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Study of biological characteristics of an improved Japanese white crucian carp lineage derived from *Carassius cuvieri* (♀) × *Megalobrama amblycephala* (♂)

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A B S T R A C T

Based on the formation of fertile allotetraploid fish (4nJB, 4n = 148) derived from female *Carassius cuvieri* (JCC, 2n = 100) × male *Megalobrama amblycephala* (BSB, 2n = 48), an improved Japanese white crucian carp (IJCC) was obtained by mating female 4nJB and male 4nJB, and thus far, the population of IJCC has reached the fifth generations (F_1–F_5). The purpose of the present study was to comparatively investigate the biological characteristics of the IJCC lineage (F_1–F_5) and their original female parent JCC. The results of DNA content and chromosome numbers showed that the IJCC lineage was diploid with 100 chromosomes. For morphological characteristics, it appears that IJCC-F_1 has smaller head and a higher body width than that of JCC, and some countable traits as well as the ratio of measurable traits were significantly different ($P < 0.05$) between these two fish. With the passing of generations, the appearance of the IJCC lineage has been stable. Regarding reproductive characteristics, the fecundity, sperm volume, sperm density, fertilization rate and hatching rate were not significantly different ($P > 0.05$) between the JCC and IJCC lineages, indicating that the reproductive capacity of the IJCC lineage was similar to that of the JCC. Furthermore, the growth performance showed that the average weight of IJCC-F_1 and IJCC-F_5 was significantly greater ($P < 0.05$) than that of JCC both at 6 months and 1 year of age. The good fertility of the IJCC lineage ensures the multigeneration utilization of its advantage, and the IJCC lineage has significant value in aquatic applications.

1. Introduction

Crucian carp is one of the most important freshwater aquaculture species in China because it has tender meat, good adaptability, fast growth, and omnivorous feeding habits. For the past ten years, the aquaculture scale and aquaculture potential of crucian carp have increased in China, the annual total output exceeds 2 million tons, and the cultured yield reached 2.7 million tons in 2018, accounting for 99.9% of global crucian carp aquaculture production (FAO). Crucian carp is not only one of the main varieties of freshwater fish, and several varieties, such as red crucian carp and goldfish, are also the main varieties of ornamental fish.

There are two native varieties (*C. auratus* and *C. carassius*) and one introduced species (*C. auratus cuuieri*) of genus *Carassius* in China. Of the existing varieties of *Carassius* species, 16 new varieties were obtained by selective breeding, hybridization and other breeding methods (Hu et al., 2021). For example, sterile triploid crucian carp ("XiangYun" crucian carp and "Xiangyun No. 2" crucian carp) were produced by mating diploid crucian carp with allotetraploid hybrids (Chen et al., 2009); and improved Allogynogenetic *carassius auratus* gibelio (gibel carp "CAS III") have been successively bred by selective breeding (Gui and Zhou, 2010). These new varieties have improved in growth speed, disease resistance and other aspects, greatly promoting the development of crucian carp aquaculture.

C. auratus cuuieri (Japanese white crucian carp, JCC), originated from Japan, and was introduced to China in the 1970s (Yuan-Dong et al., 2006). Subsequently, JCC was cultured in many regions of China. However, in recent years, the germplasm resources of JCC have been

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seriously degraded due to the limited of introduction and inbreeding under long-term artificial breeding conditions. Therefore, cultivating improved JCC varieties with fast growth and strong stress resistance has become an urgent demand for the crucian carp industry.

Hybridization is one of the most widely used methods in fish breeding, which can result in genome-level alterations and may produce offspring with heterosis, such as accelerated growth (Hu et al., 2023; Guan and Qiu, 2020; Guo et al., 2018; Tung and Shiau, 1991), improved disease resistance (Xiong et al., 2022b; Xiong et al., 2022a; Xiao et al., 2018; Meidong et al., 2017), better meat quality (Wu et al., 2019; Cai et al., 2021), and increased cold resistance (Nedoluzhko et al., 2020; Kelly and Kohler, 1999). To improve the growth speed of JCC, we conducted a subfamily cross between JCC and *Megalobrama amblycephala* (blunt snout bream, BSB). Both female and male fertile allotetraploid hybrids (4nJB) were produced in the first generation of this hybrid combination (Hu et al., 2018). Moreover, an improved white crucian carp (IJCC) lineage was produced by mating female 4nJB with male 4nJB (Liu et al., 2022). In this study, the morphological characteristics, ploidy levels, growth performances and reproductive characteristics of the IJCC lineage and their original parent JCC were compared and analyzed to determine whether the IJCC lineage has potential applications in aquaculture.

2. Materials and methods

2.1. Ethical statement

The fish involved in this study were used in strict accordance with the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the National Advisory Committee for Laboratory Animal Research in China and approved by the Animal Care Committee of Hunan Normal University (Permit Number: 4236).

2.2. Experimental fish

The JCC, BSB, 4nJB and IJCC lineages were cultured in the Fish Genetic Breeding Center at Hunan Normal University.

2.3. Morphological traits

At 1 year of age, the JCC, 4nJB and IJCC lineages (F_1–F_5) were randomly selected for morphological examination, and 20 individual fish were tested for each type of fish. The fish were first placed in the tank and photographed, and then their countable and measurable traits were determined according to a previous study (Hu et al., 2018). For both measurable and countable data, we used the SPSS Statistics 18.0 software to analyze the covariance of the data between the IJCC lineage and JCC.

2.4. Ploidy levels

The ploidy level of the IJCC lineage (F_1–F_5) was determined by measuring the mean DNA content of erythrocytes and detecting chromosome numbers. The erythrocyte DNA content of the JCC, BSB, 4nJB and IJCC lineages (F_1–F_5) was measured using a flow cytometer (cell counter/analyzer, Partec). Approximately 0.2 mL of blood was collected from the caudal vein of JCC, BSB, 4nJB and IJCC F_1–F_5 individuals into a syringe containing 100–200 units of sodium heparin. The blood samples were treated following a previously described method (Liu et al., 2001). The mean DNA contents of the IJCC lineage and JCC were compared by $\chi 2$ test using SPSS Statistics 18.0.

Chromosomal preparations were performed from kidney tissue of 10 4nJB, 10 IJCC-F_1 and 10 IJCC-F_5 at 4 months of age. The chromosomes were prepared in accordance with a previous study (Liu et al., 2001). The shape and number of chromosomes were analyzed under a microscope. For each type of fish, 200 metaphase spreads of chromosomes from 10 individuals were analyzed for further determination of ploidy.

2.5. Reproductive traits of the IJCC lineage

A previous study has shown that both female and male IJCC fish are fertile, and to date, the IJCC population has been extended to the fifth generation (F_1–F_5). To explore whether the reproductive capacity of the IJCC lineage changed, the fecundity, sperm volume, sperm concentration and fertilization rate of IJCC-F_1, IJCC-F_5 and JCC were compared and analyzed. During the breeding season, female fish were injected with a combination of human chorionic gonadotropin (HCG), luteinizing hormone-releasing hormone A2 (LHRH-A2) and domperidone (DOM) (Ningbo No. 2 hormone factory, China) at standard dose of 800 IU/kg, 10 μg/kg and 2 mg/kg, respectively. The males were injected with half the dose used for females. Approximately 8–10 h later, fish were anesthetized with 100 mg/L MS-222 (Sigma-Aldrich). Eggs were collected into clean culture dishes from 10 individuals per type of females using gentle abdominal pressure. The eggs produced by each fish were weighed and the number of eggs per 1 g of eggs was counted. The fecundity of each female individual was counted and recorded. Semen samples were collected into clean tubes from 10 individuals type of male using gentle abdominal pressure. The volume of semen collected from each fish was measured and recorded. The sperm concentration was determined following the method described (Duan et al., 2016). The mature eggs and sperm of the JCC or IJCC lineage were fertilized, and the fertilized eggs were developed in culture dishes at the water temperature of 20–22 °C. From each fish, approximately 5000 fertilized eggs were collected at random for examination fertilization rate and hatching rate.

2.6. Growth performance

The growth rates of IJCC-F_1, IJCC-F_5 and JCC were compared under the same conditions. Three days post-hatching JCC, IJCC-F_1 and IJCC-F_5 were transferred to different cement ponds. The fry were fed water fleas or fairy shrimp. At approximately 20 to 30 days, when the fry reached 3–4 cm, JCC, IJCC-F_1 and IJCC-F_5 individuals were randomly selected and transferred to ponds for the adult grow-out stage. Three ponds were prepared for each fish type. These fish were fed artificial fodder (~32% crude protein). The number of dead fish in each pond was counted to calculate the survival rate. Additionally, 30 6-month-old and 1-year-old fish from each group (JCC, IJCC-F_1 and IJCC-F_5) were randomly collected and used for growth comparisons. The total weight of each fish was measured (accurate to 0.01 g). The variances and significant differences in the data between pairs of fish types were determined by SPSS Statistics 18.0.

3. Results

3.1. Morphological traits

The IJCC lineage establishment process is shown in Fig. 1. The visual traits of JCC, 4nJB, IJCC-F_1 and IJCC-F_5 are shown in Fig. 2. The 4nJB had a pair of short barbels, making them easily distinguishable from JCC and IJCC lineage. Although JCC (Fig. 2A), 4nJB (Fig. 2B), IJCC-F_1 (Fig. 2C) and IJCC-F_5 (Fig. 2D) have similar body colors, they can be distinguished by their appearance. For example, IJCC-F_1 and IJCC-F_5 have smaller heads and higher backs than JCC.

The ratio of measurable traits and countable traits of JCC, 4nJB and IJCC F_1–F_5 were examined (Tables 1 and 2). For the ratio of measurable traits between JCC and IJCC-F_1, except for the full length/body length, body width /body length and body width/head width ratios, which were significantly different ($P < 0.05$), other ratios were not significantly different ($P > 0.05$). For the measurable traits between 4nJB and IJCC-F_1, all data were significantly different ($P < 0.05$). For the measurable traits between JCC and IJCC F_2–F_5, except for body width /body length

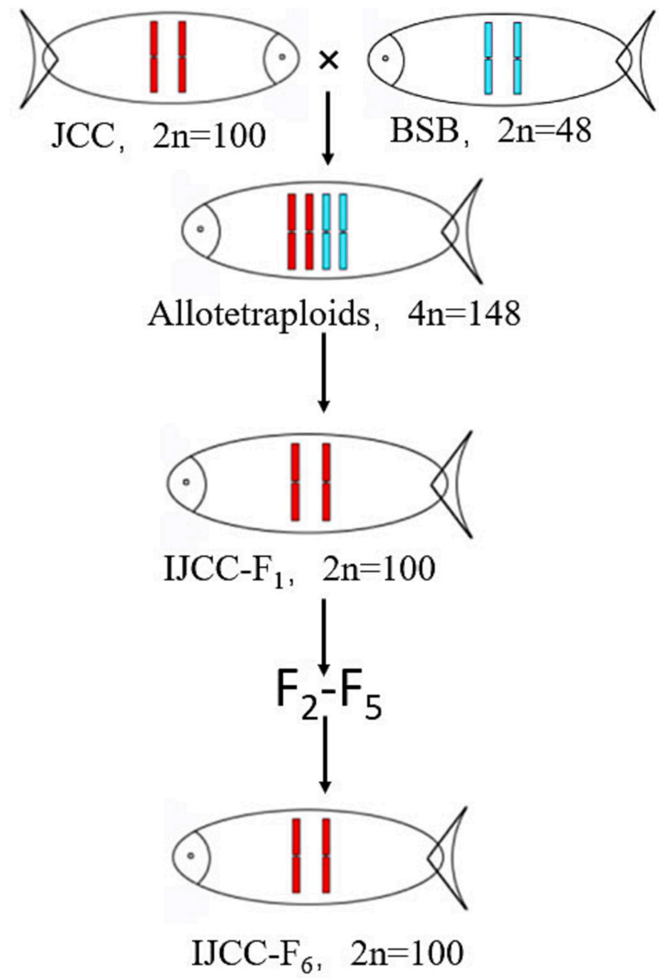

Fig. 1. The establishment of IJCC lineages. The chromosomes of Japanese white crucian carp (JCC) and blunt snout bream (BSB) are indicated by red and blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and body width/head width, which were significantly different ($P < 0.05$), other data were not significantly different ($P > 0.05$) (Table 1).

For the countable traits between JCC and IJCC-F$_1$, except for the number of lateral scales, the number of upper lateral scales and the number of pelvic fins, which were significantly different ($P < 0.05$), all other data were not significantly different ($P > 0.05$). Between 4nJB and IJCC-F$_1$, all data were significantly different ($P < 0.05$). Between JCC and IJCC F$_2$–F$_5$, except the number of lateral scales and number of upper lateral scales, which were significantly different ($P < 0.05$), all other data were not significantly different ($P > 0.05$) (Table 2).

3.2. Measurement of DNA content and examination of chromosome number

The sum DNA content of erythrocytes from JCC and BSB was used as the control, and the distribution of DNA content of erythrocytes of 4nJB and IJCC F$_1$–F$_5$ are shown in Table 3. The mean DNA content of erythrocytes from IJCC F$_1$–F$_5$ was similar to that of the JCC, indicating that IJCC F$_1$–F$_5$ were diploid.

The chromosome numbers of 4nJB, IJCC-F$_1$ and IJCC-F$_5$ were counted. Of all examined 4nJB samples, 79.0% of chromosomal metaphases had 148 chromosomes. Of all examined IJCC-F$_1$ samples, 86% of chromosomal metaphases had 100 chromosomes (Fig. 3 and Table 4). Of all examined IJCC-F$_5$ samples, 83.5.% of chromosomal metaphases had

Table 1
Comparison of the measurable traits among JCC, 4nJB, IJCC F$_1$–F$_5$.

Fish type	Full length/ body length	Body width / body length	Body length/ head length	Head length/ head width	Tail length/ tail width	Body width/ head width
JCC	1.24 ± 0.02^{a}	0.42 ± 0.01^{a}	3.70 ± 0.21^{a}	1.17 ± 0.06^{a}	0.81 ± 0.01^{a}	1.78 ± 0.09^{a}
4nJB	1.21 ± 0.03^{b}	0.42 ± 0.01^{a}	3.81 ± 0.14^{b}	1.12 ± 0.06^{b}	1.02 ± 0.08^{b}	1.83 ± 0.10^{b}
IJCC-F$_1$	1.27 ± 0.04^{c}	0.46 ± 0.02^{b}	3.71 ± 0.21^{a}	1.15 ± 0.08^{a}	0.82 ± 0.09^{a}	1.88 ± 0.09^{c}
IJCC-F$_2$	1.26 ± 0.02^{ac}	0.45 ± 0.01^{b}	3.71 ± 0.13^{a}	1.14 ± 0.07^{ab}	0.83 ± 0.06^{a}	1.87 ± 0.09^{cd}
IJCC-F$_3$	1.25 ± 0.02^{ac}	0.44 ± 0.01^{ab}	3.71 ± 0.26^{a}	1.15 ± 0.11^{a}	0.82 ± 0.06^{a}	1.87 ± 0.03^{cd}
IJCC-F$_4$	1.25 ± 0.08^{ac}	0.43 ± 0.01^{a}	3.71 ± 0.11^{a}	1.15 ± 0.10^{a}	0.81 ± 0.04^{a}	1.86 ± 0.08^{cd}
IJCC-F$_5$	1.25 ± 0.03^{ac}	0.43 ± 0.01^{a}	3.70 ± 0.09^{a}	1.15 ± 0.05^{a}	0.82 ± 0.04^{a}	1.85 ± 0.10^{bd}

The same superscript letters in the same column indicate no significant difference ($P > 0.05$); different superscript letters in the same column indicate a significant difference ($P < 0.05$). For all the values, $n = 20$.

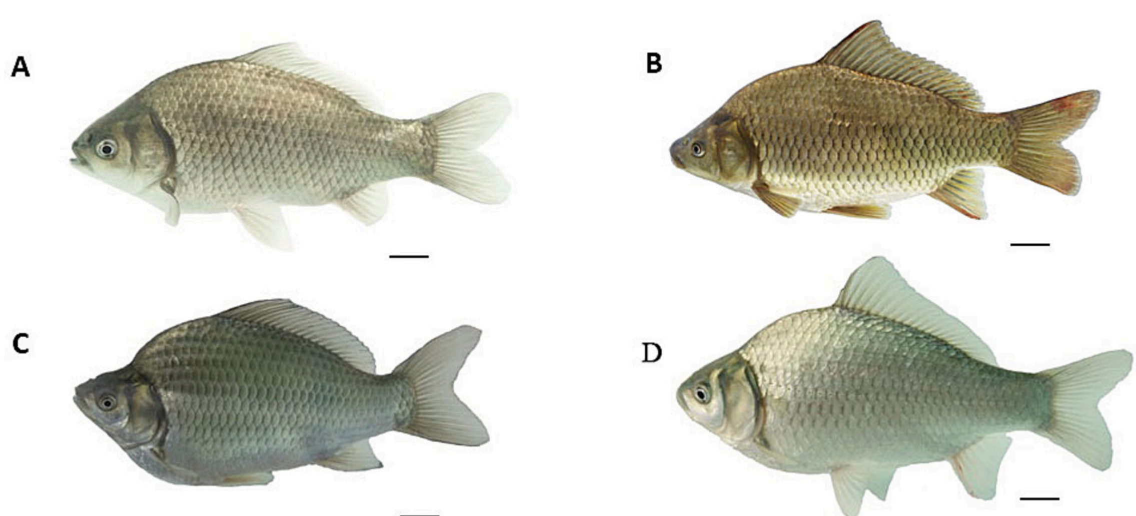

Fig. 2. The appearance of JCC, 4nJB, IJCC-F$_1$ and IJCC-F$_5$. (A) the appearance of JCC; (B) the appearance of 4nJB; (C) the appearance of IJCC-F$_1$; (D) the appearance of IJCC-F$_5$. Bar = 2 cm.

Table 2
Comparison of the countable traits among JCC, 4nJB and IJCC F_1–F_5.

Fish type	Number of lateral scales	Number of upper lateral scales	Number of lower lateral scales	Number of dorsal fins	Number of pelvic fins	Number of anal fins
JCC	33.15 ± 0.35[a] (32–34)	6.53 ± 0.42[a] (6–8)	6.60 ± 0.31[a] (5–7)	III + 19.35 ± 0.86[a] (III 18–20)	9.05 ± 0.75[a] (8–10)	III +6.05 ± 0.75[a] (III + 6–7)
4nJB	32.05 ± 0.22[b] (32–33)	7.1 ± 0.31[b] (7–8)	7.95 ± 0.22[b] (7–8)	III +17.35 ± 0.49[b] (III 17–18)	8.70 ± 0.47[a] (8–9)	III +7.15 ± 0.37[b] (III 7–8)
IJCC-F_1	30.65 ± 0.64[c] (29–31)	7.84 ± 0.42[c] (7–9)	6.66 ± 0.43[a] (6–7)	III + 19.45 ± 0.44[a] (III 18–20)	9.35 ± 0.25[b] (8–10)	III +6.35 ± 0.25[a] (III + 6–7)
IJCC-F_2	31.56 ± 0.86[b] (30–33)	7.41 ± 0.46[bc] (7–8)	6.28 ± 0.72[a] (5–7)	III + 19.56 ± 0.93[a] (III 17–20)	8.78 ± 0.68[a] (8–10)	III +6[a] (III + 6)
IJCC-F_3	31.63 ± 0.77[b] (31–33)	7.44 ± 0.49[bc] (7–8)	6.34 ± 0.50[a] (6–7)	III + 19.63 ± 0.88[a] (III 18–20)	8.72 ± 0.44[a] (8–10)	III +6[a] (III + 6)
IJCC-F_4	31.68 ± 0.84[b] (31–33)	7.37 ± 0.48[bc] (7–9)	6.30 ± 0.58[a] (6–7)	III + 19.65 ± 0.90[a] (III 17–20)	8.81 ± 0.69[a] (8–10)	III +6[a] (III + 6)
IJCC-F_5	31.42 ± 0.49[b] (31–32)	7.38 ± 0.37[bc] (7–8)	6.25 ± 0.43[a] (6–7)	III + 19.55 ± 0.44[a] (III + 18–20)	8.83 ± 0.55[a] (8–10)	III +6[a] (III + 6)

The same superscript letters in the same column indicate no significant difference ($P > 0.05$); different superscript letters in the same column indicate a significant difference ($P < 0.05$). For all the values, n = 20.

Table 3
Mean DNA content of JCC, BSB, 4nJB and IJCC F_1–F_5.

Fish type	Mean DNA content	Ratio	
		Observed	Expected
JCC	94.72		
BSB	68.4		
4nJB	158.43	4nJB/(JCC + BSB) =0.97[a]	1
IJCC-F_1	94.55	IJCC-F_1/JCC = 0.99[a]	1
IJCC-F_2	101.23	IJCC-F_2/JCC = 1.07[a]	1
IJCC-F_3	99.68	IJCC-F_3/JCC = 1.05[a]	1
IJCC-F_4	96.52	IJCC-F_4/JCC = 1.02[a]	1
IJCC-F_5	102.40	IJCC-F_5/JCC = 1.08[a]	1

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

100 chromosomes (Fig. 3 and Table 4). These results further confirmed that the IJCC lineage was diploid.

3.3. Reproductive traits of IJCC lineage

The mean fecundity values of JCC, IJCC-F_1 and IJCC-F_5 were (1.02 ± 0.29) × 10^5, (1.06 ± 0.29) × 10^5 and (1.03 ± 0.29) × 10^5 eggs kg^{-1}, respectively. The mean sperm concentrations of JCC, IJCC-F_1 and IJCC-F_5 were (1.18 ± 0.26) × 10^{10}, (1.14 ± 0.71) × 10^{10} and (1.23 ± 0.31) × 10^{10} spermatozoa mL^{-1}, respectively. The mean sperm volumes of JCC, IJCC-F_1 and IJCC-F_5 were 5.68 ± 1.51, 5.65 ± 2.99 and 5.71 ± 2.83 kg mL^{-1}, respectively. The fertilization rate and hatching rate of JCC (91.30 ± 0.53% and 80.87 ± 2.30%), IJCC-F_1 (90.70 ± 0.40% and 82.34 ± 2.62%) and IJCC-F_5 (92.2 ± 3.67% and 84.7 ± 6.84%) were not significantly different ($P > 0.05$) (Table 5).

3.4. Growth performance

The growth comparison of JCC, IJCC-F_1 and IJCC-F_5 is shown in the Table 6. During the fry stage, the survival rates of the JCC, IJCC-F_1 and IJCC-F_5 were 88.6%, 84.5% and 87.5%, respectively; during the adult grow-out stage, the survival rates of the JCC, IJCC-F_1 and IJCC-F_5 were 93.4%, 90.2% and 94.1%, respectively. At the 6-month-old stage, the average weights of IJCC-F_1 (240.55 ± 22.1 g) and IJCC-F_5 (233.6 ± 18.5 g) were significantly ($P < 0.05$) greater than that of JCC (210.4 ± 14.5 g). At the 1-year-old stage, the average weights of IJCC-F_1 (413 ± 28.4 g) and IJCC-F_5 (422.4 ± 34.7 g) were significantly ($P < 0.05$) greater than that of JCC (372.3 ± 21.4 g).

4. Discussion

As an introduced variety of crucian carp, JCC can be cultured in China on a large scale based on its growth advantages and adaptability. At present, JCC can not only be directly used for commercial production but also for hybridization with tetraploid fish or red crucian carp to produce the sterile triploid fish "XiangYun" crucian carp, "XiangYun" crucian carp No.2, "Hefang" crucian carp and "Hefang" crucian carp No. 2 (Liu et al., 2001; Liu et al., 2019; Liu et al., 2018). However, limited by the scale of introduction of the species and the mating of close relatives under the conditions of long-term artificial aquaculture, JCC is facing bottleneck problems such as insufficient quality resources and frequent diseases. Therefore, the cultivation of high-quality improved JCC varieties has become an urgent requirement for the aquaculture of crucian carp.

In our previous study, both female and male fertile allotetraploids were produced by crossing female JCC and male BSB (Hu et al., 2018). Furthermore, a new type of improved Japanese white crucian carp was produced by mating female 4nJB and male 4nJB. The production of diploid IJCC was mainly due to 4nJB producing gametes of different ploidies (Liu et al., 2022). The eggs produced by female 4nJB showed three states of size, with egg diameters of 0.13 cm, 0.17 cm and 0.2 cm. The eggs with the largest diameter were tetraploid eggs; those with intermediate diameter were diploid eggs; and the smallest diameter eggs were haploid eggs. The semen produced by male 4nJB contained haploid and diploid sperm. Thus, the male 4nJB and female 4nJB could produce viable diploid, triploid and tetraploid fish by mating.

In this study, we compared the morphological characteristics, ploidy levels, reproductive traits, and growth performances of the IJCC lineage and its original parent JCC. Regarding morphological characteristics, the IJCC lineage, especially IJCC-F_1, exhibits significant differences from those of JCC. The main difference is that IJCC-F_1 has a smaller head and larger body width than JCC (Fig. 2), which indicates a certain degree of heterozygosity in its genome. With the passing of generations, the morphological differences among different generations of the IJCC lineage gradually tended to stabilize. The measurable traits and countable traits in IJCC F_2–F_5 were closer to those of JCC than to those of IJCC-F_1 and JCC (Tables 1 and 2). Generally, the changes in appearance of hybrids were mainly concentrated in the first generation (F_1), which was mainly caused by genome incompatibility (Liu et al., 2016; Ren et al., 2019). Among the IJCC lineages, the IJCC-F_1 genome is the most unstable, and it has the most pronounced phenotypic changes.

The ploidy level of the genus *Carassius* is complex. *C. auratus* L. and *C. auratus gibelio* have been reported to coexist as diploids, triploids and tetraploids in natural waters (Xiao et al., 2011; Gui and Zhou, 2010). In addition, triploid crucian carp have been found among Dianchi high-back crucian carp and Pengze crucian carp. However, to date, no polyploid individuals have been detected in the natural population of JCC

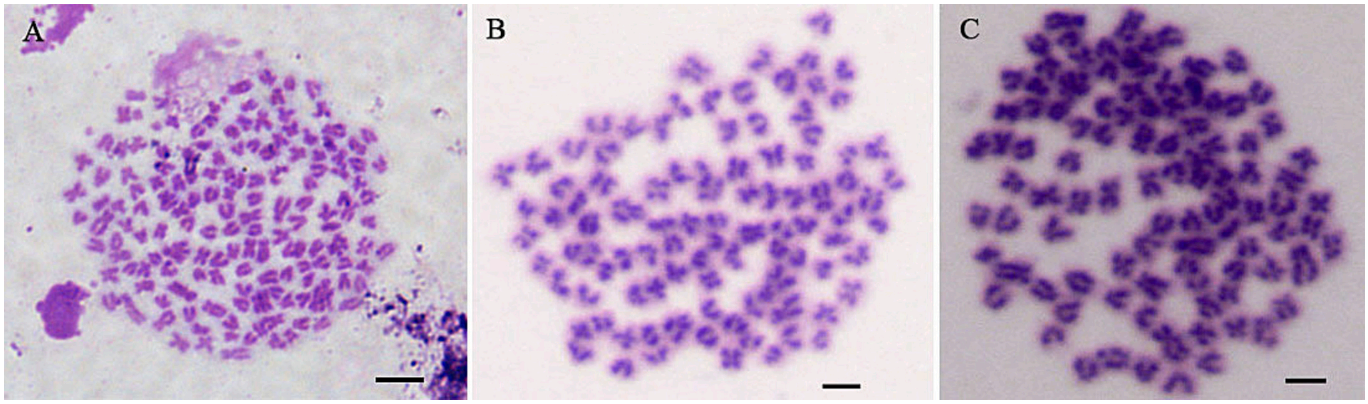

Fig. 3. Chromosomes at metaphase in 4nJB, IJCC-F_1 and IJCC-F_5. (A) the 148 chromosomes of 4nJB; (B) the 100 chromosomes of IJCC-F_1; (C) the 100 chromosomes of IJCC-F_5; Bar =20 μm.

Table 4
Examination of chromosome number in 4nJB, IJCC-F_1 and IJCC-F_5.

Fish type	Number in metaphase	Distribution of chromosome number				
		<100	100	<148	148	>148
4nJB				35	158	7
IJCC-F_1	200	26	172			
IJCC-F_5	200	33	167			

Table 5
The fecundity ($\times 10^5$ eggs kg^{-1}), sperm concentration ($\times 10^{10}$ cells mL^{-1}), semen volume (mL kg^{-1}), fertilization rate (%) and hatching rate (%) among JCC, IJCC-F_1 and IJCC-F_5.[a]

Fish type	Fecundity ($\times 10^5$ eggs kg^{-1})	Sperm concentration ($\times 10^{10}$ cells mL^{-1})	Semen volume (mL kg^{-1})	Fertilization rate (%)	Hatching rate (%)
JCC	1.02 ± 0.29	1.18 ± 0.26	5.68 ± 1.51	91.30 ± 0.53	80.87 ± 2.30
IJCC-F_1	1.06 ± 0.37	1.14 ± 0.71	5.65 ± 2.99	90.70 ± 0.40	82.34 ± 2.62
IJCC-F_5	1.03 ± 0.24	1.23 ± 0.31	5.71 ± 2.83	92.20 ± 3.67	84.70 ± 6.84

[a] Mean value ± SD, none of the traits measured significantly differed among IJCC-F_1, IJCC-F_5 and JCC ($P > 0.05$).

Table 6
Comparison of growth performance between JCC, IJCC-F_1 and IJCC-F_5.

Fish type	Weight (g)	
	6-month-old	1-year-old
JCC	210.40 ± 14.5^{bc}	372.30 ± 21.4^{bc}
IJCC-F_1	240.55 ± 22.1^{a}	413.00 ± 28.4^{a}
IJCC-F_5	233.60 ± 18.5^{a}	422.40 ± 34.7^{a}

Values in the same column with letter a, b, c for each species show significant differences with JCC, IJCC-F_1 and IJCC-F_5 ($P < 0.05$).

(Yamamoto et al., 2010). In this study, the ploidy levels of the IJCC lineage were confirmed by measuring DNA content (Table 3) and counting the chromosomal number (Fig. 3 and Table 4). All of the above results were in agreement that IJCC F_1–F_5 were diploids with 100 chromosomes.

The fertility of hybrid animal offspring has been widely studied by geneticists. It may involve many complex mechanisms, such as the complicated genetic mechanism related to the X chromosome or the abnormal operation of meiosis (Good et al., 2008; Thomsen et al., 2011; Matzuk and Lamb, 2008). In fish hybrid breeding, most F_1 hybrids are sterile or have reduced fertility, which greatly limits the use of heterosis over multiple generations (Liu et al., 2020; Wang et al., 2019). A previous study indicated that both females and males IJCC were fertility (Liu et al., 2022), but it is unknown whether the reproductive capacity of IJCC has changed compared to that of its original parent JCC. In this study, we compared and analyzed the fecundity, sperm volume, sperm concentration and fertilization rate among IJCC-F_1, IJCC-F_5 and JCC. The results showed that the IJCC lineage had good fertility, and both females and males could produce a large number of mature gametes. This result is similar to previous studies in which some of the good-fertility hybrids were observed among the offspring of *Cyprinus carpio* (♀) × BSB (♂) (Wang et al., 2017) and female BSB (♀) × male *culter alburnus* (♂) (Xiao et al., 2014). The good fertility of the IJCC lineage lays a good foundation for forming new improved JCC varieties, which can ensure the utilization of heterosis over multiple generations.

The growth phenotype is an important index to measure the economic value of a fish variety, and it is also one of the most important target traits in fish breeding (Hu et al., 2023). In China, the individual weight of JCC at the first age can reach 350 g under pond breeding conditions (Liu et al., 2019). In this study, we compared growth performance between IJCC-F_1, IJCC-F_5 and their original parent JCC. At the 6-month-old stage, the mean weights of IJCC-F_1 and IJCC-F_5 were 1.14 and 1.11 times that of JCC, respectively. At the 1-year-old stage, the mean weights of IJCC-F_1 and IJCC-F_5 were 1.11 and 1.13 times that of JCC, respectively. Our results suggest that the IJCC lineage had undergone significant improvement in growth, which can shorten the culture cycle and help expand aquaculture.

The results indicated that the IJCC lineage is an excellent improved Japanese white crucian carp variety, and the good fertility of the IJCC populations allows the establishment of the IJCC lineage (F_1–F_5). The formation of the IJCC lineage provides a new excellent germplasm resource for crucian carp improvement and aquaculture.

CRediT authorship contribution statement

Pengfei Yu: Investigation, Validation, Writing – original draft. **Haitao Zhong:** Investigation, Validation, Writing – original draft. **Hong Chen:** Investigation, Validation, Formal analysis. **Mingli Liu:** Investigation, Validation, Formal analysis. **Yi Zhou:** Visualization, Formal analysis. **Xiaoni Cao:** Investigation. **Chang Wu:** Investigation. **Yu Sun:** Investigation, Visualization. **Shi Wang:** Investigation. **Dingbin Gong:** Investigation. **Qinyong Gong:** Investigation. **Ming Wen:** Investigation. **Fangzhou Hu:** Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition. **Shaojun Liu:** Conceptualization, Supervision, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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