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The Research Advances in Distant Hybridization and Gynogenesis in Fish

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ABSTRACT

Distant hybridization and gynogenesis are two prevalent breeding techniques for fishes. Drawing from the research achievements of our team and the existing literature, we summarize the reproductive traits and genetic features of fishes derived from distant hybridizations and gynogenesis, and we deduce the fundamental mechanisms of these two methods and compare them, discerning their common and different characteristics. Both distant hybridization and gynogenesis techniques can alter genotypes and phenotypes, thus establishing them as significant breeding methods. Additionally, the genetic principles and the basic biological characteristics of distant hybridization and gynogenesis in fish have been inferred. We propose the concepts of macro-hybrid and micro-hybrid based on extensive experimental findings from fish distant hybridizations and gynogenesis. The term "macro-hybrid" refers to offspring from distant hybridization that possess two distinct subgenomes, each inherited from one of the two parental species, such as allodiploid and allotetraploid lineages. The concept of "micro-hybrid" refers to offspring, including autodiploid and autotetraploid lineages, as well as those resulting from artificial gynogenesis, whose genome almost originates solely from the maternal parent but in which certain DNA fragments derived from the paternal parent insert. Distant hybridization and gynogenesis are vital techniques in fish genetics, breeding, and evolution. We highlight the prospective paths for research and application of distant hybridization and gynogenesis in fishes.

1 | Introduction

Fish distant hybridization is a form of hybridization in which fishes from different species, genera, or even families, which are significantly genetically distant from each other, are crossbred to potentially create offspring with traits from both parents. The process of distant hybridization can be challenging due to the reproductive barriers between significantly different species, including differences in mating behaviors, habitat preferences, and timing of reproduction, as well as biological incompatibilities that prevent the sperm and egg from successfully fertilizing, the embryo from developing properly, or the larvae from surviving. Despite these challenges, various methods have been developed to overcome reproductive barriers, including artificial fertilization and hormone treatments to induce spawning.

Qizhi Liu, Shi Wang, and Chenchen Tang contributed equally to this work.

Here, we classify fish gynogenesis into artificial gynogenesis and natural gynogenesis. Artificial gynogenesis is termed fish gynogenesis in general, a reproductive method often used in aquaculture and genetics, where sperm triggers egg development, resulting in a diploid embryo with almost exclusively the maternal DNA. Artificial gynogenesis is achieved through techniques such as irradiating sperm by UV and suppressing the second meiotic division of the egg or inducing the egg to duplicate its DNA. This process results in offspring genetically identical to the maternal and almost exclusively female. In such a context, fish gynogenesis is independent of fish distant hybridization. However, in the process of artificial fertilization, a small number of natural gynogenetic offspring may appear in the absence of sperm inactivation and egg shock treatments. These gynogenetic offspring include male individuals and show some paternal traits probably due to paternal DNA integration during the gynogenesis (allo-sperm effect). Thus, distant hybridization and gynogenesis are not entirely independent concepts. Moreover, in distant hybridization, the production of natural gynogenetic offspring through artificial fertilization and the occurrence of males and the allo-sperm effect may lead one to speculate whether a similar process in nature could produce new, fertile species. This has unique significance in the field of evolutionary biology. We will summarize fish lineages produced through both artificial and natural gynogenesis.

Previously, our team has established and applied the distant hybridization technology in fish [1]. Here, on the one hand, we further refine the technology and update the reproductive traits and genetic features of fishes derived from distant hybridization. On the other hand, we further explain the gynogenesis breeding strategy established by our team and add the reproductive traits and genetic features of fishes derived from gynogenesis. Furthermore, we propose the concepts of "macro-hybrid" and "micro-hybrid" based on the extent of the genome hybrid. We hope this review offers valuable guidance for researchers interested in fish distant hybridization and gynogenesis.

2 | Research Advances in Fish Distant Hybridization

2.1 | The Reproductive Traits of Fishes Derived From Distant Hybridizations

Numerous examples suggest that distant hybridizations can lead to fertile lineages that exhibit normal gonad development in cyprinids [2–7], poeciliids [8], centrarchids [9, 10], and salmonids [11, 12]. Research shows that the hybrid F_1 derived from large yellow croaker (♀, *Larimichthys crocea*) × small yellow croaker (♂, *Larimichthys polyactis*) has normal ovary and testis development, producing fertile offspring [13]. Likewise, the hybrid grouper derived from brown-marbled grouper (♀, *Epinephelus fuscoguttatus*) × potato grouper (♂, *Epinephelus tukula*) has normal fertility [14]. The hybrid derived from orange-spotted grouper (♀, *Epinephelus coioides*) × giant grouper (♂, *Epinephelus lanceolatus*) can produce both diploids and triploids. Among them, the triploids are infertile, while the diploids have normal gonad development [15, 16]. Offspring obtained from the hybridization of female brown-marbled grouper and male giant grouper are fertile, and the gametes produced can be fertilized normally [17]. The F_1 offspring derived from blotched snakehead (♀, *Channa maculata*) × northern snakehead (♂, *Channa argus*) is fertile and can produce F_2 progeny through self-mating [18]. The easy caught carp is a new aquatic breed obtained by the Chinese Academy of Fishery Sciences through hybridization among the barbless carp (*Cyprinus pellegrini*), the German mirror carp (*Cyprinus carpio* var. mirror carp), and the Heilongjiang wild carp (*Cyprinus carpio haematopterus*). It can reproduce normally and has formed a hybrid carp lineage [19]. In contrast, the new aquatic breed "Huangyou No. 1" obtained from hybridizing the yellow catfish (♀, *Pelteobagrus fulvidraco*) and darkbarbel catfish (♂, *Pelteobagrus vachelli*) has abnormal gonad development and lacks reproductive capability [20]. Hybridization between the obscure pufferfish (♀, *Takifugu obscurus*) and the Japanese pufferfish (♂, *Takifugu rubripes*) has resulted in offspring with growth advantages. However, the development of their gonads requires further study. Luin, Fui, and Senoo [21] reported that the hybrid offspring of the brown-marbled grouper (*Epinephelus. fuscoguttatus*) and the giant grouper have normally developed gonads. Fertile offspring have been found in several distant hybridizations of tilapia. For instance, Chellappa et al. [22] reported that the gonads of hybrid offspring from the Nile tilapia (*Oreochromis niloticus*) and Mozambique tilapia (*Oreochromis mossambicus*) are normally developed and can also produce descendants. Fertile groups have been reported in the hybrid offspring of the Nile tilapia and blue tilapia (*Oreochromis aureus*), the hybrid offspring of the Nile tilapia and Wami tilapia (*Oreochromis urolepis hornorum*), as well as the hybrid offspring of the Mozambique tilapia and Wami tilapia [23]. Moreover, some of these groups have been used to establish breeding lineages [23]. These results indicate that distant hybridization can obtain a hybrid population capable of normal reproduction of offspring with normal gonadal development.

According to the findings of the authors' team, the mechanism by which fertile lineages are formed through distant hybridizations in fishes is related to the production of either reduced or unreduced gametes by distant hybridized fishes. While haploid sperm and egg originating from distant hybridization can lead to fertile diploid progeny, unreduced diploid sperm and egg from the same process can produce fertile tetraploid offspring. Another method to produce fertile tetraploid offspring is combining haploid sperm with triploid egg, which has been previously demonstrated to be effective [1, 24].

In collaboration with Hu's team, the authors' team discovered that both the allotetraploid red crucian carp (♀) × common carp (♂) and the allodiploid blunt snout bream (♀) × topmouth culter (♂) can produce primary germ cells (PGCs) that are capable of migration (unpublished data). This observation was confirmed by tracking labeled PGCs. Additional evidence indicates that fishes derived from distant hybridizations can develop mature gonads and normal gametes, validating the prediction that allotetraploid and allodiploid lineages can propagate reliably.

Distant hybridization can result in the production of gametes with varying ploidies, leading to a range of fertile hybrid lineages. From F_1–F_n, all progeny of blunt snout bream (*Megalobrama amblycephala*) × topmouth culter (*Culter alburnus*) are diploid lineages [25]. In lineages derived from red crucian carp (*Carassius auratus* red var., ♀) × common carp (*Cyprinus carpio*, ♂), F_1–F_2

are diploid while F_3–F_{31} are tetraploid [26, 27], indicating that F_2 produced unreduced gametes to form tetraploid offspring. Upon the cross of red crucian carp (♀, $2n=100$)×blunt snout bream (♂, $2n=48$), the F_1 generation exhibits an autodiploid lineage resembling the red crucian carp and this diploid lineage can consistently reproduce by self-mating [28]. From the same process, an allotetraploid hybrid ($4n=148$) was obtained from a combination of unreduced gametes [28]. Self-mating of this allotetraploid hybrid produced an autotetraploid lineage ($4n=200$). Similarly, common carp (♀)×blunt snout bream (♂) [29, 30] and koi carp (*C. carpio haematopterus*, ♀)×blunt snout bream (♂) [31] produced maternal-like autodiploid carp (grey, $2n=100$) and autodiploid carp (colorful, $2n=100$), respectively. In addition, the hybridization combination of common carp (♀)×blunt snout bream (♂) can generate autotetraploid carp ($4n=200$).

One likely reason that distant hybridization results in the production of unreduced gametes is the initial replication of DNA in the germ cell nucleus, followed by meiosis. In this section, we include examples mostly from Cyprinidae, which being a largely tetraploid family, is possibly only representative of some fish lineages.

2.2 | The Genetic Features of Fishes Derived From Distant Hybridization

2.2.1 | Genetic Laws of Fishes Derived From Distant Hybridization at the Chromosomal Level

When the parental chromosomes of distant hybridized fishes are equal in number, fertile allodiploid and allotetraploid fish lineages can be formed. When the number of chromosomes in the maternal parent is greater than that in the paternal parent, autodiploid and autotetraploid fish lineages can be formed. Conversely, when the number of chromosomes in the maternal parent is less than that in the paternal parent, it is challenging to produce surviving offspring [1, 24].

The genetic characteristics of distant hybridized fishes are much more complex than those of close hybridized fishes. It can be said that the genetic laws of distant hybridization in fishes encompass the genetic laws of intraspecific hybridization. Intraspecific hybridization occurs when both parents have the same number of chromosomes, and in this case, the kinship between the parents is relatively close, representing hybridization within the same species. Conversely, in distant hybridization, when the parents have unequal numbers of chromosomes, there are specific genetic laws. In distant hybridization, when the parents have an equal number of chromosomes since the kinship between the parents is at least a hybridization between species, its genetic laws are also very different from intraspecific hybridization.

For intraspecific hybridization, the kinship between the parents is much closer. When both parents have the same number of chromosomes, offspring from distant hybridizations can produce diploids and triploids. As generations progress, distant hybridizations can also give rise to allodiploids, allotetraploids, naturally gynogenetic offspring, and other lineages of varying ploidy levels (Figure 1), while intraspecific hybridizations only result in the formation of diploid fish lineages [1].

Distant hybridization is much more complex than intraspecific hybridization. First, the offspring resulting from distant hybridization are not limited to diploids; various types of fish with different ploidies can emerge. Therefore, exploring the genetic

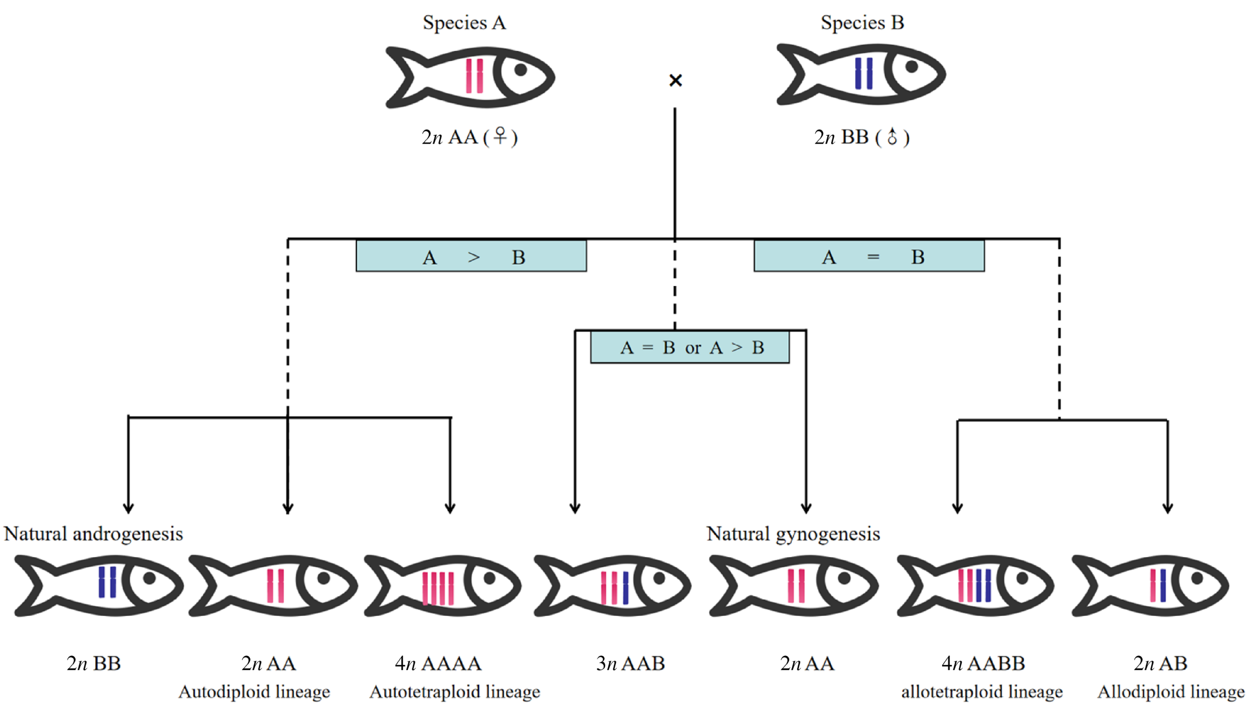

FIGURE 1 | Formation of fishes with different ploidies can occur when the number of the maternal chromosomes is greater than or equal to that of the paternal chromosomes. A and B are different species. Red and blue chromosomes represent the genetic materials from the maternal and paternal species, respectively.

laws at the chromosomal level in distant hybridization is crucial. Hence, research on distant hybridization in fishes has important implications in genetics, breeding science, and biological evolution.

2.2.2 | Genetic Mechanisms of Fishes Derived From Distant Hybridizations at the Molecular Level

In the distant hybridization of fishes, the interplay between the maternal nuclear material–paternal nuclear material, the maternal nuclear material–cytoplasm, and the paternal nuclear material–cytoplasm is associated with changes at the DNA, epigenetic, RNA, and protein levels.

At the DNA level, distant hybridized fishes undergo a hybridization process involving parents with different genomes. Regardless of whether the resulting lineages are homozygous or heterozygous, the genome of the hybrid offspring undergoes intense disruptions. As a result, their DNA structure experiences corresponding changes. A significant change is the emergence of numerous chimeric genes in the hybrid offspring, which is beneficial for combining the genetic material of both parents into one, aiding in the evolution of new species [32–36]. At the epigenetic level, sometimes epigenetic regulation can control the expression of multiple homologous genes, thus affecting the hybrid fish traits [37, 38]. At the RNA level, changes encompass nonadditive expression of duplicated genes, which includes dominant expression, overdominant expression, and bias toward homologous expression [7], along with dosage compensation effects [39, 40], nucleolar dominance (nucleolar dominance is an epigenetic phenomenon where the expression of 45S rRNA from one parent in a hybrid species is suppressed by the other parent) [41, 42], and both *cis*- and *trans*-regulatory influences from diverse parental origins. At the protein level, notable variations in growth and fertility exist among fishes with different ploidies [43–45].

2.2.3 | The Genotypes Resulting From Distant Hybridizations in Fishes

Distant hybridizations in fishes can result in different progenies having diverse ploidy levels, such as diploid and tetraploid individuals. Our recent study even achieved the formation of different polyploids by disrupting meiotic crossover frequencies following *cntd1* knockout in zebrafish [46]. Unlike typical intraspecific hybridization that yields only diploid offspring with the same species, distant hybridization involving parents from different species produces offspring of varying ploidies. This suggests a distinctive genetic mechanism at play in distant hybridization. The occurrence of fertile diploid and tetraploid offspring resulting from distant hybridization raises an intriguing question: What are the genotypes of these diploid and tetraploid descendants? Here, we compile a selection of distant hybridization crosses including red crucian carp ($2n=100$, ♀)×blunt snout bream ($2n=48$, ♂), common carp ($2n=100$, ♀)×blunt snout bream ($2n=48$, ♂), red crucian carp ($2n=100$, ♀)×common carp ($2n=100$, ♂), blunt snout bream ($2n=48$, ♀)×topmouth culter ($2n=48$, ♂), and topmouth culter ($2n=48$, ♀)×blunt snout bream ($2n=48$, ♂). We report that the progeny from such crosses can be categorized into four types: allodiploid, allotetraploid, autodiploid, and autotetraploid. The genotypes of the allodiploid and allotetraploid should be AB and AABB, respectively, while the genotypes of the autodiploid and autotetraploid should be AA and AAAA, respectively. Through analysis at the chromosomal and genomic levels, we have determined that the genetic makeup of offspring resulting from distant hybridizations reveals that allodiploids and allotetraploids, with genotypes AB and AABB respectively, contain subgenomes derived from both parent species. Conversely, autodiploids and autotetraploids, which have the genotypes AA and AAAA respectively, contain subgenomes primarily originating from the maternal parent. Into these subgenomes, DNA segments from the paternal parent are integrated. From the analyses conducted, we termed distant hybridizations leading to allodiploid and allotetraploid progenies with subgenomes from both parents as "macro-hybrid" (Figure 2). In contrast, distant hybridizations that yield autodiploid and autotetraploid progenies, which inherit their subgenome solely from the maternal parent with paternal DNA fragments inserted, are termed "micro-hybrid" (Figure 3). In addition, given the increasing evidence that gynogenetic offspring may incorporate a small amount of paternal DNA, both artificial and natural gynogenesis may also be classified as "micro-hybrid."

3 | Research Advances in Fish Gynogenesis

3.1 | Reproductive Traits of Fishes Derived From Gynogenesis

Gynogenetic offspring showing male individuals and paternal traits probably due to paternal DNA integration during the gynogenesis is termed the allo-sperm effect. Fishes developed through gynogenesis induced by allo-sperm, despite the allo-sperm effect, generally do not have their fertility altered by the subtle paternal DNA fragment insertions into the genome. As a result, the fertility of these gynogenetic fishes is typically normal. However, during actual breeding processes, few gynogenetic fishes have been observed to have poorer fertility, leading to delayed sexual maturation [47, 48]. The reasons for this remain to be further researched.

In our laboratory, using artificial gynogenesis techniques (Figure 4), we have cultivated 18 types of gynogenetic fishes. Among these, such as the gynogenetic grass carp (*Ctenopharyngodon idella*) [49, 50], red crucian carp [51], Japanese crucian carp (*Carassius cuvieri*) [52], goldfish (*Carassius auratus*) (unpublished data), blunt snout bream [53], common carp [54], largemouth bass (*Micropterus salmoides*) [55], mandarin fish (*Siniperca chuatsi*) [56], channel catfish (*Ictalurus punctatus*) (unpublished data), allodiploid blunt snout bream (♀)×topmouth culter (♂) [57], allotetraploid red crucian carp (♀)×common carp (♂) [47, 51, 57, 58], and autotetraploid red crucian carp (♀)×blunt snout bream (♂) [59] are all female. On the other hand, in our laboratory, among seven distant hybridization combinations, we have produced seven types of naturally gynogenetic fish (Figure 5). In the hybrid combinations of red crucian carp (♀)×blunt snout bream (♂) [28, 60], red crucian carp (♀)×*Xenocypris davidi Bleeker* (♂) (unpublished data), koi

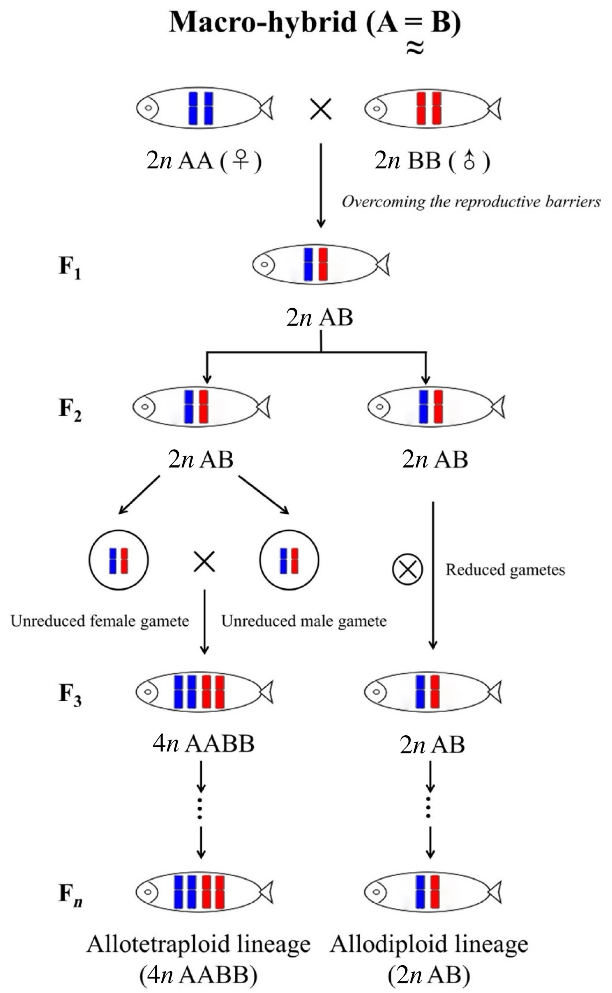

FIGURE 2 | Fertile lineages might be obtained when there is an equal count of chromosomes from each parent. A and B are two distinct species. In this cross, the resultant allodiploid (2*n* AB) and allotetraploid (4*n* AABB) from distant hybridizations, which contain subgenomes from both parents, are classified as "macro-hybrids." Blue and red chromosomes represent the genetic materials from the maternal and paternal species, respectively.

carp (♀)×blunt snout bream (♂) [31], common carp (♀)×blunt snout bream (♂) [29], blunt snout bream (♀)×*Xenocypris davidi Bleeker* (♂) [61], grass carp (♀)×topmouth culter (♂) [62], and blunt snout bream (♀)×mandarin fish (♂) [63], all resulted in naturally gynogenetic fishes. These naturally gynogenetic offspring obtained through distant hybridization breeding techniques included both females and males, and they were fertile. This gender composition might be different from that of artificial gynogenetic offspring (with an XY sex-determination mechanism).

We carried out sex reversal on gynogenetic Japanese crucian carp (by feeding them food containing methyltestosterone) and obtained male individuals (XX). These male individuals (XX) were mated with regular female Japanese crucian carp to produce all female Japanese crucian carp (XX) [64]. The female Japanese crucian carp grew faster than the male Japanese crucian carp. Therefore, this breeding technique holds significant value for production applications of Japanese crucian carp and possibly other fishes.

Gynogenetic fishes are usually female and fertile but might exhibit paternal-specific genotypes and phenotypes due to the "allo-sperm effect." To perpetuate the offspring of artificial gynogenesis, we established the gynogenesis breeding strategy in which gynogenetic females are mated to males of the normal fish to produce the progenies carrying the paternal-specific genotypes and phenotypes due to "allo-sperm effect." This breeding strategy has been applied in many combinations, including gynogenetic grass carp, mandarin fish, and largemouth bass with normal males, respectively. For example, we mate female gynogenetic grass carp [49, 50] with male grass carp. The F_1 generation showed the allo-sperm effect and disease resistance [24, 50], and this generation included both males and females. Results indicated that the allo-sperm effect can be observed in these F_1 populations. Therefore, this fertile bisexual F_1 group can be used as a new genetic resource for to produce more disease-resistant grass carp.

Natural gynogenesis can also produce male offspring, probably due to the allo-sperm effect. For example, we previously discovered that in the offspring (F_1) of distant hybridization between red crucian carp (♀, $2n=100$) and blunt snout bream (♂, $2n=48$), there are fertile natural gynogenetic F_1 fish of both sexes, and that self-crossing of F_1 led to the formation of fertile F_2 offspring of both sexes [28, 60]. The sex ratio of both F_1 and F_2 is close to 1:1. Both F_1 and F_2 exhibit the paternal unique trait of high body height. The occurrence of green body color in the F_2 is a unique characteristic of the paternal lineage, probably due to the Mendelian inheritance of the dominant pigmentation phenotype.

3.2 | Genetic Features of Fishes Derived From Gynogenesis

3.2.1 | Genetic Rules for Fishes Derived From Gynogenesis at the Chromosomal Level

Though gynogenetic offspring induced by heterologous sperm exhibit the allo-sperm effect, their chromosomal ploidy remains diploid. Due to the allo-sperm effect, their genetic material has changed compared to the original maternal parent. Usually, the offspring are all females and fertile. By mating with males, this diploid nature is heritable, and the phenotypes caused by the allo-sperm effect are transmitted.

Chen et al. [65] discovered stable paternally inherited DNA fragments in the genome of gynogenetic silver crucian carp; Fan et al. [66] detected paternal DNA fragments in the genetic material of artificially induced gynogenetic oblique-banded grouper, confirming the existence of the allo-sperm effect in their allogynogenetic population; Cao et al. [67] used sperm from the yellowcheek (*Elopichthys bambusa*) and topmouth culter to induce gynogenesis in Pengze crucian carp (*Carassius auratus* var. pengsenensis). The results of the study confirmed that different sperm sources have varying impacts on gynogenetic offspring, exhibiting a typical allo-sperm effect. These paternal

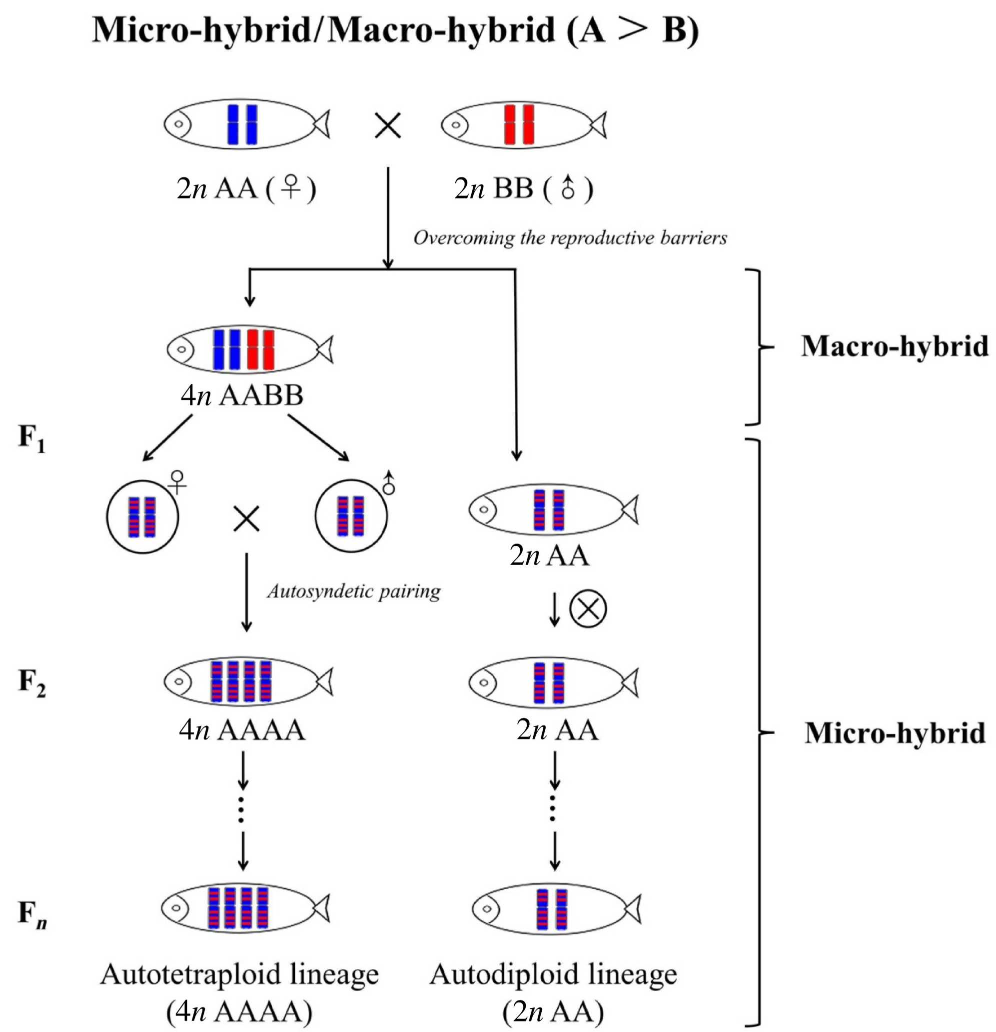

FIGURE 3 | Fertile lineages might be obtained when the number of maternal chromosomes is greater than that of paternal chromosomes. A and B are two distinct species. In this cross, the resultant allotetraploid (4*n* AABB) from distant hybridization containing the subgenomes from the different parents is called "macro-hybrid." The distant hybridizations producing autodiploid (2*n* AA) and autotetraploid (4*n* AAAA) offspring, which inherit their subgenomes exclusively from the maternal parent with the insertion of DNA fragments from the paternal parent, are termed 'micro-hybrids.' Blue and red chromosomes represent the genetic materials from the maternal and paternal species, respectively.

DNA fragments can not only serve as effective molecular markers to distinguish between gynogenetic diploids and regular diploids, but this allo-sperm effect might also endow gynogenetic offspring with superior traits such as faster growth and greater resilience [49, 50].

3.2.2 | Genetic Mechanisms of Fishes Derived From Gynogenesis

Gynogenetic fishes undergo a doubling process for the maternal chromosome set, which produces the "homozygosity effect." Relative to the typical self-breeding process, this homozygous effect can be viewed as a unique form of genetic variation. Moreover, the allo-sperm effect caused by heterologous sperm results in the insertion of paternal DNA fragments into the maternal genome, leading to genetic variation. The "homozygous effect" and the allo-sperm effect are the primary sources of genetic variation in gynogenesis. These genetic variations make gynogenetic fishes a novel and valuable resource for lineage development. These new lineages can be utilized through techniques like backcrossing, making their F_1 generation also a valuable lineage resource.

Furthermore, artificial gynogenesis undergoes cold shock or heat shock processes. Fish that survive these adverse conditions have undergone a "strict screening" or "rigorous breeding" process and possess robust resistance traits, possibly including strong disease resistance. We call this phenomenon the "selection effect" here.

Thus, gynogenetic fishes induced by heterologous sperm encompass the "homozygosity effect," "allo-sperm effect," and "selection effect" (Figure 6). All these variation factors are fundamental to their superior qualities [49, 50].

Distant hybridization also undergoes an "adverse selection" process. In the context of distant hybridization, the adversity is reflected in the fact that the parents have a distant kinship relationship. Offspring that can survive in such challenging

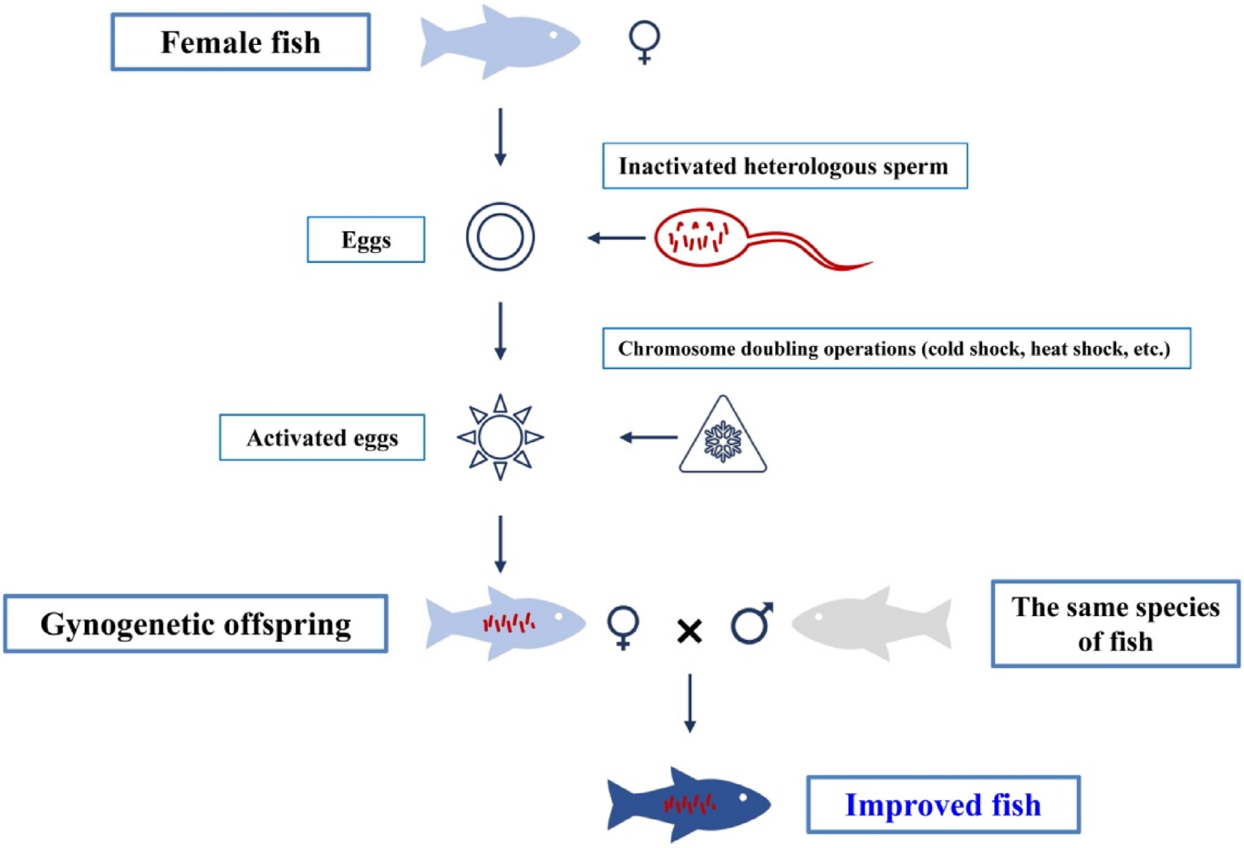

FIGURE 4 | Production of improved fish developed by backcrossing of the heterosperm-induced artificial gynogenetic fish. Both the gynogenetic offspring and the improved fish produced by backcrossing have genetic material derived from heterologous sperm in their genomes, as indicated by the short red bar in the figure, indicating the occurrence of the allo-sperm effect.

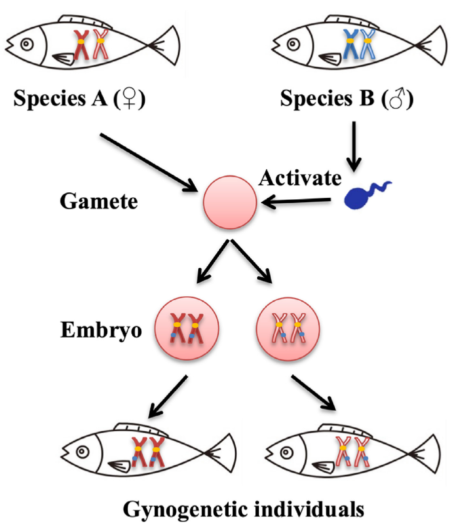

FIGURE 5 | Formation of fertile natural gynogenetic fish induced by heterologous sperm. A and B are different species. Red and blue chromosomes represent the genetic materials from the maternal and paternal species, respectively. Homozygous gynogenetic individuals are obtained after induction of development by heterologous sperm. Fragments of DNA from species B (blue) are present in the gynogenetic individuals.

"adversity" possess strong resilience. This resilience could, in some respects, surpass their parents, as they might benefit from hybrid vigor.

4 | Comparison Between Distant Hybridization and Gynogenesis in Fish

Distant hybridization refers to the crossbreeding of two or more species. Its fertile offspring carry genomes from both parents (heterogenous) or a single parent (homogenous), encompassing allo- and autodiploids and tetraploids. The scenario for autodiploids is very similar to the offspring formed by gynogenetic stimulation with heterologous sperm. Offspring formed by distant hybridizations, being autodiploids, mainly derive their genome from the maternal side but have paternal genetic material (DNA fragments) inserted. Such autodiploid offspring are also referred to as natural diploid gynogenetic descendants. Their formation is closely related to the natural chromosome doubling of the maternal haploid eggs. During artificial gynogenesis induced by heterologous sperm, chromosome sets in the eggs are doubled via cold or heat shock methods.

Formation of autodiploid fishes has been observed in crossbreeds like red crucian carp (♀)×blunt snout bream (♂) [28, 60], common carp (♀)×blunt snout bream (♂) [29], koi carp (♀)×blunt snout bream (♂) [31], blunt snout bream (♀)×*Xenocypris davidi Bleeker* (♂) [61], and blunt snout bream (♀)×mandarin fish (♂) [63]. Interestingly, both female and a minority of male individuals exist in these autodiploid fishes, laying a vital reproductive foundation for their fertile lineages. Conversely, in artificially induced gynogenetic offspring, only females generally exist, with no male presence, marking a significant difference between the two.

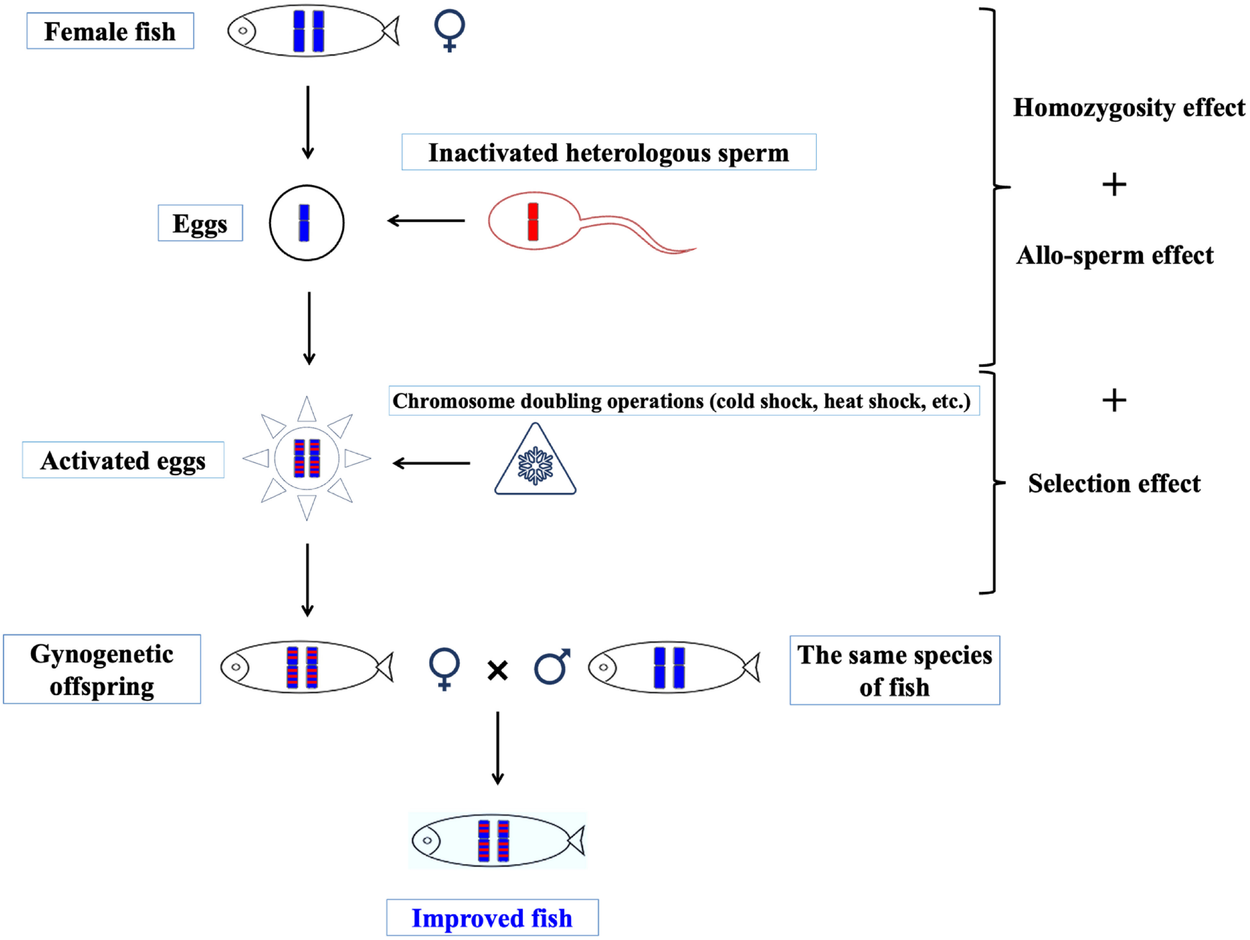

FIGURE 6 | Production of improved fish developed by backcrossing of the heterosperm-induced gynogenetic fish. Blue and red chromosomes represent the genetic materials from the maternal and paternal species (heterologous sperm), respectively. Both the gynogenetic offspring and the improved fish produced by backcrossing have genetic material derived from heterologous sperm in their genomes, as indicated by the red bar in the figure, indicating the occurrence of the allo-sperm effect. Moreover, fish gynogenesis also exhibits the homozygosity effect and the selection effect.

In offspring resulting from gynogenetic stimulation with heterologous sperm, paternal DNA fragments are inserted. This "distant hybridization" effect is quite similar to the distant hybridization effect observed in autodiploid fishes. Heterologous sperm DNA fragments can be detected in gynogenetic grass carp. Although these two methods follow different pathways, their outcomes have several similarities. Additionally, this comparison suggests that in combinations where distant hybridization struggles to yield viable offspring, like in grass carp (♀)×koi carp (♂), utilizing the gynogenetic method with heterologous sperm can achieve the "distant hybridization" effect, as seen in combinations like chub (♀)×koi carp (♂) and blunt snout bream (♀)×koi carp (♂). When the chromosome count of the mother is significantly lower than that of the father, viable offspring are challenging to produce, a significant finding obtained through the author team's long-term research [1, 68]. Although only female individuals exist in artificial gynogenetic offspring induced by heterologous sperm, mating these females with corresponding regular males can yield improved trait lineages. This method is well exemplified in disease-resistant grass carp, where the disease-resistance traits in backcrossed offspring of allogynogenetic grass carp have a close connection with the inherited DNA fragments from koi carp.

5 | Applications of Distant Hybridization and Gynogenesis in Fish

Distant hybridization and gynogenesis are essential technologies in fish breeding, serving as fundamental techniques widely applied in the fisheries industry. The author's laboratory studied 283 newly reviewed aquaculture varieties in China (only those varieties that have obtained a new variety certificate can be widely used in the industry). Among them, 58 new varieties were obtained through distant hybridizations, and 23 new varieties were obtained through multiple methods including gynogenesis, indicating that distant hybridization and gynogenesis are vital breeding techniques. Moreover, these classical breeding methods have been proven to be useful and enduring in practice. In breeding technology, there is no hierarchy. Any technique that can produce varieties with excellent traits is commendable.

Our laboratory, along with other research teams, has used distant hybridization and gynogenesis to develop a series of fertile and high-quality fishes. Some of these varieties form new fish germplasm resources, which are used for further development of superior fish varieties.

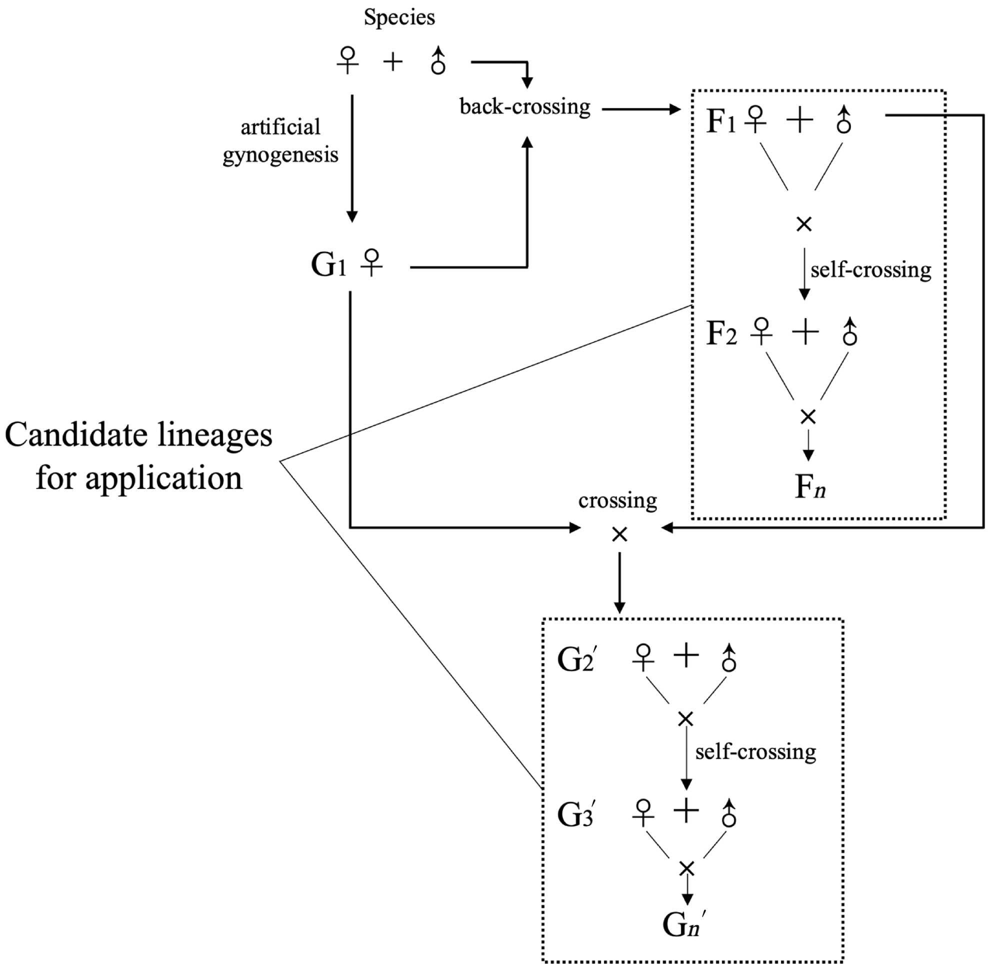

FIGURE 7 | Process for generating new lineages through artificial gynogenesis in fish.

Distant hybrids, including offspring from allotetraploid red crucian carp (♀)×common carp (♂) formed, the improved allotetraploid red crucian carp (♀)×common carp (♂) [47, 51, 57, 58], and other autotetraploid fishes have significant importance in evolutionary biology and genetics. They are also widely used in production to prepare sterile triploid fishes like the triploid Xiangyun crucian carp [69, 70], Xiangyun crucian carp No. 2 [24], Xiangyun carp [71], and Hefang crucian carp No. 3, among others. These fishes exhibit rapid growth, sterility, excellent meat quality, and strong disease resistance. Their infertility enables the nutrients typically allocated for reproduction to be redirected toward growth. Some of these triploid fishes, such as Xiangyun crucian carp and Xiangyun carp, have been widely used in China. These are examples of multistep breeding techniques explored by the authors' laboratory.

Some diploid fishes formed through distant hybridizations, such as those from the crossbreeding of Japanese crucian carp (♀)×red crucian carp (♂), have become the Hefang crucian carp [72, 73], directly used as superior fish in production. This is an example of the one-step breeding technique. Other exemplary breeds produced using the one-step breeding technique is the F_1 generation derived from blunt snout bream (♀)×*Xenocypris davidi Bleeker* (♂) [61]. In distant hybridization, natural gynogenetic diploid fishes in the F_1 can serve as new germplasm resources, forming new lineages either through their own cross or direct use. Examples are shown in the following combinations: common carp (♀)×blunt snout bream (♂) can produce natural gynogenetic carp [29] (known as Xiangjun carp); blunt snout bream (♀)×*Xenocypris davidi Bleeker* (♂) [61] results in natural gynogenetic bream; blunt snout bream (♀)×mandarin fish (♂) [63] yields natural gynogenetic bream; red crucian carp (♀)×blunt snout bream (♂) [28, 60] leads to natural gynogenetic red crucian carp; and koi carp (♀)×blunt snout bream (♂) [31] creates natural gynogenetic koi carp.

Gynogenesis, as a unique reproductive method in fish, has become an essential tool for genetic improvement in fish. Artificially induced gynogenesis accelerates the homozygosity of superior genes, rapidly fixing desirable genetic traits, and thereby shortening the breeding process. Gynogenesis has been applied extensively in various fish breeding research and practices globally, as demonstrated in various species such as Pengze crucian carp [67], blunt snout bream [74], silver barb (*Barbonymus gonionotus*) [75], rainbow trout (*Oncorhynchus mykiss*) [76], sterlet (*Acipenser ruthenus*) [77], grayling (*Thymallus thymallus*) [78], yellow-tail tetra (*Astyanax altiparanae*) [79], grouper [66], striped beakfish (*Oplegnathus fasciatus*) [80], yellow drum (*Nibea albiflora*) [81], among many others. Offspring developed from artificial gynogenesis are new fish lineage resources. These new germplasm resources can be transmitted through backcrossing, self-crossing, and regular crossbreeding, which is well exemplified in disease-resistant grass carp, thereby producing bisexual fertile candidate lineages for application (Figure 7).

The Hefang crucian carp produced using the one-step method can also be used as a new germplasm resource for further breeding. Crossbreeding male Japanese crucian carp and female Hefang crucian carp produced the superior crucian carp—Hefang crucian carp No. 2 [72, 73, 82, 83], which is now farmed in various provinces in China, yielding significant economic, social, and ecological benefits. The Hefang crucian carp exhibits rapid growth, excellent meat quality, good taste, and high resilience [49, 50]. It is not only suitable for pond cultivation, but also for paddy fields due to its high resilience. The Hefang crucian carp No. 2 is rich in savory amino acids, and its soup turns milky white, making it an excellent ingredient for fish soups and jellies.

6 | Conclusions and Future Perspectives

Based on the experimental results obtained by the authors' laboratory team and the deduced genetic laws at the chromosome level, when the chromosome numbers of both parents are the same, allodiploid and allotetraploid lineages can be formed. When the chromosome number of the mother is greater than that of the father, autodiploid and autotetraploid lineages can be formed. When the chromosome number of the mother is significantly less than that of the father, it is challenging to form surviving offspring. These laws and the vast number of fertile lineages obtained indicate that distant hybridizations can result in fertile lineages. These phenomena and rules have corrected the traditional view that distant hybridization makes it difficult to form fertile lineages, making the formation of new germplasm resources and excellent varieties through distant hybridizations possible. The distant hybridization experimental results and the corresponding rules obtained by the authors' laboratory team are consistent with the related experimental results done by other research teams. Distant hybridizations can not only produce offspring with a significant change in chromosome number (such as allotetraploid) but also offspring with the same chromosome number as the parents (such as auto- and allodiploid). This makes the formation of new lineages diverse and provides essential theoretical support for the notion that distant hybridization is an important process for species formation in nature.

Fishes in the natural world consist of more than 35,600 species, making them the most diverse vertebrates. Distant hybridizations may have contributed to the formation of new species in some lineages. The Cichlid family is the most species-rich fish family, with over 3000 species. However, they cannot overwinter in nontropical regions such as tilapia, which is prevalent worldwide and a representative fish from this family. Most fishes in this family are distributed in tropical Africa and, through evolution, have adapted to the high-temperature waters (including high-temperature climates) of Africa. Existing studies show that the numerous species in the Cichlid family are closely related to distant hybridization [84].

The Cyprinidae family contains over 2000 species, making it the second most species-rich family among fishes [85]. Among these numerous fish species, polyploidization resulting from distant hybridizations is an important mechanism for the formation of new species [58, 86].

Author Contributions

Qizhi Liu: conceptualization, writing – original draft, writing – review and editing, visualization. **Shi Wang:** conceptualization, writing – original draft, writing – review and editing, visualization. **Chenchen Tang:** conceptualization, visualization, writing – review and editing, writing – original draft. **Min Tao:** writing – review and editing. **Chun Zhang:** writing – review and editing. **Yi Zhou:** writing – review and editing. **Qinbo Qin:** writing – review and editing. **Kaikun Luo:** writing – review and editing. **Chang Wu:** writing – review and editing. **Fangzhou Hu:** writing – review and editing. **Yude Wang:** writing – review and editing. **Qingfeng Liu:** writing – review and editing. **Wuhui Li:** writing – review and editing. **Jing Wang:** writing – review and editing. **Rurong Zhao:** writing – review and editing. **Shaojun Liu:** conceptualization, investigation, supervision, funding acquisition, visualization, project administration, resources, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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