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Establishment and application of distant hybridization technology in fish

Shi Wang[1,2†], Chenchen Tang[1,2†], Min Tao[1,2], Qinbo Qin[1,2], Chun Zhang[1,2], Kaikun Luo[1,2], Rurong Zhao[1,2], Jing Wang[1,2], Li Ren[1,2], Jun Xiao[1,2], Fangzhou Hu[1,2], Rong Zhou[1,2], Wei Duan[1,2] & Shaojun Liu[1,2*]

[1]*State Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha 410081, China;*
[2]*College of Life Sciences, Hunan Normal University, Changsha 410081, China*

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Hybridization is widely used. However, for a long time, systematic theories and technologies related to hybridization in fish have been lacking. In this study, through long-term systematic research, we investigated and obtained the main rules regarding inheritance and reproduction related to fish distant hybridization. Furthermore, we established one-step and multistep breeding technologies that were suitable for interspecific hybridization and intraspecific hybridization. Simultaneously, we used these two breeding technologies to produce a batch of diploid fish lineages and tetraploid fish lineages and improved fishes. In addition, we widely discuss the methods, technologies and results of hybridization breeding, referring to the domestic and foreign literature on fish hybridization. We hope that this paper will be beneficial for the research and application of fish hybrid breeding.

fish, distant hybridization, intraspecific hybridization, breeding technology, lineage

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Introduction

Self-mating in fish easily causes species deterioration, which is characterized by decreasing growth rates, resistance abilities and fertility. At present, several genetic breeding technologies are used to prevent such negative phenomena in fish, including hybridization, gynogenesis, androgenesis, selective breeding, transgenosis and gene editing.

There were a lot of reports on the hybridization of fish (Chen et al., 2017; He et al., 2012; He et al., 2013; Hu et al., 2012; Hu et al., 2018; Liu, 2010; Liu, 2014; Liu et al., 2001; Liu et al., 2016; Liu et al., 2007b; Lou and Li, 2006; Qin et al., 2010; Qin et al., 2014; Song et al., 2012; Wang et al., 2015a; Wang et al., 2015b; Wang et al., 2017; Wang et al., 2018; Xiao et al., 2014; Xu et al., 2015; Zhang et al., 2014).

As for the gynogenesis of fish, the following studies (Felip et al., 2001; Geng et al., 2005; Gui and Zhou, 2010; Hubbs et al., 1959; Komen and Thorgaard, 2007; Liu et al., 2007a; Liu et al., 2010; Liu et al., 2004; Morgan et al., 2006; Piferrer et al., 2004; Wang et al., 2009; Wang et al., 2016; Wei et al., 2003; Xie et al., 2001; Xie et al., 1999; Yang et al., 2001; Sun et al., 2006; Sun et al., 2007; Zhang et al., 2011; Zhang et al., 2015; Zhou et al., 2000) were published.

Regarding androgenesis of fish, the related studies (Arai et al., 1995; Babiak et al., 2002; Duan et al., 2007; Komen and Thorgaard, 2007; Liu and Yang, 2009; Scheerer et al., 1986; Stanley, 1976; Sun et al., 2007; Thorgaard et al., 1990; Wang et al., 2011) were presented.

The improved tetraploid fish (Liu, 2010; Liu, 2014; Liu et al., 2007a; Liu et al., 2004), and the improved triploid fish (CAS III and CAS V) (Fang and Gui, 2017; Gheyas et al.,

†Contributed equally to this work
*Corresponding author (email: lsj@hunnu.edu.cn)

2009; Gui and Zhou, 2010; Liu and Yang, 2009; Wang et al., 2009; Wang et al., 2011) are produced by gynogenesis, and improved tetraploid fish (Duan et al., 2007; Liu, 2010; Liu, 2014; Sun et al., 2007; Xu et al., 2015) were also produced by androgenesis.

With regard to selective breeding of fish, many publications (Gheyas et al., 2009; Hong and Zhang, 2003; Kause et al., 2005; Liu et al., 2005; Moss et al., 2012; Ning et al., 2007; Rezk et al., 2003; Zhao et al., 2009) were reported.

As for the transgenosis of fish, the related studies (Cao et al., 2014; Dai et al., 2010; Dunham, 2009; Feng et al., 2011; Fu et al., 1998; Fu et al., 2005; Guan et al., 2011; Hong et al., 2004; Houdebine and Chourrout, 1991; Hu et al., 2007a; Hu et al., 2002; Hu et al., 2007b; Hu and Zhu, 2010; Li et al., 2011; Rembold et al., 2006; Wu et al., 2003; Yu et al., 2010; Zhong et al., 2012; Zhong et al., 2013) were reported.

In recent years, in terms of gene editing of fish that developed rapidly, it has been used to study not only model fishes (e.g., zebrafish (Chang et al., 2013; Chu et al., 2014; Doyon et al., 2008; Hruscha et al., 2013; Hwang et al., 2013; Liu Y et al., 2014; Shu et al., 2016; Tang et al., 2014) and medaka (Ansai et al., 2014; Ansai et al., 2013; Chiang et al., 2016)), but also commercial fishes, such as ricefield eel (Feng et al., 2017), sturgeon (Chen et al., 2018), *Tilapia mossambica* (Li M et al., 2014), catfish (Qin et al., 2016), crucian carp and common carp (Chakrapani et al., 2016; Zhong et al., 2016).

By 2017, 83 improved fish varieties received approval from the Chinese government (Table 1), including 40 hybrids (48.2%) (Table 2), 39 selective lineages (47.0%), and 4 varieties (4.8%) produced by other methods. The statistics show that hybridization is the most common breeding technology used in fish.

Hybridization is as an effective way to prevent variety degeneration and produce improved variety. Hybridization can be divided into distant (interspecific) hybridization and intraspecific hybridization. Distant hybridization, which is defined as a cross between two different species or higher-ranking taxa, can combine the genomes of different species and results in significant changes in phenotypes and genotypes of hybrid offspring. Intraspecific hybridization, which is defined as a cross between different subspecies (lineages) belonging to the same species, can result in phenotypic and genotypic changes in hybrid offspring by combining genomes from different subspecies. Apparently, as for phenotypic and genotypic changes, progenies resulting from distant hybridization have more potential to form greater changes than those resulting from intraspecific hybridization. In terms of the genetic relationship of parents, intraspecific hybridization can be regarded as a special case in distant hybridization. Revealing the rules of heredity and reproduction for distant hybridization are also beneficial for intraspecific hybridization.

It is generally considered that distant hybridization cannot result in fertile lineages because of the existence of reproductive isolation in interspecific crossing. This condition is why studies of distant hybridization for creating the fertile lineages in fish are innovative tasks. Although distant hybridization is widely applied, it is difficult to predict the type of offspring that will be given birth to because of the lack of the rules of heredity and reproduction. Some adverse outcomes can result if we consider only the complements of the phenotypic advantages of parents; for example, it will lead to the death of offspring, the birth of progenies without heterosis, or it will be difficult to form fertile lineages. To study the rules of heredity and reproduction in distant hybridization of fish will play an important role in guiding the genetic breeding of fish with the aim of bringing the phenotypic complementary of parents to the path of order genetics (genetic rules).

Previous studies indicated that the evolution of some creatures was related to hybridization, for instance, tetraploid *Raphanobrassica* (Karpechenko, 1927), hexaploidy wheat (Liu, 1991), and diploid sunflower (Rieseberg et al., 2003; Rieseberg et al., 1995) in plants. There are more than 32,500 fishes in nature, which represents the largest vertebrate population (Cossins and Crawford, 2005). We infer that the formations of many fishes are associated with hybridization. However, for a long time, it lacks of enough evidences to prove that fish hybridization will produce new species.

Many fishes (species) are important genetic resources that provide alternative parents for distant hybridization for the production of improved fishes. Different lineages or subspecies also provide abundant parents for intraspecific hybridization. However, what are the effective methods to form improved fishes? What are the rules of fish hybridization? Moreover, what is the relationship between fish hybridization and species evolution? All of these scientific questions must be answered.

The genetic rules of distant hybridization in fish

Genetic rules at the chromosomal level

Chromosomes are carriers of genomic DNA, and chromosomes can directly express genetic characteristics of organisms at the cellular level. To reveal the number of chromosomes, karyotypes, ploidy of the parents and hybridization offspring are important parts of the genetic rules of hybridization breeding.

Through long-term and systematic studies that utilized freshwater fishes ($2n$=100 and $2n$=48) as the main research objects (including common carp, crucian carp, blunt snout bream, *Culter alburnus*, Bleeker's yellowtail, grass carp, silver carp, bighead carp and other commercial fishes), we conducted 31 distant crosses and obtained 25 kinds of viable

Table 1 Introduction of new fish varieties approved by the National Aquatic Protospecies and Improved Species Examination Committee (1996–2017)

Number	Classification		Name	Year	Register number	Category	Parental origin	District	Breeding unit
1	Freshwater fish	Tilapia mossambica	Tilapia fingerlings	1996	GS-02-001-1996	Hybrids	*Oreochromis aurea* (♀)×Tilapia nilotica (♂)	Guangdong	Guangzhou Fisheries Research Institute; Freshwater Fisheries Research Center
2	Freshwater fish	Tilapia mossambica	Hybrid tilapia	1996	GS-02-002-1996	Hybrids	Tilapia nilotica (♀)×*Oreochromis mossambicus* (♂)	Guangdong	Pearl River Fisheries Research Institute of Chinese Academy of Fishery Sciences
3	Freshwater fish	Common carp	Ying common carp	1996	GS-02-003-1996	Hybrids	Scattered mirror carp (♀)×CYCA hybrid (♂)	Hubei	Yangtze River Fisheries Research Institute of Chinese Academy of Fishery Sciences
4	Freshwater fish	Common carp	Feng common carp	1996	GS-02-004-1996	Hybrids	Xiangguo red common carp (♀)×Scattered mirror carp (♂)	Hubei	Institute of Hydrobiology of Chinese Academy of Sciences
5	Freshwater fish	Common carp	Heyuan common carp	1996	GS-02-005-1996	Hybrids	*Cyprinus carpio wuyuanensis* (♀)×*Cyprinus carpio Yuanjiang* (♂)	Hubei	Yangtze River Fisheries Research Institute of Chinese Academy of Fishery Sciences
6	Freshwater fish	Common carp	Yue common carp	1996	GS-02-006-1996	Hybrids	*Cyprinus carpio wuyuanensis* (♀)×Xiangjiang wild common carp (♂)	Hunan	Department of Biology of Hunan Normal University; Yuelu Fisheries in Yangtze River
7	Freshwater fish	Common carp	Tri-hybrid common carp	1996	GS-02-007-1996	Hybrids	Heyuan common carp (♀)×Scattered mirror carp (♂)	Hubei	Yangtze River Fisheries Research Institute of Chinese Academy of Fishery Sciences
8	Freshwater fish	Common carp	Furong common carp	1996	GS-02-008-1996	Hybrids	Scattered mirror carp (♀)×Xiangguo red common carp (♂)	Hunan	Hunan Fisheries Science Institute
9	Freshwater fish	Crucian carp	*Carassius auratus gibelio*	1996	GS-02-009-1996	Hybrids	Fangzheng crucian carp (♀)×Xiangguo red common carp (♂)	Hubei	Institute of Hydrobiology of Chinese Academy of Sciences
10	Freshwater fish	Common carp	Xiangyun common carp	2001	GS-02-001-2001	Hybrids	Feng common carp (♀)×Tetraploid hybrids (♂)	Hunan	Hunan Normal University; East Lake Fisheries in Xiangyin County
11	Freshwater fish	Common carp	Xiangyun crucian carp	2001	GS-02-002-2001	Hybrids	Japanese crucian carp (♀)×Tetraploid hybrids (♂)	Hunan	Hunan Normal University; East Lake Fisheries in Xiangyin County
12	Freshwater fish	Crucian carp	Red and white long tail crucian carp	2002	GS-02-001-2002	Hybrids	Red crucian carp; White crucian carp	Tianjin	Tianjin Aquaculture Fisheries
13	Freshwater fish	Crucian carp	Long tail crucian carp with blue body color	2002	GS-02-002-2002	Hybrids	Goldfish; Color crucian carp	Tianjin	Tianjin Aquaculture Fisheries
14	Freshwater fish	Crucian carp	Hybrids of Gold crucian carp	2007	GS-02-001-2007	Hybrids	Scattered mirror carp (♀)×Red crucian carp (♂)	Tianjin	Tianjin Aquaculture Fisheries
15	Freshwater fish	Crucian carp	No. 2 of Xiangyun crucian carp	2008	GS-02-001-2008	Hybrids	Improved diploid red crucian carp (♀)×Improved tetraploid hybrids (♂)	Hunan	Hunan Normal University

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Number	Classification		Name	Year	Register number	Category	Parental origin	District	Breeding unit
16	Freshwater fish	Crucian carp	Furong hybrids derived from common carp and crucian carp	2009	GS-02-001-2009	Hybrids	[Furong common carp derived from Scattered mirror carp (♀)×Xiangguo red common carp (♂)] (♀)×Red crucian carp (♂)	Hunan	Hunan Fisheries Science Institute
17	Freshwater fish	Tilapia mossambica	“Gili” Tilapia mossambica	2009	GS-02-002-2009	Hybrids	*Sarotherodon melanotheron*; “New Gift” *Oreochromis niloticus*	Shanghai	Shanghai Ocean University; Hebei Zhongjie Tilapia Fisheries
18	Freshwater fish	*Ophicephalus argus*	Hybrid snakehead “No. 1 of Hangli”	2009	GS-02-003-2009	Hybrids	Hybrids F_1 derived from *Channa maculata* and *Ophicephalus argus*	Zhejiang	Hangzhou Academy of Agricultural Sciences
19	Freshwater fish	Hybrids from *Megalobrama* and *Xenocypris*	Hybrids derived from *Megalobrama* and *Xenocypris*	2011	GS-02-001-2011	Hybrids	*Megalobrama amblycephala* (♀)×*Xenocypris davidi* Bleeker (♂)	Hunan	Hunan Normal University
20	Freshwater fish	*Culter*	Hybrids derived from *culter* “No. 1 of Pioneer”	2012	GS-02-001-2012	Hybrids	*Erythroculter ilishaeformis* (♀)×*Ancherythroculter nigrocauda* (♂)	Hubei	Wuhan Fisheries Science Research Institute; Wuhan Pioneer Aquatic Technology Co., Ltd.
21	Freshwater fish	*Culter*	Lutai hybrids derived from *Megalobrama* and *Erythroculter*	2012	GS-02-002-2012	Hybrids	*Megalobrama amblycephala* (♀)×*Erythroculter ilishaeformis* (♂)	Tianjin	Tianjin Aquaculture Fisheries
22	Freshwater fish	Crucian carp	Jinxin crucian carp	2013	GS-02-002-2013	Hybrids	Red crucian carp (♀)×Fertile tetraploids derived from (Baihua Red crucian carp (♀)×Molong common carp (♂) F_2 (♂))	Tianjin	Tianjin Aquaculture Fisheries
23	Freshwater fish	*Ietalurus punetaus*	*Ietalurus punetaus* “No. 1 of Jiangfeng”	2013	GS-02-003-2013	Hybrids	Breeding groups of *Ietalurus Punetaus* Mississippi 2001 (♀)×*Ietalurus Punetaus* Arkansas 2003 (♂)	Jiangsu	Jiangsu Freshwater Fisheries Institute; National Fisheries Technology Extension Center; Yellow Sea Fisheries Research Institute of Chinese Academy of Fishery Sciences
24	Freshwater fish	*Ophicephalus argus*	Hybrids derived from *Ophicephalus argus* (♀)×*Channa maculate* (♂)	2014	GS-02-002-2014	Hybrids	*Ophicephalus argus* (♀)×*Channa maculate* (♂)	Guangdong	Pearl River Fisheries Research Institute of Chinese Academy of Fishery Sciences; Aquaculture Fisheries in Sanjiao, Zhongshan, Guangdong
25	Freshwater fish	Tilapia mossambica	Giao Tilapia	2014	GS-02-003-2014	Hybrids	New Gift Tilapia (♀)×*Oreochromis aurea* (♂)	Guangdong	Maoming Weiye Tilapia Fisheries; Shanghai Ocean University
26	Freshwater fish	Up-mouth bream hybrid fish	Hybrids derived from female (*Megalobrama amblycephala* (♀)×*Culter alburnus* (♂))×male *Megalobrama amblycephala*	2014	GS-02-004-2014	Hybrids	Female (*Megalobrama amblycephala* (♀)×*Culter alburnus* (♂))×male *Megalobrama amblycephala*	Hunan	Hunan Normal University

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Number	Classification		Name	Year	Register number	Category	Parental origin	District	Breeding unit
27	Freshwater fish	*Siniperca chuatsi*	Qiupu hybrids derived from *Siniperca scherzeri* (♀)×*Siniperca chuatsi* (♂)	2014	GS-02-005-2014	Hybrids	*Siniperca scherzeri* (♀)×*Siniperca chuatsi* (♂)	Anhui	Chizhou Qiupu Special Aquaculture Development Co., Ltd.; Shanghai Ocean University
28	Freshwater fish	Common carp	No. 2 of Jinxin common carp	2014	GS-02-006-2014	Hybrids	Ukraine carp (♀)×Jinxin carp (♂)	Tianjin	Tianjin Aquaculture Fisheries
29	Freshwater fish	Crucian carp	Ganchang hybrids derived from common carp and crucian carp	2015	GS-02-001-2015	Hybrids	Japanese crucian carp (♀)×Xiangguo red common carp (♂)	Jiangxi	Jiangxi Fisheries Technology Extension Center; Fish Disease Prevention and Treatment Institution of Liantang, Nanchang; Jiangxi Biological Vocational College
30	Freshwater fish	Tilapia mossambica	Mohe Tilapia “Guangfu 1”	2015	GS-02-002-2015	Hybrids	*Oreochromis mossambicus* (♀)×*Oreochromis hornorum* (♂)	Guangdong	Pearl River Fisheries Research Institute of Chinese Academy of Fishery Sciences
31	Freshwater fish	Crucian carp	Hefang crucian carp	2016	GS-02-001-2016	Hybrids	Japanese crucian carp (♀)×Red crucian carp (♂)	Hunan	Hunan Normal University
32	Freshwater fish	Sturgeon	Hybrids Sturgeon “No. 1 of Xunlong”	2016	GS-02-002-2016	Hybrids	Dauricus (♀)×Amur sturgeon (♂)	HeiLongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences; Hangzhou Qiandao Lake Xunlong Technology Co., Ltd.; Sturgeon Breeding Center of Chinese Fisheries Technology Institute
33	Freshwater fish	*Siniperca chuatsi*	Hybrids of Changzhu *Siniperca chuatsi*	2016	GS-02-003-2016	Hybrids	*Siniperca chuatsi* (♀)×*Siniperca scherzeri* (♂)	Guangdong	Sun Yat-sen University; Guangdong Haid Group Co., Ltd.; Foshan South Sea Bairong Fisheries Co., Ltd.
34	Freshwater fish	Hybrids derived from *Megalobrama* and *Erythroculter*	Taihu hybrids derived from *Megalobrama* and *Erythroculter*	2017	GS-02-001-2017	Hybrids	*Culter alburnus* (♀)×*Megalobrama terminalis* (♂)	Zhejiang	Zhejiang Freshwater Fisheries Research Institute
35	Saltwater fish	Turbot	Turbot “Danfa ping”	2010	GS-02-001-2010	Hybrids	Denmark breeding groups; French breeding groups	Qingdao	Yellow Sea Fisheries Research Institute; Shandong Haiyang Yellow Sea Aquaculture Co., Ltd.
36	Saltwater fish	*Paralichthys olivaceus*	*Paralichthys olivaceus* “No. 1 of Pingyou”	2010	GS-02-002-2010	Hybrids	(China breeding groups against Vibrio anguillarum ♀×Japanese *Paralichthys olivaceus* groups after 2 breeding generations ♂) (♀)×Breeding groups introduced from Korea (♂)	Qingdao	Yellow Sea Fisheries Research Institute; Shandong Haiyang Yellow Sea Aquaculture Co., Ltd.

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Number	Classification		Name	Year	Register number	Category	Parental origin	District	Breeding unit
37	Saltwater fish	*Paralichthys olivaceus*	No. 2 of Beiping	2013	GS-02-001-2013	Hybrids	Wild female *Paralichthys olivaceus*	Hebei	BeiDaihe Experiment Station of Chinese Academy of Fishery Sciences; Resource and Environment Research Center of China Academy of Fishery Sciences
38	Saltwater fish	Turbot	Turbot "No. 1 of Duobao"	2014	GS-02-001-2014	Hybrids	Introduced species of Turbot	Qingdao	Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences; Yantai Development Zone Tianyuan Fisheries Co., Ltd.
39	Saltwater fish	Grouper	Hulong hybrids of grouper	2016	GS-02-004-2016	Hybrids	*Epinephelus fuscoguttatus* (♀)×*Epinephelus lanceolatus* (♂)	Guangdong	Guangdong Marine Fishery Testing Center; Sun Yat-sen University; Hainan University; Hainan Chenhai Fisheries Co., Ltd.
40	Saltwater fish	*Paralichthys olivaceus*	*Paralichthys olivaceus* "No. 2 of Pingyou"	2016	GS-02-005-2016	Hybrids	Hybrids derived from disease resistant groups of *Paralichthys olivaceus* and Japanese groups (♀)×Hybrids derived from disease resistant groups of *Paralichthys olivaceus* and Korean groups (♂)	Qingdao	Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences; Haiyang Yellow Sea Fisheries Co., Ltd.
41	Freshwater fish	Common carp	Xiangguo red common carp	1996	GS-01-001-1996	Breeding species	Wild Xiangguo red common carp	Jiangxi	Red Common Carp Fisheries in Xingguo County; Department of Biology of Jiangxi University
42	Freshwater fish	Common carp	*Cyprinus carpio wuyuanensis*	1996	GS-01-002-1996	Breeding species	Wild *Cyprinus carpio wuyuanensis*	Jiangxi	*Cyprinus carpio wuyuanensis* Research Institute in Wuyuan County; Department of Biology of Jiangxi University
43	Freshwater fish	Crucian carp	Pengze crucian carp	1996	GS-01-003-1996	Breeding species	Wild Pengze crucian carp	Jiangxi	Jiangxi Fisheries Research Institute; Jiujiang Fisheries Research Institute
44	Freshwater fish	Common carp	Jian common carp	1996	GS-01-004-1996	Breeding species	*Cyprinus carpio wuyuanensis*×*Cyprinus carpio Yuanjiang*	Jiangsu	Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences
45	Freshwater fish	Crucian carp	Songpu crucian carp	1996	GS-01-005-1996	Breeding species	Fangzheng crucian carp	HeiLongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
46	Freshwater fish	Common carp	*Cyprinus carpio wuyuanensis* Cold resistance lineage	1996	GS-01-006-1996	Breeding species	HeiLongjiang wild common carp×*Cyprinus carpio wuyuanensis*	HeiLongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
47	Freshwater fish	Common carp	Breeding groups of German mirror carp	1996	GS-01-007-1996	Breeding species	German mirror carp	HeiLongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences

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Number	Classification		Name	Year	Register number	Category	Parental origin	District	Breeding unit
48	Freshwater fish	Common carp	Songpu common carp	1997	GS-01-002-1997	Breeding species	HeiLongjiang wild common carp; *Cyprinus carpio wuyuanensis*; German mirror carp; Scattered mirror carp	HeiLongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences; Harbin Fisheries Research Institute; Nen River Fisheries Research Institute of HeiLongjiang Province
49	Freshwater fish	*Megalobrama amblycephala*	*Megalobrama amblycephala* Pujiang 1	2000	GS-01-001-2000	Breeding species	*Megalobrama amblycephala* in Yuni Lake	Shanghai	Shanghai Ocean University
50	Freshwater fish	Common carp	Wan'an glassy red common carp	2000	GS-01-002-2000	Breeding species	Wild glassy red common carp	Jiangxi	Jiangxi Wan'an Glassy Red Common Carp Fisheries
51	Freshwater fish	Common carp	Songhe common carp	2003	GS-01-002-2003	Breeding species	HeiLongjiang common carp; *Cyprinus carpio wuyuanensis*; Scattered mirror carp	HeiLongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
52	Freshwater fish	*Xiphophorus hellerii*	*Xiphophorus hellerii* RP-B series	2003	GS-01-003-2003	Breeding species	Guangzhou Aquarium Market	Guangdong	Pearl River Fisheries Research Institute of Chinese Academy of Fishery Sciences
53	Freshwater fish	Common carp	Molong common carp	2003	GS-01-004-2003	Breeding species	*Cyprinus carpio haematopterus*	Tianjin	Tianjin Aquaculture Fisheries
54	Freshwater fish	Common carp	Yellow river common carp-Henan breeding species	2004	GS-01-001-2004	Breeding species	Yellow river wild common carp	Henan	Henan Fisheries Research Institute
55	Freshwater fish	Tilapia mossambica	"New Gift" Tilapia	2005	GS-01-001-2005	Breeding species	Gift species of Tilapia mossambica introduced in 1994	Shanghai	Shanghai Ocean University; Qingdao Tilapia Fisheries; Guangdong Tilapia Fisheries
56	Freshwater fish	*Oncorhynchus mykiss Walbaum*	Gansu *Oncorhynchus mykiss Walbaum*	2006	GS-01-001-2006	Breeding species	Mutant of rainbow trout 1992	Gansu	Gansu Fisheries Technology Extension Center
57	Freshwater fish	Tilapia mossambica	"Xia'ao 1"*Oreochromis aurea*	2006	GS-01-002-2006	Breeding species	*Oreochromis aurea* groups introduced from America in 1983	Jiangsu	Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences
58	Freshwater fish	Common carp	Jinxin common carp	2006	GS-01-003-2006	Breeding species	Jian common carp	Tianjin	Tianjin Aquaculture Fisheries
59	Freshwater fish	Crucian carp	Pingxiang red crucian carp	2007	GS-01-001-2007	Breeding species	Red crucian carp	Jiangxi	Pingxiang Fisheries Research Institute, Jiangxi Province; Nanchang University; Jiangxi Fisheries Research Institute
60	Freshwater fish	Crucian carp	*Carassius auratus gibelio* "No. 3 of Zhongke"	2007	GS-01-002-2007	Breeding species	*Carassius auratus gibelio*	Hubei	Institute of Hydrobiology of Chinese Academy of Sciences
61	Freshwater fish	Common carp	Songpu mirror carp	2008	GS-01-001-2008	Breeding species	Breeding species F_4 of German mirror common carp	HeiLongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences

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Number	Classification		Name	Year	Register number	Category	Parental origin	District	Breeding unit
62	Freshwater fish	Silver carp	Changfeng silver carp	2010	GS-01-001-2010	Breeding species	Wild Changfeng silver carp	Hubei	Yangtze River Fisheries Research Institute of Chinese Academy of Fishery Sciences
63	Freshwater fish	Silver carp	Jin silver carp	2010	GS-01-002-2010	Breeding species	Yangtze River wild silver carp	Tianjin	Tianjin Aquaculture Fisheries
64	Freshwater fish	Common carp	Furui common carp	2010	GS-01-003-2010	Breeding species	Jian common carp and Yellow river wild common carp	Jiangsu	Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences
65	Freshwater fish	Bass	Largemouth bass “No. 1 of Youlu”	2010	GS-01-004-2010	Breeding species	Cultured largemouth bass	Guangdong	Pearl River Fisheries Research Institute of Chinese Academy of Fishery Sciences; Agriculture and Forestry Service Center of Jiujiang county, South Sea district, Foshan, Guangdong Province
66	Freshwater fish	Common carp	Songpu red mirror carp	2011	GS-01-001-2011	Breeding species	Cold resistance species of *Cyprinus carpio wuyuanensis* and Scattered mirror carp	Heilongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
67	Freshwater fish	Common carp	Color common carp in Ou River “No. 1 of Longshen”	2011	GS-01-002-2011	Breeding species	Cultured species in Ou River, Zhejiang	Shanghai	Shanghai Ocean University; Provincial Oujiang Color Common carp Fisheries, Longquan, Zhejiang
68	Freshwater fish	*Siniperca chuatsi*	*Siniperca chuatsi* “No. 1 of Huakang”	2014	GS-01-001-2014	Breeding species	Wild *Siniperca chuatsi*	Hubei	Huazhong Agricultural University; TONGWEI CO., LTD.; Yushun Agriculture, animal husbandry and fishery science and technology service co. LTD in Qingyuan, Guangdong
69	Freshwater fish	Common carp	*Cyprinus carpio var.* Yibu	2014	GS-01-002-2014	Breeding species	*Cyprinus Pellegrini*; HeiLongjiang common carp; and Scattered mirror carp	Heilongjiang	Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
70	Freshwater fish	Tilapia mossambica	Gift Tilapia “No. 1 of Zhongwei”	2014	GS-01-003-2014	Breeding species	Gift species of Tilapia nilotica	Jiangsu	Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences; TONGWEI CO., LTD.
71	Freshwater fish	Crucian carp	Platinum high-yield crucian carp	2015	GS-01-001-2015	Breeding species	Pengze crucian carp; Wild *Cyprinus acutidorsalis*	Guangdong	Huanan Normal University; Baijin Fisheries Co. LTD, Sanshui District, Foshan; Pearl River Fisheries Research Institute of Chinese Academy of Fishery Sciences
72	Freshwater fish	*Plecoglossus altivelis*	*Plecoglossus altivelis* “No. 1 of Zhemin”	2015	GS-01-002-2015	Breeding species	Wild *Plecoglossus altivelis*	Ningbo	Ningbo University; Ningde Zhonghe Agriculture CO., LTD.

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(*Continued*)

Number	Classification		Name	Year	Register number	Category	Parental origin	District	Breeding unit
73	Freshwater fish	*Megalobrama amblycephala*	*Megalobrama amblycephala* "No. 1 of Huahai"	2016	GS-01-001-2016	Breeding species	Wild *Megalobrama amblycephala*	Hubei	Huazhong Agriculture University; Hubei Bairong Fisheries CO., LTD.; Hubei *Megalobrama amblycephala* (blunt snout bream) protospecies fisheries
74	Freshwater fish	Crucian carp	*Carassius auratus gibelio* "No. 5 of Zhongke"	2017	GS-01-001-2017	Breeding species	Gynogenesis species E of silver crucian carp marked and identified by gene; *Megalobrama amblycephala*; Xiangguo red common carp	Hubei	Institute of Hydrobiology of Chinese Academy of Sciences; Fuer Fisheries Co. LTD, Huangshi
75	Freshwater fish	*Sinocyclocheilus grahami* Regan	*Sinocyclocheilus grahami* Regan "No. 1 of Bayou"	2017	GS-01-002-2017	Breeding species	Wild species in Muyang River-*Sinocyclocheilus grahami* Regan	Yunnan	Kunming Institute of Zoology. CAS; Shenzhen Huada Ocean Technology Co. LTD; Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences
76	Freshwater fish	Common carp	No. 2 of Furui common carp	2017	GS-01-003-2017	Breeding species	Jian common carp; Yellow river common carp and Heilongjiang wild common carp	Jiangsu	Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences
77	Saltwater fish	*Larimichthys crocea*	*Larimichthys crocea* "No. 1 of Minyou"	2010	GS-01-005-2010	Breeding species	Wild *Larimichthys crocea*	Xiamen	Jimei University; Ningde Fisheries Technology Extension Station
78	Saltwater fish	*Larimichthys crocea*	*Larimichthys crocea* "No. 1 of Donghai"	2013	GS-01-001-2013	Breeding species	Wild *Larimichthys crocea*	Ningbo	Ningbo University; Gangwan Fisheries Co, LTD, Xiangshan
79	Saltwater fish	*Nibea albiflora*	*Nibea albiflora* "No. 1 of Jinling"	2016	GS-01-002-2016	Breeding species	Cultured groups of *Nibea albiflora*	Xiamen	Jimei University; Hengyudao Fisheries Co. LTD, Ningde
80	Freshwater fish	*Pelteobagrus fulvidraco*	*Pelteobagrus fulvidraco* "No. 1 of Quanxiong"	2010	GS-04-001-2010	Others	Common *Pelteobagrus fulvidraco* (♀)×Supermale *Pelteobagrus fulvidraco* (♂)	Hubei	Institute of Hydroecology, MWR & CAS; Institute of Hydrobiology of Chinese Academy of Sciences; Bairui Biotechnology Co. LTD, Wuhan
81	Freshwater fish	Tilapia mossambica	Tilapia nilotica "No. 1 of Luxiong"	2012	GS-04-001-2012	Others	Breeding groups of Tilapia nilotica (XX) ♀×Supermale Tilapia nilotica (YY) ♂	Xiamen	Xiamen Luye Fisheries Co. LTD; Guangzhou Luye Fisheries Co. LTD; Guangzhou Luye Fisheries Co. LTD; Hainan Luye Fisheries Co. LTD
82	Freshwater fish	Crucian carp	Changfeng crucian carp	2015	GS-04-001-2015	Others	*Carassius auratus gibelio* D species; CYCA hybrid	Hubei	Yangtze River Fisheries Research Institute of Chinese Academy of Fishery Sciences; Institute of Hydrobiology of Chinese Academy of Sciences
83	Saltwater fish	*Paralichthys olivaceus*	*Paralichthys olivaceus* "No. 1 of Beiping"	2011	GS-04-001-2011	Others	Wild *Paralichthys olivaceus*	Hebei	Beidaihe Experiment Center of Chinese Academy of Fishery Sciences

Table 2 Introduction of new hybrid species approved by the National Aquatic Protospecies and Improved Species Examination Committee (1996–2017)[a)]

Number	Classification		Name	Year	Register Number	Parental Origin	Hybridization Form	Breeding Technique*
1	Freshwater fish	Tilapia mossambica	Tilapia fingerlings	1996	GS-02-001-1996	*Oreochromis aurea* (♀)×Tilapia nilotica (♂)	Distant hybridization	One-step
2	Freshwater fish	Tilapia mossambica	Hybrid tilapia	1996	GS-02-002-1996	Tilapia nilotica (♀)×*Oreochromis mossambicus* (♂)	Distant hybridization	One-step
3	Freshwater fish	Crucian carp	Hybrids of Gold crucian carp	2007	GS-02-001-2007	Scattered mirror carp (♀)×Red crucian carp (♂)	Distant hybridization	One-step
4	Freshwater fish	Tilapia mossambica	"Gili" Tilapia mossambica	2009	GS-02-002-2009	*Sarotherodon melanotheron*; "New Gift" *Oreochromis niloticus*	Distant hybridization	One-step
5	Freshwater fish	Hybrids from *Megalobrama* and *Xenocypris*	Hybrids derived from *Megalobrama* and *Xenocypris*	2011	GS-02-001-2011	*Megalobrama amblycephala* (♀)×*Xenocypris davidi* Bleeker (♂)	Distant hybridization	One-step
6	Freshwater fish	*Culter*	Hybrids derived from *culter* "No. 1 of Pioneer"	2012	GS-02-001-2012	*Erythroculter ilishaeformis* (♀)×*Ancherythroculter nigrocauda* (♂)	Distant hybridization	One-step
7	Freshwater fish	*Culter*	Lutai hybrids derived from *Megalobrama* and *Erythroculter*	2012	GS-02-002-2012	*Megalobrama amblycephala* (♀)×*Erythroculter ilishaeformis* (♂)	Distant hybridization	One-step
8	Freshwater fish	Tilapia mossambica	Giao Tilapia	2014	GS-02-003-2014	New Gift Tilapia (♀)×*Oreochromis aurea* (♂)	Distant hybridization	One-step
9	Freshwater fish	*Siniperca chuatsi*	Qiupu hybrids derived from *Siniperca scherzeri* (♀)×*Siniperca chuatsi* (♂)	2014	GS-02-005-2014	*Siniperca scherzeri* (♀)×*Siniperca chuatsi* (♂)	Distant hybridization	One-step
10	Freshwater fish	Crucian carp	Ganchang hybrids derived from common carp and crucian carp	2015	GS-02-001-2015	Japanese crucian carp (♀)×Xiangguo red common carp (♂)	Distant hybridization	One-step
11	Freshwater fish	Tilapia mossambica	Mohe Tilapia "No. 1 of Guangfu"	2015	GS-02-002-2015	*Oreochromis mossambicus* (♀)×*Oreochromis hornorum* (♂)	Distant hybridization	One-step
12	Freshwater fish	*Siniperca chuatsi*	Hybrids of Changzhu *Siniperca chuatsi*	2016	GS-02-003-2016	*Siniperca chuatsi* (♀)×*Siniperca scherzeri* (♂)	Distant hybridization	One-step
13	Freshwater fish	Hybrids derived from *Megalobrama* and *Erythroculter*	Taihu hybrids derived from *Megalobrama* and *Erythroculter*	2017	GS-02-001-2017	*Culter alburnus* (♀)×*Megalobrama terminalis* (♂)	Distant hybridization	One-step
14	Saltwater fish	Grouper	Hulong hybrids of grouper	2016	GS-02-004-2016	*Epinephelus fuscoguttatus* (♀)×*Epinephelus lanceolatus* (♂)	Distant hybridization	One-step
15	Freshwater fish	Common carp	Xiangyun common carp	2001	GS-02-001-2001	Feng common carp (♀)×Tetraploid hybrids (♂)	Distant hybridization	Multistep
16	Freshwater fish	Common carp	Xiangyun crucian carp	2001	GS-02-002-2001	Japanese crucian carp (♀)×Tetraploid hybrids (♂)	Distant hybridization	Multistep
17	Freshwater fish	Crucian carp	No. 2 of Xiangyun crucian carp	2008	GS-02-001-2008	Improved diploid red crucian carp (♀)×Improved tetraploid hybrids (♂)	Distant hybridization	Multistep
18	Freshwater fish	Crucian carp	Furong hybrids derived from common carp and crucian carp	2009	GS-02-001-2009	[Furong common carp derived from Scattered mirror carp (♀)×Xiangguo red common carp(♂)] (♀)×Red crucian carp (♂)	Distant hybridization	Multistep
19	Freshwater fish	Up-mouth bream hybrid fish	Hybrids derived from female (*Megalobrama amblycephala* (♀)×*Culter alburnus* (♂))×male *Megalobrama amblycephala*	2014	GS-02-004-2014	Female (*Megalobrama amblycephala* (♀)×*Culter alburnus* (♂))×male *Megalobrama amblycephala*	Distant hybridization	Multistep
20	Freshwater fish	Crucian carp	*Carassius auratus gibelio*	1996	GS-02-009-1996	Fangzheng crucian carp (♀)×Xiangguo red common carp (♂)	Distant hybridization	Others
21	Freshwater fish	*Ophicephalus argus*	Hybrid snakehead "No. 1 of Hangli"	2009	GS-02-003-2009	Hybrids F_1 derived from *Channa maculata* and *Ophicephalus argus*	Distant hybridization	Others

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Number	Classification		Name	Year	Register Number	Parental Origin	Hybridization Form	Breeding Technique*
22	Freshwater fish	Crucian carp	Jinxin crucian carp	2013	GS-02-002-2013	Red crucian carp (♀)×Fertile tetraploids derived from (Baihua red crucian carp (♀)×Molong common carp (♂)) F_2 (♂)	Distant hybridization	Others
23	Freshwater fish	*Ophicephalus argus*	Hybrids derived from *Ophicephalus argus* (♀)×*Channa maculate* (♂)	2014	GS-02-002-2014	*Ophicephalus argus* (♀)×*Channa maculate* (♂)	Distant hybridization	Others
24	Freshwater fish	Sturgeon	Hybrids Sturgeon "No. 1 of Xunlong"	2016	GS-02-002-2016	Dauricus (♀)×Amur sturgeon (♂)	Distant hybridization	Others
25	Freshwater fish	Common carp	Feng common carp	1996	GS-02-004-1996	Xiangguo red common carp (♀)×Scattered mirror carp (♂)	Intraspecific hybridization	One-step
26	Freshwater fish	Common carp	Heyuan common carp	1996	GS-02-005-1996	*Cyprinus carpio wuyuanensis* (♀)×*Cyprinus carpio Yuanjiang* (♂)	Intraspecific hybridization	One-step
27	Freshwater fish	Common carp	Yue common carp	1996	GS-02-006-1996	*Cyprinus carpio wuyuanensis* (♀)×Xiangjiang wild common carp (♂)	Intraspecific hybridization	One-step
28	Freshwater fish	Common carp	Furong common carp	1996	GS-02-008-1996	Scattered mirror carp (♀)×Xiangguo red common carp (♂)	Intraspecific hybridization	One-step
29	Freshwater fish	*Ietalurus Punetaus*	*Ietalurus Punetaus* "No. 1 of Jiangfeng"	2013	GS-02-003-2013	Breeding groups of *Ietalurus Punetaus* Mississippi 2001 (♀)×*Ietalurus Punetaus* Arkansas 2003 (♂)	Intraspecific hybridization	One-step
30	Freshwater fish	Common carp	No. 2 of Jinxin common carp	2014	GS-02-006-2014	Ukraine carp (♀)×Jinxin carp (♂)	Intraspecific hybridization	One-step
31	Freshwater fish	Crucian carp	Hefang crucian carp	2016	GS-02-001-2016	Japanese crucian carp (♀)×Red crucian carp (♂)	Intraspecific hybridization	One-step
32	Saltwater fish	Turbot	Turbot "Danfa ping"	2010	GS-02-001-2010	Denmark breeding groups; French breeding groups	Intraspecific hybridization	One-step
33	Freshwater fish	Common carp	Tri-hybrid common carp	1996	GS-02-007-1996	Heyuan common carp (♀)×Scattered mirror carp (♂)	Intraspecific hybridization	Multistep
34	Saltwater fish	*Paralichthys olivaceus*	*Paralichthys olivaceus* "No. 1 of Pingyou"	2010	GS-02-002-2010	(China breeding groups against Vibrio anguillarum ♀×Japanese *Paralichthys olivaceus* groups after 2 generation's breeding ♂) (♀)×Breeding groups introduced from Korea (♂)	Intraspecific hybridization	Multistep
35	Freshwater fish	Crucian carp	Red and white long tail crucian carp	2002	GS-02-001-2002	Red crucian carp; White crucian carp	Intraspecific hybridization	Others
36	Freshwater fish	Crucian carp	Long tail crucian carp with blue body color	2002	GS-02-002-2002	Goldfish; Color crucian carp	Intraspecific hybridization	Others
37	Freshwater fish	Common carp	Ying common carp	1996	GS-02-003-1996	Scattered mirror carp (♀)×CYCA hybrid (♂)	Others	Others
38	Saltwater fish	*Paralichthys olivaceus*	No. 2 of Beiping	2013	GS-02-001-2013	Wild female *Paralichthys olivaceus*	Others	Others
39	Saltwater fish	Turbot	Turbot "No. 1 of Duobao"	2014	GS-02-001-2014	Introduced species of Turbot	Others	Others
40	Saltwater fish	*Paralichthys olivaceus*	*Paralichthys olivaceus* "No. 2 of Pingyou"	2016	GS-02-005-2016	Hybrids derived from disease resistant groups of *Paralichthys olivaceus* and Japanese groups (♀)×Hybrids derived from disease resistant groups of *Paralichthys olivaceus* and Korean groups (♂)	Others	Others

a)*: Classification of breeding techniques after analysis: one-step and multistep

Table 3 Distant hybridization experiments of freshwater fishes (31 crosses, 25 crosses form surviving offspring)

Phylogenetic relationship		Hybridized combination	Ploidies of F_1	Tetraploid and diploid fish lineages	Number
Different numbers of parental chromosomes (100 and 48)	Subfamily	*Cyprinus carpio* (♀)×*Megalobrama amblycephala* (♂) No survival offspring after backcross	$4n=148$; $2n=100$	Autotetraploid fish lineage (F_2–F_3, $4n=200$) Autodiploid fish lineage (F_1–F_5, $2n=100$)	1-2
		Carassius auratus red var. (♀)×*Megalobrama amblycephala* (♂) No survival offspring after backcross	$4n=148$; $3n=124$; $2n=100$	Autotetraploid fish lineage (F_2–F_{13}, $4n=200$)	3-4
		Carassius auratus cuvieri (♀)×*Megalobrama amblycephala* (♂) No survival offspring after backcross	$4n=148$; $3n=124$; $2n=100$	Autotetraploid fish lineage (F_2–F_3, $4n=200$) Autodiploid fish lineage (F_2–F_4, $2n=100$)	5-6
		Carassius auratus red var. (♀)×*Erythroculter ilishaeformis* (♂) No survival offspring after backcross	$4n=148$; $3n=124$; $2n=100$		7-8
		Carassius auratus red var. (♀)×*Xenocypris davidi* (♂) No survival offspring after backcross	$4n=148$; $3n=124$; $2n=100$		9-10
		Cyprinus carpio haematopterus (♀)×*Megalobrama amblycephala* (♂) No survival offspring after backcross	$4n=148$; $3n=124$; $2n=100$	Autodiploid fish lineage (F_1–F_3, $2n=100$)	11-12
The same number of parental chromosomes (100 or 48)	Subfamily	*Megalobrama amblycephala* (♀)×*Xenocypris davidi* (♂) Cross and Backcross	$2n=48$; $3n=72$	Allodiploid fish lineage (F_1–F_2, $2n=100$)	13-14
		Ctenopharyngodon idellus (♀)×*Megalobrama amblycephala* (♂) Cross and Backcross	$2n=48$; $3n=72$		15-16
		Xenocypris davidi (♀)×*Erythroculter ilishaeformis* (♂) Cross and Backcross	$2n=48$; $3n=72$		17-18
		Megalobrama amblycephala (♀)×*Elopichthys bambusa* (♂) Cross and Backcross	$2n=48$; $3n=72$		19-20
		Ctenopharyngodon idellus (♀)×*Erythroculter ilishaeformis* (♂)	$2n=48$; $3n=72$		21
	Genus	*Megalobrama amblycephala* (♀)×*Erythroculter ilishaeformis* (♂) Cross and Backcross	$2n=48$; $3n=72$	Two allodiploid fish lineages after cross and backcross (F_1–F_5, $2n=48$)	22-23
		Megalobrama amblycephala (♀)×*Ancherythroculter* wangi (Tchang) (♂) Cross and Backcross	$2n=48$; $3n=72$		24-25
		Hypophthalmichthys molitrix (♀)×*Aristicluthys nobilia* (♂) Cross and Backcross	$2n=48$; $3n=72$		26-27
		Ctenopharyngodon idellus (♀)×*Elopichthys bambusa* (♂) Cross and Backcross	$2n=48$; $3n=72$		28-29
		Cyprinus carpio haematopterus (♀)×*Carassius auratus* red var. (♂) Cross and Backcross	$2n=100$	Allodiploid fish lineage (F_1–F_2, $2n=100$)	30-31

offspring (Table 3), which lays a strong experimental foundation for exploring the genetic rules of distant hybridization in fish. We classified the hybridization of hybridized fish parents into two types of patterns. One pattern was that the parents of the hybridization had the same chromosome number. The other pattern was that the parents of the hybridization had different chromosome numbers. This classification covered all hybridization types, including interspecific and intraspecific hybridization. After performing many studies regarding fish hybridization (Chen et al., 2017; He et al., 2012; He et al., 2013; Hu et al., 2012; Hu et al., 2018; Liu, 2010; Liu et al., 2007b; Qin et al., 2010; Qin et al., 2014; Song et al., 2012; Wang et al., 2015a; Wang et al., 2017; Wang et al., 2018; Xiao et al., 2014; Xu et al., 2015;

Zhang et al., 2014) (referring to Table 3), combined with previous relevant studies in our laboratory (such as the study of allotetraploid hybrids) (Liu et al., 2001), we obtained the genetic rules of distant hybridization in fish as described below:

When the number of maternal chromosomes is larger than that of paternal chromosomes, autotetraploid and autodiploid lineages can be established by overcoming the reproductive barrier of hybrid F_1 (Figure 1); when the number of maternal chromosomes is equal to that of paternal chromosomes, the allotetraploid and allodiploid fish lineages can be established by breaking through the reproductive barrier of hybrid F_1 (Figure 2). When the number of maternal chromosomes is fewer than that of paternal chromosomes, the offspring of the hybridization are unlikely to survive (Liu, 2010; Liu, 2014; Song et al., 2012).

Genetic mechanisms of distant hybridization in fish

Genetic mechanisms at the chromosomal level

The genetic mechanisms at the chromosomal level in terms of chromosome number, karyotype, and composition of the parents and offspring of distant hybridization can help us understand the genetic relationship between parents and their offspring, which avoids the flaw of designing the parents based on only complementary parent phenotypes. Breeders often expect that offspring of the hybridization will exhibit

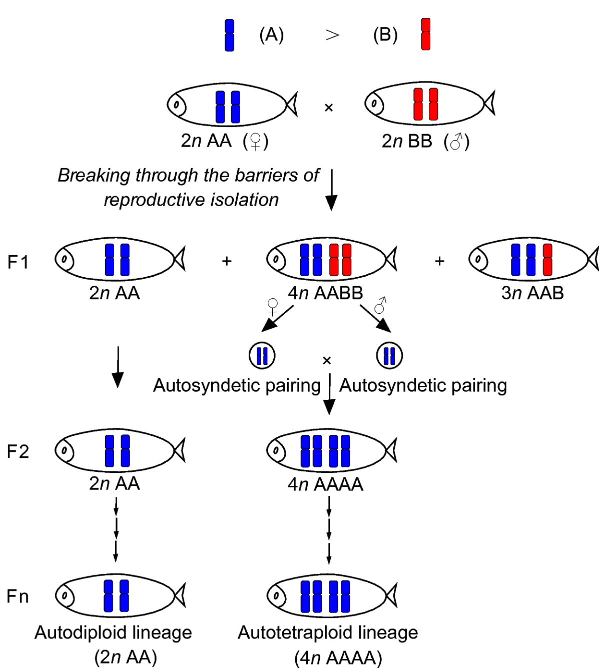

Figure 1 The formation of fertile lineages when the number of maternal chromosomes is larger than that of paternal chromosomes. In this kind of crossing, autotetraploid and autodiploid fish lineages can be produced by breaking through the reproduction barrier of hybrid F_1. However, the hybrid F_1 of this kind of crossing may present different appearances and growth rates, meaning that it is not suitable for this kind of crossing to produce hybrid F_1 with heterosis.

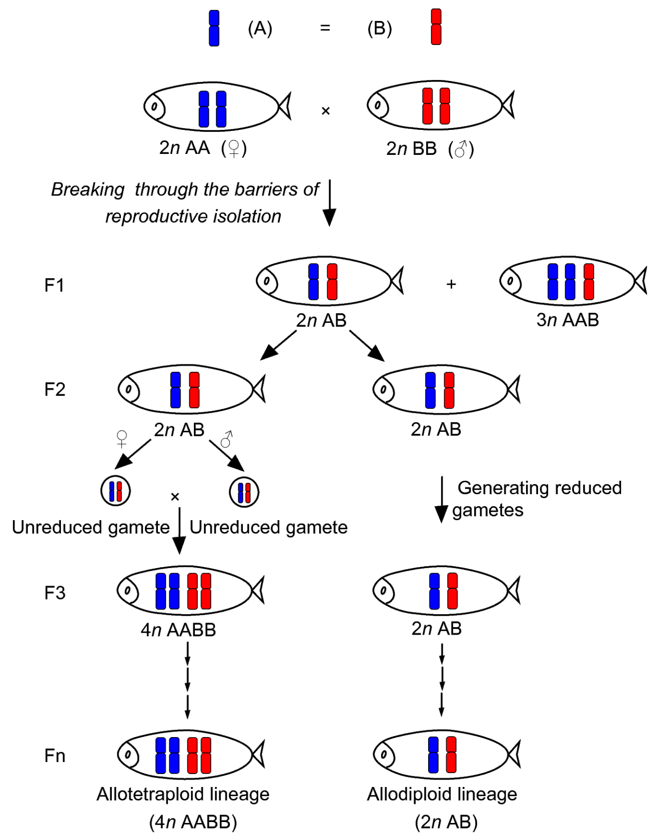

Figure 2 The formation of fertile lineages when the number of maternal chromosomes is equal to that of paternal chromosomes. In this kind of crossing, allotetraploid and allodiploid fish lineages can be produced by breaking through the reproduction barrier of hybrid F_1. In addition, hybrid F_1 of this kind of crossing may present consistent appearances and growth rates, meaning that it is suitable for this kind of crossing to produce hybrid F_1 with heterosis.

heterosis based on the phenotypic advantages of both parents. However, the lack of genetic rules to support hybrid design easily results in unexpected outcomes, such as no living offspring or fewer living offspring. Thus, designing the complementary phenotypes of the parents should be based on genetic rules such as the genetic rules of chromosomes. The above genetic rules at the chromosomal level are of great significance in guiding hybrid fish breeding.

Compatibilities of nucleus-nucleus and nucleus-cytoplasm

The genetic rules revealed at the chromosomal level involve the matching of the chromosome numbers of the parents. The degree of matching of the chromosome number of the parents will affect the nucleus-nucleus and nucleus-cytoplasm compatibilities of hybrid F_1. The nucleus-nucleus and nucleus-cytoplasm compatibilities are connected via the survival rate of hybrid F_1 (Figure 3; Table 4). In hybrid F_1, the nucleus-nucleus and nucleus-cytoplasm compatibilities include the maternal genome-paternal genome, the maternal genome-cytoplasm, and the paternal genome-cytoplasm compatibilities. Intraspecific hybridization is a special case of distant hybridization because the number of maternal

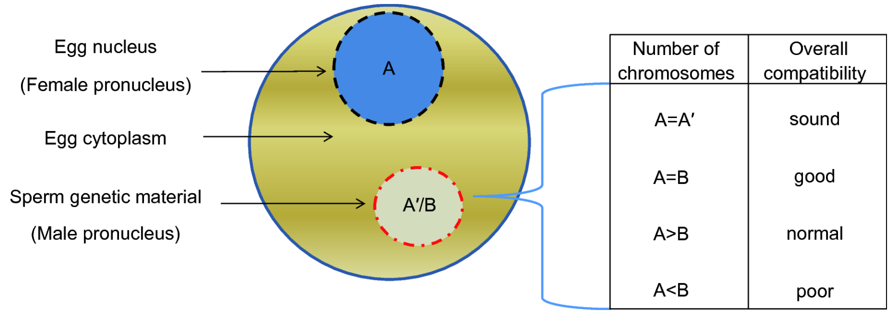

Figure 3 (Color online) The number of chromosomes and the overall compatibility of nucleus-nucleus and nucleus-cytoplasm in hybrid F_1. A refers to the number of maternal chromosomes, A' refers to the paternal chromosomes of a variety of A, B refers to the paternal chromosomes of a different species. In intraspecific hybridization, when the number of maternal chromosomes is equal to that of paternal chromosomes (A=A'), the compatibilities among the maternal nuclear material (including genome)-paternal nuclear material (including genome), the maternal nuclear material-cytoplasm, and the paternal nuclear material-cytoplasm are high, and this kind of crossing will present a very high survival rate in F_1. In interspecific hybridization, when the number of maternal chromosomes is equal to that of paternal chromosomes (A=B), the compatibilities among the maternal nuclear material-paternal nuclear material, the maternal nuclear material-cytoplasm, and the paternal nuclear material-cytoplasm are good, and this kind of crossing will present a relatively high survival rate; when the number of maternal chromosomes is larger than that of paternal chromosomes (A>B), the compatibilities among the maternal nuclear material-paternal nuclear material, the maternal nuclear material-cytoplasm, and the paternal nuclear material-cytoplasm are normal, and this kind of crossing will present a relatively normal survival rate. Conversely, when the number of maternal chromosomes is fewer than that of paternal chromosomes (A<B), the compatibilities among the above three relations are poor, and it is difficult for this kind of crossing to form surviving offspring.

Table 4 The number of chromosomes and the overall degree of nucleus-nucleus and nucleus-cytoplasm compatibility in hybrid F_1[a)]

Parental chromosomal number	Compatibility			Overall compatibility	Survival rate
	Maternal nucleus*-cytoplasm	Maternal nucleus*-paternal nucleus**	Paternal nucleus**-cytoplasm		
A=A'	++++	++++	++++	sound	high
A=B	+++	+++	+++	good	good
A>B	+++	++	++	normal	normal
A<B	++	+	+	poor	low

a) ++++: high compatibility; +++: good compatibility; ++: normal compatibility; +: poor compatibility; maternal nucleus*: the maternal nuclear material (including genome); paternal nucleus**: the paternal nuclear material (including genome).

chromosomes is equal to that of paternal chromosomes and the parents share a close genetic relationship; thus, the maternal genome-paternal genome, the maternal genome-cytoplasm, and the paternal genome-cytoplasm compatibilities are fairly good. Therefore, we can infer that the survival rate of hybrid F_1 from intraspecific hybridization is generally high.

In distant hybridization, when the number of maternal chromosomes is equal to that of paternal chromosomes, although this kind of crossing is considered interspecific hybridization and the parents of this kind crossing have relatively distant relationships, there are good compatibilities among the maternal genome-paternal genome, the maternal genome-cytoplasm, and the paternal genome-cytoplasm in hybrid F_1, so the survival rate of hybrid F_1 from this kind of crossing is high.

When the number of maternal chromosomes is larger than that of paternal chromosomes, the maternal genome occupies the dominant position and has development potential in hybrid F_1. In this case, in hybrid F_1, the compatibilities of the maternal genome-paternal genome, maternal genome-cytoplasm, and paternal genome-cytoplasm will decrease compared with the above two cases, but the overall compatibility will be normal, and the hybrid F_1 of this kind of crossing will be able to produce offspring with a certain survival rate. Conversely, when the number of maternal chromosomes is fewer than that of paternal chromosomes and the paternal genome is numerically dominant, and the above three kinds of compatibilities largely decrease, the development ability of hybrid F_1 of this kind of crossing is poor, and its survival rate is generally very low.

The compatibilities among the maternal genome-paternal genome, the maternal genome-cytoplasm, and the paternal genome-cytoplasm affect not only the survival rate of hybrid F_1 but also the genetic and reproductive characteristics of hybrid F_1.

Genetic mechanisms at the molecular level

At the molecular level in distant hybridization of fish, the compatibilities among the maternal nuclear material (including genome)-paternal nuclear material (including genome), the maternal nuclear material-cytoplasm, and the paternal nuclear material-cytoplasm involve changes in the genomic DNA. For example, chimeric genes were found in tetraploid fish lineages and diploid fish lineages derived from distant hybridization. The chimeric gene is an important genetic characteristic of hybrid fish lineages, and it can reduce the compatibilities between the genetic material of different species, which lays a firm foundation for hybrid lineages to maintain themselves from generation to generation (Liu et al., 2016; Wang et al., 2015b).

The relationships between the maternal nuclear material-paternal nuclear material, the maternal nuclear material-cytoplasm, and the paternal nuclear material-cytoplasm in the distant hybridization of fish also relate to changes at the RNA level and protein level. For example, the changes at the RNA level include nonadditive expression of duplicated genes (including dominant expression, overdominant expression, homologous expression bias) (Zhou et al., 2015), dosage-compensation effects (Li et al., 2018; Ren et al., 2017b), nucleolar dominance (Cao et al., 2018; Xiao et al., 2016) and *cis* and *trans* regulations of different parental sources. The effects of epigenetic regulation ultimately further adjust the formation of hybrid fish traits by influencing the expression patterns of some homologous genes (Ren et al., 2017a; Ren et al., 2016). At the protein level, there are significant differences in growth and fertility among different ploidy fish (Duan et al., 2016; Liu Z et al., 2014; Zhou et al., 2014). The studies on the changes in RNA and protein in fish hybridization will enrich the studies on the diversity of hybrid fish.

The reproductive rules of distant hybridization in fish

In the tetraploid lineages and diploid lineages derived from distant hybridization, the females and males are fertile. The unreduced gametes, including the diploid gametes produced by allodiploid hybrids ($2n$=100), are the key factors for the formation of allotetraploid fish lineages ($4n$=200) (Liu et al., 2001). The homologous diploid gametes and homologous triploid gametes produced by allotetraploids ($4n$=148) are important factors for the formation of autotetraploid fish lineages ($4n$=200) (Qin et al., 2014). The formation of these special gametes is related to the fusion and endoreduplication of germ cells (Wang et al., 2016). The haploid gametes produced by diploid hybrid fish are important factors for the formation of diploid fish lineages (Xiao et al., 2014).

In general, the morphology, structure, and quantity of gonadotropin secretory (GTH) cells in fish pituitary vary with reproductive activities; that is, a large number of degenerated GTH cells present vacuolar structures due to the massive release of intracellular particles after spawning (Liu, 1993). After the breeding season, numerous empty vesicular structures appeared in the GTH cells in the pituitary of the diploid red crucian carp and the allotetraploid hybrids, which indicates that they perform normal endocrine activities, thus promoting the normal development of gonads. However, in allotriploid crucian carp, only a small number of vacuolar structures were observed in the GTH cells, and most of the secretory pellets and secretory granules were not excreted. This result indicates that the allotriploid crucian carp cannot perform normal endocrine activities that were related to the abnormal gonad development. Before, during and after the breeding season, the expressions of *Fshβ* and *Lhβ* genes in the pituitary of the allotriploid crucian carp were higher than those in diploid red crucian carp and allotetraploid hybrids. The expressions of the related genes are related to the pituitary ultrastructure observations where secretory pellets and secretory granules of GTH cells in allotriploid crucian carp were not released. Additionally, at the molecular level, the expressions of *Fshβ* and *Lhβ* of the allotriploid crucian carp were not properly down-regulated after the breeding season; thus, the expressions of *Fshβ* and *Lhβ* in allotriploid crucian carp were higher than those in diploids and tetraploids. The above results explain the fertility of tetraploid fish and the sterility of allotriploid fish (Long et al., 2006; Long et al., 2009a; Long et al., 2009b).

The establishment of fertile lineages derived from distant hybridization in fish

According to the above genetic and reproductive rules, we established 10 new tetraploid fish lineages and diploid fish lineages (including 3 autotetraploid fish lineages and 7 diploid fish lineages) derived from distant hybridization by breaking through the reproductive barrier of hybrid F_1. These new lineages can be regenerated from generation to generation, and we revised the viewpoint that it is difficult to form fertile lineages via distant hybridization. These new fish lineages increase new fish germplasm resources.

The establishment of tetraploid fish lineages

We designed a series of distant crosses in which there were 100 maternal chromosomes ($2n$=100) and 48 paternal chromosomes ($2n$=48) (Table 3) and produced three autotetraploid fish lineages via the distant combination of *Carassius auratus* red var. (♀)×*Megalobrama amblycephala* (♂), *Carassius auratus cuvieri* (♀)×*Megalobrama amblycephala* (♂), and *Cyprinus carpio* (♀)×*Megalobrama amblycephala* (♂) (Qin et al., 2014). The autotetraploid fish lineage (Figure 4A) derived from *Carassius auratus* red var. (♀)×*Megalobrama amblycephala* (♂) propagated to F_{13}. In F_1 of this hybrid lineage, there were bisexual fertile allotetraploids ($4n$=148) (He et al., 2012), which produced homologous diploid gametes or homologous triploid gametes. These special gametes lead to the formation of autotetraploid fish (Qin et al., 2014).

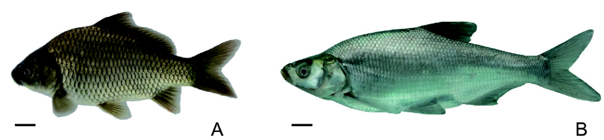

Figure 4 (Color online) The appearances of autotetraploid hybrids and allodiploid hybrids. A, F_{11} in an autotetraploid fish lineage derived from *Carassius auratus* red var. (♀)×*Megalobrama amblycephala* (♀); B, F_4 in an allodiploid fish lineage derived from *Megalobrama amblycephala* (♀)×*Xenocypris davidi Bleeker* (♂). Bar=2 cm.

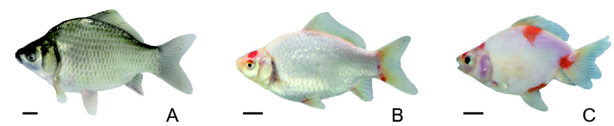

Figure 5 (Color online) The appearances of crucian carp-like fish, color crucian carp-like fish, and a new type of goldfish-like fish. A, The appearance of hybrid F_3 of the crucian carp-like fish lineage; B, the appearance of hybrid F_2 of the color crucian carp-like fish lineage; C, the appearance of hybrid F_1 of a new type of goldfish-like fish lineage. Bar =2 cm.

In addition, since the 1980s, our laboratory conducted intergeneric hybridization studies on *Carassius auratus* red var. (2*n*=100, ♀)×*Cyprinus carpio xiangjiangnensis* (2*n*=100, ♂), and in F_1, the bisexual fertile diploid individuals (2*n*=100) were found, and they produced hybrid F_2 (2*n*=100) by self-mating. The unreduced diploid eggs and unreduced diploid sperms were produced from female and male individuals of hybrid F_2, respectively, they were fertilized to form bisexual fertile tetraploid individuals (4*n*=200) in F_3, which subsequently formed an allotetraploid hybrid lineage (F_3–F_{27}) (4*n*=200) (Liu et al., 2001; Liu et al., 2016; Wang et al., 2015b).

The establishment of diploid fish lineages

We designed a series of distant crosses to establish the allodiploid fish lineages in which the parents have the same number of chromosomes (2*n*=100 or 2*n*=48) (Table 3). In this model, four fertile allodiploid fish lineages were established, including two allodiploid fish lineages derived from *Megalobrama amblycephala* (♀)×*Erythroculter ilishaeformis* (♂) (Figure 4B) and *Erythroculter ilishaeformis* (♀)×*Megalobrama amblycephala* (♂) were produced (Xiao et al., 2014), and the other two types of allodiploid fish lineages derived from *Megalobrama amblycephala* (♀)×*Xenocypris davidi* Bleeker (♂) (Hu et al., 2012) and *Cyprinus carpio* (♀)×*Carassius auratus* red var. (♂). The above four allodiploid fish lineages integrated the genomes of both parents and formed hybrid lineages with different subgenomes that presented intermediate genotypes and phenotypes.

In addition, we designed a series of distant crosses to establish the autodiploid fish lineages in which the parents have the different number of chromosomes (2*n*=100 and 2*n*=48). In this model, two autodiploid fish lineages were established including the autodiploid fish lineages of improved diploid white crucian carp lineages (Xiangjun white crucian carp) with a gray-white body derived from *Carassius auratus cuvieri* (♀)×*Megalobrama amblycephala* (♂), autodiploid crucian carp-like lineages (Xiangjun crucian carp) (Figure 5A) with a gray body derived from *Cyprinus carpio* (♀)×*Megalobrama amblycephala* (♂). The genomes of the above two fish lineages were mainly derived from the genome of the maternal parent but with some DNA fragments inserted from the paternal parent. The offspring of the above two crosses presented the genotypes derived from the paternal parent, such as a high back shape and good meat quality (Wang et al., 2017). Beside above two kinds of autodiploid fish lineage, the third autodiploid fish lineage of the color crucian carp (or red crucian carp)-like lineage (Xiangjun color crucian carp) (Figure 5B) derived from koi carp (*Cyprinus carpio haematopterus*) (♀)×*Megalobrama amblycephala* (♂) was successfully established. This lineage presented the traits of a high back and fast growth rate. Furthermore, the new type of goldfish-like fish (Xiangjun goldfish) with a twin-tail and red and white body (Figure 5C) was produced by the self-mating of the autodiploid color crucian carp-like fish, which also presented the trait of a high back, creating new goldfish germplasm resources. The crucian carp-like fish and the new type of goldfish-like fish were inherited from the genome of the maternal parent (*Cyprinus carpio haematopterus*), but some DNA fragments derived from the paternal parent (*Megalobrama amblycephala*) existed in the genomes of these two kinds of fishes. The establishment of the lineage, including the color crucian carp-like fish and the new type of goldfish-like fish, provided direct evidence of the koi carp-color crucian carp (red crucian carp)-goldfish evolutionary pathway that was triggered by distant hybridization, which has important significance in evolutionary biology and genetic breeding (Wang et al., 2018).

The establishment of one-step breeding technology and multistep breeding technology

Based on long-term and systematic studies on distant hybridization of fish, we established one-step breeding technology and multistep breeding technology (Figure 6). A series of improved diploid and triploid fish were developed using these two technologies. The applications of these technologies proved that they have a general guiding role in fish hybrid breeding.

One-step breeding technology

This breeding technology produces the hybrid F_1 with het-

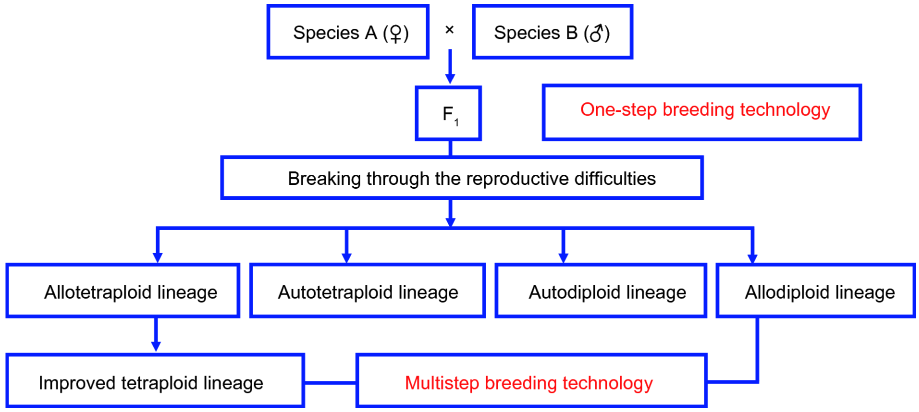

Figure 6 (Color online) Route map of one-step breeding technology and multistep breeding technology.

erosis under the conditions that both parents of the hybridization have the same number of chromosomes. In such cases, the F_1 of this kind of crossing has the potential to present consistent phenotypes such as the consistent shape and growth rate. We conducted systematic studies regarding the number of chromosomes, karyotypes, phenotypic characteristics and other biological characteristics of F_1 and its parents, and several F_1 with obvious heterosis were selected and bred (Table 3). Typical examples used by this kind of breeding technology are as follows.

(i) Hybrid F_1 derived from *Megalobrama amblycephala* (♀)×*Xenocypris davidi* Bleeker (♂) presented obvious heterosis, such as consistent body shape, small head, high meat rate, and high survival rate. The growth rate of the hybrid F_1 was 20%–40% faster than that of its parents (Figure 7A) (Hu et al., 2012).

(ii) Hybrid F_1 derived from *Xenocypris davidi* Bleeker (♀)×*Erythroculter ilishaeformis* (♂) indicated many advantages, such as consistent body shape, faster growth rate, higher survival rate and strong anti-disease ability (Figure 7B).

(iii) Diploid and triploid hybrids were found in hybrid F_1 of *Ctenopharyngodon idellus* (♀)×*Megalobrama amblycephala* (♂), and they showed the advantages such as fast growth rates and strong stress resistance. Among them, the growth rate of triploid hybrids derived from *Ctenopharyngodon idellus* (♀)×*Megalobrama amblycephala* (♂) was 30%–40% faster than that of common grass carp (He et al., 2013).

Multistep breeding technology

This breeding technology can produce the fertile lineages, including diploid and tetraploid lineages derived from distant hybridization and then the established lineages are used to produce improved diploid and triploid fish. Namely, through breaking through the reproductive barrier of hybrid F_1, the fertile diploid fish lineages and tetraploid fish lineages provide new fish germplasm resources. Using this technology, 10 fertile tetraploid and diploid hybrid fish lineages were established, and they were used as important core parents to further prepare new and improved fish.

The application of tetraploid fish lineages

The autotetraploid fish lineage derived from *Carassius auratus* red var. (♀)×*Megalobrama amblycephala* (♂) generated to F_{13}. The allotriploids (Figure 8A), which had some advantages such as infertility, rapid growth, good meat quality, and strong resistance were produced by mating male F_2–F_{12} in this lineage with female diploid common carp. The allotriploids had two sets of red crucian carp chromosome

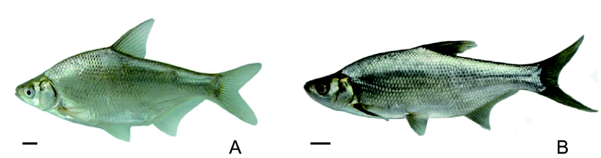

Figure 7 (Color online) The appearances of hybrids derived from *Megalobrama amblycephala* (♀)×*Xenocypris davidi* Bleeker (♂) and *Xenocypris davidi* Bleeker (♀)×*Erythroculter ilishaeformis* (♂). A, The appearance of a hybrid fish derived from *Megalobrama amblycephala* (♀)×*Xenocypris davidi* Bleeker (♂); B, the appearance of a hybrid fish derived from *Xenocypris davidi* Bleeker (♀)×*Erythroculter ilishaeformis* (♂). Bar=2 cm.

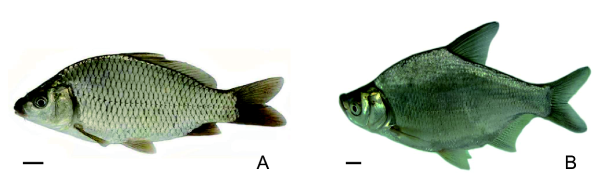

Figure 8 (Color online) The appearances of allotriploids and diploid up-mouth bream hybrid fish. A, The appearance of allotriploids produced by crossing the male autotetraploid fish lineage derived from *Carassius auratus* red var. (♀)×*Megalobrama amblycephala* (♂) with female diploid common carp; B, the appearance of diploid up-mouth bream hybrid fish produced by crossing the female allodiploid fish lineage derived from *Megalobrama amblycephala* (♀)×*Erythroculter ilishaeformis* (♂) with male *Megalobrama amblycephala*; Bar=2 cm.

groups and one set of common carp chromosomes, and the meat quality of the allotriploid was close to that of crucian carp. The infertility of allotriploids presented the function of not interfering with natural fish resources and protecting the intellectual property rights of seedling production.

The autotriploids were produced by mating male F_2–F_{12} in this lineage with female diploid red crucian carp. The autotriploids had three sets of red crucian carp chromosome groups and the advantages of beautiful shapes and good meat quality. Some fertile individuals were found in the female autotriploids, while the autotriploid males were sterile.

The application of diploid fish lineages

By crossing the allodiploid hybrid fish lineage (♀) derived from *Megalobrama amblycephala* (♀)×*Erythroculter ilishaeformis* (♂) with *Megalobrama amblycephala* (♂), a new type of improved diploid up-mouth bream hybrid (Figure 8B) was produced, which presented the advantages of herbivore feeding, tender meat, less intermuscular spines and beautiful appearance. In addition, the contents of protein, unsaturated fatty acids and flavorful amino acids in the meat of this new type of up-mouth bream hybrid were higher than those in the meat of both parents, while the carbohydrate content in the meat was lower than that in the meat of both parents (He et al., 2014). This kind of improved hybrid also had the advantages of a higher survival rate, higher low-oxygen tolerance, stronger anti-disease resistance and faster growth rate. The growth rate of the hybrid was more than 20% faster than that of its parents.

The diploid hybrid fish lineages and improved diploid hybrids mentioned above all exhibited bisexual fertility, forming new fish germplasm resources with hybrid characteristics and producing a series of new hybrids by crossing them with *Megalobrama amblycephala*, *Erythroculter ilishaeformis* and other fishes.

Applications of one-step and multistep breeding technologies in intraspecific hybridization of fish

Intraspecific hybridization can combine the genomes of different subspecies within the same species, causing changes in the phenotypes and genotypes of the offspring. From the analysis of parental relationships, intraspecific hybridization can be regarded as a special case of distant hybridization. Therefore, revealing the genetic and reproductive rules of distant hybridization is beneficial for intraspecific hybridization. One-step and multistep breeding techniques are also useful for intraspecific hybridization. Namely, F_1 with the advantage of hybridization can be used in intraspecific hybridization by means of the one-step breeding technology; it is also possible to establish a fertile lineage in intraspecific hybridization by multistep breeding technology and to prepare improved fish. However, in intraspecific hybridization, it is easier to operate in genetic breeding without breaking through the reproductive barrier of F_1. In this study, a group of improved fish was produced by using the key techniques of one-step and multistep breeding in intraspecific hybridization.

The application of one-step breeding technology in intraspecific hybridization

Japanese white crucian carp and red crucian carp are different subspecies with the same number of chromosomes. Through a systematic study including their reciprocal crosses, it was proven that the F_1 hybrids derived from Japanese white crucian carp (♀)×red crucian carp (♂) show obvious heterosis, and their phenotypes, such as shape and color, were very similar to those of the natural wild crucian carp (Figure 9A). This kind of hybrid had the advantages of a high survival rate, strong resistance, and a fast growth rate. It was not easy for this hybrid to lose its scales, which ensured that they retained a good appearance during transportation. The growth rate of the hybrid was more than 30% faster than that of its parents, and its contents of protein and flavorful amino acids were significantly higher than those of its parents (Liu et al., 2017; Wang et al., 2015a).

The application of multistep breeding technology in intraspecific hybridization

In the intraspecific hybridization, it is easy to establish a hybrid lineage because the F_1 of the intraspecific hybridization generally has good fertility and there is no reproductive barrier in the offspring of distant hybridization. The lineages (F_1–F_5) were established by the intraspecific hybridization of Japanese white crucian carp (♀)×red crucian carp (♂). The improved hybrids (No. 2 crucian carp) were produced by crossing the female of the (e.g., F_1)-Japanese white crucian carp (♀)×red crucian carp (♂) lineage with the male Japanese white crucian carp, which had the advantages of having a high back and fast growth (Figure 9B).

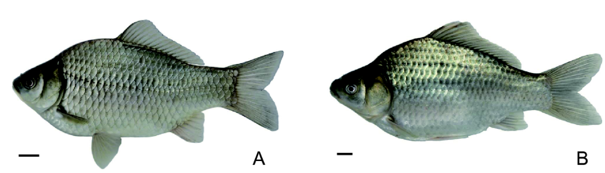

Figure 9 (Color online) The appearance of the hybrid crucian carp lineage derived from Japanese white crucian carp (♀)×red crucian carp (♂) and the improved hybrids (No. 2 crucian carp) produced by crossing the female hybrid crucian carp lineage with male Japanese white crucian carp. A, The appearance of a hybrid crucian carp; B, the appearance of the No.2 crucian carp; Bar=2 cm.

Comparison of other fish hybridization studies with one-step breeding technology and multistep breeding technology

Domestic and international scholars carried out many studies on distant and intraspecific hybridization in fish, but the basic genetic and reproductive rules were not yet established, and common hybrid breeding technologies were also lacking.

In terms of F_1 hybrids derived from distant hybridization in fish, foreign research teams conducted distant hybridization experiments on 1080 species of 56 families of fishes from 1558 to 1980, and these experiments were mainly concentrated on the families of *Centrarchidae*, *Cyprinidae*, *Poeciliidae*, and *Salmonidae* (Schwartz, 1981). For example, all-male F_1 hybrids derived from *Oreochromis niloticus* (2*n*=44, ♀)×*Oreochromis aureus* (2*n*=44, ♂) presented advantages of fast growth rates, strong resistance and high yields (Wang et al., 1989; Xu, 1984). F_1 hybrids with obvious heterosis that were created by crossing *Ictalurus furcatus* (2*n*=58, ♀) with *Ictalurus punctatus* (2*n*=58, ♂) grew more than 30% faster than their parents (Dunham and Argue, 1998). F_1 hybrids with obvious heterosis that were created by reciprocal crossings of *Morone chrysops* (2*n*=48) with *Morone saxatilis* (2*n*=48) grew more quickly and presented stronger stress resistance and stronger anti-disease resistance than their parents (Gaylord and Gatlin III, 2000).

Since the end of the 1950s, many distant hybridization experiments were carried out in China; for example, F_1 hybrids derived from *Oreochromis aureus* (2*n*=44, ♀)×*Oreochromis niloticus* (2*n*=44, ♂) (Wang et al., 1989), F_1 hybrids derived from *Oreochromis niloticus* (2*n*=44, ♀)×*Oreochromis mossambicus* (2*n*=44, ♂) (Tang et al., 2006), F_1 hybrids derived from scattered mirror carp (2*n*=100, ♀)×red crucian carp (2*n*=100, ♂) (Zhou et al., 2008), F_1 hybrids derived from *Erythroculter ilishaeformis* (2*n*=48, ♀)×*Ancherythroculter nigrocauda* (2*n*=48, ♂) (Li, 2013), F_1 hybrids derived from *Siniperca scherzeri* (2*n*=48, ♀)×*Siniperca chuatsi* (2*n*=48, ♂) (Qian et al., 2016), and F_1 hybrids derived from *Epinephelus fuscoguttatus* (2*n*=48, ♀)×*Epinephelus lanceolatus* (2*n*=48, ♂) (Zhang et al., 2018). The above distant crosses had the same number of chromosomes as their parents, which is consistent with the breeding rules stated in the one-step breeding technology proposed by us.

In terms of the F_1 hybrids derived from intraspecific hybridization of fish, foreign research teams produced F_1 hybrids with good traits by crossing Chinese common carp with large stomachs (2*n*=100, ♀) and Europe golden common carp (2*n*=100, ♂) (Hulata, 1995; Wohlfarth et al., 1983). Moreover, an F_1 hybrid was derived from Ukraine common carp (2*n*=100, ♀)×Heilongjiang wild common carp (2*n*=100, ♂), which presented the advantages such as a fast growth rate and high survival rate (Lou, 2009).

Moreover, in China, many intraspecific hybridization experiments were also performed on fish, for example, F_1 hybrids derived from *Cyprinus carpio singuonensis* (2*n*=100, ♀)×scattered scales mirror carp (2*n*=100, ♂) (Meng and Wei, 2011), F_1 hybrids derived from *Cyprinus carpio Red var. wuyuanensis* (2*n*=100, ♀)×*Cyprinus carpio Yuanjiang* (2*n*=100, ♂) (Zhang and Sun, 1988), F_1 hybrids derived from scattered scales mirror carp (2*n*=100, ♀)×*Cyprinus carpio singuonensis* (2n=100, ♂) (Li, 1994), F_1 hybrids derived from Ukraine scaled carp (2*n*=100, ♀)×Tianjin new common carp (2*n*=100, ♂) (Jin et al., 2016), F_1 hybrids derived from Denmark *Scophthalmus maximus* (2*n*=44, ♀)×France *Scophthalmus maximus* (2*n*=44, ♂) (Shi et al., 2014), F_1 hybrids derived from Mississippi *Ictalurus punctatus* (2*n*=58, ♀)×Arkansas *Ictalurus punctatus* (2*n*=58, ♂) (Yu, 2016). The above intraspecific crosses had the same number of chromosomes number as their parents, which is consistent with the design idea of the one-step breeding technology proposed by us.

The above F_1 hybrids derived from interspecific and intraspecific hybridization with the same number of chromosomes as their parents created by other experts at home and abroad showed obvious heterosis, which is consistent with the hybrid breeding rules stated in the one-step breeding technology proposed by us. In this study, the one-step breeding technology proposed by us is explored and summarized, and the corresponding genetic breeding technology has been established, which was lacking in previous studies.

In terms of the lineages derived from distant hybridization, other research teams at home and abroad established fertile hybrid lineages, for example, the F_1–F_3 lineage derived from *Oreochromis niloticus* (2*n*=44, ♀) with *Sarotherodon melanotheron* (2*n*=44, ♂) (Li et al., 2008; Wei et al., 2016), the F_1–F_2 lineage derived from *Siniperca kneri* (2*n*=48, ♀)×*Siniperca chuatsi* (2*n*=48, ♂) (Lu et al., 2013), and the F_1–F_2 lineage derived from *Siniperca chuatsi* (2*n*=48, ♀)×*Siniperca scherzeri* (2*n*=48, ♂) (Yuan et al., 2014). Some lineages derived from intraspecific hybridization were also established, for example, the F_1–F_2 lineage derived from *Siniperca scherzeri* (2*n*=48, ♀)×*Siniperca chuatsi* (2*n*=48, ♂) (Li C et al., 2014), the F_1–F_2 lineage derived from *Cyprinus pellegrini Tchang* (2*n*=100, ♀)×*Cyprinus carpio Red var. wuyuanensis* (k) (2*n*=100, ♂) (Gao et al., 2006), and the F_1–F_2 lineage derived from *Cyprinus carpio Red var. wuyuanensis* (2*n*=100, ♀)×*Cyprinus carpio Yuanjiang* (2*n*=100, ♂) (Zhang, 1985). The formations of the above fertile lineages derived from interspecific and intraspecific hybridization are consistent with the multistep breeding technology proposed by us, which was lacking in previous studies.

Application effects and prospects of one-step breeding technology and multistep breeding technology

Based on the genetic and reproductive rules of distant hybridization in fish, the one-step technology and multistep technology established by us have universal guiding functions in fish genetic breeding. Considering the situation when parents have the same or different numbers of chromosomes, this genetic rule is universal because it covers all possible hybrid types, and both interspecific and intraspecific hybridization are within this range. In this study, depending on the number of parental chromosomes, the genetic compositions of all offspring of crosses can be predicted. The genetic rules stated in this study are highly organized, and they can effectively guide the implementation of predesign by indicating the offspring that are relatively easy to form, those that are difficult to generate and those that are not feasible. If the numbers of chromosomes of the two parents are equal, it is possible to easily obtain many hybrid offspring, avoiding blindness, which can lead to the death of offspring by design mistakes. In other words, this method is a one-step breeding technology. When the number of parental chromosomes is not equal, the utilization of heterosis advantages will not occur in the first generation of hybridization but the formation of lineages is very useful for further breeding. Generally, it is necessary to break through the barrier of reproductive isolation of hybrid offspring to create fertile lineages. Once the barrier of reproductive isolation is broken, different types of tetraploid fish lineages and diploid fish lineages can be obtained and they can be used for further breeding, which is the implementation of multistep breeding technology.

With more than 32,500 species, fish are the most diverse group of vertebrates (Cossins and Crawford, 2005). Reproductive isolation between species prevails in nature, which is an important way to maintain the relative stability and balance of species. However, species in nature will change with the changes in time and space, and some species will be eliminated while others will be created. The state of relative stability and balance is not immutable. Thus, reproductive isolation can be broken in a sense. Researchers are currently attempting to produce new germplasm resources in fish by means of distant hybridization to create new species that may occur in nature under certain circumstances, that is, to form new fertile lineages through distant hybridization, which lays an important foundation for the formation of new species.

Breeding is a process of exploring individuals or groups that have mutations in phenotypes and genotypes. Some individuals or groups with changed characteristics are directly used as the improved varieties, and some individuals are used as new germplasm resources for further producing new and improved varieties. One-step breeding technology and multistep breeding technology follow such rules. For multistep breeding technology, the new germplasm resources involved are fertile lineages formed by hybridization (interspecific and intraspecific hybridization).

The genetic and reproductive rules of distant hybridization in fish have been found through long-term research, and one-step and multistep breeding technologies that are suitable for interspecific and intraspecific hybridization have been formed. A series of improved fishes have been produced using these two genetic breeding technologies, which proves that these two technologies are extensive, scientific and practical. Through comparative analyses, the two technologies proposed by us are consistent with the facts of successful hybrid breeding conducted in the past, which fully demonstrates that the two technologies have good application effects and prospects.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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