GBE

Whole Genome Incorporation and Epigenetic Stability in a Newly Synthetic Allopolyploid of Gynogenetic Gibel Carp

Guang-Ming Shao[1,2], Xi-Yin Li[1,2], Yang Wang[1,2], Zhong-Wei Wang[1,2], Zhi Li[1], Xiao-Juan Zhang[1], Li Zhou[1,2,*], and Jian-Fang Gui[1,2,*]

[1]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

[2]University of Chinese Academy of Sciences, Beijing, China

*Corresponding authors: E-mails: jfgui@ihb.ac.cn; zhouli@ihb.ac.cn

Accepted: August 1, 2018

Data deposition: This project has been deposited at NCBI GenBank under the accessions MH290789-MH290811, MH669348 and MH669349.

Abstract

Allopolyploidization plays an important role in speciation, and some natural or synthetic allopolyploid fishes have been extensively applied to aquaculture. Although genetic and epigenetic inheritance and variation associated with plant allopolyploids have been well documented, the relative research in allopolyploid animals is scarce. In this study, the genome constitution and DNA methylation inheritance in a newly synthetic allopolyploid of gynogenetic gibel carp were analyzed. The incorporation of a whole genome of paternal common carp sperm in the allopolyploid was confirmed by genomic *in situ* hybridization, chromosome localization of 45S rDNAs, and sequence comparison. Pooled sample-based methylation sensitive amplified polymorphism (MSAP) revealed that an overwhelming majority (98.82%) of cytosine methylation patterns in the allopolyploid were inherited from its parents of hexaploid gibel carp clone D and common carp. Compared to its parents, 11 DNA fragments in the allopolyploid were proved to be caused by interindividual variation, recombination, deletion, and mutation through individual sample-based MSAP and sequencing. Contrast to the rapid and remarkable epigenetic changes in most of analyzed neopolyploids, no cytosine methylation variation was detected in the gynogenetic allopolyploid. Therefore, the newly synthetic allopolyploid of gynogenetic gibel carp combined genomes from its parents and maintained genetic and epigenetic stability after its formation and subsequently seven successive gynogenetic generations. Our current results provide a paradigm for recurrent polyploidy consequences in the gynogenetic allopolyploid animals.

Key words: hybridization, synthetic allopolyploid, gibel carp, gynogenesis, epigenetics, cytosine methylation pattern.

Introduction

Allopolyploidization, via intergeneric or interspecific hybridization, has been recognized as a major evolution force in plant speciation and environmental adaptation (Comai 2000, 2005). Owing to chromosome imbalances and genome instability, the newly formed allopolyploids may undergo chaos known as "genomic shock" (Mcclintock 1984; Ng et al. 2012) and occur complicated and non-Mendelian genomic changes, including chromosomal rearrangements and chromatin remodeling (Xiong et al. 2011; He et al. 2017), gene conversion, loss or silencing of homeologs (Doyle et al. 2008; Pala et al. 2008; Jackson and Chen 2010; Salmon et al. 2010; Buggs 2012; Lashermes et al. 2016; Page et al. 2016; Wang et al. 2017), dominant and biased expression of homeologs (Grover 2012; Koh et al. 2012; Yoo et al. 2013; Hu et al. 2015; Yang et al. 2016; Wang et al. 2017), transposon reactivation (Kashkush et al. 2003; Zou et al. 2011), and epigenetic modifications (Lukens et al. 2006; Madlung and Wendel 2013; Guan et al. 2014; Song and Chen 2015; Jackson 2017; Qiu et al. 2017; Shen et al. 2017; Song et al. 2017). Although many genomic changes result in the instability of neopolyploids, some of these changes might be advantageous to help allopolyploids to pass through a bottleneck of sterility and hybrid incompatibility and subsequently become new diploids through diploidization (Comai 2005; Zhou and Gui 2017). The genomic changes or diploidization have been well documented in plant polyploids (Diez et al. 2014). However, the research on genome additive effect and variations,

© The Author(s) 2018. Published by Oxford University Press on behalf of the Society for Molecular Biology and Evolution.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

especially epigenetic changes in polyploid animals, is scarce (Zhu and Gui 2007; Koroma et al. 2011; Arkhipova and Rodriguez 2013; Stöck and Lamatsch 2013; Xiao et al. 2013; Covelo-Soto and Leunda 2015; Jiang et al. 2016; Matos et al. 2016; Zhou et al. 2016).

Allopolyploids are less prevalent in animals, but many natural or synthetic allopolyploid fishes with excellent economic traits have been extensively applied to aquaculture, such as common carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), and gible carp (*Carassius gibelio*) (Zhou and Gui 2017, 2018; Zhou et al. 2018). Gibel carp, previously nominated as a subspecies *Ca. auratus gibelio* of crucian carp (Jiang et al. 1983), has been recognized as a separate species *Ca. gibelio* owing to its polyploidization, special multiple reproduction modes and sex determination mechanisms (Gui and Zhou 2010; Rylkova et al. 2010; Kalous and Knytl 2011; Wang et al. 2011; Zhang et al. 2015; Li et al. 2017, 2018; Liu et al. 2017a, 2017b; Zhu et al. 2018). As an important aquaculture species in China, the annual production capacity of gibel carp with other crucian carps has exceeded 3 million tons. Gibel carp has been considered as evolutionary hexaploid with over 150 chromosomes (Zhou and Gui 2002; Liasko et al. 2010; Kalous and Knytl 2011). Besides unisexual gynogenesis (Jiang et al. 1983), gibel carp can reproduce through bisexual reproduction, hybrid-similar development, or even androgenesis in response to the sperm from different gibel carp clones (Gui and Zhou 2010; Wang et al. 2011; Zhang et al. 2015). Interestingly, the whole genome or chromosome fragments of heterologous sperm were found to be able to incorporate into gibel carp genome (Gui et al. 1993a, 1993b; Yi et al. 2003; Zhu and Gui 2007; Gui and Zhou 2010; Mei and Gui 2015; Li et al. 2016; Lu et al. 2018). The synthetic allopolyploids still maintain the gynogenesis ability (Gui et al. 1993a, 1993b; Li et al. 2016; Lu et al. 2018), and some of them have been applied in aquaculture practice owning to their growth superiority (Li et al. 2016; Lu et al. 2018). A novel stable allopolyploid (allo) was established from allogynogenetic hexaploid gibel carp clone D (hexa) activated by heterologous sperm from red common carp (*Cc*) and approved as a new aquatic variety by the National Certification Committee for Aquatic Varieties. Through analyzing the internal transcribed spacer-1 (ITS1) and *transferrin* (*tf*) allele sequences, the added chromosomes were supposed to originate from common carp (Li et al. 2016). However, conclusive evidence for whole genome incorporation is lacking, and little is known about its epigenetic changes.

Polyploidization-induced genomic changes seem to exhibit a species-dependent pattern. Rapid and remarkable genomic changes have been revealed in many allopolyploids (Liu and Wendel 2002; Doyle et al. 2008; Buggs 2012; Chen 2013; Madlung and Wendel 2013), but not in allopolyploid cotton (*Gossypium*) (Liu et al. 2001). In addition, most of animal polyploids are paleopolyploids and their progenitor species are already extinct or difficult to identify (Jaillon et al. 2009; Arkhipova and Rodriguez 2013). Thus, the synthetic allopolyploid of gynogenetic gibel carp provides a model system to study the epigenetic changes of nascent polyploid animals. In this study, we performed genomic *in situ* hybridization (GISH), sequence comparison and chromosome localization of 45S rDNAs, mitochondrial D-loop sequence comparison and methylation sensitive amplified polymorphism (MSAP) analysis among the allopolyploid, gibel carp clone D, and common carp. Based on the investigations, we revealed whole genome incorporation and stable DNA methylation inheritance in the allopolyploid.

Materials and Methods

Source of Experimental Samples

One-year-old individuals of allopolyploid, gibel carp clone D, and common carp were obtained from Guanqiao Experimental Station, Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China. All fishes analyzed in this study were reared at the same ponds and randomly sampled. The animal procedures were approved by the Animal Care and Use Committee of the Institute of Hydrobiology, Chinese Academy of Sciences.

Analysis of Serum Transferrin

Blood samples from two individuals of allopolyploid, gibel carp clone D, and common carp were collected. The serum transferrin was isolated according to the rivanol-treatment procedure described by Yang et al. (2001) and was applied to 10% polyacrylamide gel electrophoresis as described previously (Li and Gui 2008).

Sequence Analysis of Mitochondrial DNA (mtDNA) D-Loop and 45S rDNA

Three individuals of allopolyploid, gibel carp clone D, and common carp were sampled to analyze mtDNA D-loop and 45S rDNA. DNA was extracted according to the Genomic DNA purification Kit technical manual (Promega). DNA concentration was measured by spectrophotometer (Thermo Scientific). The quality was assessed by GelRed-stained 1% agarose gel electrophoresis. Primers CR1 and DH2, and 45S_rDNA=F and 45S_rDNA=R were used to amplify mtDNA D-loop and 45S rDNA (supplementary table S1, Supplementary Material online). DNA amplification and purification were performed as previously described (Zhu and Gui 2007; Li and Gui 2008). Multiple sequences were aligned by Dnaman 7.0.

GISH and fluorescent in situ hybridization (FISH) with 45S rDNAs Probe

GISH and FISH were performed on four individuals of each fish as previously described (Zhu et al. 2006; Zhu and Gui

2007). Genomic DNA and 45S rDNAs from gibel carp clone D were labeled by DIG-Nick Translation Mix (Roche, Mannheim, Germany) and the spectrum signal was achieved with fluorescein isothiocyanate (FITC) conjugated-antidigoxigenin antibody (Roche, Mannheim, Germany). Genomic DNA from common carp was labeled by Biotin-Nick Translation Mix (Roche, Mannheim, Germany), and the spectrum signal was obtained by ExtrAvidin-CY3 antibody. For GISH, unlabeled sheared salmon sperm DNA was used as competitor DNA. The metaphase chromosomes were counterstained with 4.6-diamino-2-phenylindole (DAPI). The images were captured under confocal microscopy (NOL-LSM; Carl Zeiss, Thornwood, NY, USA) as described previously (Li et al. 2014; Li et al. 2016). About 60 and 40 metaphase spreads from four allopolyploid individuals were selected to count positive signals in GISH or FISH analysis, respectively.

Methylation Sensitive Amplified Polymorphism Detection

MSAP is a modified version of Amplified Fragment Length Polymorphism (AFLP) and has been proved to be an effective method to detect global genomic DNA methylation (Mcclelland et al. 1994; Reyna-López et al. 1997; Fulneček and Kovařík 2014). In this study, MSAP was performed as described (Xiao et al. 2013) with modifications as follows: 1) Genomic DNA extracted from hypothalamus was digested by *Eco*RI/*Hpa*II or *Eco*RI/*Msp*I in a 20 μl reactive volume including: 1 μg of genomic DNA, 2 μl of cutsmart buffer, 40 U of *Eco*RI, and 40 U of *Hpa*II or *Msp*I. The reaction was incubated for 4 h at 37°C. 2) The adaptor ligation reaction was performed at 16°C overnight in a 20 μl solution containing 10 μl of enzyme cleavage product, 2 μl of 10× buffer, 3 pmol of *Eco*RI adapters, and 30 pmol of *Msp*I–*Hpa*II adapters. 3) The preamplification was conducted in a 40 μl solution containing 4 μl of primer E0 (10 μmol l^{-1}), 4 μl of primer M0 (10 μmol l^{-1}) (supplementary table S1, Supplementary Material online), 12.8 μl of 2×Es Taq MasterMix (Cwbio, China), 5 μl ligation products and 14.2 μl of water. The Polymerase Chain Reaction (PCR) conditions were as follows: 94°C for 5 min; 30 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1 min; and a final extension at 72°C for 7 min. 4) The preamplification products were diluted 1:20 (v/v), and 5 μl was used for selective amplification in a final volume of 20 μl which contained 2 μl of each selective amplification primers (supplementary table S1, Supplementary Material online), 10 μl of 2×Es Taq MasterMix (Cwbio, China). The PCR conditions were as following: 94°C for 2 min; 12 cycles of 94°C for 30 s, 65°C (reduce by 0.7°C each cycle) for 30 s, 72°C for 1 min; 23 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min; and extension at 72°C for 7 min. 5) The selective amplified products were denatured at 94°C for 5 min and then loaded onto 6% denaturing polyacrylamide gels. The gels were silver-stained and scanned for analysis after drying. The interpretation of MSAP profiles was listed in table 1 as described (Fulneček and Kovařík 2014).

Table 1
MSAP EcoRI/HpaII and EcoRI/MspI (HM) profiles and their interpretation in vertebrate

Types	Bands pattern		Status of CCGG
	H	M	
I	+	+	CCGG
II	−	+	CmCGG
III	+	−	GAATTC-(CmCGG)n-CCGG
IV	−	−	Nonexistence or mutation of CCGG or *Eco*RI site

Note.—The types I–IV of MSAP HM profiles were interpreted as described previously (Fulneček and Kovařík 2014). CmCGG indicates that the internal cytosine in CCGG is either hemi- or fully methylated. *n* represents the number of CmCGG, $n \geq 1$.

To exclude the variation caused by sampling error or parental heterozygosity (Zhao et al. 2007; Xu et al. 2014; Suo et al. 2015; Lauria et al. 2017), both pooled and individual DNA samples-based MSAP were performed at population level (Lauria et al. 2017). Firstly, Genomic DNA from 10 individuals of each fish was randomly divided into two groups (five individuals per group), and then genomic DNA within the same group was equally mixed to produce two bulked DNA samples of each fish (allo, hexa, and *Cc*). Then 35 selective primer combinations were used to perform pooled samples-based MSAP analysis. To further confirm variant bands, the primer combinations that produced the variant bands in pooled examples were performed MSAP analysis of other 10 allopolyploid, 10 gibel carp clone D, and 10 common carp individuals. Subsequently, the variant bands were eluted from the gel, reamplified, and sequenced as described previously (Xiao et al. 2013). By using the sequences of variant bands as the query, a BLAST search was performed in the draft genome of *Cc* (Xu et al. 2011) (Common Carp Genome Database, http://www.carpbase.org/ 2011, last accessed August 5, 2018), and the flanking sequences were obtained. According to the flanking sequences, specific primers were designed by Primer Premier 5.0 (supplementary table S1, Supplementary Material online) and amplified in allopolyploid, gibel carp clone D, and common carp. Then, the PCR products were purified and sequenced. Multiple sequences were aligned by Dnaman 7.0.

Results

Whole Genome Incorporation of Common Carp Sperm in Allopolyploid Gibel Carp

The individuals of allopolyploid, gibel carp clone D, and common carp reared in same pond were discriminated by different morphological traits and transferrin (Tf) phenotype patterns (supplementary fig. S1, Supplementary Material online). Allopolyploid exhibits similar body color and morphological body type to its female parent gibel carp clone D

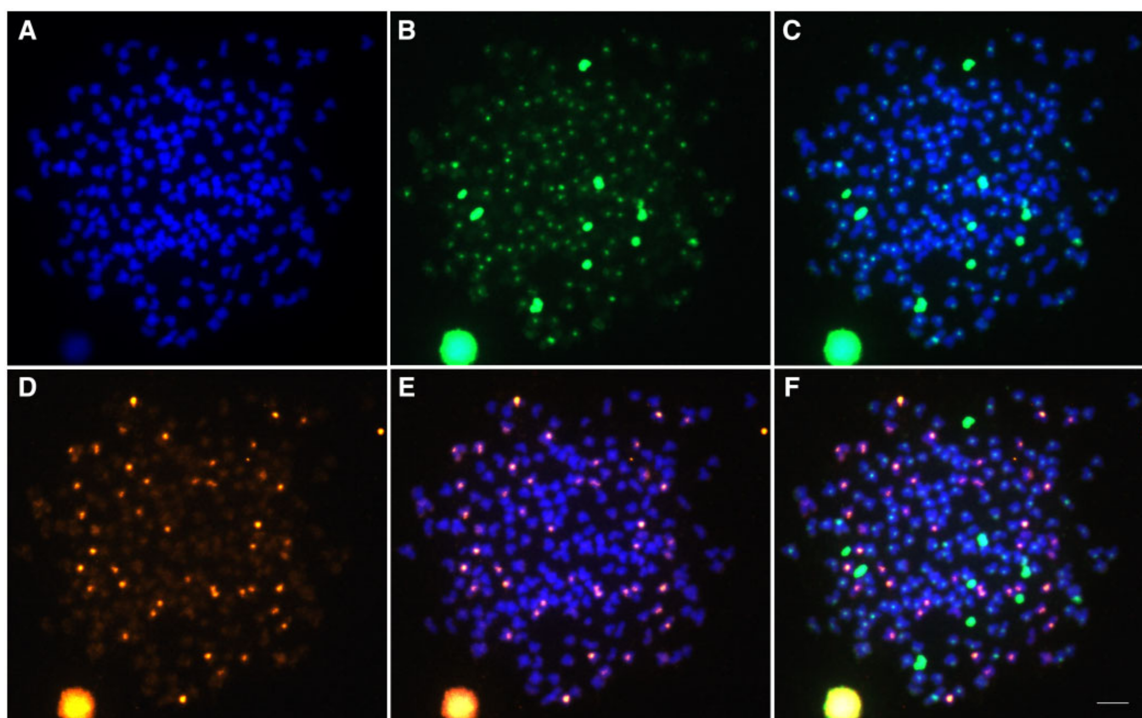

Fig. 1.—Parental chromosome discrimination in allopolyploid metaphases by GISH using DIG-labeled gibel carp clone D genomic DNA probe and biotin-labeled common carp genomic DNA probe. (*A*) A metaphase chromosome spread of allopolyploid stained by DAPI. (*B*) Chromosomes of gibel carp clone D acquired by FITC filter. (*C*) The overlapped image of (*A*) and (*B*). (*D*) Chromosomes of common carp acquired by Cy3 filter. (*E*) The overlapped image of (*A*) and (*D*). (*F*) The combined result of three colors including blue, green, and red. Scale bar represents 5 μm.

(supplementary fig. S1A, Supplementary Material online). Consistent with previous study, two denser transferrin bands were observed in gibel carp clone D. In the paternal common carp, only one transferrin band was detected. As expected, allopolyploids showed three transferrin bands, which might come from their parents, respectively (supplementary fig. S1B, Supplementary Material online).

To clearly trace the origin of chromosomes, we first performed GISH to discriminate the paternal and maternal chromosome sets in the metaphases of allopolyploid. The whole chromosomes of allopolyploid were stained into blue by DAPI, and the modal chromosome number was 208 (fig. 1*A*). When the digoxigenin-labeled genomic DNA of gibel carp clone D and biotin-labeled genomic DNA of common carp were cohybridized to allopolyploid metaphases, about 158 and 50 chromosomes were stained into green and red, respectively (fig. 1*B–E*). When the green and red fluorescent signals were synchronously overlapped with the blue chromosomes, about 158 cyan (blue plus green) and 50 pink (blue plus red) chromosomes were clearly observed (fig. 1*F*). The integrated signals confirmed the whole genome incorporation of common carp sperm in allopolyploid. Considering 162 chromosomes previously identified in gibel carp clone D (Zhou and Gui 2002), allopolyploid maintains overwhelming majority of maternal gibel carp clone D chromosomes and a whole chromosome set of paternal common carp.

Sequence Comparison and Chromosome Localization of 45S rDNAs among Allopolyploid and its Parents

Moreover, we compared the sequences and chromosome localization of 45S rDNAs among allopolyploid, gibel carp clone D, and common carp. A total of 1417 bp conservative partial sequences of 45S rDNA localized in 18S rRNA gene were amplified and compared. Two different 45S rDNA sequences (allo-45S1 and allo-45S2) were identified from allopolyploid, whereas only one sequence was amplified in gibel carp clone D and common carp, respectively (hexa-45S1 and *Cc*-45S1). Allo-45S1 and allo-45S2 possessed highly identity (99.8%) to each other, showing only three variable sites. Multiple alignments showed that allo-45S1 and allo-45S2 were completely identical to *Cc*-45S1 and hexa-45S1, respectively (fig. 2*A*).

Owing to the highly identities of 45S rDNA sequences between gibel carp clone D and common carp, the 1458 bp fragment amplified from gibel carp clone D was labeled by digoxigenin and used as a probe to detect the chromosome localization of 45S rDNAs through FISH analysis. A total of 2, 5, and 6 chromosomes displayed 45S rDNA green fluorescence signals in all analyzed metaphases from common carp, gibel carp clone D, and allopolyploid, respectively (fig. 2*B–D*). The 45S rDNA fluorescence signals are all localized to short arm terminals of the chromosomes in pairs. In order

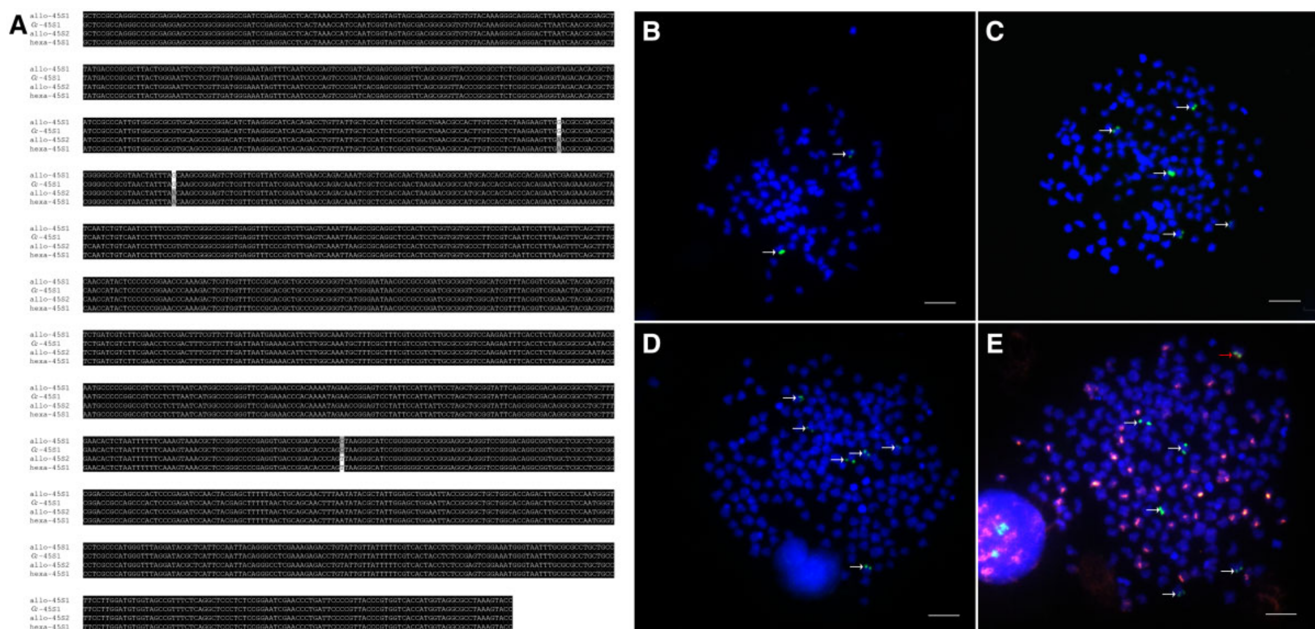

FIG. 2.—Sequence comparison and localizations of 45s rDNAs. (*A*) Sequence comparison of 45s rDNAs in allopolyploid (allo) (allo-45S1: MH290789; allo-45S2: MH290791), gibel carp clone D (hexa) (MH290792), and common carp (*Cc*) (MH290790). The primer sequences had been excluded. (*B*) Localizations of 45s rDNA in *Cc* (*B*), hex (*C*), and allo (*D*) by FISH with 45S rDNA probe. (*E*) Dual localizations of 45S rDNA labeled with digoxigenin and chromosomes from *Cc* labeled with biotin in a metaphase chromosome spread of allopolyploid. All metaphase chromosomes were counterstained with DAPI and the red fluorescene signals showed 50 chromosomes from common carp in allopolyploid. The white arrows pointing to the green fluorescence indicate the 45S rDNA loci, and the red arrow indicates colabeled loci by 45S rDNA probe and common carp genomic DNA probe.

to track the origination of the added positive chromosome in allopolyploid, the digoxigenin-labeled 45S rDNA and biotin-labeled genomic.

DNA of common carp were cohybridized to allopolyploid metaphases. Only one 45S rDNA green fluorescence signal was colocalized in one chromosome of common carp with red fluorescence signal, indicating that the added 45S rDNA positive chromosome in allopolyploid originated from haploid genome of paternal common carp (fig. 2*E*). By comparing the positive chromosome size and fluorescence intensity, the other five chromosomes with green fluorescence were supposed to be from the maternal gibel carp clone D.

Same Mitochondrial DNA Sequences between Allopolyploid and Maternal Gibel Carp Clone D

To confirm the maternal origin of allopolyploid, a total of 979, 979, and 981 bp mtDNA D-loop sequences were amplified from allopolyploid, gibel carp clone D, and common carp, respectively (fig. 3). The D-loop sequence of allopolyploid was completely identical to that of maternal hexaploid gibel carp clone D, whereas it exhibited 87.4% to 87.6% identity to that of common carp even though there existed several single nucleotide polymorphisms (SNPs) in the three common carp individuals.

Stable Cytosine Methylation Inheritance of Allopolyploid and its Parents

To investigate inheritance and variation of cytosine methylation of allopolyploid and its parents, pooled sample-based MSAP screening tests were firstly performed. Using 35 pairs of selective *Eco*RI + *Hpa*II/*Msp*I primer combinations, characteristics of cytosine methylation in two pooled DNA samples of each fish were explored. A total of 935 clear and reproducible MSAP bands scored in allopolyploid were grouped into five main patterns (class A–E) compared to those in its parents gibel carp clone D and *Cc* (fig. 4 and table 2). For inheritance, the monomorphic (class A) and additivity parental (class B and C) patterns were observed. About 10% (98/935) of total MSAP bands in allopolyploid belonged to class A which was subdivided into 3 subclasses (class A1–A3) of monomorphic patterns, exhibiting identical methylation status of same DNA fragments among allopolyploid, gibel carp clone D and common carp in either *Hpa*II (H) or *Msp*I (M) lane (fig. 4*B* and table 2). Additivity parental pattern represented the HM profile of a MSAP band in allopolyploid which was same to that in maternal gibel carp clone D (class B) or paternal common carp (class C). Class B and class C accounted for 66.10% (618/935) and 22.25% (208/935) of total MSAP bands in allopolyploid, and were subdivided into 7 and 4 subclasses, respectively. Thus, an overwhelming majority (98.82%) of cytosine methylation patterns in allopolyploid

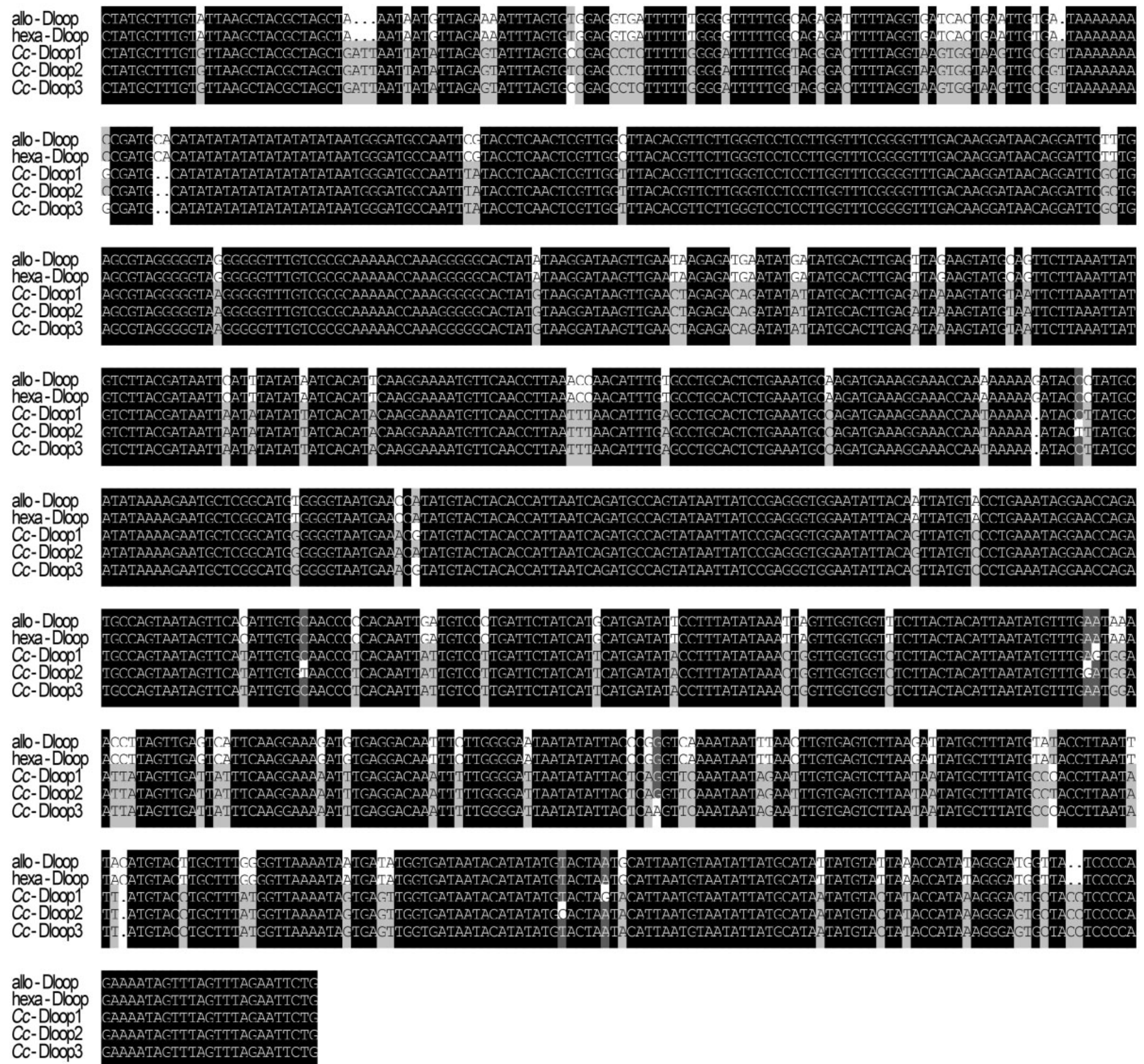

Fig. 3.—Alignments of mitochondrial D-loop sequences in allopolyploid (allo) (MH290793), gibel carp clone D (hexa) (MH290794), and common carp (*Cc*) (MH290795, MH669348 and MH669349). The D-loop sequences amplified from three individuals of allopolyploid or gibel carp clone D were identical. SNPs were detected in the three individuals of common carp.

were inherited from its parents. Additionally, very few (1.18%, 11/935) variant bands were detected in allopolyploid compared to its parents. The variation patterns were categorized into two classes which might be caused by cytosine methylation changes (class D) or mutations in nucleotide sequence (class E), respectively. Only two MSAP bands (0.21%) in allopolyploid were identified as cytosine methylation change. These methylation changes might be caused by either hypermethylation at the internal CCGG sites (5′-GAATTC-CCGG-CCGG-3′ to 5′-GAATTC-CmCGG-CCGG-3′) or hypomethylation at the external CCGG site (5′-GAATTC-CmCGG-CmCGG-3′ to 5′-GAATTC-CmCGG-CCGG-3′). Class E consisted of two HM profiles based on the methylation status at CCGG site in the mutated sequence, in which three MSAP bands showed nonmethylated CCGG sites (class E1), while six MSAP bands showed methylated CmCGG sites.

To confirm and interpret the production of 11 variant bands in class D and E, the 10 individual DNA sample of each fish (allo, hexa, and *Cc*) were performed MSAP analysis

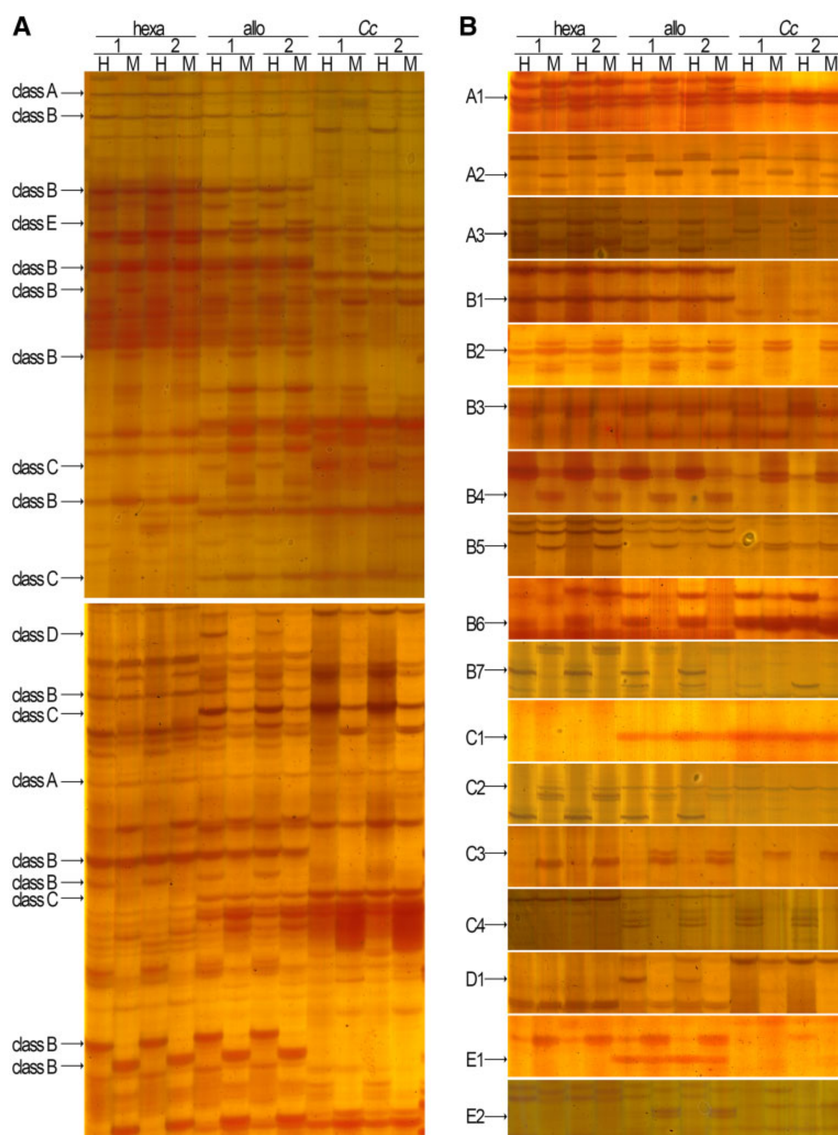

FIG. 4.—Classification (*A*) and subclassification (*B*) of band patterns in allopolyploid (allo) compared with its parents gibel carp clone D (hexa) and common carp (*Cc*) by pooled samples-based MSAP. The numbers 1 and 2 represent different pooled DNA samples from five individuals of each fish. H and M indicate genomic DNA digested by *Eco*RI/*Hpa*II or *Eco*RI/*Msp*I.

to exclude interindividual variation. Two variant bands in class D appeared to be caused by interindividual variation (fig. 5 and table 3). For MASP_E1M2_H_allo, 10, 10, and 7 individuals of gibel carp clone D, allopolyploid and common carp repeated the HM profiles (fig. 5*B*) as those in the pooled samples (fig. 5*A*). However, the other three individuals of common carp showed the same HM profiles as those in allopolyploid. The sequences amplified from individuals of allopolyploid and three individuals of common carp were identical (fig. 6*A*). So, the variant band MASP_E1M2_H_allo might be due to interindividual variation of common carp. Variant band MASP_E3M1_H_allo presented interindividual variation in the gynogenetic allopolyploid population, among which 5 individuals showed (+/−) HM profile while other individuals of allopolyploid and all individuals of gibel carp clone D and common carp exhibited (−/−) HM profile (fig. 5*B*).

The three nonmethylated variant bands in class E1 were also confirmed by individual sample-based MSAP and sequencing. The variant band MASP_E1M2_HM_allo was detected only in ten individuals of allopolyploid, not in gibel carp clone D and common carp (fig. 5*B*). Sequencing analysis showed that the 265 bp fragment MASP_E1M2_HM_allo was divided into 88 and 177 bp fragments which mapped to two different linkage groups of *Cc* reference genome (LG12 and LG13) (fig. 6*B*). Interestingly, the 177 bp fragment showed 97% identity with the same region of common carp *Helitrons* transposon, indicating the variant band MASP_E1M2_HM_allo

Table 2

Inheritance and variation of band patterns detected by pooled example-based MSAP

Classes	Subclasses	hexa	allo	*Cc*	Number	Total	Percent
		HM	HM	HM			
A (Monomorphic)	A1	++	++	++	60	98	10.48
	A2	−+	−+	−+	23		
	A3	+−	+−	+−	15		
B (Inheritance from hexa)	B1	++	++	−−	231	618	66.10
	B2	++	++	−+	6		
	B3	++	++	+−	3		
	B4	−+	−+	−−	221		
	B5	−+	−+	++	1		
	B6	+−	+−	++	3		
	B7	+−	+−	−−	153		
C (Inheritance from *Cc*)	C1	−−	++	++	82	208	22.5
	C2	−+	++	++	2		
	C3	−−	−+	−+	64		
	C4	−−	+−	+−	60		
D (Variation caused by methylation change)	D1	−−	+−	−−	2	2	0.21
E(Variation caused by mutation)	E1	−−	++	−−	3	9	0.96
	E2	−−	−+	−−	6		

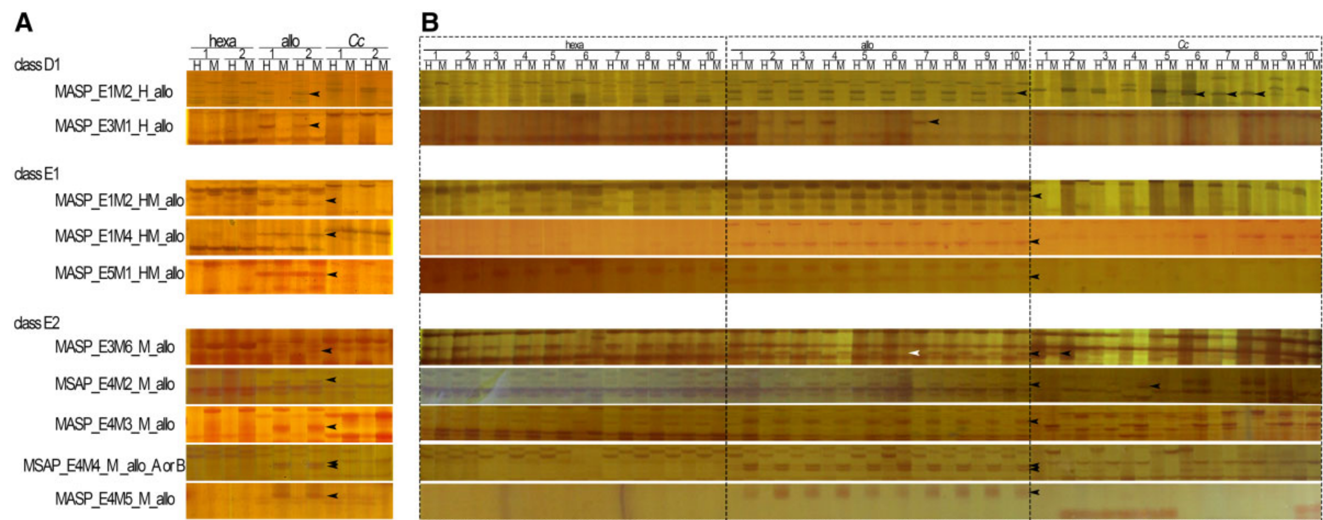

Fig. 5.—Validation of variant bands detected in pooled samples (*A*) by using individual sample-based MSAP (*B*) in allopolyploid (allo), gibel carp clone D (hexa), and common carp (*Cc*). The numbers 1–2 in (*A*) and the numbers 1–10 in (*B*) represent different pooled DNA samples from five individuals of each fish and other ten individuals of each fish, respectively. H and M indicate genomic DNA digested by *Eco*RI/*Hpa*II or *Eco*RI/*Msp*I. Black arrowheads indicate the variant bands, while white arrowhead indicates the mission of variant band in allopolyploid.

might be produced by transposition recombination. Additionally, a 299 bp fragment MASP_E1M4_HM_allo was also observed only in ten individuals of allopolyploid, not in the individuals of gibel carp clone D and common carp. According to the flanking sequences of MASP_E1M4_HM_allo searched in common carp reference genome, 452 and 458 bp fragments (allo_E1M4_HM and *Cc*_E1M4_HM) were amplified from allopolyploid and common carp respectively, and no DNA fragment was amplified from gibel carp clone D. Multiple nucleotide alignment of 299, 452, and 458 bp fragments showed that the variant band MASP_E1M4_HM_allo might be produced by a 6-bp tandem repeat unit (TAAATG) deletion of common carp corresponding DNA fragment in allopolyploid (fig. 6*C*). The HM profile of MASP_E5M1_HM_allo is same to those of MASP_E1M2_HM_allo and MASP_E1M4_HM_allo (fig. 5). Unfortunately, different sequences were amplified from allopolyploid, gibel carp clone D, and common carp (data not shown) according to the flanking sequences of

Table 3
Analysis of variant bands screened by MSAP

Classes	Subclasses	Names of variant bands in allo	MSAP HM pattern (hexa, allo, and *Cc*)	Primer combinations	Formation mechanism
Class D	D1	MASP_E1M2_H_allo	−− +− −−	E1M2	Interindividual variation in *Cc*
		MASP_E3M1_H_allo	−− +− −−	E3M1	Interindividual variation in allo
Class E	E1	MASP_E1M2_HM_allo	−− ++ −−	E1M2	Transposition recombination of *Cc* genetic materials in allo
		MASP_E1M4_HM_allo	−− ++ −−	E1M4	A 6-bp tandem repeat unit deletion of *Cc* corresponding DNA fragment in allo
		MASP_E5M1_HM_allo	−− ++ −−	E5M1	Not to be validated
	E2	MASP_E3M6_M_allo	−− −+ −−	E3M6	Interindividual variation in *Cc*
		MSAP_E4M2_M_allo	−− −+ −−	E4M2	Interindividual variation
		MASP_E4M3_M_allo	−− −+ −−	E4M3	8-bp deletion of *Cc* corresponding DNA fragment in allo
		MASP_E4M4_M_allo_A	−− −+ −−	E4M4	Not to be validated
		MASP_E4M4_M_allo_B	−− −+ −−	E4M4	Not to be validated
		MASP_E4M5_M_allo	−− −+ −−	E4M5	Mutation at EcoRI site (GAATTT to GAATTC)

Fig. 6.—Sequence alignments of variant bands amplified from allopolyploid (allo), gibel carp clone D (hexa), and common carp (*Cc*). (*A*) MASP_E1M2_H_allo (MH290796) and MASP_E1M2_H_*Cc* (MH290797). (*B*) MASP_E1M2_HM_allo (MH290798). (*C*) MASP_E1M4_HM_allo (MH290799), allo_E1M4_HM (MH290800), and *Cc*_E1M4_HM (MH290801). (*D*) MASP_E3M6_HM_allo (MH290802) and MASP_E3M6_HM_*Cc* (MH290803). (*E*) MASP_E4M2_H_allo (MH290804) and MASP_E4M2_H_*Cc* (MH290805). (*F*) MASP_E4M3_HM_allo (MH290806), allo_E4M3_HM (MH290807), and *Cc*_E4M3_HM (MH290808). (*G*) MASP_E4M5_HM_allo (MH290809), allo_E4M5_HM (MH290810), and *Cc*_ E4M5_HM (MH290811).

MASP_E5M1_HM_allo searched in common carp reference genome. Thus, we cannot speculate the reason for MASP_E5M1_HM_allo production.

The reasons for the production of six variant bands in class E2 were also analyzed. For MASP_E3M6_M_allo, 10, 9, and 6 individuals of gibel carp clone D, allopolyploid, and common carp showed the identical HM profiles (fig. 5*B*) as those in the pooled samples (fig. 5*A*). The sequences amplified from individuals of allopolyploid and four individuals of common carp showed 99% identities (fig. 6*D*). Similar to MASP_E3M6_M_allo, MSAP_E4M2_M_allo was also caused by parental heterozygosity. A total of 10, 10, and 9 individuals of gibel carp clone D, allopolyploid, and common carp repeated the HM profiles of those in the pooled sample, and the other one individual of common carp showed the same HM profiles as those in

allopolyploid, which was confirmed by comparison of the sequences between allopolyploid and common carp (fig. 6*E*). According to the flanking sequences of MASP_E4M3_M_allo searched in common carp reference genome, 219 and 227 bp DNA fragments were amplified from allopolyploid and common carp, respectively. Multiple nucleotide alignment of these sequences showed that the 219 bp MASP_E4M3_M_allo in allopolyploid might be produced by 8-bp deletion of common carp corresponding DNA fragment (fig. 6*F*). For MASP_E4M5_M_allo, 603 and 605 bp fragments were amplified from allopolyploid and common carp, respectively. Sequencing analysis showed that the 86 bp MASP_E4M5_M_allo possessed 100% identities to the DNA fragments amplified from allopolyploid and common carp. Compared to the DNA fragment amplified from common carp (*Cc*_E4M5_M), a mutation at *Eco*RI site (GAATTT to GAATTC) was detected in the DNA fragment amplified from allopolyploid (allo_E4M5_M) (fig. 6*G*), which might result in the production of variant band MASP_E4M5_M_allo. As the variant bands mentioned above in class E2 all showed (−, +) HM profile, they were expected to possess methylated CmCGG sites. In addition, E4M4_M_allo_A and E4M4_M_allo_B failed to be validated because different sequences were amplified from allopolyploid, gibel carp clone D, and common carp.

Discussion

Some gynogenetic fishes, including gibel carp (Gui et al. 1993a, 1993b; Yi et al. 2003; Zhu and Gui 2007; Knytl et al. 2013; Li et al. 2016; Lu et al. 2018), *Poeciliopsis* (Quattro et al. 1992), *Poecilia formosa* (Schartl et al. 1995), and *Squalius alburnoides* (Alves et al. 2001; Alves et al. 2004; Pala and Coelho 2005) possess the ability of integrating alien genome or subgenomic fragments into its genome (Gui et al. 1993a; Gui and Zhou 2010; Avise 2015; Zhou and Gui 2017). For example, the individuals with 206 chromosomes were identified from the offspring of *Ca. gibelio* × *Ca. carassius* (Knytl et al. 2013) or *Ca. gibelio* × *C. carpio* (Gui et al. 1993a; Lu et al. 2018), which might arise by the integration of *Ca. gibelio* whole chromosomes (156 chromosomes) and sperm genome (50 chromosomes). The novel synthetic allopolyploid hybrids still maintain their unisexual gynogenesis ability (Gui et al. 1993b; Yi et al. 2003; Zhu and Gui 2007; Li et al. 2016; Lu et al. 2018). In this study, we also confirmed that a whole chromosome set of common carp ($n = 50$) was incorporated into the stable inherited allopolyploid by GISH (fig. 1). Interestingly, we found that four chromosomes originating from gibel carp clone D were lost in allopolyploid. The mechanism by which the chromosomes were lost still remains unknown. Nonexclusive homologous pairing, multivalent formation, and subsequent chromosome mis-segregation have been considered as a major cause for aneuploidy in many newly formed allopolyploid plants (Zhang et al. 2013), and unequal distribution of genetic materials during meiosis was supposed as one of the potential mechanism of origin of the triploid *Carassius* female (Knytl et al. 2018). Moreover, the additive effects of allopolyploid were revealed through the analyses of Tf phenotype pattern (supplementary fig. S1, Supplementary Material online), 45S rDNA sequence and chromosome localization (fig. 2), and MSAP (fig. 4 and table 2) among allopolyploid, gibel carp clone D, and common carp. In addition, the same mitochondrial DNA sequences between allopolyploid and gibel carp clone D indicate that allopolyploid comes from gibel carp clone D.

DNA methylation, as one of the most important heritable epigenetic modifications, varies significantly in genomic distribution among protists, fungi, plants, and animals (Colot and Rossignol 1999; Su et al. 2011) and has been found to be involved in chromatin conformation, gene regulation, transposon activity, and genomic imprinting (Ishikawa and Kinoshita 2009). The association of DNA methylation with hybridization/polyploidization has been studied extensively in hybrid/allopolyploid plant systems (Diez et al. 2014). Compared with their parents, the proportion of methylation variation appears to be variable: 3.27%–6.29% in an diploid F1-hybrid and three allotriploid population of *Populous* (Suo et al. 2015), 8.3% in the experimentally resynthesized allotetraploid *Arabidopsis suecica* (Madlung et al. 2002), 11.3%–14.6% in the three allotriploid lines of *Senecio* (Hegarty et al. 2011), and as high as nearly 30% in *Spartina* (Salmon et al. 2005). Contrast to the rapid genomic changes in these neopolyploids, no alterations were detected in nine newly synthesized allotetraploid or allohexaploid cotton (*Gossypium*) (Liu et al. 2001). Similarly, only very few variant MSAP bands (1.18%) were detected in allopolyploid of gibel carp (table 2 and fig. 4) and were proved to be interindividual variations or DNA sequence variations (table 3 and fig. 5). Our results indicate that epigenetic changes in the newly synthetic gynogenetic allopolyploid are minimal. The variation degrees of DNA methylation in teleost also show a species-dependent characteristic with a wide range. Compared with their parents or diploids, 38.31% of 355 randomly selected CCGG sites were observed methylation changes in allotetraploid hybrids of red crucian carp and common carp (Xiao et al. 2013). Only 12 loci (2.94%) displayed significant methylation difference between diploid and synthesized triploid brown trout (*Salmo trutta* L.) (Covelo-Soto and Leunda 2015), while 73.05% and 68.17% of methylation patterns changed in naturally occurring triploid and tetraploid loach *Misgurnus anguillicaudatus* compared with diploid loach (Zhou et al. 2016).

So far, the molecular mechanism or evolutionary implications responsible for varied methylation changes in different polyploids are unknown. In our case, methylation change might not be dispensable during the formation and gynogenetic generation transmission of allopolyploid. The disadvantages of polyploidy include difficulties in meiosis, hybrid incompatibility, and epigenetic instability (Comai 2005). The

unisexual gynogenesis ability could help the newly synthetic allopolyploid to overcome meiotic difficulties, which is reminiscent of the classical view about why polyploidy is much rarer in animals than in plants (Muller 1925; Mable 2004; Liu et al. 2016). By allowing self-fertilization or unsexual reproduction, polyploids might break through the bottleneck of sterility (Comai 2005). In fact, about 60% (106/179) of analyzed insect and vertebrate polyploids reproduce by unisexual reproduction in the absence of sexual mates (Otto 2007). Additionally, postzygotic hybrid incompatibility is caused by disrupted interaction of parental divergent genomes and is supposed to be associated with species specific genes (Brideau et al. 2006; Tang and Presgraves 2009). By analyzing *Dmrt1* genes, we revealed that an early polyploidy event occurred before 18.49 Ma might result in a common tetraploid ancestor of *Ca. gibelio* and *Ca. auratus* (Li et al. 2014). Postzygotic hybrid incompatibility might not be a serious problem in allopolyploid owing to the common ancestral diploid species of its parents. In allopolyploids, extensive evidences for epigenetic remodeling have been revealed (Madlung and Wendel 2013). In synthetic *Arabidopsis* allotetraploid lines, methylation alterations were immediately induced during the first or first few generations after allopolyploidization events (Wang et al. 2004). However, many instances of epigenetic instability induced by hybridization or polyploidization have been described (O'Neill et al. 1998; Josefsson et al. 2006; Kinoshita 2007; Ishikawa and Kinoshita 2009; Wang et al. 2009; Zhao et al. 2011; Kirkbride et al. 2015; Wu et al. 2015), which is supposed to be more often deleterious than advantageous (Comai 2005). Therefore, the minimal methylation changes in gynogenetic allopolyploid maintain its stability. Future researches on the expression regulation of homeologs in the new allopolyploid with minimal methylation changes will provide new insight into polyploidy and unisexuality evolution mechanisms.

Hybridization usually companies changes of DNA sequence in respond to "genome shock" (Mcclintock 1984). For example, 9.67%–11.06% chimeric gene and 1.02%–1.16% mutation events were revealed in different generations of allopolyploids hybridized between goldfish and common carp (Liu et al. 2016). In this study, four variant MASP bands in gynogenetic allopolyploid were confirmed to be produced by changes of DNA sequences, including transposition recombination, deletion, and mutation at *Eco*RI site (table 3 and fig. 6). The genomic variations in allopolyploids might be caused by homologous recombination, transposon activation, compromise of mismatch repair system, and so on (Comai 2000; Belloch 2009; Arkhipova and Rodriguez 2013). Transposable elements (TEs), as mobile and rapidly evolving genetic units in eukaryotic genome, have significant impact on genome architecture and genetic innovations, such as generation of allelic diversity or novel genes, epigenetic effects on gene expression, and chromosomal rearrangements (Feschotte and Pritham 2007; Jurka et al. 2007; Arkhipova and Rodriguez 2013). The 265 bp fragment MASP_E1M2_HM_allo might be produced by *Helitrons*-mediated recombination (fig. 6*B*). Similar to other transposons, *Helitrons* are present in diverse eukaryotic genomes (Kapitonov and Jurka 2001; Kapitonov and Jurka 2007) and can promote rearrangements, capture or disperse gene fragments to produce chimeric transcripts (Lai et al. 2005; Choi et al. 2007). The genome influence of *Helitrons* in gynogenetic allopolyploid awaits further investigation. Interestingly, a variant band MASP_E3M1_H_allo showed interindividual methylation variation in allopolyploid (fig. 5). It is assumed that a methylation change may occur at a CCGG site in partial gynogenetic individuals of allopolyploid during generation transmission. Additionally, the different triploid asexual dandelion lineages displayed different methylation changes (Salmon et al. 2010; Verhoeven et al. 2010). Owing to the population selection during the formation process of allopolyploid, the interindividual methylation variation in allopolyploid also might be produced in different lineages.

In summary, we confirmed the whole genome incorporation of common carp and additive effect in allopolyploid of gibel carp. Significantly, we revealed that an overwhelming majority of cytosine methylation patterns in gynogenetic allopolyploid were inherited from its parents and identified a few of DNA sequence changes in the stable newly synthetic allopolyploid. Therefore, our results provide a paradigm of recurrent polyploidy consequences in unisexual polyploid animals.

Supplementary Material

Supplementary data are available at Genome Biology and Evolution online.

Acknowledgments

This work was supported by the Key Program of Frontier Sciences of the Chinese Academy of Sciences (QYZDY-SSW-SMC025), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA08030201), the earmarked fund for Modern Agro-industry Technology Research System (NYCYTX-49), and the Autonomous Project of the State Key Laboratory of Freshwater Ecology and Biotechnology (2016FBZ01).

Literature Cited

Alves MJ, Coelho MM, Collares-Pereira MJ. 2001. Evolution in action through hybridisation and polyploidy in an Iberian freshwater fish: a genetic review. Genetica 111:375–385.

Alves MJ, Gromicho M, Collares-Pereira MJ, Crespo-López E, Coelho MM. 2004. Simultaneous production of triploid and haploid eggs by triploid *Squalius alburnoides* (Teleostei: cyprinidae). J Exp Zool. 301A(7):552–558.

Arkhipova IR, Rodriguez F. 2013. Genetic and epigenetic changes involving (retro)transposons in animal hybrids and polyploids. Cytogenet Genome Res. 140(2-4):295–311.

Avise JC. 2015. Evolutionary perspectives on clonal reproduction in vertebrate animals. Proc Natl Acad Sci U S A.112(29):8867–8873.
Belloch C, et al. 2009. Chimeric genomes of natural hybrids of *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. Appl Environ Microbiol. 75(8):2534–2544.
Brideau NJ, et al. 2006. Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila*. Science 314(5803):1292–1295.
Buggs RJA. 2012. Rapid, repeated, and clustered loss of duplicate genes in allopolyploid plant populations of independent origin. Curr Biol. 22(3):248–252.
Chen ZJ. 2013. Genomic and epigenetic insights into the molecular bases of heterosis. Nat Rev Genet. 14(7):471–482.
Choi JD, Hoshino A, Park KI, Park IS, Iida S. 2007. Spontaneous mutations caused by a *Helitron* transposon, *Hel-It1*, in morning glory, *Ipomoea tricolor*. Plant J. 49(5):924–934.
Colot V, Rossignol JL. 1999. Eukaryotic DNA methylation as an evolutionary device. Bioessays 21(5):402–411.
Comai L. 2000. Genetic and epigenetic interactions in allopolyploid plants. Plant Mol Biol. 43(2-3):387–399.
Comai L. 2005. The advantages and disadvantages of being polyploid. Nat Rev Genet. 6(11):836–846.
Covelo-Soto L, Leunda PM, Pérez-Figueroa A, Morán P. 2015. Genome-wide methylation study of diploid and triploid brown trout (*Salmo trutta* L.). Anim Genet. 46(3):280–288.
Diez CM, Roessler K, Gaut BS. 2014. Epigenetics and plant genome evolution. Curr Opin Plant Biol. 18:1–8.
Doyle JJ, et al. 2008. Evolutionary genetics of genome merger and doubling in plants. Annu Rev Genet. 42:443–461.
Feschotte C, Pritham EJ. 2007. DNA transposons and the evolution of eukaryotic genomes. Annu Rev Genet. 41:331–368.
Fulneček J, Kovařík A. 2014. How to interpret methylation sensitive amplified polymorphism (MSAP) profiles? BMC Genet. 15:2.
Grover CE. 2012. Homoeolog expression bias and expression level dominance in allopolyploids. New Phytol. 196(4):966–971.
Guan XY, Song QX, Chen ZJ. 2014. Polyploidy and small RNA regulation of cotton fiber development. Trends Plant Sci. 19(8):516–528.
Gui JF, Liang SC, Zhu LF, Jiang YG. 1993a. Discovery of multiple tetraploids in artificially propagated populations of allogynogenetic silver crucian carp and their breeding potentialities. Chin Sci Bull. 38:327–331.
Gui JF, Liang SC, Zhu LF, Jiang YG. 1993b. Preliminary confirmation of gynogenetic reproductive mode in artificial multiple tetraploid allogynogenetic silver crucian carp. Chin Sci Bull. 38:67–70.
Gui JF, Zhou L. 2010. Genetic basis and breeding application of clonal diversity and dual reproduction modes in polyploid *Carassius auratus gibelio*. Sci China Life Sci. 53(4):409–415.
He ZS, et al. 2017. Extensive homoeologous genome exchanges in allopolyploid crops revealed by mRNAseq-based visualization. Plant Biotechnol J. 15(5):594–604.
Hegarty MJ, et al. 2011. Nonadditive changes to cytosine methylation as a consequence of hybridization and genome duplication in *Senecio* (Asteraceae). Mol Ecol. 20(1):105–113.
Hu GJ, Koh J, Yoo M, Chen SX, Wendel JF. 2015. Gene-expression novelty in allopolyploid cotton: a proteomic perspective. Genetics 200(1):91–104.
Ishikawa R, Kinoshita T. 2009. Epigenetic programming: the challenge to species hybridization. Mol Plant. 2(4):589–599.
Jackson SA. 2017. Epigenomics: dissecting hybridization and polyploidization. Genome Biol. 18(1):117.
Jackson SA, Chen ZJ. 2010. Genomic and expression plasticity of polyploidy. Curr Opin Plant Biol. 13(2):153–159.
Jaillon O, Aury JM, Wincker P. 2009. "Changing by doubling", the impact of whole genome duplications in the evolution of eukaryotes. C R Biol. 332(2-3):241–253.
Jiang Q, Li Q, Yu H, Kong LF. 2016. Inheritance and variation of genomic DNA methylation in diploid and triploid Pacific Oyster (*Crassostrea gigas*). Mar Biotechnol. 18(1):124–132.
Jiang YG, et al. 1983. Biological effect of heterologous sperm on gynogenetic offspring in *Carassius auratus gibelio*. Acta Hydrobiol Sin 8:1–13.
Josefsson C, Dilkes BP, Comai L. 2006. Parent-dependent loss of gene silencing during interspecies hybridization. Curr Biol. 16(13): 1322–1328.
Jurka J, Kapitonov VV, Kohany O, Jurka MV. 2007. Repetitive sequences in complex genomes: structure and evolution. Annu Rev Genom Hum Genet. 8:241–259.
Kalous L, Knytl M. 2011. Karyotype diversity of the offspring resulting from reproduction experiment between diploid male and triploid female of silver Prussian carp, *Carassius gibelio* (Cyprinidae, Actinopterygii). Folia Zool. 60(2):115–121.
Kapitonov VV, Jurka J. 2001. Rolling-circle transposons in eukaryotes. Proc Natl Acad Sci U S A. 98(15):8714–8719.
Kapitonov VV, Jurka J. 2007. *Helitrons* on a roll: eukaryotic rolling-circle transposons. Trends Genet. 23(10):521–529.
Kashkush K, Feldman M, Levy AA. 2003. Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nat Genet. 33(1):102–106.
Kinoshita T. 2007. Reproductive barrier and genomic imprinting in the endosperm of flowering plants. Genes Genet Syst. 82(3):177–186.
Kirkbride RC, et al. 2015. An epigenetic role for disrupted paternal gene expression in postzygotic seed abortion in *Arabidopsis* interspecific hybrids. Mol Plant. 8(12):1766–1775.
Knytl M, Kalous L, Symonová R, Rylková K, Ráb P. 2013. Chromosome studies of european cyprinid fishes: cross-species painting reveals natural allotetraploid origin of a carassius female with 206 chromosomes. Cytogenet Genome Res. 139(4):276–283.
Knytl M, et al. 2018. Morphologically indistinguishable hybrid Carassius female with 156 chromosomes: a threat for the threatened crucian carp, *C. carassius*, L. PLoS One 13(1):e0190924.
Koh J, et al. 2012. Comparative proteomics of the recently and recurrently formed natural allopolyploid *Tragopogon mirus* (Asteraceae) and its parents. New Phytol. 196(1):292–305.
Koroma AP, Jones R, Michalak P. 2011. Snapshot of DNA methylation changes associated with hybridization in *Xenopus*. Physiol Genomics 43(22):1276–1280.
Lai JS, Li YB, Messing J, Dooner HK. 2005. Gene movement by *Helitron* transposons contributes to the haplotype variability of maize. Proc Natl Acad Sci U S A. 102(25):9068–9073.
Lashermes P, Hueber Y, Combes MC, Severac D, Dereeper A. 2016. Inter-genomic DNA exchanges and homeologous gene silencing shaped the nascent allopolyploid coffee genome (*Coffea arabica* L.). G3-Genes Genomes Genet. 6:2937–2948.
Lauria M, Echegoyen-Nava RA, Rodríguez-Ríos D, Zaina S, Lund G. 2017. Inter-individual variation in DNA methylation is largely restricted to tissue-specific differentially methylated regions in maize. Bmc Plant Biol. 17(1):52.
Li FB, Gui JF. 2008. Clonal diversity and genealogical relationships of gibel carp in four hatcheries. Anim Genet. 39(1):28–33.
Li XY, et al. 2014. Evolutionary history of two divergent Dmrt1 genes reveals two rounds of polyploidy origins in gibel carp. Mol Phylogenet Evol. 78:96–104.
Li XY, et al. 2016. Extra microchromosomes play male determination role in polyploid gibel carp. Genetics 203(3):1415–1424.
Li XY, et al. 2017. A novel male-specific SET domain-containing gene setdm identified from extra microchromosomes of gibel carp males. Sci Bull. 62(8):528–536.
Li XY, et al. 2018. Origin and transition of sex determination mechanisms in a gynogenetic hexaploid fish. Heredity 121(1):64–74.

Li Z, Liang HW, Wang ZW, Zou GW, Gui JF. 2016. A novel allotetraploid gibel carp strain with maternal body type and growth superiority. Aquaculture 458:55–63.

Liasko R, et al. 2010. Biological traits of rare males in the population of *Carassius gibelio* (Actinopterygii: cyprinidae) from Lake Pamvotis (north-west Greece). J Fish Biol. 77(3):570–584.

Liu B, Brubaker CL, Mergeai G, Cronn RC, Wendel JF. 2001. Polyploid formation in cotton is not accompanied by rapid genomic changes. Genome 44(3):321–330.

Liu B, Wendel JF. 2002. Non-mendelian phenomena in allopolyploid genome evolution. Curr Genomics 3(6):489–505.

Liu SJ, et al. 2016. Genomic incompatibilities in the diploid and tetraploid offspring of the goldfish x common carp cross. Proc Natl Acad Sci U S A. 113(5):1327–1332.

Liu XL, et al. 2017a. Numerous mtDNA haplotypes reveal multiple independent polyploidy origins of hexaploids in Carassius species complex. Ecol Evol. 7(24):10604–10615.

Liu XL, et al. 2017b. Wider geographic distribution and higher diversity of hexaploids than tetraploids in Carassius species complex reveal recurrent polyploidy effects on adaptive evolution. Sci Rep. 7(1):5395.

Lu M, Wang ZW, Hu CJ, Zhou L, Gui JF. 2018. Genetic identification of a newly synthetic allopolyploid strain with 206 chromosomes in polyploid gibel carp. Aquac Res. 49(1):1–10.

Lukens LN, et al. 2006. Patterns of sequence loss and cytosine methylation within a population of newly resynthesized *Brassica napus* allopolyploids. Plant Physiol. 140(1):336–348.

Madlung A, et al. 2002. Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic *Arabidopsis* allotetraploids. Plant Physiol. 129(2):733–746.

Madlung A, Wendel JF. 2013. Genetic and epigenetic aspects of polyploid evolution in plants. Cytogenet Genome Res. 140(2-4):270–285.

Matos IM, Coelho MM, Schartl M. 2016. Gene copy silencing and DNA methylation in natural and artificially produced allopolyploid fish. J Exp Biol. 219(Pt 19):3072–3081.

Mcclelland M, Nelson M, Raschke E. 1994. Effect of site-specific modification on restriction endonucleases and DNA modification methyltransferases. Nucleic Acids Res. 22(17):3640–3659.

Mcclintock B. 1984. The significance of responses of the genome to challenge. Science 226(4676):792–801.

Mei J, Gui JF. 2015. Genetic basis and biotechnological manipulation of sexual dimorphism and sex determination in fish. Sci China-Life Sc.i 58(2):124–136.

Muller HJ. 1925. Why polyploidy is rare in animals than in plants. Am Nat. 59(663):346–353.

Mable BK. 2004. 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. Biol J Linnean Soc. 82(4):453–466.

Ng DWK, Lu J, Chen ZJ. 2012. Big roles for small RNAs in polyploidy, hybrid vigor, and hybrid incompatibility. Curr Opin Plant Biol. 15(2):154–161.

O'Neill RJ, O'Neill MJ, Graves JA. 1998. Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. Nature 393(6680):68–72.

Otto SP. 2007. The evolutionary consequences of polyploidy. Cell 131(3):452–462.

Page JT, et al. 2016. DNA sequence evolution and rare homoeologous conversion in tetraploid cotton. PLos Genet. 12(5): e1006012.

Pala I, Coelho MM. 2005. Contrasting views over a hybrid complex: between speciation and evolutionary 'dead - end'. Gene 347(2): 283–294.

Pala I, Coelho MM, Schartl M. 2008. Dosage compensation by gene-copy silencing in a triploid hybrid fish. Curr Biol. 18(17):1344–1348.

Qiu T, Dong YZ, Yu XM, Zhao N, Yang YF. 2017. Analysis of allopolyploidy-induced rapid genetic and epigenetic changes and their relationship in wheat. Genet Mol Res. 16(2):1–14.

Quattro JM, Avise JC, Vrijenhoek RC. 1992. Mode of origin and sources of genotypic diversity in triploid gynogenetic GISH clones (Poeciliopsis: poeciliidae). Genetics 130:621–628.

Reyna-López GE, Simpson J, Ruiz-Herrera J. 1997. Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. Mol Gen Genet 253(6):703–710.

Rylkova K, Kalous L, Slechtova V, Bohlen J. 2010. Many branches, one root: first evidence for a monophyly of the morphologically highly diverse goldfish (*Carassius auratus*). Aquaculture 302(1-2):36–41.

Salmon A, Ainouche ML, Wendel JF. 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). Mol Ecol. 14(4):1163–1175.

Salmon A, Flagel LE, Ying B, Udall JA, Wendel JF. 2010. Homoeologous nonreciprocal recombination in polyploid cotton. New Phytol. 186(1):123–134.

Schartl M, et al. 1995. Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. Nature 374(6518):196–171.

Shen YF, et al. 2017. Analysis of transcriptional and epigenetic changes in hybrid vigor of allopolyploid *Brassica napus* uncovers key roles for small RNAs. Plant J. 91(5):874–893.

Song QX, Chen ZJ. 2015. Epigenetic and developmental regulation in plant polyploids. Curr Opin Plant Biol. 24:101–109.

Song QX, Zhang TZ, Stelly DM, Chen ZJ. 2017. Epigenomic and functional analyses reveal roles of epialleles in the loss of photoperiod sensitivity during domestication of allotetraploid cottons. Genome Biol. 18(1):99.

Stöck M, Lamatsch DK. 2013. Why comparing polyploidy research in animals and plants? Cytogenet Genome Res. 140(2-4):75–78.

Su ZX, Han L, Zhao ZM. 2011. Conservation and divergence of DNA methylation in eukaryotes. Epigenetics 6(2):134–140.

Suo YJ, Dong CB, Kang XY. 2015. Inheritance and variation of cytosine methylation in three populus allotriploid populations with different heterozygosity. PLoS One 10(4):e0126491.

Tang SW, Presgraves DC. 2009. Evolution of the *Drosophila* nuclear pore complex results in multiple hybrid incompatibilities. Science 323(5915):779–782.

Verhoeven KJ, Van Dijk PJ, Biere A. 2010. Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages. Mol Ecol. 19(2):315–324.

Wang HY, et al. 2009. Molecular characterization of a rice mutator-phenotype derived from an incompatible cross-pollination reveals transgenerational mobilization of multiple transposable elements and extensive epigenetic instability. BMC Plant Biol. 9(1):63.

Wang JL, et al. 2004. Stochastic and epigenetic changes of gene expression in *Arabidopsis* polyploids. Genetics 167(4):1961–1973.

Wang XF, et al. 2017. Cytonuclear variation of rubisco in synthesized rice hybrids and allotetraploids. Plant Genome 10(3):1–11.

Wang ZW, et al. 2011. A novel nucleo-cytoplasmic hybrid clone formed via androgenesis in polyploid gibel carp. BMC Res Notes 4:82.

Wu Y, et al. 2015. Immediate genetic and epigenetic changes in F1 hybrids parented by species with divergent genomes in the rice genus (*Oryza*). PLoS One 10(7):e0132911.

Xiao J, et al. 2013. DNA methylation analysis of allotetraploid hybrids of red crucian carp (*Carassius auratus* red var.) and common carp (*Cyprinus carpio* L.). PLoS One 8(2):e56409.

Xiong ZY, Gaeta RT, Pires JC. 2011. Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. Proc Natl Acad Sci U S A. 108(19):7908–7913.

Xu P, et al. 2011. Generation of the first BAC-based physical map of the common carp genome. BMC Genomics 12:537.

Xu P, et al. 2014. Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. Nature Genet. 46(11):1212–1219.

Yang JH, et al. 2016. The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. Nat Genet. 48(10):1225–1232.

Yang L, Yang ST, Wei XH, Gui JF. 2001. Genetic diversity among different clones of the gynogenetic silver crucian carp, *Carassius auratus gibelio*, revealed by transferrin and isozyme markers. Biochem Genet. 39(5–6):213–225.

Yi MS, et al. 2003. Molecular cytogenetic detection of paternal chromosome fragments in allogynogenetic gibel carp, *Carassius auratus gibelio* Bloch. Chromosome Res. 11(7):665–671.

Yoo M, Szadkowski E, Wendel JF. 2013. Homoeolog expression bias and expression level dominance in allopolyploid cotton. Heredity 110(2):171–180.

Zhang HK, et al. 2013. Persistent whole-chromosome aneuploidy is generally associated with nascent allohexaploid wheat. Proc Natl Acad Sci U S A. 110(9):3447–3452.

Zhang J, et al. 2015. Meiosis completion and various sperm responses lead to unisexual and sexual reproduction modes in one clone of polyploid *Carassius gibelio*. Sci Rep. 5(1):1–14.

Zhao N, et al. 2011. Extensive and heritable epigenetic remodeling and genetic stability accompany allohexaploidization of wheat. Genetics 188(3):499–510.

Zhao X, Chai Y, Liu B. 2007. Epigenetic inheritance and variation of DNA methylation level and pattern in maize intra-specific hybrids. Plant Sci. 172(5):930–938.

Zhou H, et al. 2016. Analysis of different ploidy and parent-offspring genomic DNA methylation in the loach *Misgurnus anguillicaudatus*. Int J Mol Sci. 17(8):1299.

Zhou L, Gui JF. 2002. Karyotypic diversity in polyploid gibel carp, *Carassius auratus gibelio* bloch. Genetica 115(2):223–232.

Zhou L, Gui JF. 2017. Natural and artificial polyploids in aquaculture. Aquacult Fish. 2(3):103–111.

Zhou L, Gui JF. 2018. Applications of genetic breeding biotechnologies in Chinese aquaculture. In: Gui JF, Tang QS, Li ZJ, Liu JS, De Silva SS, editors. Aquaculture in China: success stories and modern trends. Oxford: John Wiley & Sons Ltd. p. 465-496.

Zhou L, Wang ZW, Wang Y, Gui JF. 2018. Crucian carp and gibel carp culture. In: Gui JF, Tang QS, Li ZJ, Liu JS, De Silva SS, editors. Aquaculture in China: success stories and modern trends. Oxford: John Wiley & Sons Ltd. p.149-157.

Zhu HP, Gui JF. 2007. Identification of genome organization in the unusual allotetraploid form of *Carassius auratus gibelio*. Aquaculture 265(1–4):109–117.

Zhu HP, Ma DM, Gui JF. 2006. Triploid origin of the gibel carp as revealed by 5S rDNA localization and chromosome painting. Chromosome Res. 14(7):767–776.

Zhu YJ, et al. 2018. Distinct sperm nucleus behaviors between genotypic and temperature-dependent sex determination males are associated with replication and expression-related pathways in a gynogenetic fish. BMC Genomics 19(1):437.

Zou J, et al. 2011. De novo genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. Plant J. 68(2):212–224.

Associate editor: Takashi Gojobori