Aquaculture Reports 50 (2026) 103771



Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep



Production of a novel all-male tetraploid fish via androgenesis

Xu Huang [a,1], Qian Xiao [a,1], Yanling Fu [a], Chang Wu [a], Chongqing Wang [a], Xiaowei Xu [a], Yali Long [a], Xinyu Yao [a], Yuhan Yang [a], Zeyang Li [a], Xidan Xu [a], Kun Zhang [a], Yue Zhou [a], Jinhai Bai [a], Zhengkun Liu [a], Yuchen Jiang [a], Enkui Hu [a], Qinbo Qin [a,b,*], Shaojun Liu [a,b]

[a] Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry, College of Life Sciences, Hunan Normal University, Changsha 410081, China
[b] Yuelushan Laboratory, Changsha 410128, China

ARTICLE INFO

Keywords:
Artificial androgenesis
Cytoplasmic–nuclear hybridization
Unreduced gametes
All-male tetraploid fish

ABSTRACT

Male tetraploid fish exhibit significant advantages in polyploid breeding owing to their capacity for producing abundant diploid sperm to produce triploid fish. In this study, androgenesis was induced by fertilizing UV-irradiated haploid eggs (n = 50) from Japanese white crucian carp (*Carassius cuvieri*, WCC, 2 n = 100) with diploid sperm (2 n = 100) obtained from autotetraploid fish (4nRR, 4 n = 200). This procedure gave rise to a diploid androgenetic population (2nAR, 2 n = 100), representing a typical cytoplasmic-nuclear hybrid that harbors cytoplasmic genetic material from WCC and nuclear genetic material from 4nRR. Notably, 2nAR is bisexual fertile and capable of producing unreduced gametes. Through the self-crossing of 2nAR, a novel all-male tetraploid fish (AM4nAR, 4 n = 200) was generated. AM4nAR reaches sexual maturity at approximately 6.5 months of age and produces abundant diploid sperm, with total sperm motility exceeding that of 4nRR, indicating robust reproductive performance and providing a valuable diploid gamete resource for polyploid fish production. Overall, this study enhances the understanding of androgenesis in polyploid fish and expands the approaches for generating all-male tetraploid fish.

1. Introduction

Fertile tetraploid fish constitute valuable germplasm resources for aquaculture (Zhong, et al., 2025). Crucially, male tetraploid fish exhibit superior practical utility by generating abundant diploid gametes, serving as indispensable sources for large-scale triploid fry production (Jung, et al., 2026). Additionally, the all-male tetraploid population can also provide valuable models for exploring mechanisms of species evolution and sex determination. Contemporary methods for tetraploid acquisition encompass artificial induction (hydrostatic pressure application or temperature shock), genetic breeding techniques, and gene editing, among others (Refstie, 1981; Chourrout, et al., 1986; Zou, et al., 2004; Ou, et al., 2024). Numerous genetic breeding techniques, such as hybridization, gynogenesis and androgenesis, have been applied to improve traits in fish (Wang, et al., 2018; Liu, et al., 2025). Hybridization, especially distant hybridization, is considered effective in the generation of bisexual fertile tetraploid and diploid lineages (Liu, 2010; Qin, et al., 2014b; Liu, et al., 2016, 2020; Ren, et al., 2019). Researchers have produced tetraploid fish with improved traits via gynogenesis (Liu, et al., 2004, 2007a; Liu, 2010; Xu, et al., 2015) and androgenesis (Duan, et al., 2007; Sun, et al., 2007; Huang, et al., 2024).

Androgenesis is widely acknowledged as a uniparental developmental mode, wherein progeny exclusively inherit the paternal nuclear genome (Pigneur, et al., 2012). Artificial androgenesis refers to the fertilization of normal sperm with genetically inactivated eggs, making it a unique method of reproduction. Analogous to artificial gynogenesis, this technique primarily functions to inactivate maternal genetic material while enabling embryonic development solely under paternal genomic regulation. Its applications span various fields, including unisexual population production, elucidation of sex determination mechanisms, rapid establishment of inbred lines, conservation of endangered species, and assessment of mitochondrial genome influences on development (Thorgaard, et al., 1990; Babiak, et al., 2002; Ezaz, et al., 2004; Brown, et al., 2006; Liu, et al., 2013, 2022d). Given the rarity of natural

* Corresponding author at: Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry, College of Life Sciences, Hunan Normal University, Changsha 410081, China.
E-mail address: qqb@hunnu.edu.cn (Q. Qin).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.aqrep.2026.103771
Received 17 March 2026; Received in revised form 23 June 2026; Accepted 29 July 2026
Available online 6 August 2026
2352-5134/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

androgenesis, artificial induction constitutes a vital approach for generating androgenetic progeny and represents a crucial chromosome or gamete manipulation technology. To date, artificially induced androgenesis has been reported in fish including rainbow trout, brook trout, common carp, Nile tilapia, loach, and zebrafish (Parsons, Thorgaard, 1984; Thorgaard, et al., 1990; Myers, et al., 1995; Corley-Smith, et al., 1996; Morishima, et al., 2011; Kašpar, et al., 2022).

Artificial androgenesis can be divided into diploid and tetraploid pathways. In the diploid pathway, artificial androgenesis in fish is based on the fertilization of genetically inactivated haploid eggs with normal haploid sperm, followed by treatment (e.g., cold shock), to double the chromosomes and ultimately form the diploid offspring (Liu, et al., 2022a). However, cold shock treatment can compromise embryonic viability and substantially diminish androgenetic success rates. In the tetraploid pathway, diploid sperm produced by male tetraploid fish are used to fertilize genetically inactivated haploid eggs, allowing normally developed androgenetic diploid offspring to be obtained directly without chromosome doubling treatment (Arai, et al., 1995; Duan, et al., 2007; Liu, et al., 2022a). This latter methodology demonstrates significantly superior survival outcomes compared to the diploid pathway.

In previous studies, diploid androgenetic individuals (2 n = 100) were produced by fertilizing UV-irradiated haploid eggs (n = 50) of goldfish (GF, *Carassius auratus*, 2 n = 100) with diploid sperm (2 n = 100) from male allotetraploid hybrid fish (4 n = 200, F_{13}) (Sun, et al., 2007). These allotetraploids were generated via distant hybridization between *Carassius auratus* red var. (RCC, ♀, 2 n = 100) and *Cyprinus carpio* L. (CC, ♂, 2 n = 100) (Liu, et al., 2022b). Separately, a small number of diploid androgenetic fish were obtained by fertilizing UV-inactivated haploid eggs (n = 50) of RCC with diploid sperm (2 n = 100) produced by autotetraploid crucian carp (4nRR, 4 n = 200, F_8), which originated from distant hybridization between RCC (♀, 2 n = 100) and *Megalobrama amblycephala* (BSB, ♂, 2 n = 48) (Zhou, et al., 2015). Although neither of these two types of androgenetic offspring underwent chromosome doubling treatment, the hatching rates were only 4.1% and 2.1%, respectively (Sun, et al., 2007; Zhou, et al., 2015). Mass and efficient production of androgenetic fish therefore remains challenging. Consequently, the present study focused on refining existing androgenesis protocols to overcome the bottleneck of low hatching rates and generate a large number of androgenetic offspring.

The present study induced androgenesis by fertilizing haploid eggs (n = 50) from Japanese white crucian carp (*Carassius cuvieri*, WCC, 2 n = 100) with diploid sperm (2 n = 100) derived from autotetraploid crucian carp (4nRR, 4 n = 200, F_{16}-F_{20}). Concurrently, the efficacy of genetically inactivated eggs from common carp (*Cyprinus carpio*, CC) and Hefang crucian carp (HF) was evaluated. HF is a hybrid population derived from female WCC × male RCC (Wang, et al., 2015). The ploidy, fertility, and genetic characteristics of androgenetic offspring (2nAR, 2 n = 100) were examined. Subsequent self-crossing of sexually mature 2nAR generated a novel all-male tetraploid fish (AM4nAR, 4 n = 200), which, at 6.5 months of age, produced abundant diploid sperm (2 n = 100) with significantly superior motility compared to 4nRR. These breakthroughs resolve longstanding efficiency barriers in large-scale androgenesis and pioneer a high-performance tetraploid germplasm for aquaculture.

2. Material and methods

2.1. Ethics statement

The Administration of Affairs Concerning Animal Experimentation Guidelines states that approval from the Science and Technology Bureau of China and the Department of Wildlife Administration is not necessary when the fish in question are not rare or near extinction (first-class or second-class state protection level). Therefore, approval was not required for the experiments conducted in this study. All specimens were raised in natural aquatic environments and humanely euthanized using 2-phenoxyethanol (P1953, Sigma-Aldrich, Shanghai, China) prior to dissection. Research personnel possessed valid certification from the accredited Laboratory Animal Practitioners Training Program administered by Hunan Province's Institute of Experimental Animals, China.

2.2. Animals and crosses

The fish used in this study were cultivated at Hunan Normal University. The flowchart of breeding procedure for 2nAR and AM4nAR was shown in Fig. 1. During the reproductive season (from April to May) in 2021, androgenic offspring (2nAR) were obtained from the diploid sperm (2 n = 100) of autotetraploid fish (4nRR, 4 n = 200, F_{16}) via artificial androgenesis. The heterologous haploid eggs (n = 50) of female WCC were irradiated with UV rays to inactivate the maternal genome. In the dark environment, a scaffold was constructed with a low-speed shaker (set at 100 r/min) (SCI−0180-S, Scilogex, Beijing, China) placed at the bottom to gently rotate the eggs during ultraviolet irradiation, ensuring uniform exposure. The UV lamp (ZW15S19W, Huanya, Jiangyin, China) was positioned at the top of the scaffold, 15 cm above the shaker. Approximately 6000 eggs (2000 eggs × 3 replicates) were divided into three groups and stored in glass Petri dishes (1177–180, Huaou, Jiangsu, China) containing approximately 5 mL of ovarian fluid. The dishes were placed on the ice plate and irradiated with 300 mJ/cm^2 of ultraviolet (UV) rays for 180 s (Zhou, et al., 2015). To verify complete inactivation of the maternal genome, under the same conditions, UV-irradiated haploid eggs were activated with UV-irradiated diploid sperm as a negative control. If embryonic development was observed to fail, the treatment was considered successful. Meanwhile, artificial fertilization using non-UV-irradiated haploid eggs with diploid sperm (4nRR) was performed as a positive control, and the fertilization rate, hatching rate, and survival rate of each group were respectively recorded. Ovarian fluid was a liquid obtained by the precipitation or filtration of eggs and liquid extruded from the cloaca of naturally purebred female fish after induced spawning. These irradiated eggs were promptly fertilized with untreated semen from 4nRR and the resulting embryos were incubated until hatching. Temperature was continuously monitored throughout the embryonic incubation period and maintained at 22–24°C. Concurrently, androgenetic efficiency was comparatively assessed using genetically inactivated eggs from common carp (CC) and Hefang crucian carp (HF). Fertilization rate (no. of embryos at the gastrula stage/no. of eggs), hatching rate (no. of hatched fry/no. of eggs), and survival rates (no. of adult fish/no. of eggs) were systematically quantified. Approximately 2000 eggs were used per experimental replicate, and three biological replicates were conducted for each egg source group (WCC, CC, and HF). During the reproductive season (from April to May) in 2023, sexually mature 2nAR individuals were self-crossed. The hatching conditions of embryos were identical to those described previously. To assess the fertilization and hatching rates of 2nAR self-crossing, a total of approximately 1800 eggs were allocated into three batches of 600 eggs each. Fertilization success was evaluated at the gastrula stage, and hatching events were recorded following the completion of embryogenesis, which typically occurred at approximately 80 h post-fertilization. All fry (AM4nAR) were transferred to microbially matured ponds at densities of 2000 individuals per 667 m^2 for subsequent cultivation.

2.3. Analysis of ploidy

To measure the mean DNA content of erythrocytes in 4nRR, WCC, 2nAR, and AM4nAR, blood cells were collected from 10 individuals for 4nRR and WCC, 150 individuals for 2nAR and 124 individuals for AM4nAR. The larger sample sizes for 2nAR and AM4nAR were necessary because their mean DNA content was unknown and required adequate population representation, whereas the DNA content of 4nRR and WCC was already known. Semen specimens were isolated from sexually

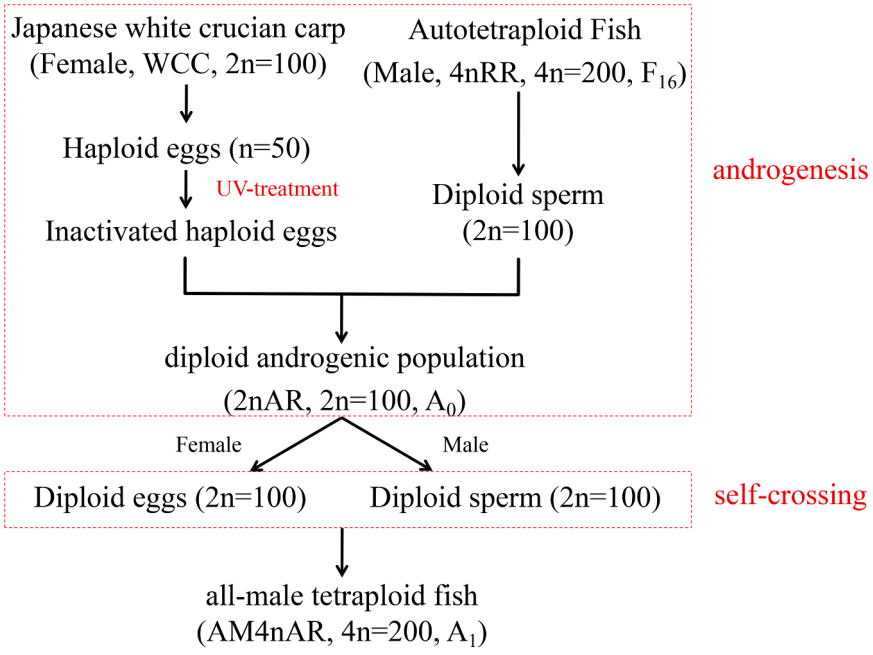


Fig. 1. The flowchart of breeding procedure of 2nAR and AM4nAR.

mature males of 4nRR, 2nAR, and AM4nAR. All samples were processed following previously published methods (Peng, et al., 2020; Huang, et al., 2024).

Chromosome preparation was performed using kidney tissue from 10 4nRR, 10 WCC, 30 2nAR and 30 AM4nAR individuals, following previously published methods (Liu, et al., 2001). Twenty metaphase spreads from each fish were counted and analyzed. The sample sizes were chosen based on empirical experience and practical feasibility in fish breeding research.

2.4. Analysis of genetics

2.4.1. DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was isolated from blood samples using a commercial extraction kit (9765, TaKaRa, Beijing, China), strictly following the manufacturer's protocols. Polymerase chain reaction amplification was performed using degenerate primers (5′-TGAAGCGACCCATGAA(C/T)G−3′ and 5′-AGGTCG(A/G)TACTT(A/G)TA(A/G)T−3′) targeting the conserved HMG box domain of *Sox* genes (Chen, et al., 2009). The amplification conditions were the same as those described in a previous paper (Fu, et al., 2020).

2.4.2. Fluorescence in situ hybridization (FISH)

Species-specific centromeric probes (263 bp, GenBank JQ086761) for red crucian carp (RCC) were synthesized via PCR amplification using primers 5′-TTCGAAAAGAGAGAATAATCTA−3′ and 5′-AACTCGTCTAAACCCGAACTA−3′ (Qin, et al., 2014a). This probe has been shown to have 100 signal points in RCC and 100 signal points in 4nRR (Xu, et al., 2023). FISH procedures were performed following the protocol described by He *et al.* (He, et al., 2012), with twenty metaphase spreads per specimen subjected to quantitative signal enumeration and analysis.

2.4.3. Mitochondrial genome sequencing and analysis

Mitochondrial genome sequencing and analysis were performed by Beijing Tsingke Biotech Co., Ltd., strictly following Illumina's standardized workflow, including sample quality assessment, library construction, library quality verification, and sequencing. The procedure was as follows: Genomic DNA was extracted and purified, then subjected to end-repair and 3′-terminal adenylation. Subsequently, sequencing adapters were ligated to the processed fragments. Target insert-sized fragments were selectively isolated by agarose gel electrophoresis. The sequencing libraries were generated by PCR amplification using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina®. The constructed library was inspected, and qualified libraries were sequenced on the Illumina NovaSeq platform. Clean reads were obtained by filtering low-quality data with the following criteria: reads with $>$ 5% N bases, reads with $\geq$ 50% low-quality bases (quality value $\leq$5), and reads with adapter contamination were removed. Subsequently, the clean data were *de novo* assembled using SPAdes v.3.14.1, with k-mers 31, 55, 77, 99 and 127 used to generate graph files. Moreover, the graph files were visualized by the Bandage software to facilitate further assembly of the mitochondrial genome. The assembled complete mitochondrial genome was annotated using MITOS (http://mitos.bioinf.uni-leipzig.de/index.py), and the annotation results were manually corrected. Finally, the mitochondrial genome circular map was generated online using Organellar Genome DRAW v1.2.

2.5. Histological analysis of gonadal structure and gamete

For histological assessment of gonadal structure, specimens were randomly sampled as follows: sixty 12-month-old 2nAR individuals (30 male, 30 female), sixty 24-month-old 2nAR individuals (30 male, 30 female), ten 12-month-old 4nRR individuals (5 male, 5 female), and fifty 6.5-month-old AM4nAR individuals. All procedures adhered to the methodology of Qin *et al.* (Qin, et al., 2018). For 2nAR, eggs were collected in sterile glass Petri dishes and observed directly under a stereomicroscope (S9i, Leica, Singapore). Semen samples were fixed in 2.5% glutaraldehyde (G916054, Macklin, Shanghai, China) and then stained with Giemsa (DM0002, Solarbio, Beijing, China) to examine sperm head and tail morphology under a light microscope (DM1000, Leica, Shanghai, China). For AM4nAR, a portion of the semen samples was fixed in 2.5% glutaraldehyde and dehydrated for ultrastructural imaging by scanning electron microscopy (SEM) (JSM−6360, JEOL, Tokyo, Japan). Remaining samples were activated and evaluated for total sperm motility using the CASA II system (Hamilton-Thorne Research, Beverly, USA) and the Animal Motility Software Manual Version 1.4. The indicators of this instrument include beat cross

frequency (BCF), velocity average path (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), and linearity (LIN). Testicular tissues were fixed in 2.5% glutaraldehyde for transmission electron microscopy (TEM) (HT7800, Hitachi High-Technologies, Tokyo, Japan) following the protocol of Zhou *et al.* (Zhou, et al., 2026).

2.6. Statistical analysis

Data were expressed as mean ± Standard Deviation (SD). Statistical analyses were conducted using SPSS 20.0 after verifying data normality (Shapiro-Wilk test) and variance homogeneity (Levene's test). Comparisons between two groups were performed using independent samples *t*-test, while comparisons among multiple groups were analyzed by one-way ANOVA followed by Tukey's HSD test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Generation of androgenic offspring

UV-inactivated haploid eggs from WCC (Fig. 2A) were fertilized with untreated diploid sperm derived from 4nRR (Fig. 2B), yielding diploid embryos that developed into androgenetic fish (2nAR, Fig. 2C). Under the same breeding conditions, the shape and size of 2 nAR are similar to those of the parental 4 nRR, and it inherits a pair of short barbels from 4nRR. The body color of WCC is silvery white, and that of 4nRR is greenish-brown. Notably, 2 nAR displays a yellowish-brown hue with a whitish tinge, which is distinct from the coloration of both its parents. The DNA content in the blood and chromosomes at metaphase of 2nAR was determined, with WCC and 4nRR serving as controls (Fig. 2D–2I, Table 1). The mean DNA content of 2nAR was 102.27 (peak 1), which was equal to the DNA content in WCC (peak 1: 101.64) and was approximately half that in 4nRR (peak 2: 203.00). 2nAR individuals exhibited 100 chromosomes at metaphase, which were the same as those in WCC (2n=100) and half of those in 4nRR (4n=200). These results revealed that 2nAR was a diploid (2n=100).

3.2. Comparative analysis of the induction efficiency of androgenesis

In addition to the eggs of WCC, the genetically inactivated eggs of CC and HF were used for artificial androgenesis. The fertilization, hatching,

Table 1
Examination of chromosome number in WCC, 4nRR, 2nAR (WCC) and AM4nAR.

Fish type	No. in metaphase	Distribution of chromosome number					
		< 100	100	> 100	< 200	200	> 200
WCC	200	5	194	1	/	/	/
4nRR	200	/	/	/	15	181	4
2nAR (WCC)	600	18	575	7	/	/	/
AM4nAR	600	/	/	/	24	568	8

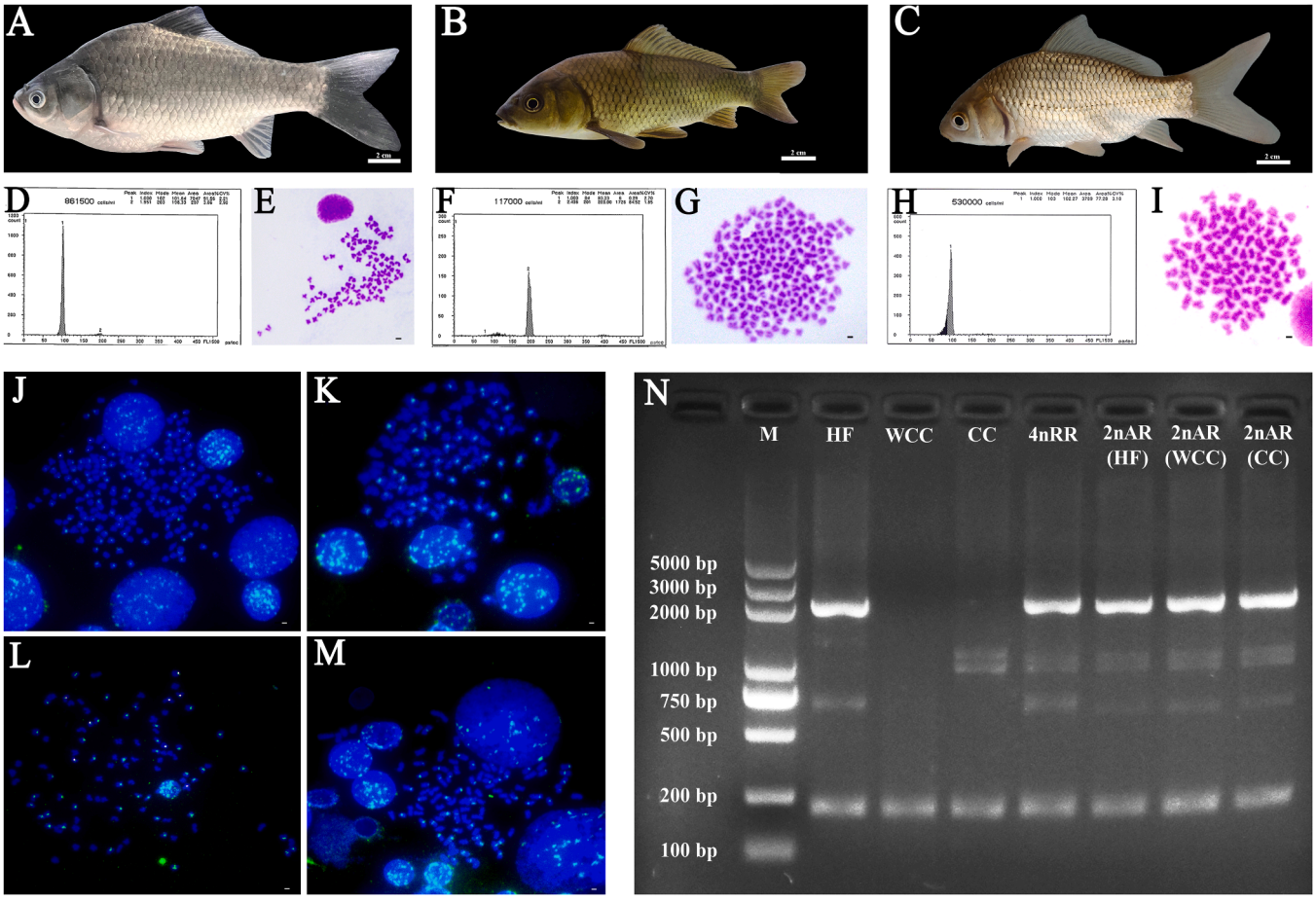


Fig. 2. The appearance, mean DNA content in blood, metaphase chromosomes, and nuclear material identification of 2nAR and its parents. The appearance of WCC, (B) The appearance of 4nRR, (C) The appearance of 2nAR; (D, F, H) The DNA content in WCC (n = 10), 4nRR (n = 10) and 2nAR (n = 150). (E, G, I) the chromosomes in WCC (n = 10), 4nRR (n = 10) and 2nAR (n = 30), Bar= 3 μm; (J) One hundred chromosomes were marked with florescent signals in 4nRR, (K) Fifty chromosomes were marked with florescent signals in 2nAR (HF), (L) Fifty chromosomes were marked with florescent signals in 2nAR (WCC), (M) Fifty chromosomes were marked with florescent signals in 2nAR (CC), Bar= 3 μm; (N) DNA-band patterns of *Sox*-HMG. M, DL5000-bp DNA marker.

and survival rates of artificial androgenesis induced by three different inactivated haploid eggs are presented in Table 2. The negative control group showed 0% fertilization, hatching, and survival rates, and no embryonic development was observed, confirming complete inactivation of the maternal genome. Comparative analysis revealed that the fertilization rate (75.20 ± 0.26%), hatching rate (28.27 ± 0.25%) and survival rate (23.33 ± 0.23%) of androgenesis induced by HF-inactivated eggs were much higher than those induced by WCC and CC ($P < 0.05$). These results suggest that the eggs of hybrid fish exhibit superior developmental competence and enhanced compatibility with androgenesis. Furthermore, the stability of the androgenesis system was evaluated over three consecutive years (2021–2023). The fertilization, hatching, and survival rates were presented in Supplementary Table 1. Statistical analysis revealed no significant differences among the three years ($P > 0.05$), confirming the reproducibility and stability of the system.

3.3. Nuclear material identification using FISH and Sox-HMG markers

The 263 probe was hybridized to the metaphase chromosomes of 4nRR, 2nAR (HF), 2nAR (WCC) and 2nAR (CC) for FISH analysis, and the results were shown in Fig. 2J-2M. One hundred specific centromere repeat loci in the chromosomes were marked with fluorescent signals in 4nRR. Fifty specific centromere repeat loci in the metaphase chromosomes were marked with fluorescent signals in 2nAR (HF), 2nAR (WCC) and 2nAR (CC). The full-length, uncropped FISH images were presented in Supplementary Figures 1–4. The *Sox*-HMG marker can amplify different DNA fragments in different species, and was used to analyze the genetic composition of 2nAR. As shown in Fig. 2N, three bands were observed in HF, only one band was observed in WCC, two bands were observed in CC, and four bands were observed in 4nRR and the three 2nAR groups. The full-length, uncropped PCR gel image was presented in Supplementary Figure 5. The above results indicated that 2nAR possessed half the chromosome number of 4nRR and its nuclear material was predominantly derived from 4nRR.

3.4. Cytoplasmic material identification using the complete mitochondrial genome

The mitochondrial genomes of 4nRR (16,580 bp, GenBank accession number: PQ240357), HF (16,580 bp, GenBank accession number: PQ278909), 2nAR (HF) (16,580 bp, GenBank accession number: PQ278910), 2nAR (WCC) (16,580 bp, GenBank accession number: PQ240358), and 2nAR (CC) (16,580 bp, GenBank accession number: PQ240359) were obtained via sequencing and analysis (Fig. 3). The mitochondrial genome sequences of WCC (16,581 bp, AB045144.1) and CC (16,581 bp, KF856965.1) were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). To explore the origin of the mitochondrial genome in 2nAR, we compared the sequence similarities between 2nAR and its parents (Table 3). The results revealed that the full-length mitochondrial genome sequences and individual gene sequences of 2nAR shared higher similarity with their respective maternal parents (HF, WCC, and CC) than with the paternal parent (4nRR), indicating that the mitochondrial genome of 2nAR was maternally inherited.

3.5. Fertility assessment of 2nAR and generation of all-male tetraploid fish

The 12-month-old 4nRR contained normally developing oocytes in phase III and IV in the ovaries (Fig. 4A) and abundant sperm in the testes (Fig. 4D). Moreover, only a small number of oocytes in phase II were observed in the ovaries of 12-month-old 2nAR (WCC) (Fig. 4B), and many spermatogonia and spermatocytes were observed in the testes (Fig. 4E). At 24 months of age, the ovaries contained many phase III and

Table 2
Fertilization, hatching, and survival rates of androgenesis induced by different inactivated haploid eggs.

Experiment Category	Experimental Batch		1	2	3	Total
	eggs (n)		2000	2000	2000	6000
Experimental group	UV-irradiated eggs (WCC) × sperm (4nRR)	Fertilization rate (%)	30.20	30.30	30.35	30.28 ± 0.04[b]
		Hatching rate (%)	5.85	5.90	5.90	5.88 ± 0.02[b]
		Survival rate (%)	4.05	4.15	4.10	4.10 ± 0.03[b]
	UV-irradiated eggs (CC) × sperm (4nRR)	Fertilization rate (%)	25.40	25.80	25.60	25.60 ± 0.20[c]
		Hatching rate (%)	5.50	5.70	5.60	5.60 ± 0.10[c]
		Survival rate (%)	3.15	3.25	3.35	3.25 ± 0.10[c]
	UV-irradiated eggs (HF) × sperm (4nRR)	Fertilization rate (%)	75.00	75.50	75.10	75.20 ± 0.26[a]
		Hatching rate (%)	28.10	28.55	28.15	28.27 ± 0.25[a]
		Survival rate (%)	23.10	23.55	23.35	23.33 ± 0.23[a]
Negative control group	UV-irradiated eggs (WCC) × UV-irradiated sperm (4nRR)	Fertilization rate (%)	0.00	0.00	0.00	0.00
		Hatching rate (%)	0.00	0.00	0.00	0.00
		Survival rate (%)	0.00	0.00	0.00	0.00
	UV-irradiated eggs (CC) × UV-irradiated sperm (4nRR)	Fertilization rate (%)	0.00	0.00	0.00	0.00
		Hatching rate (%)	0.00	0.00	0.00	0.00
		Survival rate (%)	0.00	0.00	0.00	0.00
	UV-irradiated eggs (HF) × UV-irradiated sperm (4nRR)	Fertilization rate (%)	0.00	0.00	0.00	0.00
		Hatching rate (%)	0.00	0.00	0.00	0.00
		Survival rate (%)	0.00	0.00	0.00	0.00
Positive control group	non-UV-irradiated eggs (WCC) × non-UV-irradiated sperm (4nRR)	Fertilization rate (%)	97.60	97.75	97.70	97.68 ± 0.08[b]
		Hatching rate (%)	92.50	92.55	92.60	92.55 ± 0.05[b]
		Survival rate (%)	86.30	86.35	86.30	86.32 ± 0.03[b]
	non-UV-irradiated eggs (CC) × non-UV-irradiated sperm (4nRR)	Fertilization rate (%)	95.20	95.55	95.90	95.55 ± 0.35[c]
		Hatching rate (%)	90.00	90.75	90.35	90.37 ± 0.38[c]
		Survival rate (%)	82.75	83.50	83.60	83.28 ± 0.46[c]
	non-UV-irradiated eggs (HF) × non-UV-irradiated sperm (4nRR)	Fertilization rate (%)	98.70	99.10	98.85	98.88 ± 0.20[a]
		Hatching rate (%)	96.10	96.40	96.25	96.25 ± 0.15[a]
		Survival rate (%)	90.45	90.90	90.40	90.58 ± 0.28[a]

Note: Fertilization rate = no. of embryos at the gastrula stage/no.of eggs.
Hatching rate = no. of hatched fry/no. of eggs.
Survival rates = no. of adult fish/no. of eggs.
Values are presented as mean ± SD (n = 3). Different superscript letters (a, b, c) in the same column indicate significant differences among groups ($P < 0.05$, one-way ANOVA followed by Tukey's HSD test).

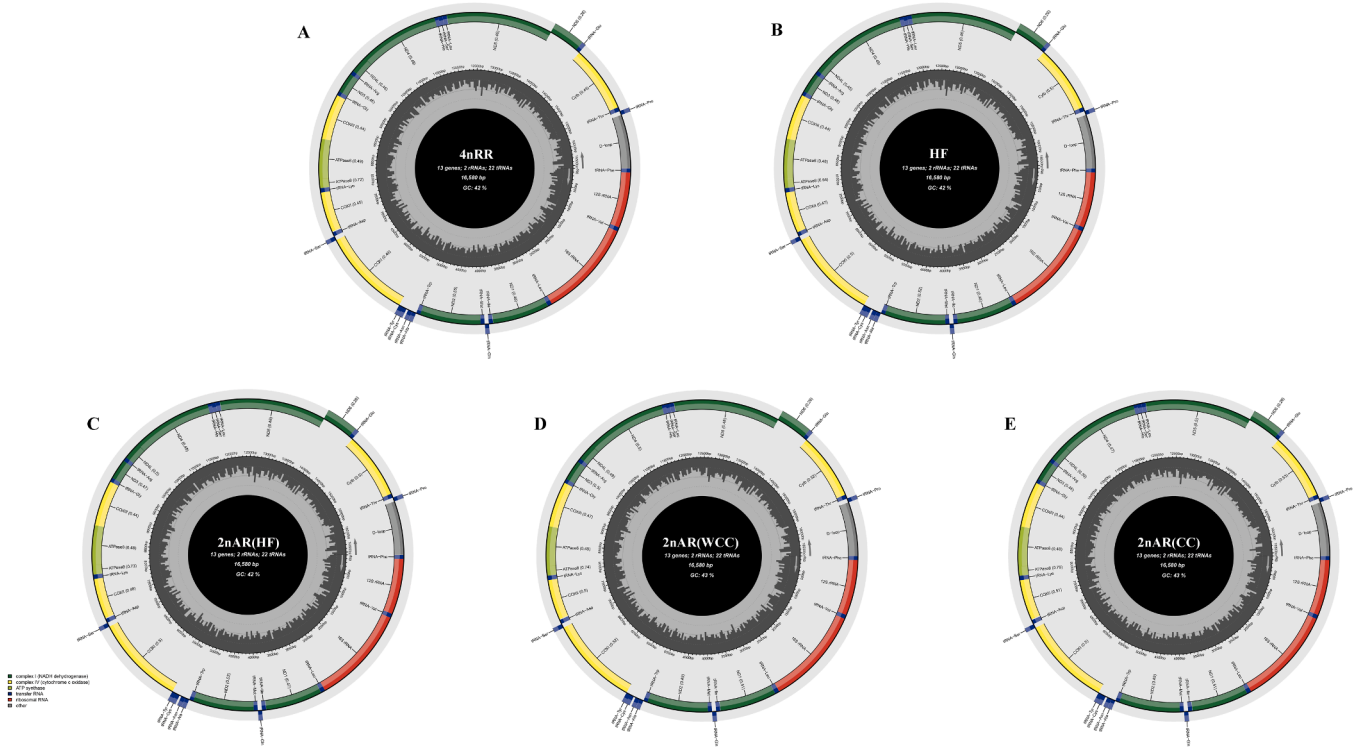


Fig. 3. Complete mitochondrial structures of 4nRR (A), HF (B), 2nAR (HF) (C), 2nAR (WCC) (D) and 2nAR (CC) (E).

IV oocytes (Fig. 4C), while the testes contained many spermatocytes, along with a small number of spermatids and sperm (Fig. 4F). Among the 2nAR population examined (n = 150), there were 72 females and 78 males, yielding a sex ratio of approximately 1:1. Mature eggs of three different size classes were observed in the 24-month-old 2nAR (Fig. 4G). Water-like semen was collected from the 24-month-old 2nAR, and the mean DNA content of the semen was 102.21 (Fig. 4H, peak 1), which was equal to the DNA content of the blood in the 2nAR (Fig. 2H). The sperm count of 2nAR is extremely low, but they possess a normal head-tail structure (Fig. 4I). These results revealed that 2nAR did not produce mature gametes until 24 months of age.

The fertilization and hatching rates of 2nAR self-crossing were approximately 15.17 ± 0.60% and 12.67 ± 0.58%, respectively. Among 1800 eggs divided into three batches, 273 eggs were fertilized (95, 88, and 90 per batch) and 228 hatched (80, 74, and 74 per batch). The DNA content of 124 self-crossing progeny (Fig. 5A) of 2nAR was detected, with values all around 200 (Fig. 5B, peak 2: 200.81). Chromosome number analysis was performed on 30 of these offspring, revealing that their chromosome count was also 200 (Fig. 5C, Table 1). This indicated that the self-crossing progeny of 2nAR were tetraploid fish. Furthermore, it demonstrated that 2nAR was bisexually fertile and could produce unreduced diploid gametes.

3.6. Testicular structure and sperm ultrastructure in AM4nAR

This novel tetraploid fish was an all-male population, designated as AM4nAR (Fig. 5A), and had similar appearance with 4nRR. Growth performance was evaluated by measuring body weight and body length at 6.5 months of age. The mean body weight and body length of AM4nAR (n = 30) were 97.79 ± 13.12 g and 10.62 ± 0.92 cm, respectively, while those of 4nRR (n = 30) were 96.69 ± 10.82 g and 10.59 ± 0.68 cm, respectively. No significant differences were detected between the two groups in either parameter ($P > 0.05$). The deformity rate of AM4nAR (n = 124) was 4.03 ± 1.37%, while that of 4nRR (n = 120) was 1.67 ± 1.44%. In addition, from 1 day post-hatching to 6.5 months of age, the mean cumulative survival rate of AM4nAR was 92.0% (Supplementary Figure 6). Testes of 6.5-month specimens contained copious diploid sperm (Figs. 5D and 5G, peak 1: 101.73) with typical morphological structure: spherical heads, midpieces (neck), and flagella (tail) via scanning electron microscopy (Fig. 5E). Meanwhile, TEM analysis revealed intact sperm ultrastructure in AM4nAR (Figs. 5F and 5H). The head exhibited an oval or round shape, with highly condensed nuclear chromatin showing uniform electron density. Numerous mitochondria were observed in the neck region, and the axoneme exhibited the typical "9 + 2" microtubule fibril structure in the tail (Fig. 5F). The relative semen volume was 2.52 ± 0.03 mL/kg body weight (n = 10) for 4nRR and 3.42 ± 0.04 mL/kg body weight (n = 10) for AM4nAR. The sperm motility duration of AM4nAR and 4nRR was approximately 20 s after activation. Noteworthy was the significantly enhanced sperm motility in AM4nAR (89.6 ± 3.82%) versus 4nRR (66.2 ± 2.65%, $P < 0.05$, Fig. 5I). Additionally, as shown in Table 4, significant differences were detected in multiple kinematic parameters, including progressive motility, static, VAP, VCL, and VSL ($P < 0.05$), suggesting that AM4nAR may possess superior reproductive capacity. During the reproductive season (from April to May) in 2024 and 2025, diploid sperm from AM4nAR were used to fertilize haploid eggs from RCC and CC, yielding 100% triploid offspring. Consequently, it was confirmed that the sperm produced by AM4nAR were diploid, and were demonstrated to have excellent application value in the large-scale production of triploids.

4. Discussion

Androgenesis is considered a powerful tool for manipulating fish genomes, as it produces organisms with exclusively paternal chromosomes. Key concerns in fish androgenesis technology include the induction of androgenesis using diploid sperm, the application of highly effective UV irradiation for egg inactivation, and the use of heterologous eggs (BHISE, KHAN, 2002; Hou, et al., 2013). In the implementation and application of androgenesis, most researchers have focused on the selection of methods for inactivation of maternal genetic material and chromosome doubling in the paternal parent; however, these

Table 3
Comparison of the similarity of the mitochondrial genomes of three kinds of androgenetic fish and their parents.

Gene sequence similarity		2nAR (HF) VS HF	2nAR (HF) VS 4nRR	2nAR (WCC) VS WCC	2nAR (WCC) VS 4nRR	2nAR (CC) VS CC	2nAR (CC) VS 4nRR
Control region	*D-loop*	1	0.979	1	0.934	0.996	0.866
2 ribosomal RNA (rRNA) genes	*12S rRNA*	1	0.995	1	0.987	0.998	0.976
	16S rRNA	0.998	0.992	0.998	0.976	0.997	0.953
13 protein coding genes	*ND1*	1	0.981	1	0.922	0.995	0.893
	ND2	1	1	1	0.917	1	0.860
	ND3	0.997	0.988	1	0.957	0.991	0.862
	ND4	1	0.985	1	0.945	0.994	0.862
	ND4L	1	0.989	1	0.946	1	0.858
	ND5	1	0.984	0.999	0.929	0.995	0.866
	ND6	1	0.986	1	0.921	0.996	0.865
	COI	1	0.989	1	0.956	0.996	0.884
	COII	1	0.997	1	0.973	0.997	0.897
	COIII	1	0.992	1	0.956	0.993	0.910
	ATPase8	1	1	1	0.987	1	0.963
	ATPase6	1	0.985	1	0.963	0.994	0.879
	CYTB	1	0.978	0.999	0.936	1	0.883
22 transfer RNA (tRNA) genes	*tRNA-Phe*	1	1	1	1	1	0.956
	tRNA-Val	1	1	1	0.985	1	1
	tRNA-Leu	1	1	1	1	1	0.986
	tRNA-Ile	1	1	1	0.986	1	0.986
	tRNA-Gln	1	0.985	1	1	1	0.971
	tRNA-Met	1	0.985	1	1	1	0.985
	tRNA-Trp	1	1	1	1	1	0.957
	tRNA-Ala	1	1	1	1	1	0.913
	tRNA-Asn	1	1	1	1	1	0.972
	tRNA-Cys	1	1	1	0.985	0.985	0.913
	tRNA-Tyr	1	1	1	1	1	0.971
	tRNA-Ser	1	1	1	1	1	1
	tRNA-Asp	1	1	1	0.972	1	0.994
	tRNA-Lys	1	1	1	0.986	1	1
	tRNA-Gly	1	1	1	1	1	0.986
	tRNA-Arg	1	1	1	1	1	0.942
	tRNA-His	1	0.971	1	0.971	1	0.898
	tRNA-Ser2	1	0.956	1	0.985	1	0.913
	tRNA-Leu2	1	1	1	1	1	0.972
	tRNA-Glu	1	1	1	1	1	1
	tRNA-Thr	1	0.986	1	0.986	1	0.986
	tRNA-Pro	1	1	1	1	1	1
Full-length		0.999	0.987	0.999	0.952	0.996	0.899

technologies are also very mature (Komen, Thorgaard, 2007). Notably, when diploid sperm is used, androgenetic diploids can be produced without any treatment for chromosome doubling (Thorgaard, et al., 1990; Arai, et al., 1995). Furthermore, UV irradiation, an essential step for maternal genome inactivation during androgenesis, inevitably compromises egg cytoplasmic integrity, thereby resulting in low hatching rates. Previous studies have obtained several high-quality germplasm resources capable of producing diploid sperm, such as the allotetraploid lineage of RCC × CC, the autotetraploid crucian carp (4nRR) lineage of RCC × BSB, and the autotetraploid carp lineage of CC × BSB (Liu, et al., 2007b; Qin, et al., 2014b; Wang, et al., 2020). This has increased the convenience of studying artificial androgenesis in fish. Indeed, androgenetic offspring are produced by using these tetraploid lineages (Sun, et al., 2007; Zhou, et al., 2015; Gu, et al., 2024). Notably, in those studies, egg selection was often random, with a tendency to use the original maternal parents of these tetraploid lineages, while the potential effects of heterologous eggs on androgenesis received little attention. In this study, the fertilization rate, hatching rate, and survival rate of androgenesis with diploid sperm produced by 4nRR induced by three different sources of inactivated heterologous haploid eggs, namely, WCC, CC, and HF, were compared. Surprisingly, compared with previous studies in which inactivated homologous haploid eggs from RCC were used to induce the androgenesis of 4nRR (Zhou, et al., 2015), heterologous eggs have obvious advantages in the application of androgenesis. RCC is the original maternal parent of 4nRR. The fertilization, hatching and survival rates of androgenesis induced by inactivated homologous eggs from RCC were only 10.01%, 2.10% and 1.66%, respectively. However, the rates induced by inactivated heterologous haploid eggs from HF, WCC and CC are much higher than the above data (Table 2). Moreover, the fertilization, hatching and survival rate of androgenesis induced by eggs from HF were much higher than those induced by WCC and CC ($P < 0.05$). We speculate that these improvements may be related to the large number of eggs carried by HF and the presence of heterologous genomes in these eggs, increasing their compatibility and improving the fertilization, hatching and survival rates of androgenic offspring. Ihssen argued that the optimal breeding strategy in fish would be to maximize heterozygosity to achieve heterosis or hybrid vigor (Ihssen, 1976). In long-term and systematic studies of distant hybridization in fish, our team reported that one-step and multi-step breeding technologies can yield offspring with excellent traits (Wang, et al., 2018). Moreover, in multi-step breeding technology, these hybrid fish from established lineages have obvious advantages as maternal parents, and the traits of the offspring obtained by using the female individuals of these hybrid lineages as maternal parents and hybridizing them with the original parents (or other fish) are better than those of the reciprocal cross offspring (Wu, et al., 2020; Gong, et al., 2021; Hu, et al., 2021). These results suggest that the eggs of hybrid fish exhibit potential and greater compatibility and may be more suitable for androgenesis. Nevertheless, the mechanisms underlying this improved hatching rate will be further investigated in our future studies.

Androgenesis might be used for the rapid generation of inbred lines, the recovery of genotypes from cryopreserved sperm, and the study of the effects of the mitochondrial genotype on performance (Thorgaard, 1986). Androgenetic fish are essentially cytoplasmic-nuclear hybrid

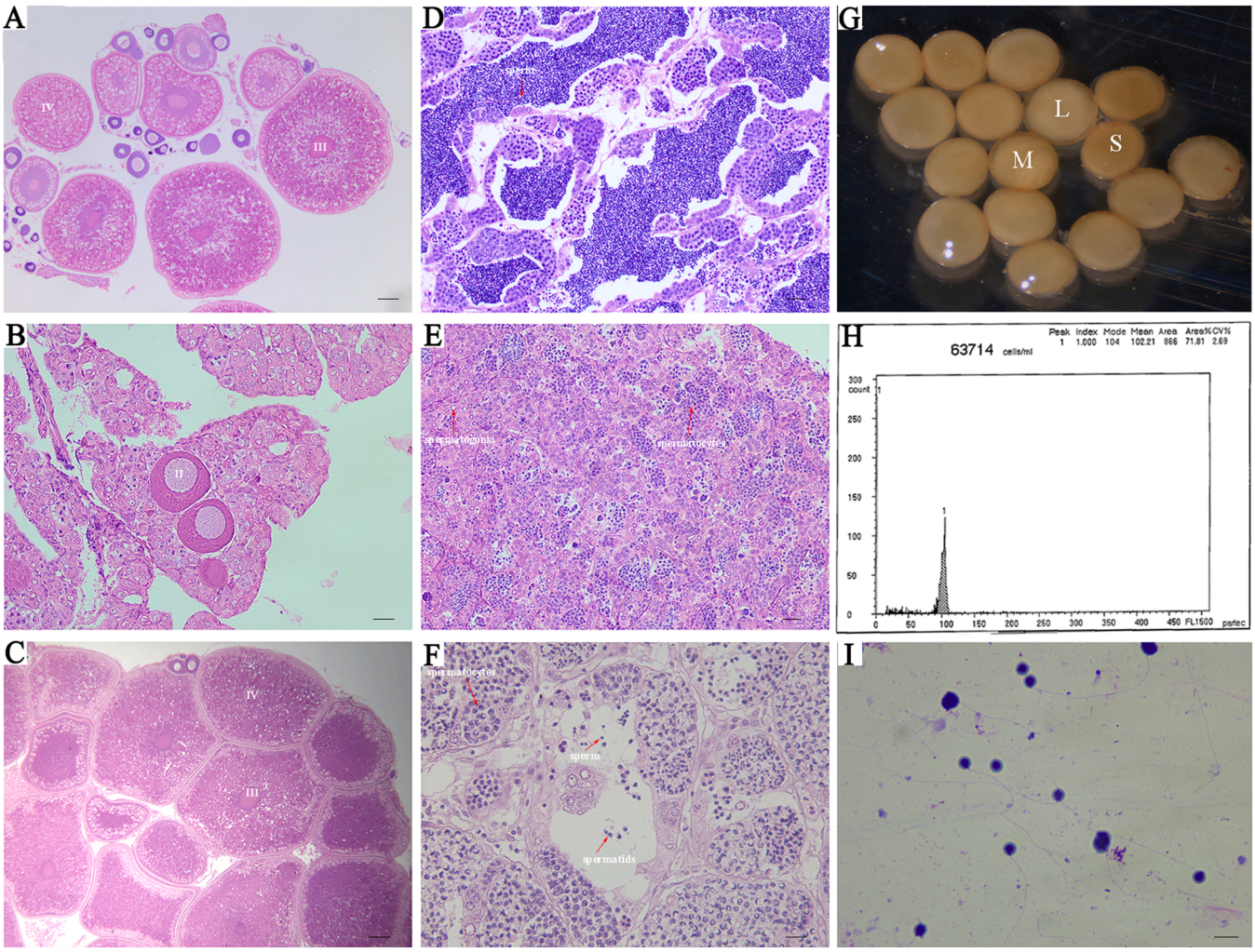


Fig. 4. Gonadal microstructure of 4nRR and 2nAR, together with gamete morphology and mean DNA content of water-like semen from 2nAR. Ovary of 4nRR at 12 months of age (n = 5), showing normally developed oocytes in phases III and IV, (B) Ovary of 2nAR at 12 months of age (n = 30), showing many oogonia and a small number of oocytes in phase II, (C) Ovary of 2nAR at 24 months of age (n = 30), showing normally developed oocytes in phase III and phase IV, Bar = 100 μm; (D) Testis of 4nRR at 12 months of age (n = 5), showing many sperm, (E) Testis of 2nAR at 12 months of age (n = 30), showing many spermatogonia and spermatocytes, (F) Testis of 2nAR at 24 months of age (n = 30), showing many spermatocytes and a small number of spermatids and sperm, Bar = 50 μm; (G) Mature eggs from 2nAR (n = 30). (H) The mean DNA content in the semen of 2nAR (n = 30). (I) Sperm from 2nAR (n = 30), Bar = 20 μm.

fish (Liu, et al., 2022a). It has been reported that a new nuclear-cytoplasmic hybrid clone was formed through androgenesis in polyploid gibel carp (Wang, et al., 2011), and androgenetic nucleocytoplasmic hybrids were obtained via dispermic androgenesis and the eggs of interspecific hybrids in sturgeon fishes (Grunina, Recoubratsky, 2005). If the gametes of two different species are used in artificial androgenesis, the process can be considered a unique type of hybridization. Although the nuclear genetic material of the maternal parents is inactivated, the genetic material from the cytoplasm cannot be completely inactivated. As shown in Fig. 6, the haploid eggs produced by female diploid fish after genetic inactivation were fertilized with diploid sperm produced by male tetraploid fish, and the embryos could directly develop into androgenetic diploid fish. During this process, the nuclear genetic material from the egg is inactivated, but its cytoplasmic genetic material (mitochondrial genome) cannot be fully inactivated. Although sperm carry a small number of mitochondria, these are concentrated mainly in the tail (providing energy for flagellar movement), while the tail (together with mitochondria) of the sperm during fertilization usually does not enter the egg. Even if a small number of paternal mitochondria enter the egg, the number is far from being able to compete with many maternal mitochondria in the egg. Therefore, we believe that the cytoplasmic genetic material of the offspring of androgenetic diploid fish comes mainly from the maternal parent, and that the nuclear genetic material comes mainly from the paternal parent. The similarities between the mitochondrial genomes of the three 2nAR groups and their respective maternal parents (HF, WCC, and CC) exceeded 99.6%, which was higher than those with the paternal parent (4nRR) (Table 3), suggesting maternal inheritance of the mitochondrial genomes in 2nAR. However, the functional roles of these cytoplasmic factors in development, survival, and fertility remain unknown. To address this, future studies will compare 2nAR individuals with different mitochondrial backgrounds across various traits, including androgenic development efficiency, embryonic survival, gonadal differentiation, unreduced gamete formation, growth rate and muscle development, to elucidate the functional roles of nucleo-cytoplasmic interactions. Moreover, the nuclear genetic composition of 2nAR was analyzed via a species-specific centromere probe (263 bp, JQ086761) for FISH and a conserved *Sox*-HMG molecular marker. For the 263 probe (Fig. 2), 100 fluorescence signals were detected in 4nRR, and 50 fluorescence signals were detected in 2nAR. Similarly, Qin et al. detected 100 and 50 fluorescent signals in 4nRR and gynogenetic progeny generated by 4nRR, respectively (Qin, et al., 2019; Xu, et al., 2023). Given the bivalent-like pairing behavior in 4nRR during meiosis, the 50 fluorescence signals in 2nAR are derived from 4nRR, further confirming that 2nAR originated from

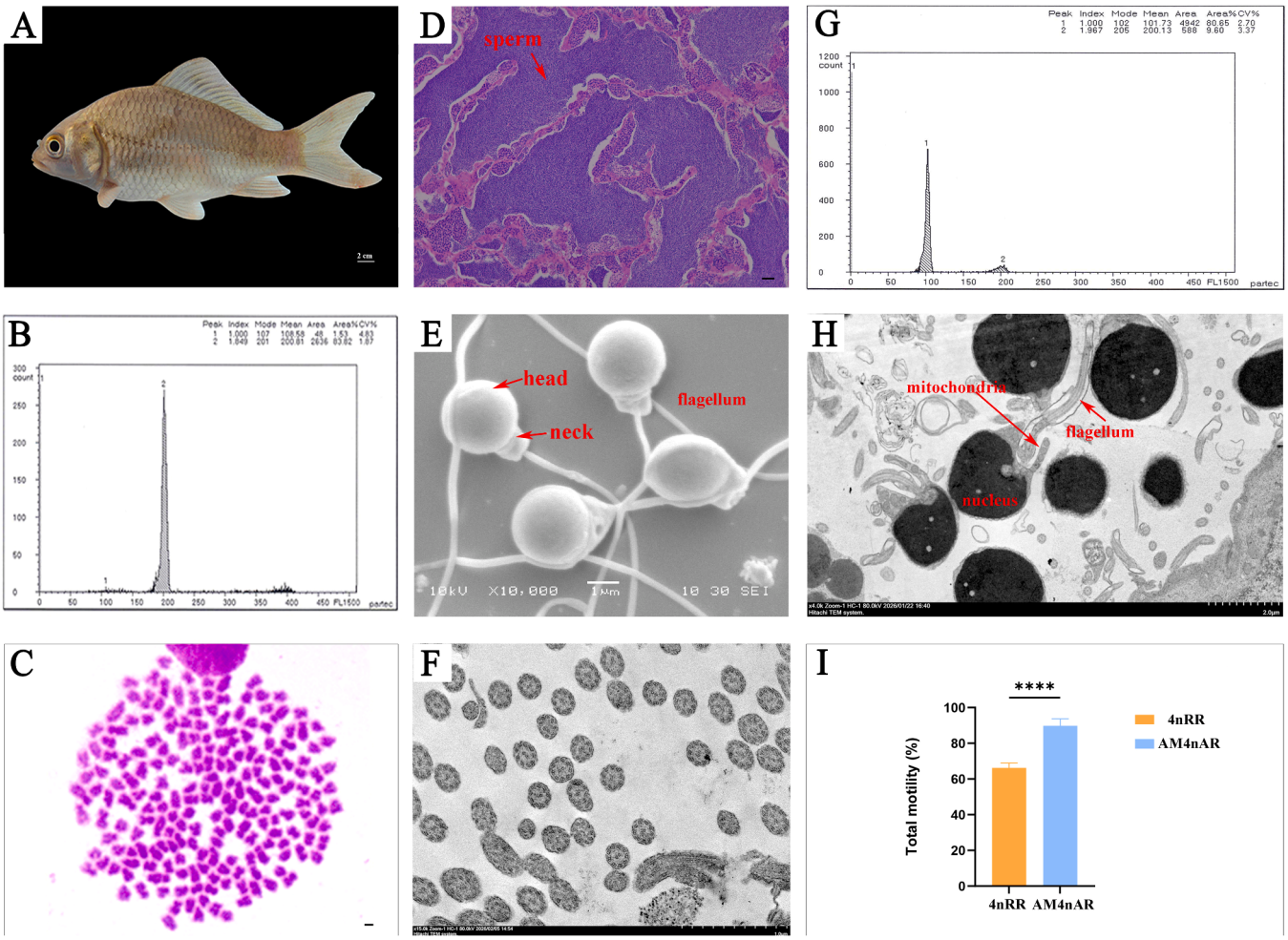


Fig. 5. The appearance, DNA content in the blood, chromosomes at metaphase and structure of testis and sperm of AM4nAR. (A) The appearance of AM4nAR; (B) The DNA content in AM4nAR (n = 124); (C) the chromosomes in AM4nAR (n = 30), Bar= 3 μm; (D) Testis of AM4nAR at 6.5 months of age (n = 50), showing many sperm, Bar= 50 μm; (E) Sperm morphology under SEM (n = 10); (G) The mean DNA content in the semen (n = 50); (F and H) Testicular structure under TEM (n = 10); (I) Total sperm motility of AM4nAR (n = 10) and 4nRR (n = 10), **** indicates $P < 0.05$.

Table 4
Sperm motility parameters detection of AM4nAR and 4nRR.

Fish type	Total Motility (%)	Progressive (%)	Static (%)	VAP (μm/s)	VSL(μm/s)	VCL (μm/s)	BCF (Hz)	LIN (%)
4nRR	66.2 ± 2.65^{b}	16.11 ± 2.71^{b}	33.8 ± 2.79^{a}	68.94 ± 8.78^{b}	53.51 ± 7.66^{b}	77.53 ± 13.05^{b}	16.84 ± 2.97^{a}	70.5 ± 13.51^{a}
AM4nAR	89.6 ± 3.82^{a}	36.23 ± 8.75^{a}	10.4 ± 3.82^{b}	115.3 ± 10.66^{a}	92.56 ± 7.99^{a}	123.52 ± 7.7^{a}	17.22 ± 2.34^{a}	75.26 ± 8.37^{a}

Note: Values are presented as mean±SD (n = 10). Different superscript letters (a, b) in the same column indicate significant differences ($P < 0.05$, independent samples *t*-test).

the diploid sperm of 4nRR via androgenesis. For the *Sox*-HMG marker, the banding patterns of 2nAR were consistent with those of 4nRR and clearly distinct from HF, WCC, and CC. These results provide strong support for further study of the genetic background of 2nAR and its potential application in breeding. Moreover, androgenesis has been used to investigate the role of mitochondrial DNA during embryonic development in fish (Brown, Thorgaard, 2002; Ocalewicz, 2024). The preparation of these three types of androgenetic fish lays a foundation for further exploration of their nuclear-cytoplasmic interactions.

Despite widespread androgenesis attempts in fish, few studies have detailed gonadal development and offspring fertility (Parsons, Thorgaard, 1985; Bongers et al., 1994; Corley-Smith, et al., 1996; Sarder, et al., 1999). Documented cases include sexually mature androgenetic rainbow trout and common carp, with YY supermale rainbow trout producing functional sperm (Scheerer, et al., 1991; Grunina, et al., 1995). Such populations generate normal haploid gametes. We previously obtained two bisexual fertile diploid groups producing unreduced gametes at sexual maturity (two years) (Duan, et al., 2007; Sun, et al., 2007; Zhou, et al., 2015). Similarly, bisexual fertile 2nAR were obtained in this study, and self-crossing rapidly yielded an all-male tetraploid population (AM4nAR), confirming the capacity for unreduced diploid gamete production. However, it should be emphasized that although 2nAR can serve as a good source of diploid gametes, it requires 24 months to reach sexual maturity, which represents a significant limitation for aquaculture applications. Moreover, the mechanistic basis for unreduced gametogenesis remains elusive. We speculate that this phenomenon may be the result of a combination of genetic and developmental regulatory abnormalities or special adaptive mechanisms. One possible cause is the variation in cytological mechanisms, such as pre-meiotic endoreplication or the fusion of germ cells, as well as spindle

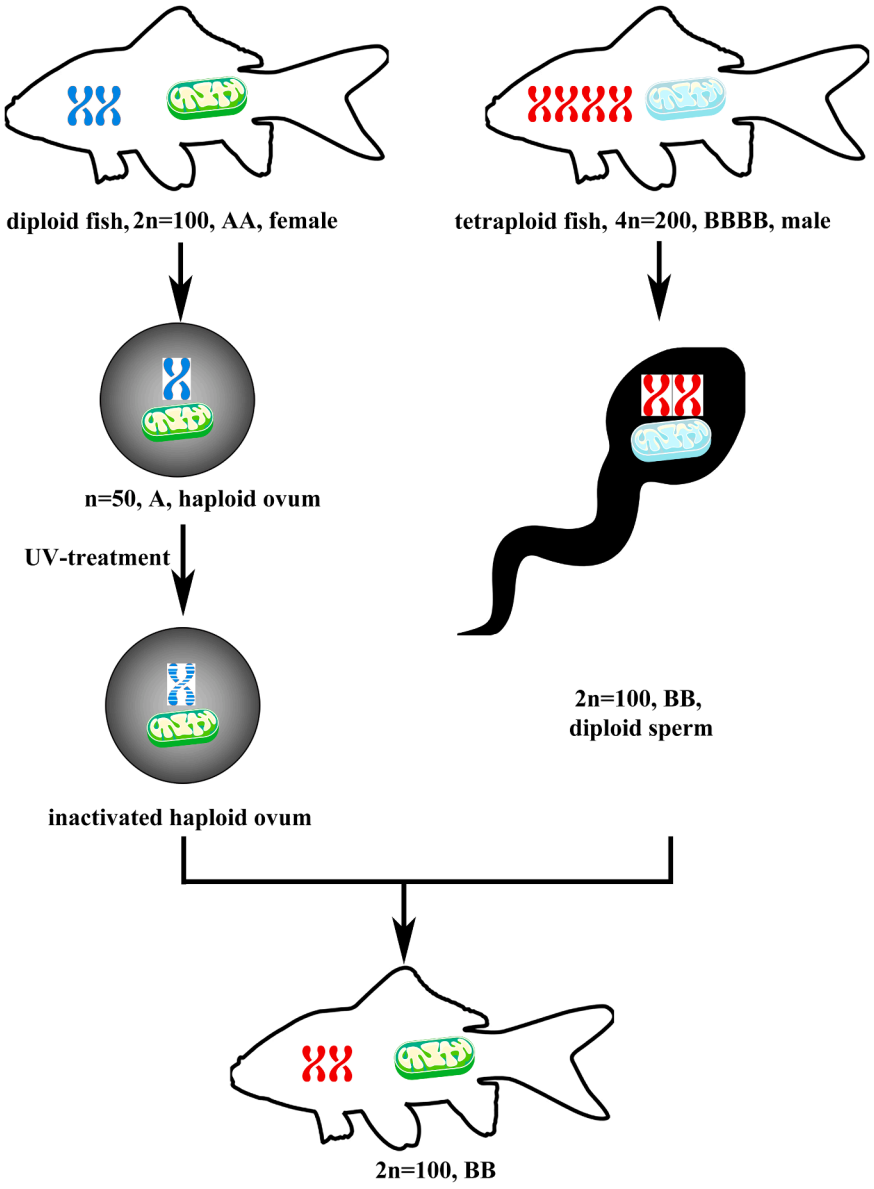


Fig. 6. The genetic composition of androgenic fish.

dysfunction during meiosis, which directly leads to the production of unreduced gametes (Yoshikawa, et al., 2009; Wang, et al., 2016; Liu, et al., 2022c). Another possible cause is mutations in key genes, such as, mutations in genes related to cell cycle regulation, homologous chromosome pairing, or sister chromatid separation, which directly disrupt the fidelity of meiosis (Zhang, et al., 2023, 2025; Ou, et al., 2024). Notably, unreduced gametes are generally believed to be formed by meiotic defects. Previous studies have found that knockout of *Cntd1* (cyclin N-terminal domain containing 1) gene in zebrafish and RCC affects the crossover frequency during meiosis, generating unreduced eggs and polyploid offspring (Ou, et al., 2024; Li, et al., 2025). These findings represent a classical meiotic defect pathway for unreduced gamete formation. However, recent studies have demonstrated that mitotic defects in spermatogonia can also lead to unreduced sperm formation in fish. Deletion of *Cdk1* (cyclin-dependent kinase 1) in diploid loach (*Misgurnus anguillicaudatus*) has been shown to lead to chromosome doubling in some spermatogonia, thereby inducing the production of unreduced diploid sperm (Zhang, et al., 2023). Likewise, heterozygous *espl1* (extra spindle pole bodies like 1) deficiency in diploid loach impaired mitotic sister chromatid separation of some spermatogonia, causing chromosome number doubling and subsequent unreduced sperm production (Zhang, et al., 2025). Similar results were also obtained in zebrafish in both studies, suggesting the universality of this mechanism across fish species. Collectively, these findings demonstrate that mitotic defects in spermatogonia mediated by genes such as *cdk1* and *espl1* represent an alternative mechanism for unreduced gamete formation in fish, complementing the classical meiotic defect pathway. These findings provide more directions for subsequent research on the generation of unreduced diploid gametes and provide new strategies for aquaculture-oriented polyploid breeding.

We previously identified Chr46B as the candidate sex chromosome in 4nRR, with the sex determination region spanning 22.50–22.80 Mb. A 26-bp Indel (GGTTCAATAATGACTGATCACAGGTT) was identified between males and females within this interval, leading to the development of a specific molecular marker for accurate genetic sex identification (Zhang, et al., 2024) and designed specific primers (F: AACTGGGCTCCATTGACGAG, R: GCACGTACTCCCTAAGCTCT). Validation in 4nRR males and females, as well as in gynogenetic (all-female) and androgenetic (2nAR, both sexes) offspring, revealed that all male 4nRR individuals produced two distinct fragments (differing by 26 bp),

whereas females, including gynogenetic offspring, produced only a single shorter band. In 2nAR, both sexes showed a single band. However, the band length in males was longer than that in females. Sanger sequencing further confirmed that this length difference corresponded precisely to the 26 bp Indel sequence, confirming that the sex determination system of 4nRR is of the XX/XY type (these data are unpublished). Based on these results, we hypothesize that among the androgenetic offspring, males possess a Y0 sex chromosome composition (containing only a single Y chromosome without an X), while females possess an X0 sex chromosome composition (containing only a single X chromosome). 2nAR exhibit the characteristic of producing unreduced diploid gametes: X0 females produce unreduced diploid eggs (carrying an X chromosome), and Y0 males produce unreduced diploid sperm (carrying a Y chromosome). Through artificial fertilization, the diploid eggs from X0 females were fertilized with the diploid sperm from Y0 males, yielding offspring with an XY sex chromosome composition, which formed an all-male tetraploid population (AM4nAR). The results of multiple artificial insemination experiments conducted in 2024 and 2025 demonstrated that crosses between males and females within the 2nAR indeed produced an all-male tetraploid population. Nevertheless, the mechanism underlying the formation of the AM4nAR is still under further investigation in our team. Furthermore, AM4nAR obtained through androgenesis is also of great significance for revealing the molecular mechanisms of sex determination in polyploid fish, dosage compensation effects, and the impact of polyploidization on sex differentiation pathways.

In summary, as a breeding technique, androgenesis has both potential and limitations in aquaculture. It allows rapid production of all-male tetraploids, but faces several challenges. In this study, androgenesis was induced by fertilizing diploid sperm from autotetraploid fish with three different sources of heterologous haploid eggs. The fertilization, hatching and survival rates of androgenesis induced by inactivated eggs from HF were significantly higher than those induced by WCC and CC, suggesting the potential and greater compatibility of the eggs of hybrid fish and may be more suitable for androgenesis. Three androgenic diploid offspring (2nAR) were obtained, with nuclear material derived from 4nRR and cytoplasmic material from the corresponding maternal parent. Moreover, we used the bisexual fertile 2nAR to generate an all-male tetraploid population (AM4nAR) with enhanced reproductive performance. This study provides valuable insights into androgenesis and broadens the way to obtain all-male tetraploid fish.

CRediT authorship contribution statement

Xidan Xu: Resources. **Zeyang Li:** Investigation. **Yue Zhou:** Resources. **Qian Xiao:** Investigation. **Kun Zhang:** Resources. **Xu Huang:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Data curation. **Qinbo Qin:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Yali Long:** Investigation. **Enkui Hu:** Resources. **Xiaowei Xu:** Resources. **Yuhan Yang:** Investigation. **Shaojun Liu:** Resources, Conceptualization. **Xinyu Yao:** Investigation. **Yanling Fu:** Investigation. **Jinhai Bai:** Resources. **Yuchen Jiang:** Resources. **Chongqing Wang:** Resources. **Zhengkun Liu:** Resources. **Chang Wu:** Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Qinbo qin reports financial support was provided by Yuelushan Laboratory. Xu huang reports financial support was provided by the Natural Science Foundation of Hunan Province, China. Xu huang reports was provided by Hunan Provincial Education Department. Chang wu reports financial support was provided by the Special funds for the construction of innovative province in Hunan. Xu huang reports financial support was provided by Changsha Municipal Natural Science Foundation. Qinbo qin reports financial support was provided by the earmarked fund for HARS. Qinbo qin reports financial support was provided by the Aid Program for Science and Technology Innovative Research Team in Higher Educational Institutions of Hunan Province. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by Yuelushan Laboratory Breeding Program (Grant No. YLS−2025-ZY02043), the Natural Science Foundation of Hunan Province, China (Grant No. 2024JJ6314), the Project of Hunan Provincial Education Department (Grant No. 25B0073), the Special funds for the construction of innovative province in Hunan (Grant No. 2023WK2001), Changsha Municipal Natural Science Foundation (Grant No. kq2402160), the earmarked fund for HARS (Grant No. HARS−07) and the Aid Program for Science and Technology Innovative Research Team in Higher Educational Institutions of Hunan Province.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2026.103771.

Data Availability

Data will be made available on request.

References

Arai, K., Ikeno, M., Suzuki, R., 1995. Production of androgenetic diploid loach Misgurnus anguillicaudatus using spermatozoa of natural tetraploids. Aquaculture 137, 131–138.
Babiak, I., Dobosz, S., Goryczko, K., Kuzminski, H., Brzuzan, P., Ciesielski, S., 2002. Androgenesis in rainbow trout using cryopreserved spermatozoa: the effect of processing and biological factors. Theriogenology 57, 1229–1249.
BHISE, M.P., KHAN, T.A., 2002. Androgenesis: the best tool for manipulation of fish genomes. Turk. J. Zool. 26, 317–325.
Bongers, A., In't Veld, E., Abo-Hashema, K., Bremmer, I., Eding, E., Komen, J., Richter, C., 1994. Androgenesis in common carp (Cyprinus carpio L.) using UV irradiation in a synthetic ovarian fluid and heat shocks. Aquaculture 122, 119–132.
Brown, K., Lee, R., Thorgaard, G., 2006. Use of androgenesis for estimating maternal and mitochondrial genome effects on development and oxygen consumption in rainbow trout, Oncorhynchus mykiss. Comparative Biochemistry Physiology Part B Biochemistry Molecular Biology 143, 415–421.
Brown, K.H., Thorgaard, G.H., 2002. Mitochondrial and nuclear inheritance in an androgenetic line of rainbow trout, Oncorhynchus mykiss. Aquaculture 204, 323–335.
Chen, L., Li, W., Liu, S., Tao, M., Long, Y., Duan, W., Zhang, C., Xiao, J., Qin, Q., Luo, K., Liu, J., Liu, Y., 2009. Novel genetic markers derived from the DNA fragments of Sox genes. Mol. Cell. probes 23, 157–165.
Chourrout, D., Chevassus, B., Krieg, F., Happe, A., Burger, G., Renard, P., 1986. Production of second generation triploid and tetraploid rainbow trout by mating tetraploid males and diploid females—potential of tetraploid fish. Theor. Appl. Genet. 72, 193–206.
Corley-Smith, G.E., Lim, C.J., Brandhorst, B.P., 1996. Production of androgenetic zebrafish (Danio rerio). Genetics 142, 1265–1276.
Duan, W., Qin, Q., Chen, S., Liu, S., Wang, J., Zhang, C., Sun, Y., Liu, Y., 2007. The formation of improved tetraploid population of red crucian carp× common carp hybrids by androgenesis. Science China Series C Life Sciences 50, 753–761.
Ezaz, M.T., Myers, J.M., Powell, S.F., McAndrew, B.J., Penman, D.J., 2004. Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia, Oreochromis niloticus L. Aquaculture 232, 205–214.
Fu, W., Peng, L., Wu, X., He, S., Zhao, H., Liu, J., Liu, W., Xiao, Y., 2020. Triploidization of hybrids (female zebrafish× male blunt snout bream) by heat-shock can improve survival rate. Aquaculture 517, 734786.
Gong, D., Xu, L., Liu, Q., Wang, S., Wang, Y., Hu, F., Wu, C., Luo, K., Tang, C., Zhou, R., 2021. A new type of hybrid bream derived from a hybrid lineage of Megalobrama amblycephala (♀)× Culter alburnus (♂). Aquaculture 534, 736194.
Grunina, A., Recoubratsky, A., 2005. Induced androgenesis in fish: obtaining viable nucleocytoplasmic hybrids. Russ. J. Dev. Biol. 36, 208–217.
Grunina, A., Neyfakh, A., Gomelsky, B., 1995. Investigations on diploid androgenesis in fish. Aquaculture 129, 218–219.

Gu, H., Liu, Q., Fan, Y., Liu, Q., Zhu, Y., Yang, C., Wang, S., Liu, S., 2024. A new method for creating androgenetic diploid fish from tetraploid carp sperm and their genetic composition analysis. Aquaculture 590, 741077.
He, W., Qin, Q., Liu, S., Li, T., Wang, J., Xiao, J., Xie, L., Zhang, C., Liu, Y., 2012. Organization and variation analysis of 5S rDNA in different ploidy-level hybrids of red crucian carp× topmouth culter. PloS. One 7, e38976.
Hou, J., Fujimoto, T., Yamaha, E., Arai, K., 2013. Production of androgenetic diploid loach by cold-shock of eggs fertilized with diploid sperm. Theriogenology 80, 125–130.
Hu, F., Zhong, H., Wu, C., Wang, S., Guo, Z., Tao, M., Zhang, C., Gong, D., Gao, X., Tang, C., Wei, Z., Wen, M., Liu, S., 2021. Development of fisheries in China. Reprod. Breed. 1, 64–79.
Huang, X., Xiao, Q., Wu, C., Wang, C., Xu, X., Long, Y., Xu, X., Zhou, Y., Ma, M., Qin, Q., Liu, S., 2024. Characteristics of testis development in autotetraploid fish. Aquaculture 579, 740269.
Ihssen, P., 1976. Selective breeding and hybridization in fisheries management. J. Fish. Board. Can. 33, 316–321.
Jung, H.S., Kim, J., Lee, S.H., Choi, J.H., Kang, J.-H., 2026. First report of tetraploid induction by cold-shock treatment in olive flounder Paralichthys olivaceus. Aquac. Rep. 46, 103397.
Kašpar, V., Hubalek, M., Pšenička, M., Arai, K., Taggart, J.B., Franěk, R., 2022. Cold-shock androgenesis in common carp (Cyprinus carpio). Aquaculture 548, 737610.
Komen, H., Thorgaard, G.H., 2007. Androgenesis, gynogenesis and the production of clones in fishes: A review. Aquaculture 269, 150–173.
Li, H., Zhang, Y., Zhang, L., Li, J., Ou, Y., Wen, M., Wei, Z., Wang, J., Deng, Y., Jiang, Y., 2025. Triploidy induction through cntd1 knockout in red crucian carp (Carassius auratus red var.). Aquaculture 602, 742347.
Liu, H., Guan, B., Xu, J., Hou, C., Tian, H., Chen, H., 2013. Genetic manipulation of sex ratio for the large-scale breeding of YY super-male and XY all-male yellow catfish (Pelteobagrus fulvidraco (Richardson)). Mar. Biotechnol. 15, 321–328.
Liu, Q., Liu, J., Yuan, L., Li, L., Tao, M., Zhang, C., Qin, Q., Chen, B., Ma, M., Tang, C., 2020. The establishment of the fertile fish lineages derived from distant hybridization by overcoming the reproductive barriers. Reprod. (Camb. Engl.) 159, R237–R249.
Liu, Q., Wang, S., Tang, C., Tao, M., Zhang, C., Zhou, Y., Qin, Q., Luo, K., Wu, C., Hu, F., 2025. The Research Advances in Distant Hybridization and Gynogenesis in Fish. Rev. Aquac. 17, e12972.
Liu, S., 2010. Distant hybridization leads to different ploidy fishes. Sci. China Life Sci. 53, 416–425.
Liu, S., Liu, Y., Zhou, G., Zhang, X., Yang, H., 2001. The formation of tetraploid stocks of red crucian carp × common carp hybrids as an effect of interspecific hybridization. Aquaculture 192, 171–186.
Liu, S., Sun, Y., Zhang, C., Luo, K., Liu, Y., 2004. Production of gynogenetic progeny from allotetraploid hybrids red crucian carp×common carp. Aquaculture 236, 193–200.
Liu, S., Duan, W., Tao, M., Zhang, C., Sun, Y., Shen, J., Wang, J., Luo, K., Liu, Y., 2007a. Establishment of the diploid gynogenetic hybrid clonal line of red crucian carp x common carp. Sci. China Ser. C. Life Sci. 50, 186–193.
Liu, S., Qin, Q., Xiao, J., Lu, W., Shen, J., Li, W., Liu, J., Duan, W., Zhang, C., Tao, M., Zhao, R., Yan, J., Liu, Y., 2007b. The formation of the polyploid hybrids from different subfamily fish crossings and its evolutionary significance. Genetics 176, 1023–1034.
Liu, S., Luo, J., Chai, J., Ren, L., Zhou, Y., Huang, F., Liu, X., Chen, Y., Zhang, C., Tao, M., Lu, B., Zhou, W., Lin, G., Mai, C., Yuan, S., Wang, J., Li, T., Qin, Q., Feng, H., Luo, K., Xiao, J., Zhong, H., Zhao, R., Duan, W., Song, Z., Wang, Y., Wang, J., Zhong, L., Wang, L., Ding, Z., Du, Z., Lu, X., Gao, Y., Murphy, R.W., Liu, Y., Meyer, A., Zhang, Y. P., 2016. Genomic incompatibilities in the diploid and tetraploid offspring of the goldfish x common carp cross. Proc. Natl. Acad. Sci. USA 113, 1327–1332.
Liu, S., Wang, S., Liu, Q., Wu, C., Zhou, Y., Tao, M., Zhang, C., Qin, Q., Luo, K., 2022c. The research advances in animal distant hybridization and polyploid organisms. Fish. Distant Hybrid. 1–37.
Liu, S., Zhang, C., Zhou, Y., Wang, S., Mao, Z., Gong, D., Han, L., Gao, X., 2022a. The Gynogenesis and Androgenesis of the Diploid Gametes Derived from the Allotetraploid Fish. Fish Distant Hybridization. Springer, pp. 103–143.
Liu, S., Zhou, Y., Tao, M., Zhang, C., Qin, Q., Zhao, R., Tang, C., Luo, K., 2022b. The Formation of Allotetraploids of Red Crucian Carp× Common Carp. Fish Distant Hybridization. Springer, pp. 39–45.
Liu, S., Wen, M., Huang, X., Zhou, Y., Qin, Q., Luo, K., Hu, F., Wang, C., Tan, H., Wang, Y., 2022d. The Formation and Biological Characteristics of the Different Ploidy Fishes Derived from the Hybridization of Red Crucian Carp× Blunt Snout Bream. Fish distant hybridization. Springer, pp. 145–187.
Morishima, K., Fujimoto, T., Sato, M., Kawae, A., Zhao, Y., Yamaha, E., Arai, K., 2011. Cold-shock eliminates female nucleus in fertilized eggs to induce androgenesis in the loach (Misgurnus anguillicaudatus), a teleost fish. BMC Biotechnol. 11, 116.
Myers, J.M., Penman, D., Basavaraju, Y., Powell, S.F., Baoprasertkul, P., Rana, K., Bromage, N., McAndrew, B., 1995. Induction of diploid androgenetic and mitotic gynogenetic Nile tilapia (Oreochromis niloticus L.). Theor. Appl. Genet. 90, 205–210.
Ocalewicz, K., 2024. Quality of fish eggs and production of androgenetic and gynogenetic doubled haploids (DHs). Fish. Physiol. Biochem. 50, 1947–1957.
Ou, Y., Li, H., Li, J., Dai, X., He, J., Wang, S., Liu, Q., Yang, C., Wang, J., Zhao, R., 2024. Formation of different polyploids through disrupting meiotic crossover frequencies based on cntd1 knockout in zebrafish. Mol. Biol. Evol. 41, msae047.
Parsons, J.E., Thorgaard, G.H., 1984. Induced androgenesis in rainbow trout. J. Exp. Zool. 231, 407–412.
Parsons, J.E., Thorgaard, G.H., 1985. Production of androgenetic diploid rainbow trout. J. Hered. 76, 177–181.
Peng, L., Fu, W., Wu, X., He, S., Zhao, H., Liu, J., Liu, W., Xiao, Y., 2020. Bisexual Fertile Triploid Zebrafish (Danio rerio): a Rare Case. Mar. Biotechnol. 22, 443–455.
Pigneur, L.M., Hedtke, S., Etoundi, E., Van Doninck, K., 2012. Androgenesis: a review through the study of the selfish shellfish Corbicula spp. Heredity 108, 581–591.
Qin, Q., Wang, Y., Wang, J., Dai, J., Xiao, J., Hu, F., Luo, K., Tao, M., Zhang, C., Liu, Y., Liu, S., 2014b. The Autotetraploid Fish Derived from Hybridization of Carassius auratus red var. (Female) × Megalobrama amblycephala (Male)1. Biol. Reprod. 91.
Qin, Q., Wang, Y., Wang, J., Dai, J., Liu, Y., Liu, S., 2014a. Abnormal chromosome behavior during meiosis in the allotetraploid of Carassius auratus red var. (♀) × Megalobrama amblycephala (♂). BMC Genet. 15, 95. -95.
Qin, Q., Cao, L., Wang, Y., Ren, L., Liu, Q., Zhou, Y., Wang, C., Qin, H., Zhao, C., Liu, S., 2019. Rapid genomic and genetic changes in the first generation of autotetraploid lineages derived from distant hybridization of Carassius auratus red Var.(♀)× Megalobrama amblycephala (♂). Mar. Biotechnol. 21, 139–149.
Qin, Q.B., Liu, Q.W., Zhou, Y.W., Wang, C.Q., Qin, H., Zhao, C., Liu, S.J., 2018. Differential expression of HPG-axis genes in autotetraploids derived from red crucian carp Carassius auratus ♀ × blunt snout bream Megalobrama amblycephala. J. Fish. Biol. 93.
Refstie, T., 1981. Tetraploid rainbow trout produced by cytochalasin B. Aquaculture 25, 51–58.
Ren, L., Li, W., Qin, Q., Dai, H., Han, F., Xiao, J., Gao, X., Cui, J., Wu, C., Yan, X., Wang, G., Liu, G., Liu, J., Li, J., Wan, Z., Yang, C., Zhang, C., Tao, M., Wang, J., Luo, K., Wang, S., Hu, F., Zhao, R., Li, X., Liu, M., Zheng, H., Zhou, R., Shu, Y., Wang, Y., Liu, Q., Tang, C., Duan, W., Liu, S., 2019. The subgenomes show asymmetric expression of alleles in hybrid lineages of Megalobrama amblycephala x Culter alburnus. Genome Res. 29, 1805–1815.
Sarder, M.R.I., Penman, D., Myers, J.M., McAndrew, B., 1999. Production and propagation of fully inbred clonal lines in the Nile tilapia (Oreochromis niloticus L.). J. Exp. Zool. 284, 675–685.
Scheerer, P.D., Thorgaard, G.H., Allendorf, F.W., 1991. Genetic analysis of androgenetic rainbow trout. J. Exp. Zool. 260, 382–390.
Sun, Y., Zhang, C., Liu, S., Duan, W., Liu, Y., 2007. Induced interspecific androgenesis using diploid sperm from allotetraploid hybrids of common carp×red crucian carp. Aquaculture 264, 47–53.
Thorgaard, G.H., 1986. Ploidy manipulation and performance. Aquaculture 57, 57–64.
Thorgaard, G.H., Scheerer, P.D., Hershberger, W.K., Myers, J.M., 1990. Androgenetic rainbow trout produced using sperm from tetraploid males show improved survival. Aquaculture 85, 215–221.
Wang, J., Xiao, J., Zeng, M., Xu, K., Tao, M., Zhang, C., Duan, W., Liu, W., Luo, K., Liu, Y., Liu, S., 2015. Genomic variation in the hybrids of white crucian carp and red crucian carp: evidence from ribosomal DNA. Sci. China Life Sci. 58, 590–601.
Wang, J., Liu, Q., Luo, K., Chen, X., Xiao, J., Zhang, C., Tao, M., Zhao, R., Liu, S., 2016. Cell fusion as the formation mechanism of unreduced gametes in the gynogenetic diploid hybrid fish. Sci. Rep. 6, 31658.
Wang, S., Tang, C., Tao, M., Qin, Q., Zhang, C., Luo, K., Zhao, R., Wang, J., Ren, L., Xiao, J., Hu, F., Zhou, R., Duan, W., Liu, S., 2018. Establishment and application of distant hybridization technology in fish. Sci. China Life Sci. 62, 22–45.
Wang, S., Zhou, P., Huang, X., Liu, Q., Lin, B., Fu, Y., Gu, Q., Hu, F., Luo, K., Zhang, C., 2020. The establishment of an autotetraploid fish lineage produced by female allotetraploid hybrids× male homodiploid hybrids derived from Cyprinus carpio (♀)× Megalobrama amblycephala (♂). Aquaculture 515, 734583.
Wang, Z.-W., Zhu, H.-P., Wang, D., Jiang, F.-F., Guo, W., Zhou, L., Gui, J.-F., 2011. A novel nucleo-cytoplasmic hybrid clone formed via androgenesis in polyploid gibel carp. BMC Res. Notes 4, 1–13.
Wu, C., Huang, X., Chen, Q., Hu, F., Zhou, L., Gong, K., Fu, W., Gong, D., Zhao, R., Zhang, C., Qin, Q., Tao, M., Liu, S., 2020. The formation of a new type of hybrid culter derived from a hybrid lineage of Megalobrama amblycephala (♀) × Culter alburnus (♂). Aquaculture 525, 735328.
Xu, K., Duan, W., Xiao, J., Tao, M., Zhang, C., Liu, Y., Liu, S., 2015. Development and application of biological technologies in fish genetic breeding. Sci. China Life Sci. 58, 187–201.
Xu, X., Wang, C., Xiao, Q., Huang, X., Zhou, Y., Luo, X., Zhang, Y., Xu, X., Qin, Q., Liu, S., 2023. The alternative transcription and expression characterization of Dmc1 in autotetraploid Carassius auratus. Front. Genet. 14, 1135006.
Yoshikawa, H., Morishima, K., Fujimoto, T., Saito, T., Kobayashi, T., Yamaha, E., Arai, K., 2009. Chromosome Doubling in Early Spermatogonia Produces Diploid Spermatozoa in a Natural Clonal Fish. Biol. Reprod. 80, 973–979.
Zhang, K., Huang, X., Wang, C., Xu, X., Xu, X., Dong, X., Xiao, Q., Bai, J., Zhou, Y., Liu, Z., Deng, X., Tang, Y., Li, S., Hu, E., Peng, W., Xiong, L., Qin, Q., Liu, S., 2024. Unveiling potential sex-determining genes and sex-specific markers in autotetraploid Carassius auratus. Sci. China Life Sci. 67, 2444–2458.
Zhang, Y., Lv, M., Jiang, H., Li, H., Li, R., Yang, C., Huang, Y., Zhou, H., Mei, Y., Gao, J., Cao, X., 2023. Mitotic defects lead to unreduced sperm formation in cdk1−/− mutants. Int. J. Biol. Macromol. 242, 125171.
Zhang, Y., Mei, Y., Fujimoto, T., Gao, J., Huang, Y., Zheng, Y., Li, R., Guo, Y., Zhang, N., Jiang, Y., Ouyang, L., Jiang, H., Arai, K., Cao, X., 2025. Polyploid Formation Through Disrupting Mitotic Sister Chromatid Separation of Spermatogonia based on espl1 Heterozygous Knockout in Fish. Mol. Biol. Evol. 42.
Zhong, H., Chen, H., Liu, M., Zhao, C., Luo, C., Xiong, Z., Li, Y., Wu, Y., Wang, Y., Zhang, C., Wu, C., Liu, Q., Sun, Y., Wang, S., Wen, M., Hu, F., Liu, S., 2025. DNA damage may cause the reproductive differences between the male tetraploid fish and diploid red crucian carp. Aquaculture 601, 742283.

Zhou, R., Xiao, J., Qin, Q., Zhu, B., Zhao, R., Zhang, C., Tao, M., Luo, K., Wang, J., Peng, L., 2015. YY super sperm lead to all male triploids and tetraploids. BMC Genet. 16, 1–9.
Zhou, Y., Xiong, L., Huang, X., Xu, X.D., Wang, C., Xu, X., Zhang, K., Bai, J., Liu, Z., Jiang, Y., Peng, W., Hu, E., Li, S., Xiao, Q., Li, Z., Yang, Y., Qin, Q., Liu, S., 2026. An improved autotetraploid fish derived from the hybridization of gynogenetic homologous diploid crucian carp (♀) × autotetraploid Carassius auratus (♂). Aquaculture 613, 743448.
Zou, S., Li, S., Cai, W., Zhao, J., Yang, H., 2004. Establishment of fertile tetraploid population of blunt snout bream (Megalobrama amblycephala). Aquaculture 238, 155–164.