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Triploidization of hybrids (female zebrafish × male blunt snout bream) by heat-shock can improve survival rate

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ABSTRACT

The high mortality rate of hybrid offspring in distant hybridization is believed to be caused by distant genetic relationships. In this study, the hybrids from female zebrafish (*Barchydanio rerio var*, 2n = 50, ZF) and male blunt snout bream (*Megalobrama amblycephala*, 2n = 48, BSB) could not survive into adulthood. However, by heat shock treatment of hybrid embryos shortly after fertilization, we have obtained a large number of hybrid offspring that can survive to adulthood. These offspring have been confirmed to be all-male hybrid triploids (n = 74) and can develop to sexual maturity. Our results demonstrate that artificial induction of triploids may improve the survival rate of distant hybrids. Our knowledge from this study may extend to other distant hybridization of fish and may have implications for the practice of hybrid fish breeding.

1. Introduction

Hybridization is an important breeding method for obtaining new varietie (Abbott et al., 2013; Barton, 2001; Grant et al., 2005; Mallet, 2007; Seehausen, 2004; Williamson, 2006). Hybrid breeding can increase genetic diversity, and different genomic combinations can change the genetic composition of hybrid offspring. It can combine favorable traits, achieve heterosis and obtain new varieties with superior phenotypes relative to parents (Fitzpatrick and Shaffer, 2007; Lippman and Zamir, 2007; Longin et al., 2012; Shull, 1908). Hybridization between different species of the same genus or different genera of the same family is also called distant hybridization. Distant hybridization can break through species boundaries, expand genetic variation and create new variation types or species (Chen et al., 2018; Engle et al., 2017; Hermsen, 1992; Tseng and Poon, 1983; Zhang et al., 2014).

Due to the huge genetic and physiological differences of parents, there are difficulties in distant hybridization breeding, such as the incompatibility between the alleles from parents and the disorder of gene regulation which cause the death of the hybrid offspring (Chen et al., 2018; Lou and Li, 2006). Based on a large body of distant hybridization breeding experience, Liu (2010) proposed a hypothesis: in general, when the chromosome number of maternal fish is less than that of paternal fish, a distant hybridization hardly produces living offspring, such as for the male common carp hybrid with female blunt snout bream (Wang et al., 2017) and the male Japanese crucian carp hybrid with female blunt snout bream (Hu et al., 2018). It has been suggested that even though the parents have the same number of chromosomes, the offspring of the distant hybridization are still difficult to survive if their karyotypes are strikingly different (Lou and Li, 2006; Wang et al., 1986; Zhang and Jin, 1984). It is critical to further understand how to improve the survival rate of distant hybrid offspring.

Blunt snout bream (*Megalobrama amblycephala*, 2n = 48, BSB) is an economic fish unique to China (Gong et al., 2019). In this study, BSB and zebrafish (Barchydanio rerio var., 2n = 50, ZF) were chosen as the parent fish for hybridization. We founded that the hybrid offspring could not survive. How-

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ever, when the hybrid embryos were treated by heat shock, some of hybrid offspring (TZB, ZF (♀) × BSB (♂)) could survive. These TZB were not different from ZF in appearance and somatotype, but ploidy analysis showed that they were triploids with karyotype consisting of two sets of chromosomes from ZF and one set from BSB. In addition, TZBs were a male group that they reached sexual maturity at 3 months of age. Our data suggest that artificial induction of triploids may increase the survival rate of hybrids from distant hybridizations.

2. Materials and methods

2.1. Ethics statement

All experimental procedures involving fish were approved by the Institutional Animal Care and Use Committee of Hunan Normal University. Procedures were conducted following the regulations of the Administration of Affairs Concerning Experimental Animals for the Science and Technology Bureau of China.

2.2. Fish

BSB and ZF were maintained at the State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University. The offspring of ZF (♀) × BSB (♂) crossing were obtained by artificial insemination. Heat shock was conducted as previously described (Kavumpurath and Pandian, 1990; Pandian and Koteeswaran, 1998). Briefly, embryos were transferred into 41.4 °C for 2 min at 2 min after fertilization, and then cultured in 28 °C water.

2.3. Ploidy analysis

Fish were anesthetized with 100 mg/l MS-222 (Sigma-Aldrich, China) before dissection. Pieces of caudal fin were obtained, washed with phosphate buffer solution (PBS), shred and filtered through a 30 μm cell strainer (Sysmex, Germany), and then incubated with DAPI (Sysmex, Germany) for 10 min in dark. Finally, they were measured by flow cytometry (Sysmex-partec, Germany).

Nuclear measurements of erythrocytes were used to further ascertain ploidy. Fish blood samples were stained with Wright's solution (Solarbio, China) following the manufacturer's protocol. About 20 erythrocytes per fish were measured using an OLYMPUS inverted microscope (BX63, Japan). The volume of nuclear was calculated by (4/3) πabˆ2, where “a” was the major semi-axis and “b” was the minor semi-axis of normal ellipsoid. The results of measurement are slightly biased due to the flattening of cells. χ^2 test with Yate's correction was used for quantifying deviations from expected ratios (Liu et al., 2007).

2.4. Karyotyping

The chromosomal preparations of ZF and BSB were performed using kidney tissue, the method was described in Qin et al. (2015). Fish were injected with concanavalin at a dose of 5 mg/g body weight. After 12 h, each fish was injected with colchicine at a dose of 4 mg/g body weight. Six hours after injection, fish was anesthetized with 100 mg/l MS-222 (Sigma-Aldrich, China) before dissection. Pieces of kidney tissue were obtained, washed with PBS, then shred in 0.9% NaCl.

The chromosomes of TZB were prepared with short term cultured caudal fin cells. Pieces of caudal fin (~ 0.2 cm^2) were washed with PBS after a quick rinse with 70% alcohol, then digested with 0.25% trypsin (Invitrogen, China) for 15–30 min. Fin cells were cultured in a complete growth medium at 28 °C, as shown by Zhou et al. (2016). Confluent cells were treated with 0.2 μg/ml colchicine for 4–6 h before chromosome preparation.

The cells, from kidney or fin, were subjected to hypotonic treatment with 0.075 mol/L KCl at 28 °C for 40–60 min, fixed twice with cold Carnoy's fixative (methanol/ glacial acetic acid, 3:1, v /v) for 1 h each time, then dropped onto slides. The chromosomes were stained with 5% Giemsa solution (Solarbio, China) for 20 min. >30 metaphases were examined per sample.

2.5. Histology of gonad

Gonad tissue was surgically excised from fish and fixed in Bouin's solution. The fixed tissues were first dehydrated, then embedded in paraffin. Sections were cut at 5–6 μm using a Leica RM2015 Microtome (Leica, Germany), then transferred to slides, which were processed for haematoxylin and eosin staining according to procedures described in Xiao et al. (2013).

2.6. DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from the caudal fin of fish using a kit (OMEGA, USA) following the manufacturer's protocol. PCR was performed with degenerate primers for the HMG box of *sox* gene: P (+) [5'-TGAAGCGACCCATGAA(C/T)G-3′] and P (–) [5′- AGGTCG (A/G)TACTT(A/G)TA (A/G)T-3′] (Lin et al., 2009). A 20 μl of reaction contained 1 μl (~ 100 ng) of genomic DNA, 10 μl of high-fidelity DNA polymerase PCR mix (2×) (TsingKe, Beijing, China), 0.5 μl (10 μm) of each primer and 8 μl of ddH_2O. The thermal cycling program consisted of 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 50 s, with a final extension of 7 min at 72 °C. The PCR products were analyzed by electrophoresis on 1.5% agarose gels. PCR fragments with expected size were purified using a gel extraction kit (OMEGA, USA) and cloned into the pMD18-T cloning vector (TaKaRa, Dalian, China). The cloned PCR fragments were sequenced by Sagon (Shanghai, China). Sequencing results were analyzed using programs BioEdit (Hall, 1999) and Clustal W (Larkin et al., 2007).

3. Results

3.1. Generation of hybrid fish

The hybridization of ZF (♀) × BSB (♂) resulted in a large number of malformed embryos with low hatching rate (about 14.98%), and no adult hybrid was obtained (Table 1, Fig. 1C, E). When the hybrid embryos from ZF (♀) × BSB (♂) were treated with heat shock at 41.4 °C for 2 min at 2 min post fertilization (Kavumpurath and Pandian, 1990; Pandian and Koteeswaran, 1998), the hatching rate of hybrid offspring significantly increased to 28.01%. About 20.86% of them developed into normal larvae after 7 days of hatched (Table 1, Fig. 1F). These heat shock treated hybrid fish, named as TZB, were able to survive to adulthoods. They were indistinguishable from ZF in terms of body size and shape (Fig. 1G, H).

Table 1
Fertilization and hatching rates of ZF (♀) × BSB (♂) and TZB.

Group		No.of eggs	Fertilization, (%)	Hatching, (%)	7 days after hatched larvae with normal phenotype, (%)
ZF (♀) × BSB (♂) (untreated)	1	234	210 (89.74%)	27 (12.85%)	0
	2	278	262 (94.24%)	46 (17.55%)	0
	3	328	296 (90.24%)	42 (14.18%)	0
	4	381	347 (91.08%)	54 (15.56%)	0
	5	264	237 (89.77%)	35 (14.77%)	0
	Average		90.91%	14.98%	0
TZB (Treated with heat shock)	1	439	386 (87.93%)	144 (37.31%)	38 (26.39%)
	2	505	462 (91.46%)	133 (28.79%)	21 (15.79%)
	3	671	624 (93.00%)	164 (26.28%)	35 (21.34%)
	4	554	496 (89.53%)	105 (21.17%)	18 (17.14%)
	5	466	415 (89.06%)	110 (26.51%)	26 (23.64%)
	Average		90.20%	28.01%	20.86%

3.2. Ploidy analysis of hybrid offspring treated with heat shock

Peripheral blood smears showed that erythrocytes of all the three fish were oval, but the size of TZB erythrocyte was larger than that of ZF (Fig. 2A, C), but smaller than that of BSB (Fig. 2B, C). Nuclear measurements (Table 2) showed that the nuclear volume of ZF erythrocytes was similar to that of BSB, while that of TZB was significantly larger. Really, the ratio of nuclear volume of TZB erythrocytes is almost equal to the sum of that of ZF and half of BSB ($P > .05$).

The DNA contents of TZB erythrocytes were determined by flow cytometry analysis. As shown in Fig. 2, the average relative fluorescence intensity of ZF, BSB and TZB on DNA histogram were 120.36, 92.31, and 155.13, respectively (Fig. 2D, E, F). The data showed that DNA content of TZB was equal ($P > .05$) to the sum of that of ZF and half of BSB (Table 3). The results of erythrocytes measurement and flow cytometry showed that TZBs were triploid.

3.3. Karyotype analysis

Chromosomes preparations were made from ZF, BSB and TZB. 30 metaphase phases of each sample were counted under optical microscope. As shown in Table 4, there were 90.83% of metaphases with 50 chromosomes in ZF (Fig. 3A), their karyotype formula is 12 m + 26 sm + 12 st (Fig. 3B); In BSB, 93.33% of metaphases had 48 chromosomes (Fig. 3C), their karyotype formula is 18 m + 22 sm + 8 st (Fig. 3D). These results were consistent with previous studies (Hu et al., 2018; Liu et al., 2007; Pijnacker and Ferwerda, 1995). In TZB, 89.16% of metaphases had 74 chromosomes with karyotype formula of 19 m + 41 sm + 14 st (Fig. 3E, F). Notably, a largest submetacentric chromosome was presented in the metaphase chromosome set of TZB, it was used as a marker chromosome for BSB species (Liu et al., 2007). By comparing the karyotypes of these three fish species, we further demonstrated that TZB was triploid with two sets of chromosomes from ZF and one set from BSB.

3.4. Genetic identification using the Sox-HMG marker

Sox-HMG genetic markers are DNA fragments amplified from different species by degenerate PCR. PCR primers were designed to amplify a conserved HMG domain of the *Sox* genes of different species (Lin et al., 2009). Liu et al. (2007) used *sox*-HMG to rapidly identify the genetic background of different hybrid lines, and confirmed t hat t wo specific DNA fragments could be amplified from the BSB genome with *sox*-HMG primers. We performed genetic analysis of ZF, BSB and TZB by PCR with *sox*-HMG primers. As shown in Fig. 4A, only one DNA band (215 bp) was seen in ZF, while two DNA bands (215 bp and 714 bp) were presented in BSB. The two DNA bands, 215 bp and 714 bp, could also be seen in TZBs. The 714 bp fragments from TZB and BSB were sequenced. DNA alignment results indicated a similarity of 99.86% between the two DNA fragments (Fig. 4B).

3.5. Fertility of ploidy-treated hybrid fish

Paraffin s ections o f t he g onads o f 1 4 T ZB fi sh (3 -month-old) were prepared. Histology analysis revealed that all the gonads were typical testis with a large number of spermatogenic cells at different development stages, such as spermatogonia, sper-

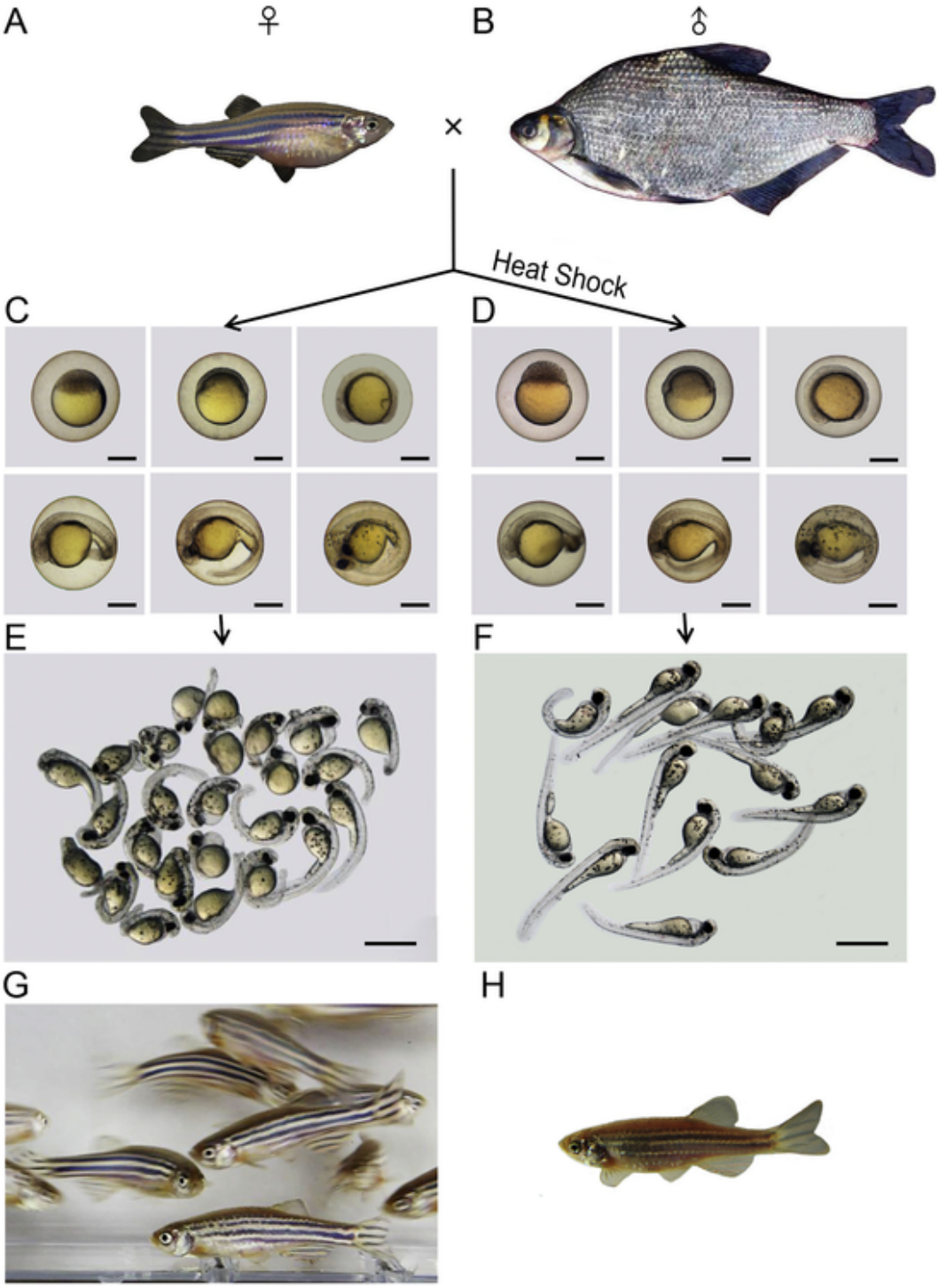

Fig. 1. Hybridzation of female ZF and male BSB, and their heat shock treated hybrid offspring TZB. (A) Female zebrafish. (B) Male blunt snout bream. (C) hybrid embryos from ZF (♀) × BSB (♂), untreated. And (D) shown the hybrid embryos treated with heat shock. The embryos were at the stages of blastula, gastrula, optical vesicle, muscle effection, eye pigmentation, and body pigmentation, respectively. The larvae of untreated hybrid offspring (E) and hybrids treated with heat shock (TZB) (F) (G, H) shown the adult TZB. The scale bars in (C, D) represent 0.2 mm and those in (E, F) represent 1 mm.

matocytes, spermatids, even a small amount of sperm (Fig. 5A, B).

We further determined the DNA content of sperm of 3-month-old TZBs by flow cytometry. Results showed that the DNA content of TZB sperms, higher than that of ZF and BSB sperm, was about half that of TZB erythrocyte (Fig. 5G—F). Furthermore, when TZB was crossed with female ZF, the fertilization rate was low (< 20%), and the most embryos were malformed (Fig. 5G-I), no larvae could survive beyond 7 days after hatching (Fig. 5J).

4. Discussion

In distant hybridization, there are distant genetic relationship between hybridization parents, such as the large differ-

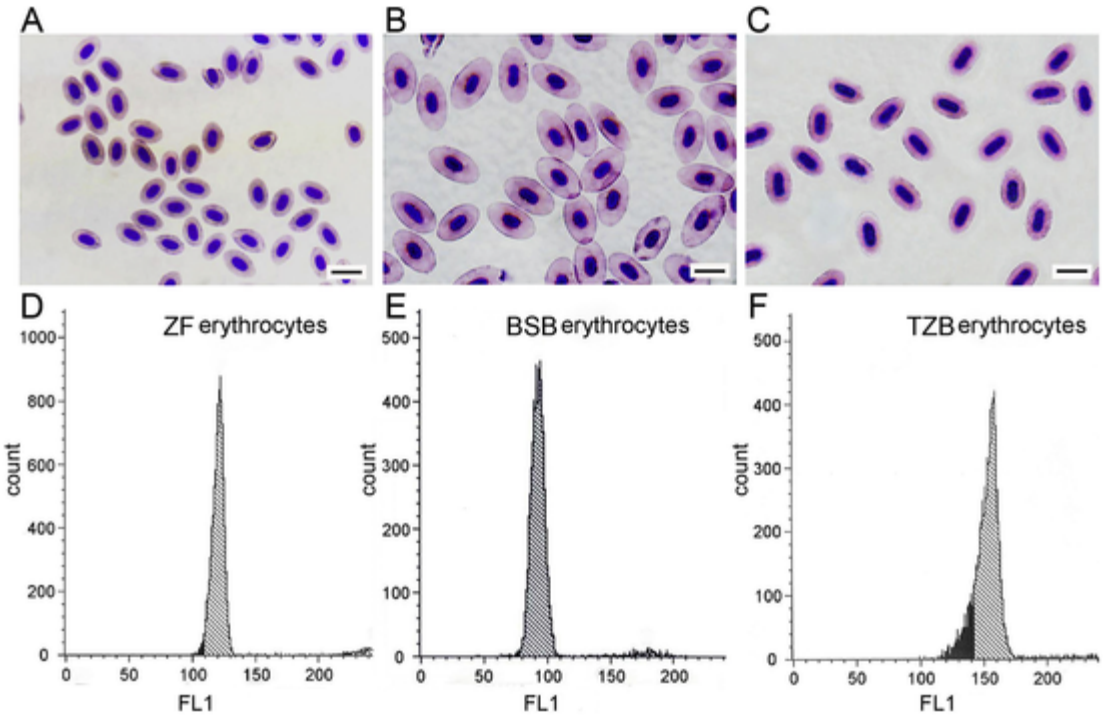

Fig. 2. Ploidy analysis. Erythrocytes morphology of ZF (A), BSB (B) and TZB (C), Scale bars represent 10 μm. Flow cytometry for detection of ZF (D), BSB (E) and TZB (F) DNA content, the X-axis FL1 shows the relative fluorescence intensity (represents the DNA content), the Y-axis represents the number of cells. The results were obtained from at least three independent experiments.

Table 2
Nuclear size of ZF, BSB, and TZB erythrocytes.

Fish type	Nuclear			Volume ratio	
	Major axis (μm)	Minor axis (μm)	Volume (μm^3)	Observed	Expected
ZF	5.352 ± 0.31	2.47 ± 0.16	17.18 ± 2.40		
BSB	5.53 ± 0.50	2.52 ± 0.19	18.59 ± 3.58		
TZB	6.13 ± 0.43	2.75 ± 0.21	24.30 ± 3.01	TZB/(ZF + 0.5BSB) = 0.92 [a]	1

[a] The observed ratio was not significantly different ($P > .05$) from the expected ratio. In each sample, 50 erythrocytes were measured.

ence in chromosomes number or karyotypes of parents, the incompatibility between alleles from parents and the disorder of gene regulation cause the death of most hybrid embryos before hatching, and the hybrid offspring with low hatching rate (Lou and Li, 2006; Wang et al., 1986; Yu et al., 1989; Zhang and Jin, 1984). The distant relationship may also disrupt the temporal ordering of allelic gene expression, lead to asynchronized or mutually inhibited allelic gene expression. Qing et al. (1997) examined the expression of isozymes in the hybrids of grass carp and blunt snout bream, they found that genes derived from the female parent in the hybrids were expressed early, whereas genes derived from the male parent were inhibited. These evidences suggest that incompatibility of enzymes could lead to abnormal embryonic tissues and organ formation, resulting in malformation or death of hybrid embryos. Meanwhile, it has also been reported that maternal gene products in the egg cytoplasm affected gene expression in hybrid embryos. If the cytoplasm of egg does not properly coordinate with the nuclear DNA of sperm, it would inhibit or accelerate the expression of certain genes, resulting in the dealth of embryos before hatching, and even if a small part of them could be hatched, it was difficult for them to develop into adulthood (Lou and Li, 2006; Whitt, 1981).

Most of the distant hybridization of fish were carried out between different subfamilies or genera of the Cyprinidae (Lou and Li, 2006), such as the grass carp × blunt snout bream (He et al., 2013), the grass carp and topmouth culter (Wu et al., 2019) and the Japanese crucian carp × blunt snout bream (Hu et al., 2018). Some previous studies also suggested that the triploid hybrids might survive better than the diploid hybrids. In hybridization experiments between female *Rana catesbeiana* and male *Rana clamitans melanota*, some major embryonic developmental events were blocked in the gastrulation stage, resulting in few survived hybrids. Further investigation revealed that these survived offspring were triploid hybrids (Elinson and Briedis, 1981). In fish, Qing et al. (1997) also found that the survived hybrid offspring were triploid and no viable diploids in the cross between female grass carp and male blunt snout bream. Evidence from a serial studies also strongly support the hypothesis that triploid hybrids survived better than diploid in the cross between grass carp and Bighead carp (Márián and Krasznai, 1978, Sutton et al., 1981 and Beck and Biggers, 1982). In distant hybridization, in-

Table 3
DNA contents of ZF, BSB and TZB erythrocytes.

Fish	Fluorescence intensity	Observed ratio	Expected ratio
ZF	120.36		
BSB	92.31		
TZB-1	155.13	TZB/(ZF + 0.5BSB) = 0.93 [a]	1
TZB-2	161.42	TZB/(ZF + 0.5BSB) = 0.97 [a]	1
TZB-3	160.28	TZB/(ZF + 0.5BSB) = 0.96 [a]	1
TZB-4	158.46	TZB/(ZF + 0.5BSB) = 0.95 [a]	1
TZB-5	163.29	TZB/(ZF + 0.5BSB) = 0.98 [a]	1
TZB-6	161.27	TZB/(ZF + 0.5BSB) = 0.97 [a]	1
TZB-7	159.56	TZB/(ZF + 0.5BSB) = 0.96 [a]	1
TZB-8	169.17	TZB/(ZF + 0.5BSB) = 1.02 [a]	1
TZB-9	166.23	TZB/(ZF + 0.5BSB) = 1.00 [a]	1
TZB-10	163.49	TZB/(ZF + 0.5BSB) = 0.98 [a]	1
TZB-11	164.32	TZB/(ZF + 0.5BSB) = 0.99 [a]	1
TZB-12	163.61	TZB/(ZF + 0.5BSB) = 0.98 [a]	1
TZB-13	165.57	TZB/(ZF + 0.5BSB) = 0.99 [a]	1
TZB-14	164.29	TZB/(ZF + 0.5BSB) = 0.99 [a]	1

[a] The observed ratio was not significantly different ($P > .05$) from the expected ratio. ZF and BSB as controls and 14 TZBs were detected.

Table 4
Chromosome numbers in ZF, BSB, and TZB.

Fish type	No. in metaphase	Distribution of chromosome number					
		<50	50	<48	48	<74	74
ZF	120	11	109				
BSB	120			8	112		
TZB	120					13	107

The chromosomal preparations were performed from 4 ZF, 4 BSB and 4 TZB.

compatibility, not only between the female pronucleus and male pronucleus, but also between the nuclei and cytoplasm, led to developmental disorders in hybrids. The triploid hybrids, with two maternal or paternal alleles, might compensate the reduced gene expression of the third allele and ensure normal synthesis of genetic materials and metabolism, which is critical for the survival of hybrid offspring.

In this paper, we selected a distant taxonomic relationship between zebrafish (ZF) and blunt snout sea bream (BSB) for hybridization. They belong to different subfamilies and have similar chromosome numbers, but they are distinct in morphological characteristics and karyotypes (Liu et al., 2007; Pijnacker and Ferwerda, 1995). We hybridized female ZF with male BSB, as expected, did not obtain surviving hybrid offspring. When treated by heat shock, a large proportion of hybrid embryos can survive into adulthood. These surviving offspring were identified as hybrid triploids. In other fish hybrids, for example, female blunt snout bream × male red crucian carp, female red crucian carp × male zebrafish. Their hybrid offspring could not survive, but some of them survived after heat shock treatment (data have not yet been published). These results showed that the hybrid fish survived after heat shock treatment were triploids. Our data further demonstrated that triploid induction can improve the survival rate of distant hybrids.

At the molecular level, compatibility of genes between species is very important in cross-breeding. In this study, that the ZF (♀) × BSB (♂) hybrid offspring could not survive indicated that the gene between ZF and BSB were incompatible. Miraculously, this incompatibility can be overcome when the hybrid offspring were induced into triploid. TZBs have two sets of chromosomes from ZF and one set from BSB, which were morphologically closer to ZF. However, it is unclear what kind of relationship between the parental genetic materials in hybrid offspring. Cross-breeding is one of the most effective ways to cultivate improved varieties. It is necessary to further under-

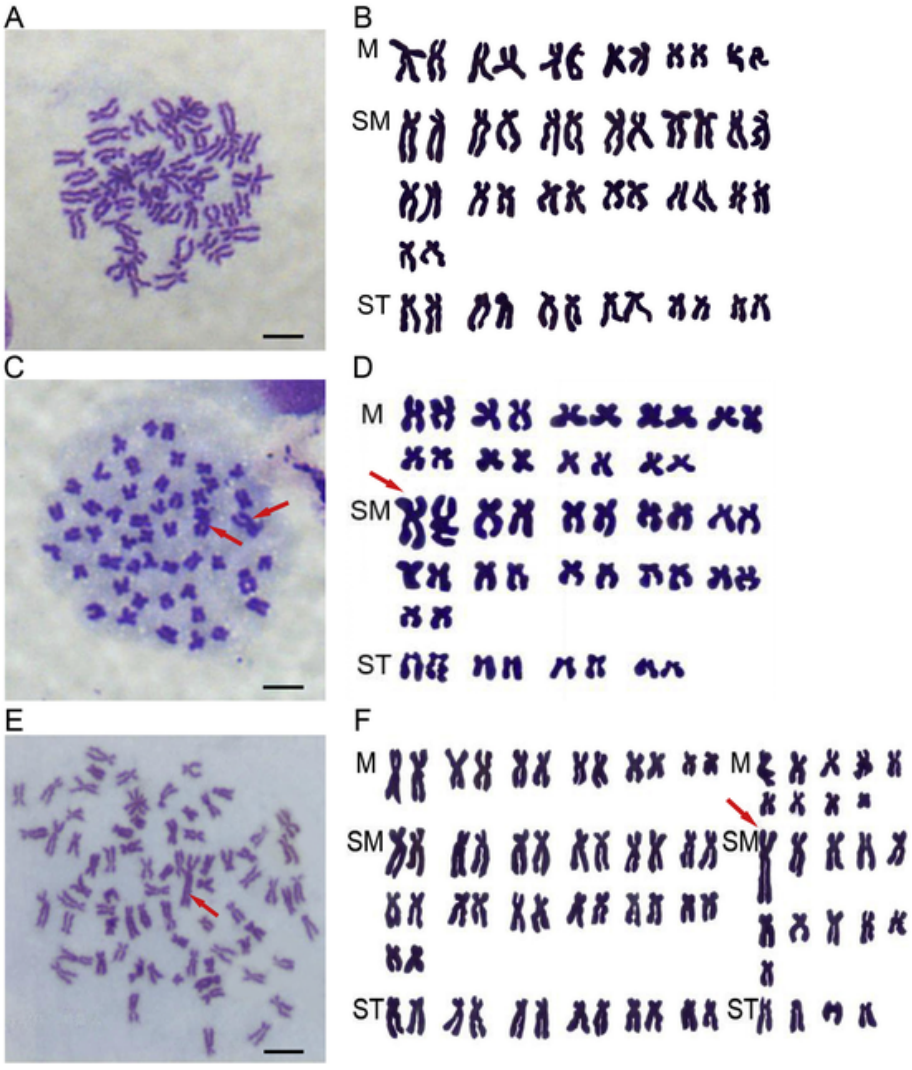

Fig. 3. Chromosome metaphase and karyotypes of ZF (A, B), BSB (C, D) and TZB (E, F). The arrows indicate the BSB-specific large chromosome, scale bars represent 10 μm.

stand the genetic effects of parents and the mechanisms of their interaction during the formation of hybrid triploids, including the introgressive hybridization between species in distant hybridization.

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Declaration of Competing Interest

The authors have declared that no competing interest exists.

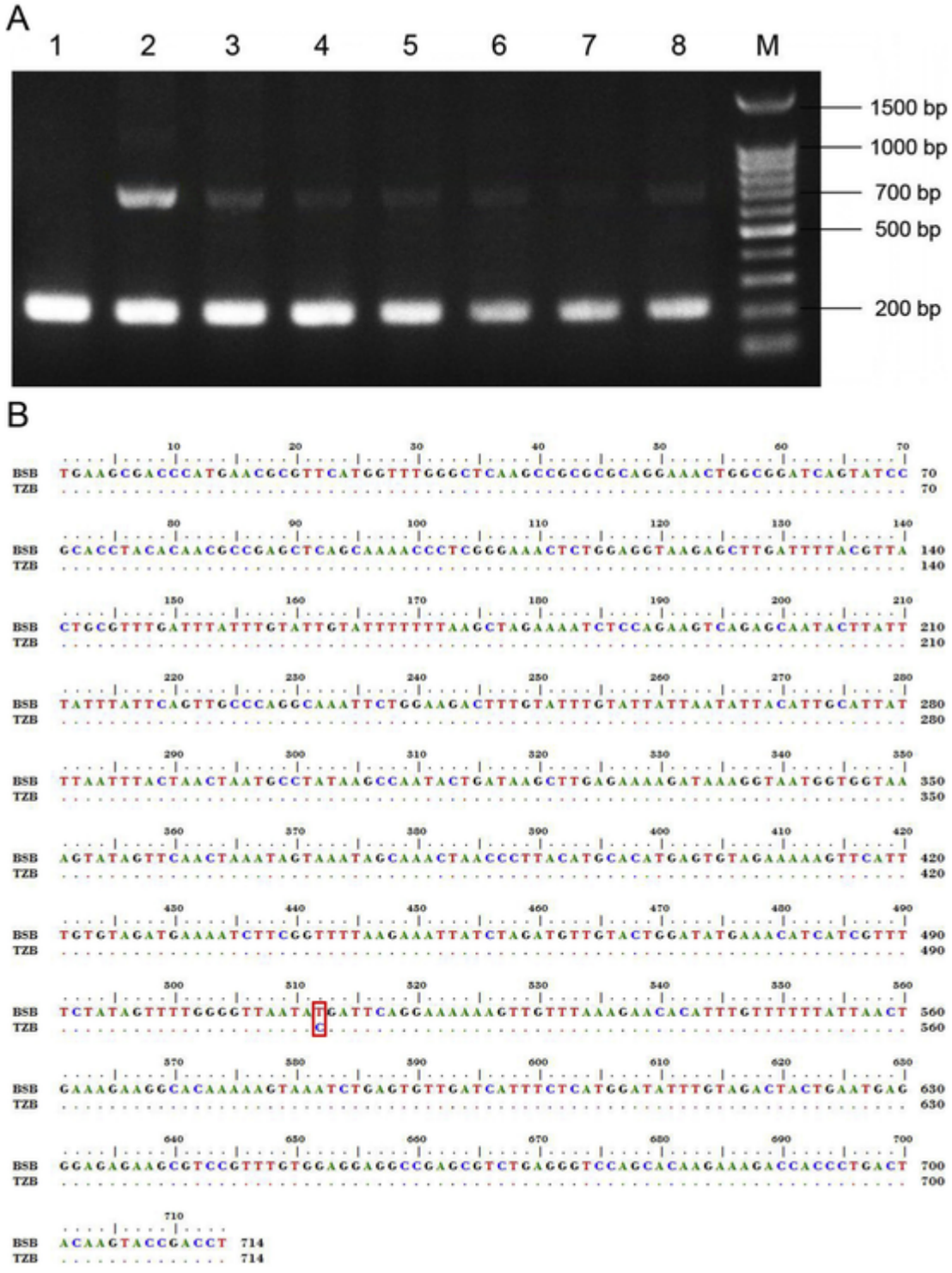

Fig. 4. Genetic analysis by PCR and sequence alignment. (A) Degenerate PCR with sox-HMG primers. 1:ZF, 2:BSB, 3–8:TZBs, M: 100 bp DNA marker. The results were obtained from at least three independent experiments. (B) Sequence alignment of the 714 bp DNA fragment from BSB and TZB.

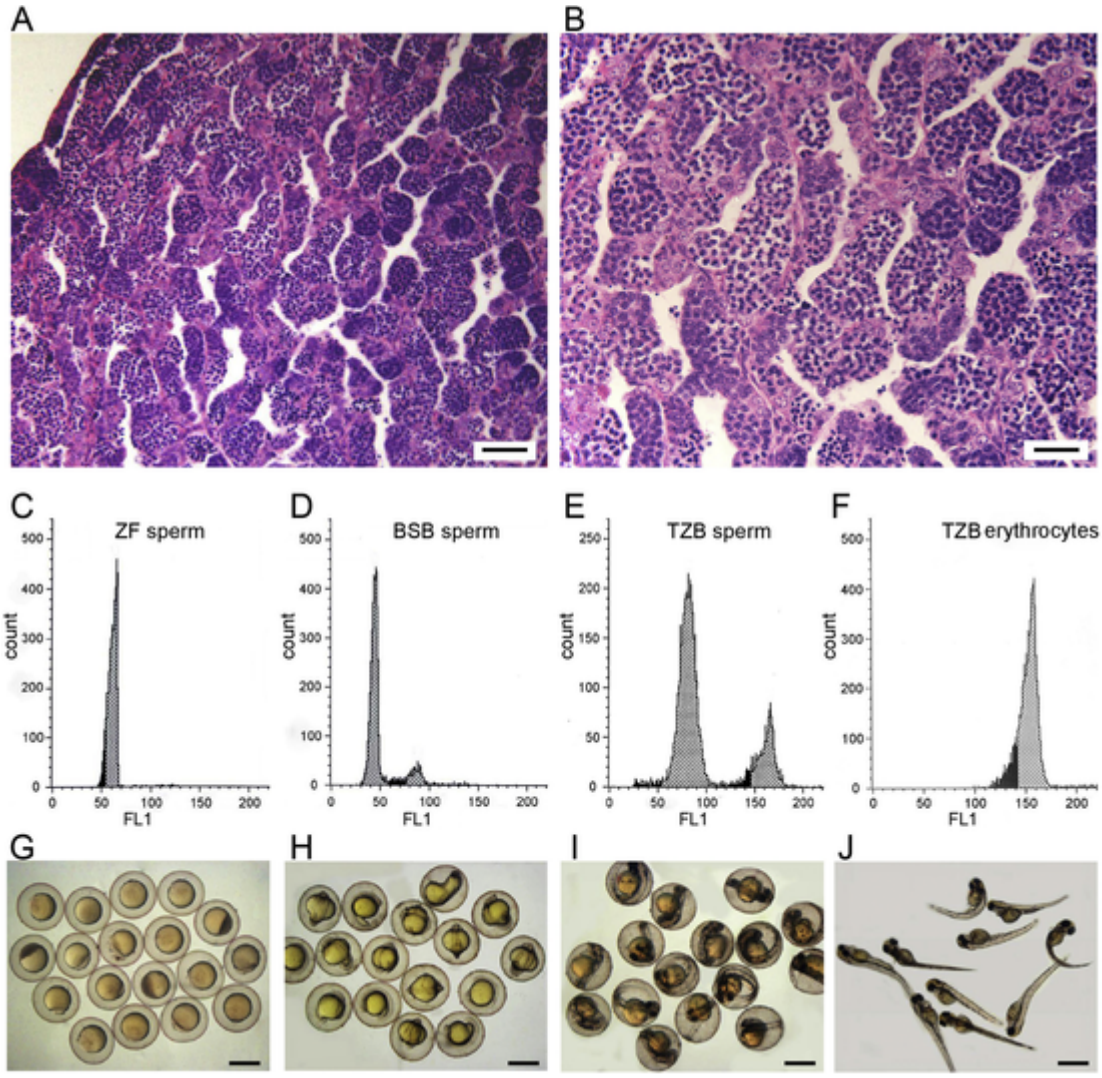

Fig. 5. Fertility analysis of TZBs. Histology of gonads of 3-month-old TZBs, The scale bars in (A) represents 50 μm and that in (B) represents 20 μm. (C-E) Flow cytometry analysis of sperm DNA contents of 3-month-old ZF, BSB, and TZB, respectively. (F) Flow cytometry analysis of DNA contents of TZB erythrocytes, the X-axis FL1 is the relative fluorescence intensity, the Y-axis represents the number of cells. (G-J) shown the backcrossing offspring from female ZF × male TZB: the embryos of blastula(G), optical vesicle stage (H), body pigmentation stage (I), and larvae (J), scale bars represent 1 mm. The results were obtained from at least three independent experiments.

References

Abbott, R., Albach, D., Ansell, S., Arrntzen, J.W., Baird, S.J., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Möst, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice, A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., Stelkens, R., Szymura, J.M., Väinölä, R., Wolf, J.B.W., Zinner, D., 2013. Hybridization and speciation. J. Evol. Biol. 26 (2), 229–246.

Barton, N.H., 2001. The role of hybridization in evolution. Mol. Ecol. 10, 551–568.

Beck, M.L., Biggers, C.J., 1982. Chromosomal investigation of Ctenopharyngodon idella × Aristichthys nobilis hybrids. Cell. Mol. Life Sci. 38 (3), 319.

Chen, J., Luo, M., Li, S., Tao, M., Ye, X., Duan, W., Zhang, C., Qin, Q., Xiao, J., Liu, S.J., 2018. A comparative study of distant hybridization in plants and animals. Sci. China Life Sci. 61 (3), 285–309.

Elinson, R.P., Briedis, A., 1981. Triploidy permits survival of an inviable amphibian hybrid. Dev. Genet. 2 (4), 357–367.

Engle, C., Renukdas, N., Park, J., Rees, P., 2017. Hybrid catfish (♂Ictalurus punctatus × ♀Ictalurus furcatus) production: does mixed-size production make economic sense? J. Appl. Aquac. 29 (3–4), 245–255.

Fitzpatrick, B.M., Shaffer, H.B., 2007. Hybrid vigor between native and introduced salamanders raises new challenges for conservation. Proc. Natl. Acad. Sci. 104 (40), 15793–15798.

Gong, D., Xu, L., Wu, C., Wang, S., Liu, Q., Cao, L., Mao, Z., Wang, Y., Hu, F., Zhou, R., Zhang, C., Tao, M., Luo, K., Zhao, R., Wang, Y., Liu, S.J., 2019. Two types of gynogenetic blunt snout bream derived from different sperm. Aquaculture. 734250.

Grant, P.R., Grant, B., Petren, K., 2005. Hybridization in the recent past. Am. Nat. 166 (1), 56–67.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. 95–98.

He, W., Xie, L., Li, T., Liu, S., Xiao, J., Hu, J., Jing, W., 2013. The formation of diploid and triploid hybrids of female grass carp × male blunt snout bream and their 5S rDNA analysis. BMC Genet. 14 (1).

Hermsen, J.T., 1992. Introductory considerations on distant hybridization. Distant Hybridization of Crop Plants. 1–14.

Hu, F., Wu, C., Zhou, Y., Cao, L., Xiao, J., Wang, S., Wu, Y., Ren, L., Liu, Q., Li, W., Wen, M., Tao, M., Qin, Q., Zhao, R., Luo, K., Liu, S.J., 2018. Production of androgenetic, triploid and tetraploid hybrids from the interspecific hybridization of female Japanese crucian carp and male blunt snout bream. Aquaculture 491, 50–58.

Kavumpurath, S., Pandian, T.J., 1990. Induction of triploidy in the zebrafish, Brachydanio rerio (Hamilton). Aquac. Res. 21 (3), 299–306.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., 2007. Clustal W and Clustal X version 2.0. Bioinformatics. 23 (21), 2947–2948.
Lin, C., Wei, L., Liu, S., Min, T., Yu, L., Wei, D., Chun, Z., Jun, X., Qin, Q., Luo, K., Ji, L., Yun, L., 2009. Novel genetic markers derived from the DNA fragments of sox genes. Mol. Cell. Probes 23 (3–4), 157–165.
Lippman, Z.B., Zamir, D., 2007. Heterosis: revisiting the magic. Trends Genet. 23 (2), 60–66.
Liu, S., 2010. Distant hybridization leads to different ploidy fishes. Sci. China Life Sci. 53 (4), 416–425.
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., 2007. The formation of the polyploid hybrids from different subfamily fish crossings and its evolutionary significance. Genetics. 176 (2), 1023–1034.
Longin, C.F.H., Mühleisen, J., Maurer, H.P., Zhang, H., Gowda, M., Reif, J.C., 2012. Hybrid breeding in autogamous cereals. Theor. Appl. Genet. 125 (6), 1087–1096.
Lou, Y., Li, X., 2006. Distant hybridization of fish and its application in aquaculture in China. J. Fish. Sci. China 1.
Mallet, J., 2007. Hybrid speciation. Nature. 446 (7133), 279–283.
Márián, T., Krasznai, Z., 1978. Kariological investigations on Ctenopharyngodon idella and Hypophthalmichthys nobilis and their cross-breeding. Aquac. Hung. 1, 44.
Pandian, T., Koteeswaran, R., 1998. Ploidy induction and sex control in fish. Hydrobiologia. 384 (1–3), 167–243.
Pijnacker, L.P., Ferwerda, M.A., 1995. Zebrafish chromosome banding. Génome. 38 (5), 1052–1055.
Qin, Q.B., Wang, J., Wang, Y.D., Liu, Y., Liu, S.J., 2015. Organization and variation analysis of 5S rDNA in gynogenetic offspring of Carassius auratus red var. (♀) × Megalobrama amblycephala (♂). BMC Genet. 16 (1), 26.
Qing, W., Hong, F., Yu, Y., 1997. Effect of enzymic gene dosage on the distant hybridisation of fishes. Acta Hydrobiol. 21, 143–151.
Seehausen, O., 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19 (4), 198–207.
Shull, G.H., 1908. The composition of a field of maize. J. Hered. (1), 296–301.
Sutton, D.L., Stanley, J., Miley, W., 1981. Grass carp hybridization and observations of a grass carp x bighead hybrid. Aquat. Plant Manage. 19, 37–39.
Tseng, W.Y., Poon, C.T., 1983. Hybridization of Epinephelus species. Aquaculture. 34 (1), 177–182.
Wang, Z., Zhang, J.X., Jin, G.Q., 1986. Study on the incompatibility of fish hybridization. Acta Hydrobiologica Sinica. 10 (2), 171–179.
Wang, S., Ye, X., Wang, Y., Chen, Y., Lin, B., Yi, Z., Wang, J., Liu, S.J., 2017. A new type of homodiploid fish derived from the interspecific hybridization of female common carp × male blunt snout bream. Sci. Rep. 7 (1), 4189.
Whitt, G.S., 1981. Developmental genetics of fishes: isozymic analyses of differential gene expression. Am. Zool. 21 (2), 549–572.
Williamson, D.I., 2006. Hybridization in the evolution of animal form and life-cycle. Zool. J. Linnean Soc. 148 (4), 585–602.
Wu, C., Huang, X., Hu, F., Ouyang, Y., Zhao, L., Wang, S., Ren, L., 2019. Production of diploid gynogenetic grass carp and triploid hybrids derived from the distant hybridization of female grass carp and male topmouth culter. Aquaculture 504, 462–470.
Xiao, Y., Zhou, Y., Xiong, Z., Zou, L., Jiang, M., Luo, Z., Wen, S., Liu, W., Liu, S., Li, W., 2013. Involvement of JNK in the embryonic development and organogenesis in zebrafish. Mar. Biotechnol. 15 (6), 716–725.
Yu, Y., Qing, W., Rong, C., 1989. Studies on cytology or crosses between grass carp and carp asynchronization between nucleus and cytoplasm in distant hybridization of fishes. Acta Hydrobiologica Sinica. 13 (3), 234–239.
Zhang, J., Liu, X.F., Wang, Z.X., Jin, G.Q., 1984. A comparative study on the karyotype among the hybrid fish (Sinilabeo decorus♂× Cirrhinus molitorella♀) and its parental fishes. Acta Hydrobiol Sin. 8, 313–322.
Zhang, Z., Chen, J., Li, L., Tao, M., Zhang, C., Qin, Q., Xiao, J., Liu, Y., Liu, S., 2014. Research advances in animal distant hybridization. Sci. China Life Sci. 57 (9), 889–902.
Zhou, Y., Mei, W., Jiang, M., Peng, L., Cong, W., Liu, J., Liu, W., Zhao, R., Zhao, X., Hu, W., Liu, S., Xiao, Y., 2016. Autotetraploid cell line induced by SP600125 from crucian carp and its developmental potentiality. Sci. Rep. 6, 21814.