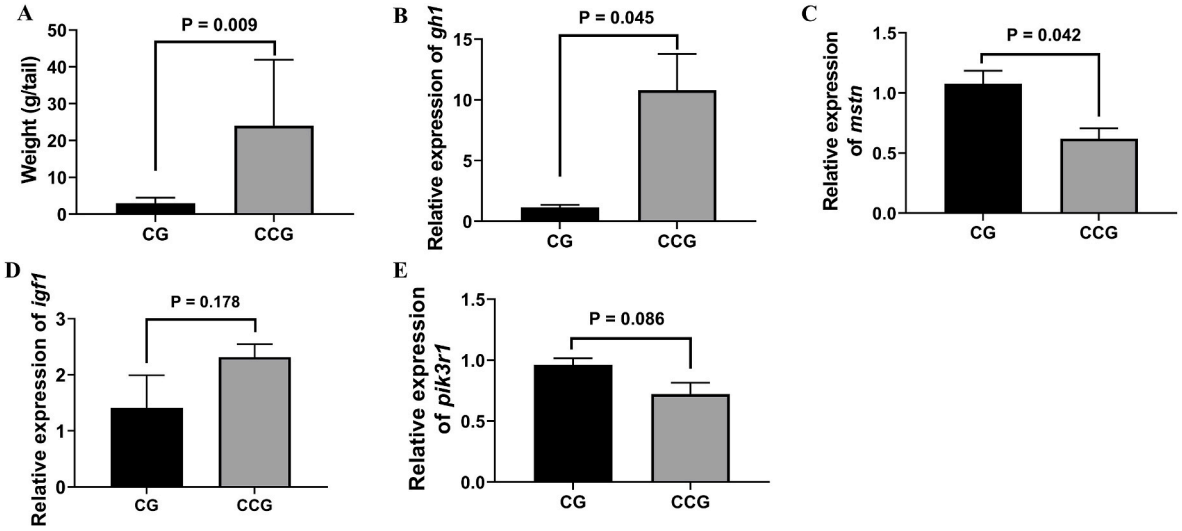

Fig. 5. Comparison of growth characteristics between CG and CCG. (A) Analysis of body weight between CG and CCG. (B–E) Analysis of expression levels of *gh*, *mstn*, *igf1*, and *pik3r1* between CG and CCG. Six individuals were used for each group.

amplified 12 fragments from mitochondrial genes, sequenced them, and found that the mitochondrial gene sequences of CG and CCG hybrids were largely consistent with the maternal mtDNA, indicating maternal inheritance of the mitochondrial genome in the hybrid offspring. However, we observed one sequence of ND5 in CCG and a few sites in ND4 and ND5 of both hybrids that were identical to the paternal-specific sequence or base (Fig. 4), suggesting paternal leakage in the hybrid offspring. These findings align with previous reports of paternal leakage of mtDNA in hybrid zones, which are thought to have less stringent mechanisms for preventing paternal leakage [43,69].

Triploid fish have been reported to exhibit faster growth rates in systemic studies, but the underlying mechanism remains unclear as they are influenced by both polyploidization and hybridization [70–73]. In this study, both CG and CCG hybrids were affected by the same genetic background as well as the effects of hybridization. However, there was a significant difference in the terminal body weights between CG and CCG individuals cultured in the same pool (Fig. 5). These results suggest that the growth advantage observed in CCG hybrids can be primarily attributed to polyploidy rather than hybridization. To further investigate this hypothesis, we examined the expression of growth-related genes, such as *gh*, *igf1*, *pik3r1,* and *mstn* in the pituitary, liver, brain, and muscle, respectively [74–76]. It was found that CCG hybrids exhibited higher expression of *gh* and *igf1* with a positive impact on growth, but lower expression of growth-inhibited genes, such as *pik3r1* and *mstn* (Fig. 5). The differential expression of these growth-related genes may contribute to the disparity in body size between the two hybrids with different ploidies. The upregulation of *gh* and *igf1* probably results from the extra genome in CCG hybrids, while the underlying mechanism for the downregulation of *pik3r1* and *mstn* in CCG remains unclear and requires further exploration.

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CRediT authorship contribution statement

Min Wang: Writing – review & editing. **Yuan Ou:** Investigation, Writing – original draft. **Zijian Guo:** Data curation, Investigation. **Juan Li:** Data curation, Investigation. **Huilin Li:** Data curation. **Xinyi Li:** Data curation. **Jingyang Li:** Data curation. **Shi Wang:** Methodology. **Qingfeng Liu:** Methodology. **Jing Wang:** Methodology. **Yuqin Shu:** Conceptualization, Funding acquisition, Investigation, Writing – original draft. **Shaojun Liu:** Conceptualization, Supervision.

Declaration of competing interest

Shaojun Liu is the editor-in-chief for Reproduction and Breeding and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests. The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.repbre.2023.12.005.

References

[1] J. Mallet, Hybridization as an invasion of the genome, Trends Ecol. Evol. 20 (5) (2005) 229–237.
[2] J. Mallet, Hybrid speciation, Nature 446 (7133) (2007) 279–283.
[3] M.L. Arnold, A. Meyer, Natural hybridization in primates: one evolutionary mechanism, Zoology 109 (4) (2006) 261–276.
[4] R. Abbott, D. Albach, S. Ansell, J.W. Arntzen, S.J. Baird, N. Bierne, J. Boughman, A. Brelsford, C.A. Buerkle, R. Buggs, et al., Hybridization and speciation, J. Evol. Biol. 26 (2) (2013) 229–246.
[5] D.Y. Li, Z.Y. Huang, S.H. Song, Y.Y. Xin, D.H. Mao, Q.M. Lv, M. Zhou, D.M. Tian, M.F. Tang, Q. Wu, et al., Integrated analysis of phenome, genome, and transcriptome of hybrid rice uncovered multiple heterosis-related loci for yield increase, Proc. Natl. Acad. Sci. U. S. A. 113 (41) (2016) E6026–E6035.
[6] S. Wang, C.C. Tang, M. Tao, Q.B. Qin, C. Zhang, K.K. Luo, R.R. Zhao, J. Wang, L. Ren, J. Xiao, et al., Establishment and application of distant hybridization technology in fish, Sci. China Life Sci. 62 (1) (2019) 22–45.
[7] S.J. Liu, Distant hybridization leads to different ploidy fishes, Sci. China Life Sci. 53 (4) (2010) 416–425.
[8] C. Song, S. Liu, J. Xiao, W. He, Y. Zhou, Q. Qin, C. Zhang, Y. Liu, Polyploid organisms, Sci. China Life Sci. 55 (4) (2012) 301–311.
[9] J. Chen, M. Luo, S. Li, M. Tao, X. Ye, W. Duan, C. Zhang, Q. Qin, J. Xiao, S. Liu, A comparative study of distant hybridization in plants and animals, Sci. China Life Sci. 61 (3) (2018) 285–309.
[10] M. Kocour, O. Linhart, D. Gela, M. Rodina, Growth performance of all-female and mixed-Sex common carp *Cyprinus carpio L.* Populations in the central europe climatic conditions, J. World Aquacult. Soc. 36 (1) (2007) 103–113.
[11] X. Zhang, Y. Zhang, X. Zheng, Y. Kuang, Z. Zhao, L. Zhao, C. Li, L. Jiang, D. Cao, C. Lu, et al., A consensus linkage map provides insights on genome character and evolution in common carp (*Cyprinus carpio L.*), Mar. Biotechnol. 15 (3) (2013) 275–312.
[12] S. Wang, X. Ye, Y. Wang, Y. Chen, B. Lin, Z. Yi, Z. Mao, F. Hu, R. Zhao, J. Wang, et al., A new type of homodiploid fish derived from the interspecific hybridization of female common carp × male blunt snout bream, Sci. Rep. 7 (1) (2017) 4189.
[13] R.B.G. Mcnicoll, Longevity records, life spans of mammals, birds, Amphibians, reptiles, and fishby james R. Carey; debra S. Judge, Popul. Dev. Rev. 27 (2) (2001) 381.
[14] G.W. Tempero, N. Ling, B.J. Hicks, M.W. Osborne, Age composition, growth, and reproduction of koi carp (*Cyprinus carpio*) in the lower Waikato region, New Zealand, N. Z. J. Mar. Freshw. Res. 40 (4) (2006) 571–583.
[15] A.J. Crivelli, The biology of the common carp, *Cyprinus carpio L.* in the Camargue, southern France, J. Fish. Biol. 18 (3) (1981) 271–290.
[16] Prochelle Oscar, Campos Hugo, The biology of the introduced carp *cyprinus carpio L.*, in the river Cayumapu, Valdivia, Chile, Stud. Neotrop. Fauna 20 (2) (1985) 65–82.
[17] C. Fernández-Delgado, Life history patterns of the common carp, *Cyprinus carpio*, in the estuary of the Guadalquivir river in south-west Spain, Hydrobiologia 206 (1) (1990) 19–28.
[18] L. Vilizzi, K.F. Walker, Age and growth of the common carp, *Cyprinus carpio*, in the River Murray, Australia: validation, consistency of age interpretation, and growth models, Environ. Biol. Fish. 54 (1) (1999) 77–106.
[19] M.R. Re, T.Y. Fu, Description of a new genus and species of *danioninae* from China (*cypriniformes: cyprinidae*), Acta Zootaxonomica Sin. 8 (1983) 434–437.
[20] H.E. Shunping, L. Huanzhang, C. Yiyu, M. Kuwahara, T. Nakajima, Z. Yang, Molecular phylogenetic relationships of Eastern Asian Cyprinidae (Pisces: *cypriniformes*) inferred from cytochrome b sequences, Sci. China Life Sci. 47 (2) (2004) 130–138.
[21] Q. Liu, X. Zhang, J. Liu, F. Liu, S. Liu, A new type of allodiploid hybrids derived from female Megalobrama amblycephala × male gobiocypris rarus, Front. Genet. 19 (12) (2021) 685914.
[22] J. Zha, L. Sun, Y. Zhou, P.A. Spear, M. Ma, Z. Wang, Assessment of 17alpha-ethinylestradiol effects and underlying mechanisms in a continuous, multigeneration exposure of the Chinese rare minnow (*Gobiocypris rarus*), Toxicol. Appl. Pharmacol. 226 (3) (2008) 298–308.
[23] J.M. Chen, O.Q. C, Y.Y. Li, Y.Y. Wang, C. Liu, J.Y. Yin, S.M. Bergmannn, W. W. Zeng, Q. W, Establishment of a rare minnow (*Gobiocypris rams*) disease model for grass carp reovirus genotype II, Aquaculture 533 (1) (2021).
[24] M. Zhou, B. Wang, Y. Lin, C. Zhang, H. Cao, Molecular cloning, functional characterization and expression analysis of P65 subunit in response to GCRV infection in rare minnow (*Gobiocypris rarus*), Dev. Comp. Immunol. 113 (2020) 103786.
[25] Bin Zhu, Gong Yu-Xin, Liu Lei, Li Dong-Liang, Yuan Wang, Toxic effects of triazophos on rare minnow (*Gobiocypris rarus*) embryos and larvae, Chemosphere 108 (2014) 46–54.
[26] L. Liu, Y.Y. Xiao, Y.H. Ji, M.Z. Liu, Y. Chen, Y.L. Zeng, Y.G. Zhang, L. Jin, CuInS 2/ZnS QD exposure induces developmental toxicity, oxidative stress and DNA damage in rare minnow (*Gobiocypris rarus*) embryos and larvae, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 198 (2017) 19–27.
[27] Jiliang Zhang, Ping Sun, Shao Xian Chunnuan, Tributyltin affects shoaling and anxiety behavior in female rare minnow (*Gobiocypris rarus*), Aquat. Toxicol. 178 (2016) 80–87.
[28] H.J. Wang, Temporal variation in genetic structure of the Chinese rare minnow (*Gobiocypris rarus*) in its type locality revealed by microsatellite markers, Biochem. Genet. 48 (3–4) (2010) 312–325.
[29] J. Wang, P. Li, Y. Zhang, Z. Peng, The complete mitochondrial genome of Chinese rare minnow, *Gobiocypris rarus* (Teleostei: *cypriniformes*), Mitochondrial DNA 22 (5–6) (2011) 178–180.
[30] J.E. Martinez, J.R. Beck, W.C. Allsbrook Jr., C.G. Pantazis, Flow cytometric DNA analysis, Clin. Lab. Sci. 3 (3) (1990) 180–183.
[31] D. Gong, M. Tao, L. Xu, F. Hu, Z. Wei, S. Wang, Y. Wang, Q. Liu, C. Wu, K. Luo, et al., An improved hybrid bream derived from a hybrid lineage of *Megalobrama amblycephala* (♀)×*Culter alburnus* (♂), Sci. China Life Sci. 65 (6) (2022) 1213–1221.
[32] H.W. Thompson, R. Mera, C. Prasad, A description of the appropriate use of student's t-test, Nutr. Neurosci. 1 (2) (1998) 165–172.
[33] P. Mishra, C.M. Pandey, U. Singh, A. Keshri, M. Sabaretnam, Selection of appropriate statistical methods for data analysis, Ann. Card Anaesth. 22 (3) (2019) 297–301.
[34] C.P. You, R.R. Zhao, J. Hu, S.J. Liu, M. Tao, C. Zhang, Y.B. Chen, Q.B. Qin, J. Xiao, W. Duan, et al., Inheritance of the complete mitochondrial genomes in three different ploidy fishes, Curr. Mol. Med. 14 (10) (2014) 1322–1330.
[35] S. Wang, N. Jiao, L. Zhao, M. Zhang, P. Zhou, X. Huang, F. Hu, C. Yang, Y. Shu, W. Li, et al., Evidence for the paternal mitochondrial DNA in the crucian carp-like fish lineage with hybrid origin, Sci. China Life Sci. 63 (1) (2020) 102–115.

[36] Q. Qin, W. He, S. Liu, J. Wang, J. Xiao, Y. Liu, Analysis of 5S rDNA organization and variation in polyploid hybrids from crosses of different fish subfamilies, J. Exp. Zool. B Mol. Dev. Evol. 314 (5) (2010) 403–411.
[37] Y.D. Wang, Q.B. Qin, R. Yang, W.Z. Sun, Q.W. Liu, Y.Y. Huo, X. Huang, M. Tao, C. Zhang, T. Li, et al., Hox genes reveal genomic DNA variation in tetraploid hybrids derived from *Carassius auratus red var*, (female) × *Megalobrama amblycephala* (male), BMC Genet. 18 (1) (2017) 86.
[38] K. Luo, S. Wang, Y. Fu, P. Zhou, X. Huang, Q. Gu, W. Li, Y. Wang, F. Hu, S. Liu, Rapid genomic DNA variation in newly hybridized carp lineages derived from *Cyprinus carpio* (♀) × *Megalobrama amblycephala* (♂), BMC Genet. 20 (1) (2019) 8720.
[39] Q. Qin, Y. Wang, J. Wang, J. Dai, J. Xiao, F. Hu, K. Luo, M. Tao, C. Zhang, Y. Liu, et al., The autotetraploid fish derived from hybridization of *Carassius auratus red var.* (female) × *Megalobrama amblycephala* (male), Biol. Reprod. 91 (4) (2014) 93.
[40] W. Lu, S. Liu, Y. Long, M. Tao, C. Zhang, J. Wang, J. Xiao, S. Chen, J. Liu, Y. Liu, Comparative study of erythrocytes of polyploid hybrids from various fish subfamily crossings, Cell Tissue Res. 336 (1) (2009) 159–163.
[41] Q.F. Liu, Y.H. Qi, Q.L. Liang, X.J. Xu, F.Z. Hu, J. Wang, J. Xiao, S. Wang, W.H. Li, M. Tao, et al., The chimeric genes in the hybrid lineage of Carassius auratus cuvieri (f)xCarassius auratus red var. (o), Sci. China Life Sci. 61 (9) (2018) 1079–1089.
[42] S. Liu, J. Luo, J. Chai, L. Ren, Y. Zhou, F. Huang, X. Liu, Y. Chen, C. Zhang, M. Tao, et al., Genomic incompatibilities in the diploid and tetraploid offspring of the goldfish × common carp cross, Proc. Natl. Acad. Sci. U. S. A. 113 (5) (2016) 1327–1332.
[43] J.A. Morgan, M. Macbeth, D. Broderick, P. Whatmore, R. Street, D.J. Welch, J. R. Ovenden, Hybridisation, paternal leakage and mitochondrial DNA linearization in three anomalous fish (*Scombridae*), Mitochondrion 13 (6) (2013) 852–861.
[44] Y. Wang, W. Sun, Q. Gu, J. Yao, H. Tan, X. Huang, Q. Qin, M. Tao, C. Zhang, S. Liu, Variations in the mitochondrial genome of a goldfish-like hybrid [koi carp (♀) × blunt snout bream (♂)] indicate paternal leakage, Front. Genet. 11 (2020) 613520.
[45] Y. Wang, W. Hu, G. Wu, Y. Sun, S. Chen, F. Zhang, Z. Zhu, J. Feng, X. Zhang, Genetic analysis of "all-fish" growth hormone gene trans ferred carp (*Cyprinus carpio L.*) and its F1 generation, Sci. Bull. 46 (14) (2001) a1–a4.
[46] F. Le Gac, O. Blaise, A. Fostier, P.Y. Le Bail, M. Loir, B. Mourot, C. Weil, Growth hormone (GH) and reproduction: a review, Fish Physiol. Biochem. 11 (1–6) (1993) 219–232.
[47] J.C. Gabillard, P.R. Biga, P.Y. Rescan, I. Seiliez, Revisiting the paradigm of myostatin in vertebrates: insights from fishes, Gen. Comp. Endocrinol. 194 (2013) 45–54.
[48] Y. Sun, G.D. Zheng, M. Nissa, J. Chen, S.M. Zou, Disruption of mstna and mstnb gene through CRISPR/Cas9 leads to elevated muscle mass in blunt snout bream (*Megalobrama amblycephala*), Aquaculture 528 (2020) 735597.
[49] B. Tao, J. Tan, L. Chen, Y. Xu, X. Liao, Y. Li, J. Chen, Y. Song, W. Hu, CRISPR/Cas9 system-based myostatin-targeted disruption promotes somatic growth and adipogenesis in loath, Misgurnus anguillicaudatus, Aquaculture 544 (15) (2021) 737097.
[50] J. Xiao, X.W. Kang, L.H. Xie, Q.B. Qin, Z.L. He, F.Z. Hu, C. Zhang, R.R. Zhao, J. Wang, K.K. Luo, et al., The fertility of the hybrid lineage derived from female *Megalobrama amblycephala* x male *Culter alburnus*, Anim. Reprod. Sci. 151 (1–2) (2014) 61–70.
[51] J. Hu, S.J. Liu, J. Xiao, Y. Zhou, C.P. You, W.G. He, R.R. Zhao, C. Song, Y. Liu, Characteristics of diploid and triploid hybrids derived from female *Megalobrama amblycephala* Yih x male *Xenocypris davidi* Bleeker, Aquaculture 364–365 (2012) 157–164.
[52] Q. Liu, J. Liu, Q. Liang, Y. Qi, M. Tao, C. Zhang, Q. Qin, R. Zhao, B. Chen, S. Liu, A hybrid lineage derived from hybridization of *Carassius cuvieri* and *Carassius auratus red var.* and a new type of improved fish obtained by back-crossing, Aquaculture 505 (2019) 173–182.
[53] Q. Qin, Y. Wang, J. Wang, J. Dai, J. Xiao, F. Hu, K. Luo, M. Tao, C. Zhang, Y. Liu, et al., The autotetraploid fish derived from hybridization of *Carassius auratus red var.* (female) x *Megalobrama amblycephala* (male), Biol. Reprod. 91 (4) (2014) 93.
[54] J. Wang, W. He, W. Wang, Z. Luo, L. Han, C. Xiang, M. Chai, T. Li, J. Li, K. Luo, et al., A novel allotriploid hybrid derived from female goldfish × male bleeker's yellow tail, Front. Genet. 13 (2022) 880591.
[55] Q. Liu, X. Zhang, J. Liu, F. Liu, F. Shi, Q. Qin, M. Tao, C. Tang, S. Liu, A new type of allodiploid hybrids derived from female *Megalobrama amblycephala* × male *Gobiocypris rarus*, Front. Genet. 12 (2021) 685914.
[56] Y. Wang, J. Yao, Y. Luo, H. Tan, X. Huang, S. Wang, Q. Qin, C. Zhang, M. Tao, K. Dabrowski, et al., Two new types of homodiploid fish and polyploid hybrids derived from the distant hybridization of female koi carp and male bighead carp, Mar. Biotechnol. 23 (4) (2021) 628–640.
[57] S. Wang, X. Xu, K. Luo, Q. Liu, L. Chen, Z. Wei, P. Zhou, F. Hu, Z. Liu, M. Tao, Two new types of triploid hybrids derived from *Cyprinus carpio* (female) x *Megalobrama amblycephala* (male), Aquaculture 528 (2020) 735448.
[58] Y. Han, Comparative Study on Gonad and Gamete Development and Haemocytes of Different Ploid Rainbow Trout, Northeast Agricultural University, 2008.
[59] A.H. Houston, A. Murad, Erythrodynamics in fish: recovery of the goldfish *Carassius auratus* from acute anemia, Can. J. Zool. 73 (3) (1995) 411–418.
[60] S.J. Liu, Y.D. Sun, C. Zhang, K.K. Luo, Y. Liu, Triploid crucain carp-allotetraploid Hybrids(♂)×Goldfish(♀), Acta Genetica Sin. 31 (1) (2004) 31–38.
[61] B. Wang, Y. Liu, X. Chen, Z. Fan, Amitosis-like nuclear division in erythrocytes of triploid rainbow trout Oncorhynchus mykiss, J. Fish. Biol. 76 (5) (2010) 1205–1211.
[62] S. Wang, X. Xu, K. Luo, Q. Liu, L. Chen, Z. Wei, P. Zhou, F. Hu, Z. Liu, M. Tao, Two new types of triploid hybrids derived from *Cyprinus carpio* (female) x *Megalobrama amblycephala* (male), Aquaculture 528 (2020) 735448.
[63] D.B. Gong, L.H. Liu, Q.F. Liu, S. Wang, Y.D. Wang, F.Z. Hu, C. W, K.K. Luo, C. C. Tang, R. Zhou, C. Zhang, M. Tao, Y.Q. Wang, S.J. Liu, A new type of hybrid bream derived from a hybrid lineage of *Megalobrama amblycephala* (female) x *Culter alburnus* (male), Aquaculture 534 (2021) 736194.
[64] S. Wang, P. Zhou, X.X. Huang, Q.L. Liu, B.W. Lin, Y.Q. Fu, Q.H. Gu, F.Z. Hu, K. K. Luo, C. Zhang, et al., The establishment of an autotetraploid fish lineage produced by female allotetraploid hybrids x male homodiploid hybrids derived from *Cyprinus carpio* (female) x *Megalobrama amblycephala* (male), Aquaculture 515 (2020) 734583.
[65] C. Belloch, R. Perez-Torrado, S.S. Gonzalez, J.E. Perez-Ortin, J. Garcia-Martinez, A. Querol, E. Barrio, Chimeric genomes of natural hybrids of Saccharomyces cerevisiae and Saccharomyces kudriavzevii, Appl. Environ. Microbiol. 75 (8) (2009) 2534–2544.
[66] E.S. Polovina, M.E. Parakatselaki, E.D. Ladoukakis, Paternal leakage of mitochondrial DNA and maternal inheritance of heteroplasmy in Drosophila hybrids, Sci. Rep. 10 (1) (2020) 2599.
[67] S. Liu, Q. Qin, Y. Wang, H. Zhang, R. Zhao, C. Zhang, J. Wang, W. Li, L. Chen, J. Xiao, et al., Evidence for the formation of the male gynogenetic fish, Mar. Biotechnol. 12 (2) (2010) 160–172.
[68] Y. Wang, W. Sun, Q. Gu, J. Yao, H. Tan, X. Huang, Q. Qin, M. Tao, C. Zhang, S. Liu, Variations in the mitochondrial genome of a goldfish-like hybrid [koi carp (♀) × blunt snout bream (♂)] indicate paternal leakage, Front. Genet. 11 (2020) 613520.
[69] J.N. Wolff, S. Gandre, A. Kalinin, N.J. Gemmell, Delimiting the frequency of paternal leakage of mitochondrial DNA in chinook salmon, Genetics 179 (2) (2008) 1029–1032.
[70] E.H. Ignatz, L.M. Braden, T.J. Benfey, A. Caballero-Solares, T.S. Hori, C. D. Runighan, M.D. Fast, J.D. Westcott, M.L. Rise, Impact of rearing temperature on the innate antiviral immune response of growth hormone transgenic female triploid Atlantic salmon (Salmo salar), Fish Shellfish Immunol. 97 (2020) 656–668.
[71] F. Yu, J. Xiao, X.Y. Liang, S.J. Liu, G.J. Zhou, K.K. Luo, Y. Liu, W. Hu, Y.P. Wang, Z. Y. Zhu, Rapid growth and sterility of growth hormone gene transgenic triploid carp, Chin. Sci. Bull. 56 (16) (2011) 1679–1684.
[72] I.S. Park, H.W. Gil, D.S. Kim, Morphometric characteristics of diploid and triploid marine medaka, Oryzias dancena, Dev Reprod 22 (2) (2018) 183–192.
[73] B.M. Cleveland, G.M. Weber, Effects of triploidy on growth and protein degradation in skeletal muscle during recovery from feed deprivation in juvenile rainbow trout (Oncorhynchus mykiss), Comp. Biochem. Physiol. Mol. Integr. Physiol. 166 (1) (2013) 128–137.
[74] R.G. Rosenfeld, Endocrinology of growth, Nestle Nutr Workshop Ser Pediatr Program 65 (2010) 225–234. ; discussion 234-227.
[75] Y. Gao, Z. Dai, C. Shi, G. Zhai, X. Jin, J. He, Q. Lou, Z. Yin, Depletion of myostatin b promotes somatic growth and lipid metabolism in zebrafish, Front. Endocrinol. 7 (2016) 88.
[76] J.F. Huang, C. Shi, Y.P. Gao, J.Z. Su, Y.Q. Shu, N.M. Zeng, G. Zhai, X. Jin, D. Han, J. Y. He, et al., Heterozygous depletion of pik3r1 improves growth and feed conversion efficiency in Gibel carp (Carassius gibelio), Aquaculture 545 (2021) 737207.