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Establishment of a hybrid lineage via *Megalobrama amblycephala* (♀) × *Culter alburnus* (♂) and Genetic Analysis

Chang Wu [a,c,d,1,*], Qian Chen [a,1], Yuxiang Wang [a,1], Jinhui Huang [a], Lang Qin [a], Ming Wen [a], Liang Kuai [a], Yang Lu [a], Jiayi Chen [a], Jiawang Huang [a], Xu Huang [a], Fangzhou Hu [a], Shi Wang [a], Wuhui Li [a], Li Ren [a], Jun Xiao [a], Shaojun Liu [a,b,d,*]

[a] *Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry, College of Life Sciences, Hunan Normal University, Changsha, China*
[b] *Yuelushan Laboratory, Changsha, China*
[c] *Hunan Province Fish Fry Breeding Technology Co., Ltd., Shaoyang, China*
[d] *Institute of Interdisciplinary Studies, Hunan Normal University, Changsha, China*

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ABSTRACT

Distant hybridization is a significant method for creating new hybrid lineages and potentially hybrid species. In the past ten years, we have established a hybrid lineage population, BTF_1-BTF_6 (six generations), via crosses between *Megalobrama amblycephala* (blunt snout bream, BSB, 2 n = 48, ♀) and *Culter alburnus* (topmouth culter, TC, 2 n = 48, ♂). The DNA content and the chromosome number showed that the hybrid lineage (BTF_1-BTF_6) was diploid with 48 chromosomes. Furthermore, examining the genomes of BSB and TC, we identified 10 *Hox* genes from the *Hox* family that exhibited significant single-nucleotide polymorphisms (SNPs), which we utilized to differentiate between the two parental genomes. Subsequent cloning and analysis of these 10 *Hox* genes in the hybrid lineage (BTF_1-BTF_6) indicated that most of the heterologous genes stably coexist within the hybrids. However, a small fraction of genes inherited from the male parent may be lost during the establishment of the hybrid lineage. Additionally, chimeric genes were detected in the BTF_1 population, demonstrating stable inheritance within the hybrid lineage. The establishment of this hybrid lineage from female BSB and male TC not only serves as a vital model for investigating genetic variation in early hybrid speciation but is also crucial for advancing fish genetic breeding.

1. Introduction

Distant hybridization, defined as crosses between different species, enables gene transfer between species, thereby altering hybrid genotypes and phenotypes (Chen, et al., 2018). These phenotypic improvements, such as accelerated growth or improved disease resistance in hybrids compared to their parental species, are commonly referred to as heterosis or hybrid vigor. Moreover, if the hybrids are bisexually fertile, it becomes possible to establish a hybrid lineage through self-mating, facilitating the transmission of heterosis (Goncearuc, 2008; Zhang, et al., 2014). A notable example is the allotetraploid lineage produced from *Carassius auratus* red *var.* (2 n = 100, ♀) crossed with *Cyprinus*

Abbreviations: BSB, blunt snout bream; TC, topmouth culter; BTF_1-BTF_6, a hybrid population representing F_1 to F_6 filial generations was developed via crosses between BSB ♀ and TC ♂; TBF_1-TBF_3, a hybrid population representing F_1 to F_3 filial generations was developed via crosses between TC ♀ and BSB ♂; SNPs, single-nucleotide polymorphisms; FL/BL, the ratio of full length to body length; BL/BH, the ratio of body length to body height; BH/HH, the ratio of body height to head height; HL/HH, the ratio of head length to head height; BTF_n–T, the sequence of the *Hox* gene in hybrids was derived from TC and n represents the generation number of the hybrid lineage; BTF_n–B, the sequence of the *Hox* gene in hybrids was derived from BSB and n represents the generation number of the hybrid lineage; BTF_n–C, the sequence of the *Hox* gene in hybrids was a chimeric gene containing the gene fragment of BSB and TC and n represents the generation number of the hybrid lineage.

* Correspondence to: 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 addresses: wuchang@hunnu.edu.cn (C. Wu), lsj@hunnu.edu.cn (S. Liu).
[1] These authors contributed equally to this work.

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Table 1
The primers for the amplification of 10 *Hox* genes.

Gene name	Forward primer	Reverse primer	Annealing temperature (℃)	Poduct size (bp)
HoxA1a	ATGAGCACATTCTTAGATTTCTCG	TTAATTCGAGGAGTAGGCATCAAC	57.90	993
HoxA3a	ATGCAAAAAGCGACCTACTGCGACG	CTATAGATGCGTCAGTTTGGGCG	64.02	1236
HoxA9a	ATGTCGACATCTGGAACTCTGACAG	TCATTCTTGGTCGTCCTTYGTTCC	62.08	762
HoxA13b	ATGACAGCGTCTTTACTCCTCC	TTAACTGATGCCCTTGTACTTGTTG	59.80	870
HoxB1a	ATGGACAATTCCAGAATGAACTC	TTATGAATCCGGACTTGGAGAAGC	58.76	951
HoxB3a	ATGCAGAAAACGACCTACTACGAC	CTACAGATGAGTCAACTTGGGTGC	61.15	1251
HoxB8b	ATGAGTTCTTACTTCGTCAATTCCC	CTATTTAGAATTASTGGAAGTCTCC	56.95	744
HoxC6b	ATGAATTCCTATTTTACGAACCCG	TCAGTTATGCTCTTTTTTTTCTG	55.61	696
HoxC12b	ATGGGCGAGCATAATYTCYTTAATC	TTAAAAGTAATACAACGCCTGTTCC	58.96	855
Hoxd12a	GCACAATCTTCTTAGTTCTGGC	TAGTAAATARTGAAGGTATGGTCG	55.60	780

Note: R=A/G, S=G/C, Y=C/T.

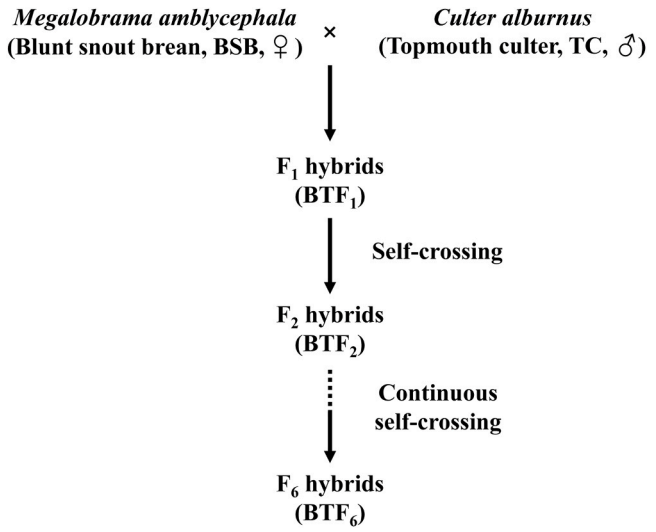


Fig. 1. The crossing procedure regarding the establishment of the hybrid lineage.

carpio L. (2 n = 100, ♂), which can generate allotriploid hybrids exhibiting enhanced growth rates and disease resistance by crossing with diploid crucian carp or common carp (Chen, et al., 2009; Liu, et al., 2016). Moreover, these hybrid lineages can create new germplasm resources, potentially leading to the development of further hybrid species through multistep breeding technologies (Wang, et al., 2019).

Megalobrama amblycephala (blunt snout bream, BSB, 2 n = 48) ranks among the primary herbivorous fish species globally and is considered a high-quality freshwater fish in China. In contrast, *Culter alburnus* (topmouth culter, TC, 2 n = 48) is a carnivorous fish recognized for its flavorful meat and is commonly found in major river systems throughout China. Following intergeneric reciprocal hybridization between BSB and TC, researchers successfully produced two types of fertile hybrids (Xiao, et al., 2014). A decade later, two hybrid lineages, BTF_1-BTF_6 (BSB ♀ × TC ♂) and TBF_1-TBF_3 (TC ♀ × BSB ♂), were established respectively (Liu, 2014). These hybrid lineages not only represent new germplasm resources but also offer valuable materials for investigating the genetic and reproductive principles underlying hybrid lineage establishment.

The *Hox* gene family is characterized by a specific DNA sequence known as a homeobox sequence, which encodes a conserved homeodomain protein in vertebrates (Holland, et al., 2007; Santini, et al., 2003). These genes play a critical role in the development of axial bones and appendages in vertebrates (Ahn, Ho, 2008; McIntyre, et al., 2007; Wellik, 2007, 2009). In addition to their developmental functions, *Hox* genes are also involved in essential physiological processes, such as maintaining reproductive homeostasis and regulating fluid balance (Alharbi, et al., 2013; Du and Taylor, 2016; Sheth, et al., 2012). A research indicates that *Hox* genes are present in nearly all eukaryotic organisms and are classified into 11 gene classes and over 100 gene families throughout the evolutionary history of animals (Holland, 2013). The variation and evolution of vertebrate morphology appear to be closely linked to the ongoing evolution and duplication of *Hox* genes over hundreds of millions of years (Geng, Sun, 2012). The conservation and evolutionary diversity of *Hox* genes, alongside their strong association with vertebrate morphology, make them ideal candidates for studying genetic variation in hybrid species. In contrast to mice, humans, and other organisms, the genome of fish *Hox* gene clusters shows a significantly accelerated evolution, potentially accounting for the diversity and variability observed in fish morphology (Meyer, Mélaga-Trillo, 1999). Furthermore, numerous studies have established that gene loss, chimerism, recombination, and genomic pseudogenization occur in hybrid offspring (Luo, et al., 2019; Zhao, et al., 2020). However, investigations into the relationships between *Hox* gene variation and phenotypic traits in distantly related fish hybrid offspring remain limited. Analyzing genetic variation in the *Hox* gene family within the FBF_1-F_6 hybrid lineage may offer valuable insights and data references.

In this study, we first reported a hybrid lineage (BTF_1-BTF_6) from the hybrid of BSB ♀ and TC ♂, and conducted a screening of the BSB and TC genomes, selecting 10 specific *Hox* genes (*HoxA1a*, *HoxA3a*, *HoxA9a*, *HoxA13b*, *HoxB1a*, *HoxB3a*, *HoxB8b*, *HoxC6b*, *HoxC12b*, and *HoxD12a*). We amplified these genes from BTF_1-BTF_6 hybrids to assess genetic variation within this hybrid lineage. The findings from this research provide significant insights into the genetic variations that occur during hybrid lineage formation or hybrid speciation.

2. Methods

2.1. Ethics statement

The guidelines established by the Administration of Affairs Concerning Animal Experimentation state 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 neither rare nor near extinction (first- or second-class state protection level). The fish involved in this experiment were approved by the Biomedical Research Ethics Committee of Hunan Normal University (approval number: 2023 No. (610)). The fish were strongly anesthetized with 100 mg/L MS-222 (Sigma—Aldrich, St. Louis, MO, USA) prior to dissection. The fish care and experimenters were certified under a professional training course for laboratory animal practitioners held by the Institute of Experimental Animals, Hunan Province, China.

2.2. Establishment and collection of samples of the hybrid lineage

For the establishment of the hybrid lineage, female BSB and male TC were selected from Hunan Normal University, Hunan, China. During the spawning seasons in May to July of 2009 and 2010, 20 mature female BSB and 20 mature male TC, each over two years old and weighing

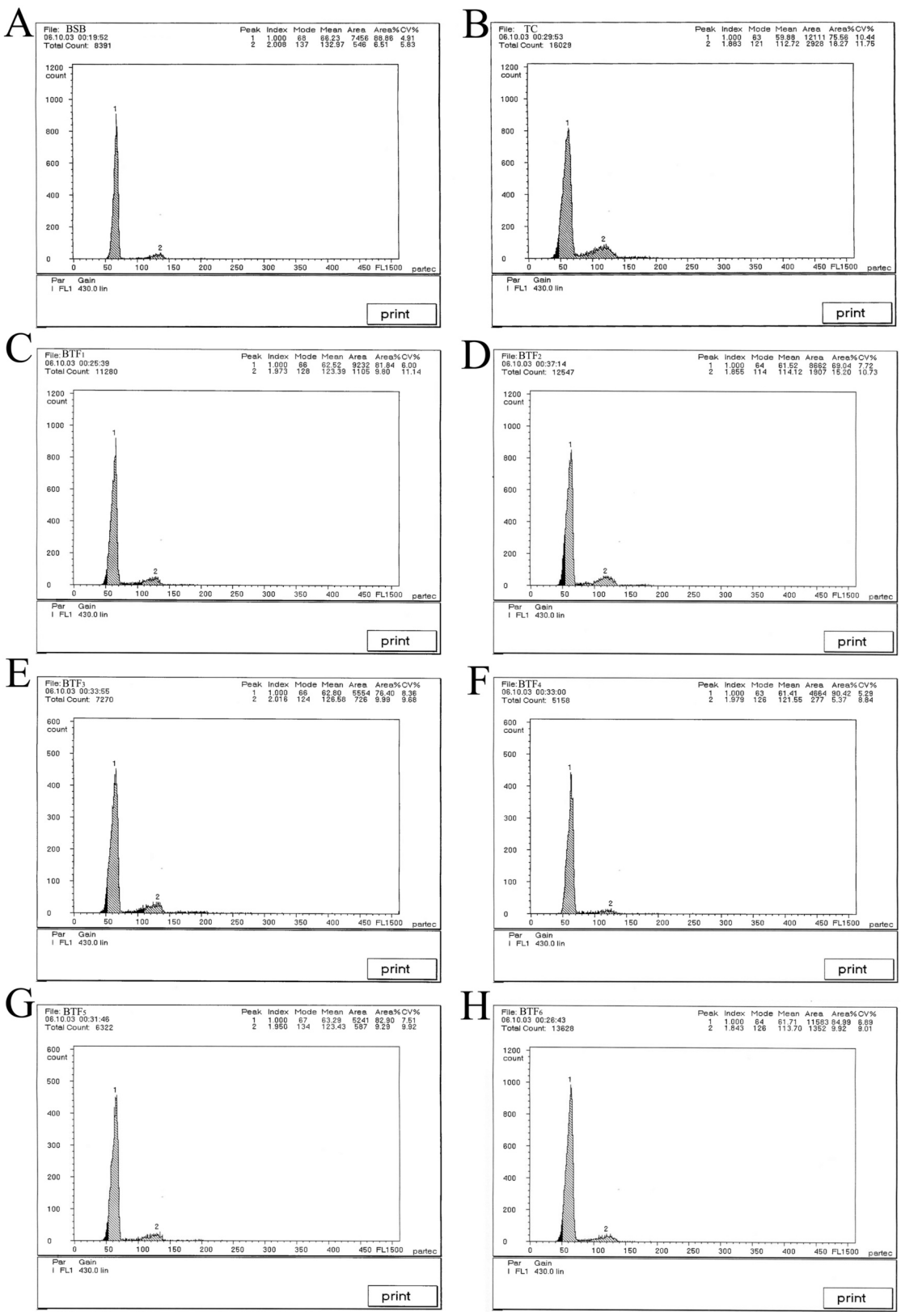


Fig. 2. Cytometric histogram of DNA fluorescence for BSB, TC and their hybrid lineage (BTF_1-BTF_6). (A) The mean DNA content of BSB (peak 1: 66.23). (B) The mean DNA content of TC (peak 1: 59.88). (C) The mean DNA content of BTF_1 (peak 1: 62.52). (D) The mean DNA content of BTF_2 (peak 1: 61.52). (E) The mean DNA content of BTF_3 (peak 1: 62.80). (F) The mean DNA content of BTF_4 (peak 1: 61.41). (G) The mean DNA content of BTF_5 (peak 1: 63.29). (H) The mean DNA content of BTF_6 (peak 1: 61.71).

Table 2
Mean DNA content of BSB, TC and their hybrid lineage (BTF_1-BTF_6).

Fish type	Mean DNA content	Ratio	
		Observed	Expected
BSB	66.23		
TC	59.88		
BTF_1	62.52		1
		BTF_1/(0.5BSB+0.5TC) = 0.99[a]	
BTF_2	61.52		1
		BTF_2/(0.5BSB+0.5TC) = 0.98[a]	
BTF_3	62.80		1
		BTF_3/(0.5BSB+0.5TC) = 1.00[a]	
BTF_4	61.41		1
		BTF_4/(0.5BSB+0.5TC) = 0.97[a]	
BTF_5	63.29		1
		BTF_5/(0.5BSB+0.5TC) = 1.00[a]	
BTF_6	61.71		1
		BTF_6/(0.5BSB+0.5TC) = 0.98[a]	

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

approximately 1 kg, were injected with oxytocin to facilitate artificial insemination. The resulting first filial generation was designated as the BTF_1 population, which reached sexual maturity at two years of age. In 2011 and 2012, 2-year-old BTF_1 males and females were crossed to produce the BTF_2 generation (Xiao, et al., 2014). Subsequent years, the continued self-crossing was performed to further establish the hybrid lineage.

2.3. Determination of ploidy level

To ascertain the ploidy levels of the BTF_1-BTF_6 fish, DNA content detection and chromosome preparation techniques were employed. For each generation from BTF_1 to BTF_6, along with BSB and TC, 30 individuals aged 18 months, balanced for sex, were selected for erythrocyte sampling and kidney chromosome preparation following the methodologies outlined by (Liu, et al., 2019, 2007). The DNA content of BSB and TC served as the control. Measurements of DNA content for all samples were conducted using a flow cytometer (Cell Counter Analyzer, Partec, Germany). A $\chi 2$ test with Yate's correction was applied to assess deviations in the DNA content ratios of BTF_1-BTF_6 fish compared to the combined DNA content of BSB and TC against expected ratios. Additionally, the chromosome numbers of ten metaphase spreads from each kidney chromosome sample were counted under a microscope, with a total of 300 spreads analysed per generation for BTF_1-BTF_6 and their parent fish.

2.4. Morphological traits

A total of 160 fish, including BTF_1-BTF_6, BSB and TC (20 18-month-old fish each), with an equal male-to-female ratio, were randomly selected for morphological trait examinations. The countable traits assessed included the number of lateral line scales, upper lateral line scales, lower lateral line scales, dorsal fin rays, abdominal fin rays, and anal fin rays. Measurable traits included the average ratios of full length to body length (FL/BL), body length to body height (BL/BH), body length to head height (BL/HH), and head length to head height (HL/HH). Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS; Chicago, IL, USA, version 17.0), employing analysis of variance (ANOVA) and pairwise comparisons to evaluate the data, with significance determined by the P value.

2.5. DNA extraction

Ten 18-month-old individuals with the same proportion of males and females were randomly selected from each generation of BTF_1-BTF_6 for a genetic survey. We followed the manufacturer's (Sangon, Shanghai, China) instructions to extract genomic DNA from prepared blood samples of BTF_1-BTF_6. DNA quality was tested via agarose gel electrophoresis, and the eligible genomic DNA was used as a template for amplification.

2.6. Screening and amplification of the Hox gene family

To identify members of the *Hox* gene family in the BSB and TC genomes, we employed the Blast Several Sequences to A Big Database function of TBtools software with an E-value threshold of 1e-5 (Chen, et al., 2020). This analysis was based on known zebrafish sequences available in the NCBI database and the genomes from (Ren, et al., 2019). Subsequently, homologous *Hox* gene clusters from BSB and TC were selected, and pairwise alignment was performed using the ClustalW multiple alignment function in BioEdit software. *Hox* genes exhibiting significant single-nucleotide polymorphism (SNPs) differences between BSB and TC were chosen for further analysis. For these candidate genes, specific primers targeting conserved regions were designed using Primer Premier 5 software.

The amplification primers used in this study are detailed in Table 1. Each PCR reaction was conducted in a 20 μL system, comprising 10 μL of LA mix (TaKaRa, Dalian, China), 7.4 μL of ultrapure water, 0.8 μL of forward primer, 0.8 μL of reverse primer, and 1 μL of DNA template. The thermal cycling protocol included an initial denaturation at 94 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50–60 °C for 60 s, and extension at 72 °C for 2 min. The program concluded with an additional extension at 72 °C for 10 min, followed by storage at 4 °C. The PCR products were then separated using 1.2 % agarose gels, stained with ethidium bromide (EB), and purified using a kit from Sangon (Shanghai, China). The purified products were subsequently cloned into a pMD18-T vector (TaKaRa, Dalian, China). A total of 10 positive clones from each sample were sequenced using Sanger sequencing on an automated DNA sequencer (ABI PRISM3730, Applied Biosystems, Carlsbad, CA), and the resulting *Hox* gene sequences were analyzed for homology and variations using the ClustalW multiple alignment function in BioEdit software.

2.7. Genetic analysis

Following the processes of amplification, purification, recovery, ligation, transformation, and sequencing, we successfully obtained the *Hox* gene sequences from BTF_1 to BTF_6 fish. Utilizing the sequences of BSB and TC as controls, we performed a comparative analysis of genetic changes within individual genes across generations using BioEdit software. By examining the differences in SNP sites at corresponding positions in the homologous genes of BSB and TC, we determined the parental origin of these sites in the hybrid offspring. This assessment allowed us to infer whether the SNP sites were inherited from the female parent or the male parent. Furthermore, based on these parental SNP origins, we evaluated the occurrence of chimerism or genetic recombination in the offspring. Ultimately, the *Hox* gene sequences of the hybrid lineage fish were deposited in the NCBI public database.

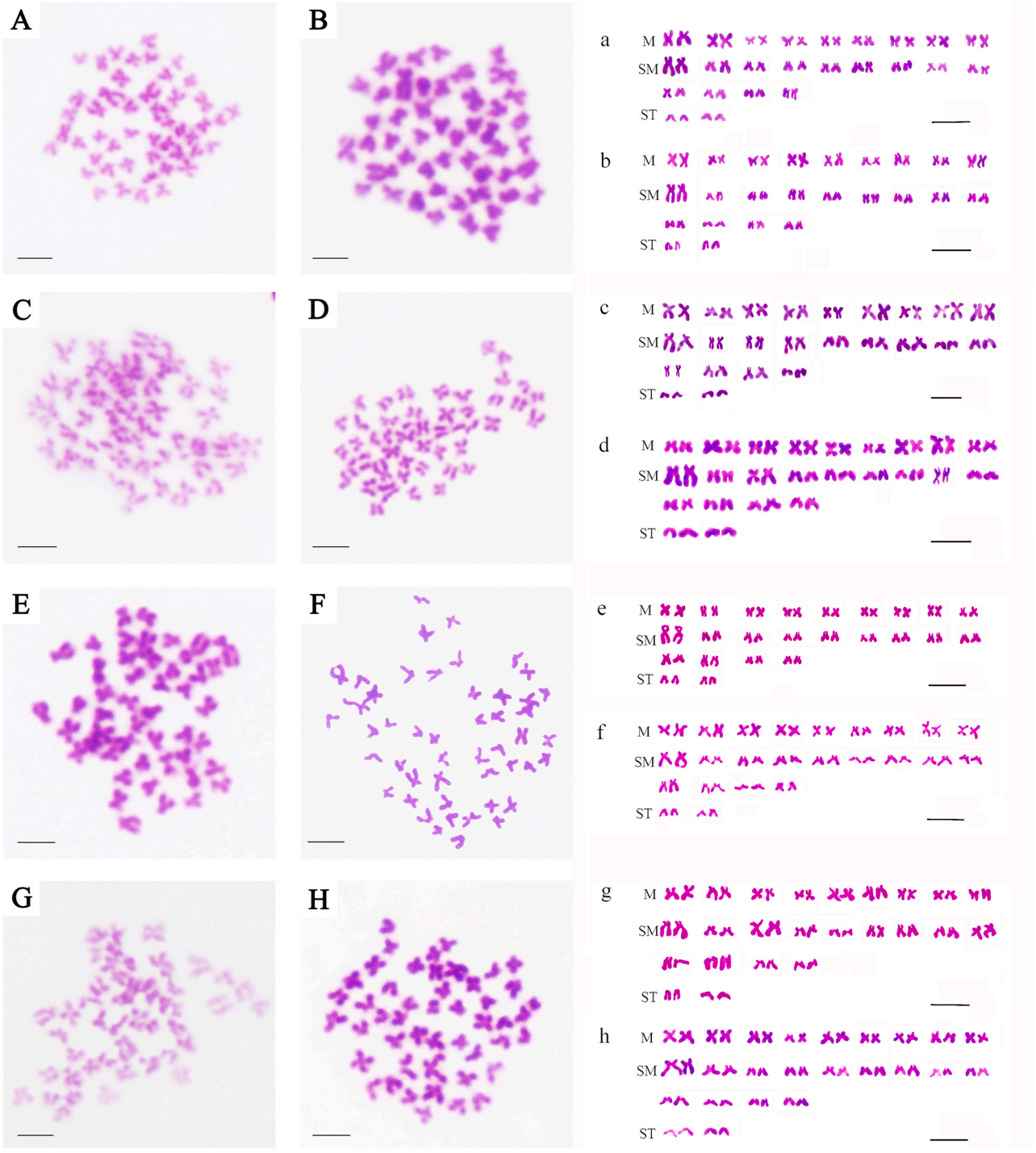


Fig. 3. The chromosome spreads and karyotype at metaphase in BSB, TC and their hybrid lineage (BTF_1-BTF_6). (A) The chromosome spreads of BSB. (B) The chromosome spreads of TC. (C) The chromosome spreads of BTF_1. (D) The chromosome spreads of BTF_2. (E) The chromosome spreads of BTF_3. (F) The chromosome spreads of BTF_4. (G) The chromosome spreads of BTF_5. (H) The chromosome spreads of BTF_6. (a) the of BSB (18 m + 26 sm + 4 st). (b) the karyotype of TC (18 m + 26 sm + 4 st). (c) the karyotype of BTF_1 (18 m + 26 sm + 4 st). (d) the karyotype of BTF_2 (18 m + 26 sm + 4 st). (e) the karyotype of BTF_3 (18 m + 26 sm + 4 st). (f) the karyotype of BTF_4 (18 m + 26 sm + 4 st). (g) the karyotype of BTF_5 (18 m + 26 sm + 4 st). (h) the karyotype of BTF_6 (18 m + 26 sm + 4 st). Bar= 20 μm.

3. Results

3.1. Establishment of hybrid lineage

BTF_1 hybrids were generated through the intergeneric crossing of BSB (2 n = 48, ♀) and TC (2 n = 48, ♂), BTF_2 hybrids were produced via the self-crossing of fertile BTF_1 fish. This process culminated in the establishment of a new allodiploid hybrid lineage (BTF_1-BTF_6, 2 n = 48) through successive self-crossing (Fig. 1).

3.2. Determination of ploidy in the hybrid lineage

Based on the establishment of the hybrid lineage, the DNA content of BTF_1-BTF_6 fish and their parents is illustrated in Fig. 2 and Table 2, while the chromosomal distribution is presented in Fig. 3 and Table 3. The mean DNA content of BSB and TC served as control groups, and

Table 3
Examination of chromosome number of BSB, TC and their hybrid lineage (BTF_1-BTF_6).

Fish type	No. in metaphase	Distribution of chromosome number	
		< 48	48
BSB	150	12	138
TC	150	9	141
BTF_1	300	14	286
BTF_2	300	8	292
BTF_3	300	12	288
BTF_4	300	10	290
BTF_5	300	15	285
BTF_6	300	12	288

Table 4
The countable traits of BSB, TC and their hybrid lineage (BTF_1-BTF_6).

Fish type	No. of lateral scales	No. of upper lateral scales	No. of lower lateral scales	No. of dorsal fins	No. of abdominal fins	No. of anal fins
BSB	49–52	9–10	9–11	III+ 8–9	8–10	III+ 25–27
TC	80–92	16–19	6–7	III+ 7	9	III+ 20–23
BTF_1	59–64	11–13	9–11	III+ 8	9–10	III+ 22–24
BTF_2	57–64	12–14	9–11	III+ 8–9	9–10	III+ 20–25
BTF_3	61–65	11–13	9–10	III+ 8	9	III+ 23–25
BTF_4	60–62	11–13	10–11	III+ 8	9–10	III+ 21–24
BTF_5	60–62	10–12	9–11	III+ 8	9–10	III+ 22–24
BTF_6	58–63	10–12	9–11	III+ 8	9–10	III+ 21–24

III. The number of fin spines.

Table 5
The measurable traits of BSB, TC and their hybrid lineage (BTF_1-BTF_6).

Fish type	FL/BL	BL/BH	BH/HH	HL/HH
BSB	1.18 ± 0.02	2.36 ± 0.04	2.08 ± 0.05	1.19 ± 0.06
TC	1.18 ± 0.03	3.97 ± 0.23	2.27 ± 0.01	2.18 ± 0.27
BTF_1	1.19 ± 0.01	3.33 ± 0.11	2.73 ± 0.04	1.83 ± 0.54
BTF_2	1.17 ± 0.02	3.34 ± 0.16	2.53 ± 0.14	1.68 ± 0.09
BTF_3	1.16 ± 0.02	3.26 ± 0.05	2.29 ± 0.03	1.59 ± 0.06
BTF_4	1.17 ± 0.02	3.21 ± 0.09	2.39 ± 0.05	1.55 ± 0.04
BTF_5	1.21 ± 0.03	3.23 ± 0.18	2.23 ± 0.13	1.47 ± 0.08
BTF_6	1.18 ± 0.04	3.25 ± 0.12	2.34 ± 0.08	1.57 ± 0.06

statistical analysis indicated that the mean DNA content of BTF_1-BTF_6 fish did not significantly differ from that of their parents ($P > 0.05$). Chromosome separation analysis revealed that 92.45 % of BSB and 95.67 % of TC exhibited 48 chromosomes. Furthermore, between 90.84 % and 96.75 % of cells in metaphase from the BTF_1-BTF_6 fish also possessed 48 chromosomes with the karyotype of 18 m + 26 sm + 4 st. Collectively, these findings confirm that the hybrid lineage (BTF_1-BTF_6) is a diploid lineage with a consistent chromosomal count of 48.

3.3. Morphological traits

The morphological traits of BTF_1-BTF_6 fish were assessed and compared with those of the control groups, BSB and TC. Regarding countable traits (Table 4), the number of lateral line scales, upper lateral line rays, and abdominal fin rays in the BTF_1-BTF_6 fish fell between the values observed in BSB and TC. The anal fin rays were also intermediate, leaning towards the counts found in TC. Conversely, the lower lateral line counts in BTF_1-BTF_6 fish were more similar to those of BSB. For measurable traits (Table 5), the FL/BL ratio showed no significant difference ($P > 0.05$) between BTF_1-BTF_6 fish and their parental lines. The BL/BH and HL/HH ratios for BTF_1-BTF_6 fish were situated between those of BSB and TC. Notably, the BH/HH ratio in BTF_1-BTF_6 fish was significantly greater than that of either BSB or TC.

3.4. Screening and amplification of the Hox gene family

Based on the published *Hox* gene clusters of zebrafish and the annotated genomes of BSB and TC, we extracted all homologous full-length sequences of the *Hox* gene family from the BSB and TC genomes. Following homologous alignment of the genes, we identified 10 *Hox* genes (*HoxA1a*, *HoxA3a*, *HoxA9a*, *HoxA13b*, *HoxB1a*, *HoxB3a*, *HoxB8b*, *HoxC6b*, *HoxC12b*, and *HoxD12a*) that exhibited significant differences in SNP sites between the sequences from BSB and TC (Fig. 4). We designed ten pairs of PCR primers to amplify these *Hox* genes from the genomic DNA of BTF_1-BTF_6 fish. A total of 600 cloned *Hox* gene sequences were obtained through Sanger sequencing from the BTF_1-BTF_6 hybrids, with 10 samples analyzed per generation. All sequences from the BSB, TC, and BTF_1-BTF_6 fish were submitted to the GenBank database, with accession numbers detailed in Supplementary Table 1.

3.5. Genetic analysis

Using BSB and TC as controls, we performed multiple sequence alignments and comparative analyses of each *Hox* gene across BSB, TC, and each generation of the hybrid lineage. The *Hox* genes of the hybrids were classified into three categories: BTF_n–T, indicating that the *Hox* gene sequence in the hybrids originated from TC; BTF_n–B, indicating that the sequence derived from BSB; and BTF_n–C, signifying that the hybrid *Hox* gene sequence was chimeric, containing fragments from both BSB and TC (where n represents the generation number of the hybrid lineage). The changes in gene structure for each *Hox* gene among BSB, TC, and the hybrid lineage (BTF_1-BTF_6) fish are illustrated in Fig. 4. Our findings revealed the presence of both paternal and maternal *Hox* genes within the BTF_1-BTF_3 population, suggesting stable inheritance of these genes from both parental genomes in the first three generations. Notably, the absence of TC-derived *HoxB1a* and *HoxB3a* genes in the BTF_4 group and its subsequent offspring indicates a potential loss of paternal genes. Interestingly, the maternal *Hox* genes demonstrated stable inheritance from BTF_1 to BTF_6.

Hybrids, or hybrid lineages with a composite genome, serve as an exemplary model for investigating subgenome fusion, particularly in the context of homologous gene fusion. In this study, we analyzed the paternal- and maternal-specific SNPs within the hybrid lineage to explore the subgenome fusion process. During the hybridization of BSB and TC, seven genes (*HoxA1a*, *HoxA3a*, *HoxB1a*, *HoxB3a*, *HoxC6b*, *HoxC12b*, and *HoxD12a*) in the BTF_1 hybrids exhibited chimerism. Notably, three of these chimeric genes (*HoxA1a*, *HoxA3a*, and *HoxC12b*) were stably inherited through generations BTF_2 to BTF_6. Conversely, the remaining four chimeric genes (*HoxB1a*, *HoxB3a*, *HoxC6b*, and *HoxD12a*) were lost by BTF_3 or BTF_4. Additionally, no instances of chimerism were observed for *HoxA9a*, *HoxA13b*, and *HoxB8b* across the BTF_1 to BTF_6 populations.

4. Discussion

Distant hybridization is a common strategy employed in fish genetics and breeding (Liu, 2010). When F_1 hybrids are fertile, they have the capability to overcome reproductive isolation and initiate a new hybrid lineage. Fish, being lower vertebrates, typically face fewer reproductive isolation barriers compared to higher animals, facilitating the establishment of novel lineages through distant hybridization. A notable example is the creation of the world's first allotetraploid fish lineage, achieved by crossing goldfish (maternal) with common carp (paternal). This lineage has successfully reproduced for up to 29 generations (Liu, et al., 2016) and possesses sufficient diploid gametes to generate sterile triploid fish with rapid growth rates. BSB and TC are prominent freshwater varieties in China, belonging to the genera *Megalobrama* and *Culter*, respectively. Despite being from different genera, the F_1 hybrids between BSB and TC were fertile and subsequently formed a new hybrid lineage through continuous self-crossing, thereby providing a novel

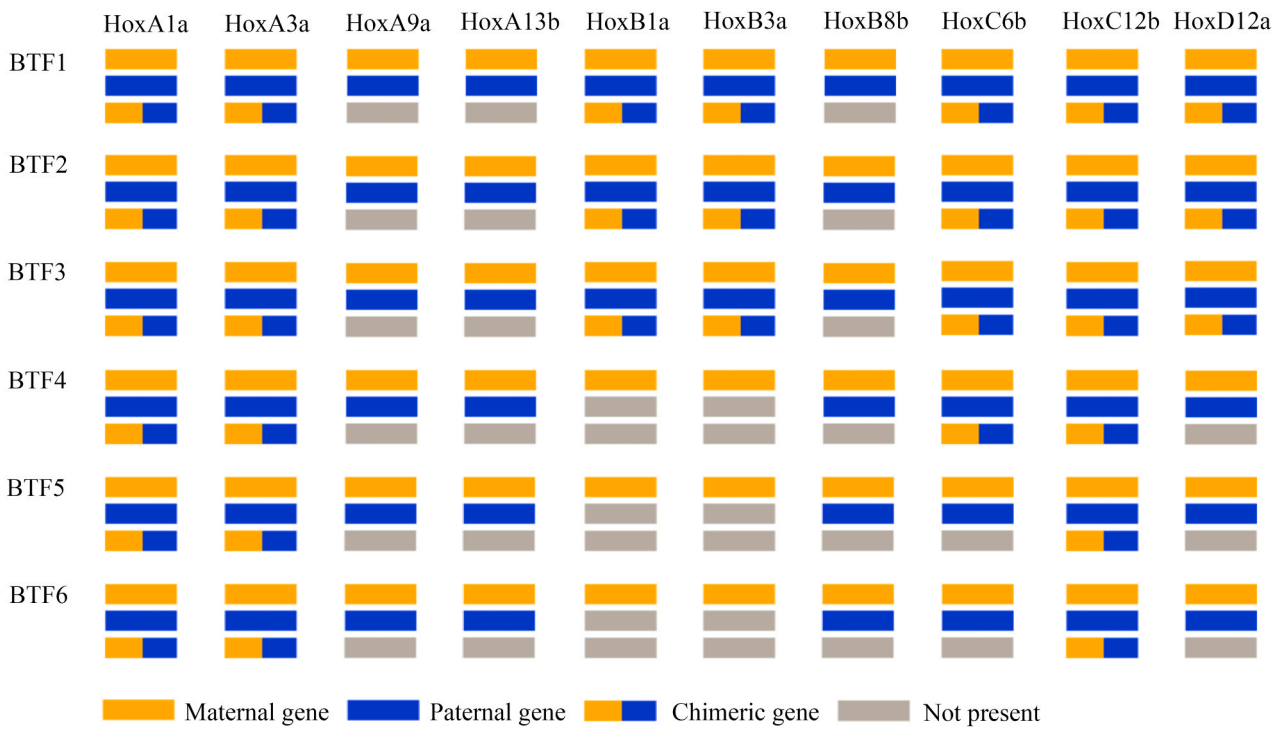


Fig. 4. Diagram regarding genetic variation of hox genes in BTF_1-BTF_6.

source of gametes for future fish breeding endeavors.

The macro-hybrid with significant phenotypic changes refers to the allo-progeny derived from distant hybridization, including the allo-progenies (Liu, et al., 2025). Due to macro-hybridization (Liu, et al., 2024), F_1 hybrids inherit two subgenomes from distinct species, which can lead to instability in subsequent generations. In the cross between *Carassius auratus* red *var.* (♀) and *Megalobrama amblycephala* (♂), allo-tetraploid hybrids in the F_1 generation exhibited 148 chromosomes and were capable of producing autotetraploid offspring with 200 chromosomes (Qin, et al., 2018, 2014). In this study, both parental ITS1 sequences of 45S rDNA were absent in the F_1 hybrids; however, distinct genetic models emerged in the F_2 hybrids, indicating notable genetic differentiation (Xiao, et al., 2016). Consequently, the distribution of differentially expressed genes and alternative splicing in the BSB- and TC-subgenomes showed significant variations between the BTF_3 and TBF_3 hybrids (Ren, et al., 2020). This research investigated the genetic variation within the F_1-F_6 hybrid populations through the analysis of 10 *Hox* genes. Although paternal homologous genes were not identified in the F_4-F_6 hybrid populations, gene flow remained relatively stable in the F_1-F_3 populations. The coexistence and exclusion of subgenomes may be a prevalent mechanism for generating new hybrid lineages or sources.

Mutual chimerism of homologous genes is a prevalent occurrence in hybridization, particularly in cases of distant hybridization. The distinct differences between heterologous genomes facilitate the detection of chimeric genes. In the hybrids of *Carassius auratus* cuvieri (♀) and *Carassius auratus* red *var.* (♂), 19.04 % chimeric genes in the F_1 hybrids and 4.17 % chimeric genes in F_2 hybrids were detected by RNA-seq (Liu, et al., 2018). In liver transcriptomes spanning four generations, over 9 % of chimeric genes were identified, encompassing 16 distinct chimeric gene patterns in the offspring (Liu, et al., 2016). Based on the different SNPs found in the BSB and TC genomes, seven chimeric genes were validated in the F_1 hybrid population; however, only four of these chimeric genes persisted in the F_6 population. Although the molecular mechanisms underlying chimeric gene formation remain unclear, analogous phenomena have been documented in hybrid crucian carp. For instance, the chimeric ferritin H present in hybrids of *Carassius auratus* cuvieri (♀) and *Carassius auratus* red *var.* (♂) plays a regulatory role in inflammatory signaling pathways associated with lipopolysaccharide-induced NF-kappa B activation. Despite the fluctuating proportion of chimeric genes in this hybrid lineage, self-crossing still yields viable offspring. This stability may be attributed to the high genomic similarity between BSB and TC, which allows for the maintenance of normal biological functions even when the two genomes are combined in varying proportions (Wu, et al., 2020). Additionally, the emergence of chimeric genes may represent a novel strategy to mitigate genomic incompatibilities in hybrid genomes.

In this study, we established a hybrid lineage (BTF_1-BTF_6) from the hybrid of BSB ♀ and TC ♂, and further conducted a screening of the BSB and TC genomes, selecting ten specific *Hox* genes (*HoxA1a*, *HoxA3a*, *HoxA9a*, *HoxA13b*, *HoxB1a*, *HoxB3a*, *HoxB8b*, *HoxC6b*, *HoxC12b*, and *HoxD12a*) to differentiate between the BSB and TC genomes. We then investigated rapid genomic changes in the BTF_1-BTF_6 hybrid population through these ten specific *Hox* genes, analyzing the alterations in chimeric gene formation. These findings offer valuable insights into genetic variation during hybrid lineage formation and the process of hybrid speciation.

CRediT authorship contribution statement

Xu Huang: Software, Funding acquisition. **Jiawang Huang:** Resources. **Shi Wang:** Visualization, Conceptualization. **Qian Chen:** Methodology, Formal analysis, Data curation. **Fangzhou Hu:** Supervision, Conceptualization. **Chang Wu:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition. **Jiayi Chen:** Methodology. **Yang Lu:** Investigation. **Liang Kuai:** Visualization. **Li Ren:** Software. **Jinhui Huang:** Resources, Methodology. **Wuhui Li:** Resources, Methodology. **Yuxiang Wang:** Validation, Software. **Shaojun Liu:** Writing – review & editing, Project administration, Funding acquisition. **Ming Wen:** Formal analysis. **Jun Xiao:** Resources. **Lang Qin:** Investigation, Data curation.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2026.103368.

Data availability

Data will be made available on request.

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