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Genetic basis and biotechnological manipulation of sexual dimorphism and sex determination in fish

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Aquaculture has made an enormous contribution to the world food production, especially to the sustainable supply of animal proteins. The utility of diverse reproduction strategies in fish, such as the exploiting use of unisexual gynogenesis, has created a typical case of fish genetic breeding. A number of fish species show substantial sexual dimorphism that is closely linked to multiple economic traits including growth rate and body size, and the efficient development of sex-linked genetic markers and sex control biotechnologies has provided significant approaches to increase the production and value for commercial purposes. Along with the rapid development of genomics and molecular genetic techniques, the genetic basis of sexual dimorphism has been gradually deciphered, and great progress has been made in the mechanisms of fish sex determination and identification of sex-determining genes. This review summarizes the progress to provide some directive and objective thinking for further research in this field.

reproduction strategies, sexual dimorphism, sex determination, sex-determining gene, sex chromosome, sex control breeding

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For a long time, sex is always one of the important propositions in life sciences, because most vertebrates are gonochoristic and show significant sexual differences in morphology and physiology, which makes life more complex and wonderful and promotes some evolutionary biologists to believe “sex is the queen of problems in evolutionary biology” [1]. Since the phenomenon of hermaphrodite and sex reversal in rice field eel (*Monopterus albus*) was firstly reported by famous fish biologist Liu Jian-Kang in 1944 [2], which opened a fresh field for research into fish sex mechanism [3], a large number of significant achievements had been obtained in basic studies on fish sex and the related fields, and applied to the aquaculture practice [4]. Fishes are the most species-rich group of vertebrates with nearly half of all extant vertebrate species, which serve as an evolutionary link between invertebrates and higher vertebrates [5], so that the studies on genetic basis and mechanisms for sexual dimorphism and sex determination have been extensively noticed, and some significant progress, especially in identification of sex chromosomes and sex determination genes, has been achieved [6–8].

According to the UN Food and Agriculture Organization (FAO) (2014), world fish food supply has outpaced global population growth during the last five decades, and the aquaculture fishes have become an important animal protein source for most of the world’s population [9]. Since the

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*Corresponding author (email: jfgui@ihb.ac.cn) entry of the 21st century, a global consensus on effect and contribution of aquaculture to world fish supplies has emerged [10,11], and significant breakthroughs have resulted from the basic and applied studies on sex control breeding biotechnologies for sexual dimorphism [12,13]. Here, we will review recent breakthrough advancements in the studies on genetic basis and regulation mechanism of fish sexual dimorphism and sex determination, introduce several successful cases of biotechnological manipulations for sex control breeding, and thereby look forward to the promising prospects for future research in the field.

1 Diversity of fish reproduction strategies and their application in genetic breeding

1.1 Diversity of reproduction strategies in fish

Fish is the most abundant and successful group of vertebrates in the world, with about 32,700 documented species (www.fishbase.org). As primitive vertebrates, their reproduction strategies have evolved high particularities and abundant diversities to adapt to water environment change in the habitat [14]. According to the difference of reproduction strategy, fish can be grouped into unisexuals, hermaphrodites and gonochorists [15]. The majority of fishes are gonochorism, and hermaphroditic phenomena are also common, which have been reported in some fishes including rice field eel, black sea bream (*Acanlhopagrus schegeli*) and grouper (*Epinephelus coioides*) [2,16–18]. Even in gonochoristic rainbow trout (*Oncorhynchus mykiss*), it was found that the sex steroid level change could induce hermaphrodites, and the hermaphrodites produced YY males by self-fertilization of the generated sperm and egg [19].

1.2 Discovery of multiple modes of unisexual gynogenesis and sexual reproduction and the genetic breeding application in polyploid gibel carp

Since the first unisexual fish, the Amazon molly *Poecilia formosa*, was found by American ichthyologist Hubbs et al. in 1932, unisexual all-female populations had been reported in about 30 fish species, and these unisexual fishes had been demonstrated to reproduce by gynogenesis, hybridogenesis or parthenogenesis [20–23]. Polyploid gibel carp, *Carassius auratus gibelio*, because of the existence of a minor but significant portion of males in natural populations and the discovery of multiple modes of unisexual gynogenesis and sexual reproduction in some gynogenetic clones [23], has been noted extensively [12,24]. Through a series of studies, various gynogenetic clones and the clone-specific genetic markers have been identified, and used to perform studies on the evolutionary and ecological genetics [25–33]. Moreover, some important functional genes have been screened, and utilized to study the developmental genetics and regulative mechanism on reproduction [34–40]. In addition, a novel nucleo-cytoplasmic hybrid clone A+ has been created by sexual mating between clone D female and clone A male, and rapidly multiplied up to several hundred millions by subsequent more than five generations of unisexual gynogenesis [41]. Owing to the significant growth superiority and better resistance to myxosporean pathogen parasitized in the liver [41,42], the novel clone variety, as nominated “CAS III”, has been cultured throughout China.

2 Sexual dimorphism and its genetic basis in fish

2.1 Diversity of sexual dimorphism in fish

Sexual dimorphism is commonly defined as the difference between male and female individuals. Sexual dimorphism in fish not only includes size dimorphism, shape dimorphism and color dimorphism, but also comprises sexual differences in physiology and behavior [43,44]. Long-term field surveys and aquaculture practices have observed significant size dimorphism between male and female individuals in more than 20 fish species. As listed in Table 1, in more than 10 fish species including common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), Japanese flounder (*Paralichthys olivaceus*), and half-smooth tongue sole (*Cynoglossus semilaevis*), the females mature later than the males so that the nutrient substance in females is able to transform into body composition and body weight during maturation and reach a larger size than males. On the contrary, males mature later and grow faster in more than 10 fish species, such as tilapia (*Oreochromis niloticus*), yellow catfish (*Pelteobagrus fulvidraco*), channel catfish (*Ictalurus punctatus*) and rusty parrotfish (*Scarus ferrugineus*), in which males are bigger than females. Especially in some sex-changing fishes from females to males, the males grow much faster than females because large males have been believed to monopolize the right to mate with many females [70]. Additionally, sexual dimorphism was also observed in body shape and color in some ornamental fishes. For example, male swordtail fish (*Xiphophorus helleri*) uniquely has a needle-like fin extended from caudal fin margin, which owns more ornamental value than the female [71]. The skin of male bitterling fish (*Rhodeus ocellatus*) becomes colorful during its breeding season, while the female bitterling fish is just silver white in the body [72]. Recent studies have indicated that the physiological responses of the nervous system and sense organs also have significant difference between males and females in some fish species, and lead to sexual dimorphism in behavior [73].

2.2 Genetic basis of sexual dimorphism in fish

Sexual dimorphism commonly exists throughout the animal

Table 1 The known fish species with sexual dimorphism in size

Size dimorphism	Species	Sex chromosome system	References
Bigger in males	Yellow catfish (*Pelteobagrus fulvidraco*)	XX-XY	[45]
	Nile tilapia (*Oreochromis niloticus*)	XX-XY	[46]
	Blue tilapia (*Oreochromis aureus*)	ZW-ZZ	[47]
	Ussuri catfish (*Pseudobagrus ussuriensis*)	XX-XY	[48]
	Channel catfish (*Ictalurus punctatus*)	XX-XY	[49]
	Snakehead (*Channa argus Cantor*)	XX-XY	[50]
	Bluegill sunfish (*Lepomis macrochirus*)	XX-XY	[51]
	Blackfin sandperch (*Parapercis snyderi*)	?	[52]
	Bluefin tuna (*Thunnus maccoyii*)	?	[53]
	Dark sleeper (*Odontobutis obscura*)	?	[54]
	Rusty parrotfish (*Scarus ferrugineus*)	?	[55]
Bigger in females	Common carp (*Cyprinus carpio*)	XX-XY	[56]
	Bastard halibut (*Paralichthys olivaceus*)	XX-XY	[57]
	Southern Flounder (*Paralichthys lethostigma*)	XX-XY	[58]
	Rainbow trout (*Oncorhynchus mykiss*)	XX-XY	[59]
	Atlantic salmon (*Salmo salar*)	XX-XY	[60]
	Sea bass (*Dicentrarchus labrax*)	XX-XY	[61]
	Yellow perch (*Perca flavescens*)	XX-XY	[62]
	Atlantic halibut (*Hippoglossus hippoglossus*)	XX-XY	[63]
	Chinook salmon (*Oncorhynchus tshawytscha*)	XX-XY	[64]
	Herring smelt (*Oncorhynchus kisutch*)	XX-XY	[65]
	Half-smooth tongue sole (*Cynoglossus semilaevis*)	ZW-ZZ	[66]
	European eels (*Anguilla Anguilla*)	?	[67]
	Spotted scat (*Scatophagus argus*)	?	[68]
	Silver barb (*Puntius gonionotus*)	?	[69]

kingdom, but our knowledge for its mechanism is very limited. A large number of studies have shown that sexual dimorphism in vertebrates mainly results from genetic selection during the evolutionary process [74], and is the consequence of differential expression of sex-biased genes in development and growth of embryo, larval and adult [75,76]. Theoretically, sex chromosomes and the genes located on them may play critical roles in sexual dimorphism [77,78]. In some cases, however, sex chromosomes are not the leader of sexual dimorphism, because quantitative genetic studies have observed discordance between sex chromosomes and phenotypic data [79]. Genetic architecture of threespine stickleback fish (*Gasterosteus aculeatus*) is very similar between males and females, but many traits show significant sexual difference, suggesting that genetic constraints for evolution of sex dimorphism might not be so severe and absolute as generally thought [80].

A great deal of data accumulation of the completely sequenced genomes, transcriptomes, and proteomics from model and farmed fishes provides a previous basis for us to explore the genetic and molecular mechanism of sex dimorphism [12]. As we have known, size dimorphism in vertebrates mainly results from the different growth rate between male and female individuals, and the growth rate is regulated by growth hormone (GH)/insulin-like growth factor (IGF) and other related factors secreted by the hypothalamic-pituitary-gonad axis and other tissues [81]. So far, the discovered factors in fish growth hormone axis include GH/growth hormone receptor (GHR)/IGFs [82] and corticotropinreleasing hormone (CRH)/pro-opiomelanocortin (POMC)/melanocortin receptors (MCRs) [83]. In swordtails of the genus *Xiphophorus*, the expression level of *mc4r* gene was found to accordingly increase in XX females or males, small XY males, intermediate and large XY males. In addition, its B allele lacking two cysteine residues is only expressed in XY males, and its expression level is closely related to male body size [84]. In Nile tilapia, comparative transcriptome analysis in ovary and testis also revealed differential expression genes during sex differentiation [85]. Recently, Jing et al. [86] used solexa high-throughput sequencing technology to compare gene and microRNA expression patterns in gonads of XX females, XY males and YY super-males, and attempted to reveal the molecular basis of sexual dimorphism in body size through analyzing expression difference of growth-related genes between adult males and females in the hypothalamus and pituitary (unpublished data).

In addition, it has been found in some fishes that sexual dimorphism in color, body shape, physiology and behavior is also controlled by some key genes. For example, in Lake Malawi cichlid fishes, the color dimorphism between males and females is probably controlled by the expressional and functional difference of *pax7* gene [87], and the neo-sex chromosome in stickleback has significant correlation with the physiology and reproductive behavior [88].

3 Genetic basis of sex determination in fish

3.1 Diversity of sex determination systems in fish

In teleost fish, sex determination is a plastic process that is usually governed by the interaction between genetic (genetic sex determination) and environmental (environmental sex determination) factors [89,90]. A variety of environmental factors, such as hormones, temperature, pH, density, light intensity and hypoxia, may affect sex determination pathway. Much evidence indicates that in many fish species with genetic sex determination, environmental factors such as temperature may overwhelm the effect of genetic factors at the edge of temperature tolerance threshold [91,92].

For most gonochoristic fish species, the mechanism of genetic sex determination is usually determined by either a critical gene or polygene on sex chromosomes or autosomes [8]. In single gene system, sex is determined by a critical gene on sex chromosome [6]. XX/XY male heterogametic system and ZZ/ZW female heterogametic system are two main sex determination systems in fish species, and several variants including XX/XO, XX/XY1Y2, X1X2X1X2/X1X2Y, X1X2X1X2/X1X2X1, ZZ/ZO and ZZ/ZW1W2 also exist in a few of fish species, whereas no heteromorphic sex chromosomes have been found in most of fish species [93]. Even in fish with heteromorphic sex chromosomes, it is difficult to identify sex chromosomes by cytogenetics and fluorescence *in situ* hybridization techniques, since the differentiation and divergent degree of sex chromosome is still very low [4,94].

Many current data from most studies have shown that sex of some fish species is determined by either several loci dispersed throughout the genome, or multiple allele combinations located on a preferential pair of chromosomes, which has been designated as polygenic sex determination (PSD) system [95,96]. In zebrafish [97,98], Lake Malawi cichlid fish [99] and European sea bass [100] that do not have typical XX/XY or ZZ/ZW sex determination system, sex is determined by multiple genes on several chromosomes. At whiles, sex determination locus is variable in different strains or subspecies of the same fish species, in which there are more than two types of sex determination systems. These situations may be caused by several reasons including recombination or fusions between the extant sex chromosomes and autosomes [101–103].

3.2 Plasticity of sex differentiation in fish

In most gonochoristic fish, such as yellow catfish, medaka(*Oryzias latipes*) and common carp, the gonad development process initiates from undifferentiated state, and then differentiates into testis or ovary. However, in a small number of gonochoristic fish including zebrafish and European eel, the undifferentiated gonad first develops into ovary-like structure. At juvenile stage, the ovarian structure is regressed in near half of the population and eventually develops into a normal testis [104,105]. Sex differentiation is dependent on sex determination, but the whole process of embryonic development, sex determination and differentiation is exposed to the outside environment with many variable factors, so that fish must utilize diverse types of sex determination mechanisms and plastic patterns of sex differentiation to gradually adapt to the habitat environment during the evolution [6,92,106].

In most of fish species, no matter whether there is a sex determining gene or not, the change of sex steroid concentration or temperature is able to induce sex reversal, and thereby leads to the discordance between genetic and physiological phenotypes [44,92,93]. Temperature is the main factor affecting sex differentiation, because a lot of observations have shown in most of temperature-sensitive fishes that the male proportion increases along with elevation of hatching temperature, and low temperature is able to induce ovarian development [107–109]. Estrogen and androgen are essential for female and male sexual differentiation and gonadal development. At early stage of fish sex differentiation, when endogenous steroids are not produced or not enough *in vivo*, optimal dose of exogenous steroids can change the direction of gonadal sex differentiation [6,92]. In protogynous hermaphrodite groupers, 17α-methyltestosterone could induce sex reversal from females to males [110], which lead to up-regulation of *dmrt1* expression [111,112], and down-regulation of *cyp19a1a* and *sox3* expression [113,114]. On the other hand, estrogen treatment could promote male-to-female sex reversal in tilapia, and resulted in the inhibition of *dmrt1* expression [115]. When XX medaka was exposed to high water temperature, the physiological males could be induced along with decreasing expression of *cyp19a1a*, whereas co-treatment with 17β-estradiol (E2) inhibited the sex reversal [116]. Besides sex hormone, aromatase inhibitors (Fadrozole and Letrozole) could reduce the enzyme activity and thereby led to masculinization, which had been used to induce female-to-male sex reversal in tilapia and medaka [117,118]. Godwin analyzed the influence of social factors on sex determination and differentiation of reef fish, and explored the advantages of this plasticity mechanism in evolution [119]. In a sex-changing fish *Thalassoma bifasciatum*, it was found that whether the males either directly differentiated or firstly produced as females and then sex-reversed to males largely depended on the social environment signals that they received at early stage of sex determination [120].

3.3 Sex-determining (SD) genes in fish

3.3.1 SD genes in fish with XX/XY sex determination system

SD genes mainly refer to the genes that transiently express in the undifferentiated gonad and directly determine the bipotential gonad into either a testis or an ovary. Sry gene, the first SD gene discovered in vertebrates, is located on Y-chromosome and initiates testicular differentiation [121,122]. *Dmy/dmrt1bY* is the first SD gene identified in fish, and it is also located on Y-chromosome in *Oryzias latipes*, a species of medaka [123]. Subsequently, five other SD-related genes, such as *gsdf* in *Oryzias luzonensis*, *sox3* in *Oryzias dancena*, *amhr2* in *Takifugu rubripes*, *Amhy* in *Odontesthes hatchery* and *sdY* in *Oncorhynchus mykiss*, were respectively identified from several teleost fish with XX/XY sex determination system. During testis differentiation, their expressed products were also localized on early differentiating cells of testis tissue. Moreover, the mutation of *dmy*, *sox3*, *amhr2* and *sdY* or knockdown of *amhy* led to male-to-female sex reversal in XY males, while transgenic over-expression of *sox3*, *sdY* and *gsdf* genes caused female-to-male sex reversal in XX females, suggesting that they should be male SD genes and play critical roles in male sex determination [124–128]. A large number of studies have confirmed that most male SD genes including *dmy* in medaka, are the duplications of *Dmrt1* (*dsx* and *mab-3* related transcription factor 1) [129,130]. It had been detected in medaka that *dmrt1* mutation caused a male-to-female sex reversal, suggesting that *dmrt1* should be essential for maintaining testis differentiation in the *Dmy*-triggered male sex determination pathway [131,132].

Actually, *dmy* has not been revealed as a universal SD gene in any other kind of fish, soon after it was identified as the first SD gene in medaka *Oryzias latipes*, suggesting that SD genes are diverse in fish. In other medaka fish *Oryzias luzonensis* and *Oryzias dancena*, *gsdf* and *sox3* take the place of *dmy* as new SD genes [124,125]. Using genome-wide linkage analysis and association mapping strategy, Kamiya et al. [126] have found that only a SNP (C/G) polymorphism in the kinase domain of *Amhr2* gene is associated with sex phenotype in fugu and two other species of Takifugu, but not in Tetraodon. These data suggest that sex determination is diverse among different species even in the same genus. In addition, the hotei homogenous mutation in the kinase domain of Amhr2 also led to male-to-female sex reversal in XY medaka males [133]. Therefore, Amhr2 plays a pivotal role in sex determination of fugu and medaka, mainly through the function of its kinase domain.

3.3.2 Sex-determining genes in fish with ZZ/ZW sex determination system

Sex determination system in birds is ZZ (male)/ZW (female) system, and the first male SD gene, *Dmrt1* on the Z-chromosome, is identified in chickens [134]. *DM-W*, a W-linked paralogue of *Dmrt1* that antagonizes the transcriptional activity of DMRT1, was further identified as an ovary-determining gene from frog *Xenopus laevis* with a ZZ/ZW SD system [135,136]. In teleost fish with ZZ/ZW sex determination system, no definitive SD genes have been detected, but many candidate SD genes have already appeared.

Recently, Chen et al. [137] reported the whole genome sequencing of a ZZ/ZW type fish, the half-smooth tongue sole. Through phylogenetic analysis, a high level of homology was detected between half-smooth tongue sole and avian W and Z chromosomes. They found a functional *dmrt1* located on the Z-chromosome and its homologous pseudogene on the W chromosome. Similar to the expression pattern of other SD genes, the *dmrt1* was revealed to specifically express in male germ cells and pre-somatic cells of the undifferentiated gonad at sex-determination stage and persists at high levels during testis development. In ZW females, the *dmrt1* promoter was hypermethylated and silenced, whereas it was demethylated and activated in ZW pseudomales, which resulted in the upregulation of *dmrt1* expression to a level as normally developing ZZ males by dosage compensation. Moreover, female-specific genes on W chromosome were found to be inhibited by methylation regulation in pseudomales [138,139]. All the above data suggest that, similar to avian, not only part of the genes on fish Z chromosome has effective and variable dosage compensation, but also W chromosome contains a part of sex determination or the dosage compensation mechanism [139]. Obviously, the novel understanding from whole genome sequence analysis of the farmed fish is an exciting stimulus for further study on SD genes of ZZ/ZW system [140,141].

In addition, two potential SD genes, *amh* and *dmrta2*, were mapped to the sex-determination loci in ZZ/ZW type turbot (*Scophthalmus maximus*) and tilapia by applying sex-associated markers and QTL analysis [142,143]. Xu et al. [144] also observed regulative role of dmrta2 in zebrafish spermatogenesis.

3.4 Network modules of sex determination in fish

The known SD genes in fish, such as *dmy* and *amhy*, have been demonstrated to be respective duplicated gene of the autosomal *dmrt1* and *amh* on Y chromosome [123,127]. Gene duplication is a common phenomenon because all teleosts are hypothesized to have experienced another “fish-specific” (3R) whole genome duplication event during the ancestral lineage evolution [32,145–147]. Actually, the master SD genes in vertebrates, such as Sry, *dmy*, *amhr2*, *amhy* and *gsdf* are either key factors for gonadal differentiation or their duplications. However, *sdY*, a male-specific gene on Y chromosome in rainbow trout and most species of salmonids, is a truncated copy of autosomal *irf9* (interferon regulatory factor 9) [148]. Recent continuous discovery about new SD genes strengthens the hypothesis that not only downstream key factors or related genes for gonadal differentiation but also some other genes involved in different developmental process are able to recruit to the top signal of SD pathway through either directness or duplication [15,149].

Undifferentiated gonad in fish is commonly thought to have double potentials to trigger testis development or ovary development. When sex determination "switch" is initiated by master sex-determining gene, a genetic network of sex determination and differentiation that is composed by some conserved sex differentiation genes will be activated (Figure 1). These downstream sex-related genes can regulate the expression of sex steroid hormones, and thereby direct the development of functional gonads with the sex phenotype [150–152]. A new idea believes that fish sex determination is neither a single genetic cascade reaction nor a result of hierarchical cascades by genetic network, but the reciprocal links between different genetic modules which functionally interact with each other [15,153]. As shown in Figure 1, in fish with XX/XY sex determination system, high expression of the master male sex determination gene on Y chromosome, such as *dmy*, *sox3*, *amhr2*, *sdY*, or *amhy*, is able to initiate different modules in genetic network of sex determination and testis differentiation so that activate the essential genes for testis development, such as *dmrt1*, *sox9* and *amh*, and thereby completes male formation. Conversely, the absence of master male sex determination gene on X chromosomes leads to other module activation in genetic network of female sex determination and ovary differentiation, and thereby induces the expression of ovary formation-related genes, such as *cyp19a1*, *foxl2*, *sf1* and *wnt4* [154,155].

Recent findings in sex-changing fish indicate that some sex differentiation-related genes are also regulated by epigenetic modifications [156,157]. In juvenile males of European sea bass, the *cyp19a* promoter was found to have double levels of DNA methylation than the females, in which exposure to high temperature increased the promoter methylation levels in females and suppressed the ability of SF-1 and Foxl2 to stimulate its transcription [156]. Similarly, in hermaphrodite and sex-changing rice field eel, the *cyp19a1a* promoter was also discovered to be hypermethylated in the ovotestis and testis compared with the ovary, and the natural sex changing could be reversed by DNA methylation inhibitor 5-aza-2′-deoxycytidine [157].

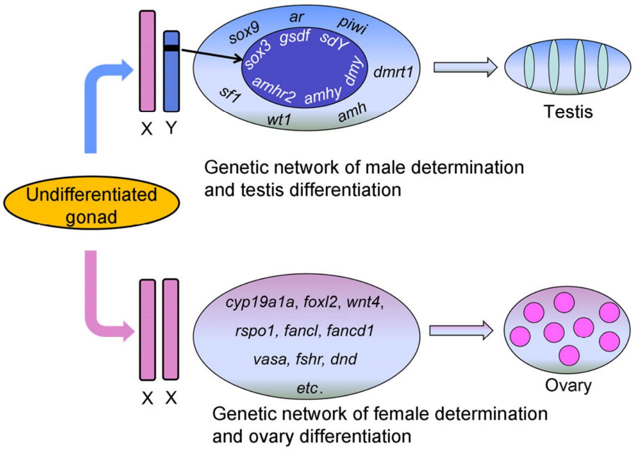

Figure 1 A schematic diagram of sex determination and gonad (testis or ovary) differentiation in fish with XX/XY sex determination system.

4 Sex control biotechnology and its breeding application in fish

Growth is one of the most valuable economic traits for fish genetic improvement. Because some fish species display different growth rate and body size, all-female or all-male population production has significant economic implications in aquaculture. As mentioned above, fish genetic breeding scientists have performed a lot of studies in some aquaculture fish species with growth and size dimorphism between females and males. Through these studies, sex-linked genetic markers or X chromosome-linked and Y chromosome-linked genetic markers and the marker-assisted sex control breeding biotechnology have been successfully exploited, and thereby provided convenient and practical technological approaches for sex control breeding application in fish [12,158,159]. In the past five years, only Chinese scientists have successfully produced many mono-sex novel varieties in some farmed fish species, such as Yellow catfish "all-male No.1", all-female "North flounder No.1", all-female "North flounder No.2" and Tilapia "Luxiong No.1". Based on these significant breakthrough advances, this section will highlight some novel ideas and new biotechnological approaches for future sex control breeding in fish.

4.1 Interspecific hybridization and production of all-males and all-females in fish

Interspecific hybridization not only improves fish economic traits including growth rate, survival rate, disease resistance, cold tolerance and hypoxia tolerance [160,161], but also utilizes to produce high ratio offspring of males or females. The most representative case is massive production of all-male population by interspecific hybridization between different tilapia species. As early as 1960, Hickling [162] firstly reported the generation of all-male tilapia population by crossing female *O. mossambicus* (XX) with male *O. hornorum* (ZZ). Subsequently, a large number of interspecific crossings were performed between multiple tilapia species, and the hybrid offspring between Nile tilapia and Aureus tilapia was found to have significant advantages in growth, survival and male proportion [163,164]. Moreover, when Nile tilapia was hybridized with the variety "Xiaao No.1" selected from blue tilapia by population breeding, the hybrid offspring produced more than 93% males that were applied to massive aquaculture in China [165]. In bass, 100% all-female offspring could be generated by specific hybridization between different species [166], but the sex determination system and mechanism for monosex generation remain unclear [167].

4.2 Artificial gynogenesis and all-female population production

As another important approach for producing monosex fish population, artificial gynogenesis generally refers to the haploid egg activation by genetically inactivated sperm and the activated egg development into diploid offspring after chromosome doubling. Therefore, the genetic information of these offspring is almost from their female parent [4]. After artificial gynogenesis, a XX all-female population will be produced if the parents have a XY/XX sex determination system, whereas ZZ male and WW female, not all-female offspring will be theoretically produced from the parents with ZZ/ZW sex determination system.

Gibel carp has the capacity of unisexual gynogenesis, and it is feasible to produce all-female offspring when its eggs are activated by heterologous common carp sperm, in which the aquaculture of all-female gynogenetic offspring has become a typical case of sex control breeding application in fish aquaculture [23]. Through a combining approach of artificial gynogenesis and sex reversal, all-female population was also produced in common carp and used for aquaculture practice [60]. Recently, artificial gynogenesis has been performed in many marine fish species, such as flounder [168], red sea bream (*Pagrosomus major*) [169], sea bass [170], spotted halibut (*Verasper variegatus*) [171], and half-smooth tongue soles [172]. Especially in flounder, several gynogenetic clones have been obtained [173–175]. Based on these artificial clone strains, the Beidaihe experiment station of the Chinese Academy of Fishery Sciences have successively bred two aquaculture varieties of flounder, “north flounder No.1” and “north flounder No.2”.

Generally, the gynogenetic offspring is composed of all-females. However, a certain part of male individuals have also been recorded in some gynogenetic fishes resulting from distant hybridization, such as in *Poecilia formosa* and in *Carassius auratus* red var. [176–178], which may have exploitable significance and potential in fish genetic breeding.

4.3 Identification of sex-specific markers and sex chromosome-specific markers

For most fish species, the degree of sex chromosome differentiation is very low and it is difficult to discriminate through sex chromosome morphology. In addition, fish sex is easily influenced by environment factors, especially by temperature [179], which often results in inconstancy between physiology phenotype and sex genotype. So it is important and meaningful to search for a convenient and forthright method to identify fish genetic sex in aquaculture. In some fish species, fish genetic scientists have successfully identified a great number of sex-specific or sex chromosome-specific markers by multiple techniques including AFLP (amplified fragment length polymorphism), SNP (single nucleotide polymorphism), RAPD (random amplified polymorphic DNA), SSR (simple sequence repeats) and QTL (quantitative trait locus) (Table 2). Obviously, these markers establish a profound foundation for identifying fish genetic sex and sex chromosomes, and offer a high efficient technology approach for massive production of all-male or all-female populations [12,180].

4.4 Biotechnological approaches of sex control breeding in fish

During early gonadal differentiation, exogenous steroid hormone treatment could effectively induce sex reversal in fish. So far, the hormone-induced sex reversal has been prohibited to directly produce monosexual fish population, because we are still uncertain whether the hormone derivatives generated during the metabolic process can quickly degenerate or not [4,12,197]. In consideration of food security, the feasible way is to use the hormone-induced sex reversal fish as parents to breed monosexual offspring in aquaculture.

In common carp and crucian carp, Chinese scientists have successfully incorporated artificial gynogenesis and sex reversal techniques to quickly establish all-female populations [198,199]. Based on the identification of X chromosome-specific and Y chromosome-specific markers [49,180], Yellow catfish “all-male No. 1”, a novel aquatic variety, has been produced by using a biotechnological approach of sex control breeding [12,200]. Accordingly, we have proposed an integrative sex control breeding approach for mass production of all-male population in XX/XY sex determination system fish by 17α-ethinyloestradiol (EE2) treatment and by Y chromosome-specific marker (YSM) and X chromosome-specific marker (XSM) selection [12]. The technological approach for production of all-female population is simpler than that for all-male population in XX/XY fish. The XX physiological males can be either produced by sex reversal after artificial gynogenesis, or selected by the Y chromosome-specific marker (YSM) and X chromosome-specific marker (XSM) from sex reversal progeny through 17α-methyltestosterone (MT) treatment. And, the batch XX physiological males can be obtained from the MT treated offspring between XX male and XX female mating. Finally, mass production of all-females can be produced by the mating of XX males and females (Figure 2).

5 Conclusion and outlook

Over the past few decades, the slow progress in discovery of SD genes and sex chromosome in fish mainly results from the shortness of genetic information and genomic resources. Recently, rapid development of high-throughput DNA sequencing technique, massive accumulation of ge-

Table 2 A list of sex-specific markers or sex chromosome-specific markers and their associated genes

Species	Sex determination system	The identified tech niques	Marker type and quantity	Associated genes	References
Yellow catfish (*Pelteobagrus fulvidraco*)	XX/XY	AFLP	Two X- and Y-specific markers		[49]
		genomic walking	Two X- and Y-specific markers; One different length X and Y markers		[180]
Channel catfish (*Ictalurus punctatus*)	XX/XY	Sequencing and analysis	One male-specific marker		[181]
African catfish (*Clarias gariepinus*)	XX/XY	RAPD	Two male-specific markers		[182]
Rainbow trout (*Oncorhynchus mykiss*)	XX/XY	AFLP	Fifteen male-specific markers		[183]
		gene expression	One Y-linked marker	*sdY*	[128]
Patagonian pejerrey (*Odontesthes hatcheri*)	XX/XY	AFLP	One male-specific marker		[184]
		gene expression	One Y-linked marker	*amhy*	[127]
Japanese pufferfish (*Takifugu rubripes*)	XX/XY	SNP	One Y-linked marker	*Amhr2*	[126]
Japanese medaka (*Oryzias latipes*)	XX/XY	gene expression	One Y-linked marker	*dmy*	[123]
Philippine medaka (*Oryzias luzonensis*)	XX/XY	gene expression	One Y-linked marker	*gsdf*	[124]
Indian ricefish (*Oryzias dancena*)	XX/XY	gene expression	One Y-linked marker	*sox3*	[125]
Platyfish (*Xiphophorus maculates*)	XX/XY	Sequence analysis	One Y-linked marker	*MC4R*	[84]
Fathead minnow (*Pimephales promelas*)	XX/XY	AFLP	Eight male-specific markers One male-specific marker		[185]
Nine-spined stickleback (*Pungitius pungitius*)	XX/XY	SSR	One male-specific marker		[186]
Common carp (*Cyprinus carpio*)	XX/XY	RAPD	One male-specific marker		[187]
Nile tilapia (*Oreochromis niloticus*)	XX/XY	AFLP	Three male-specific markers		[188]
		RAPD, AFLP	Four X- linked markers Five Y-linked markers		[189]
Turbot (*Scophthalmus maximus*)	ZZ/ZW	RAPD	One female-specific marker	*wnt4*, *foxl2*	[190]
		RAPD	One male-specific marker	*amh*, *dmrta2*	[191]
Spotted halibut (*Verasper variegatus*)	ZZ/ZW	AFLP	Two female-specific markers		[192]
Atlantic halibut (*Hippoglossus stenolepis*)	XX/XY	SSR	Three female-specific markers		[193]
Half-smooth tongue sole (*Cynoglossus semilaevis*)	ZZ/ZW	AFLP, SSR	Seven and one female-specific markers		[70,174]
		Genomic sequencing	One Z-linked marker	*dmrt1*	[137]
Yellowtail (*Seriola quinqueradiata*)	ZZ/ZW	SSR	One female-specific marker		[194]
Rock bream (*Oplegnathus fasciatus*)	$X_1X_1X_2X_2/X_1X_2Y$	AFLP	Four male-specific markers		[195]
matrinxã (*Brycon amazonicus*)		RAPD	One female-specific marker	*PIGW*	[196]

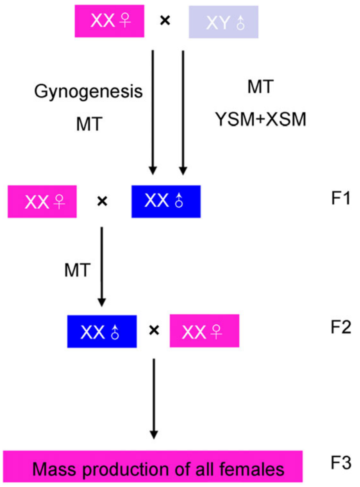

Figure 2 A schematic diagram of mass production of all-female population through an integration approach of gynogenesis, methyltestosterone (MT) treatment, and X chromosome-specific marker (XSM) and Y chromosome-specific marker (YSM) identification in fish with XX/XY sex determination system.

nomic and transcriptomic data, and extensive utilization of comparative phylogenetic methods have greatly promoted the pace of genetic basis studies on fish sex determination [201]. Many sex-linked or sex chromosome-specific molecular markers have been rapidly developed and start to be efficiently applied to aquaculture. Moreover, the gene targeting and editing approaches, such as TALEN (transcription activator-like effector nuclease) and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated 9), have made it easier to perform functional experiments in fish [202–204]. It is expected that a new era is coming for studying genetic basis of sex dimorphism and sex determination and biotechnological manipulation of sex control breeding.

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