

## Molecular basis and genetic improvement of economically important traits in aquaculture animals

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Aquaculture has been believed to be a major Chinese contribution to the world. In recent 20 years, genome and other genetic technologies have promoted significant advances in basic studies on molecular basis and genetic improvement of aquaculture animals, and complete genomes of some main aquaculture animals have been sequenced or announced to be sequenced since the beginning of this century. Here, we review some significant breakthrough progress of aquaculture genetic improvement technologies including genome technologies, somatic cell nuclear transfer and stem cell technologies, outline the molecular basis of several economically important traits including reproduction, sex, growth, disease resistance, cold tolerance and hypoxia tolerance, and present a series of candidate trait-related genes. Finally, some application cases of genetic improvement are introduced in aquaculture animals, especially in China, and several development trends are highlighted in the near future.

**aquaculture genome technology, somatic cell nuclear transfer, stem cell technology, reproduction-related gene, sex determination gene, growth-related gene, disease resistance-related genes, cold tolerance-related gene, hypoxia tolerance-related gene, genetic improvement**

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Aquaculture has a history of about 4000 years, and China is the earliest country for raising fish in fishponds. However, it is only in the recent 50 years that aquaculture has rapidly developed and become a true production industry because artificial propagation technique has been successfully established since the early 1960s for the “four important domestic fish” including silver carp, bighead carp, grass carp and black carp [1] and aquaculture biotechnology for genetic breeding and disease control has been quickly developed since the 1980s [2]. In China, the annual aquaculture production capacity has increased to 38.3 million tons in 2010 from less than 1 million tons in 1960s, and possesses the fastest growing rate among agriculture and food industry. The aquaculture proportion has risen to more than 71.3% of the food fish, and reaches about 70% of the world’s aquaculture production. Aquaculture industry has become a major power to promote sustainable, rapid and stable devel-

opment of China’s fishery, and played an important role in guaranteeing supply, stabilizing the market, ensuring national food security and promoting trade development. As an optimal method for exchanging animal proteins by minimum plant proteins, aquaculture has been believed as the most efficient technology by some authoritative economists, which is a major Chinese contribution to the world.

Just entering the 21st century, sustainable fisheries have become a common theme for the world, and the effect and contribution of aquaculture on world fish supplies have been widely recognized by many scientists in the developed countries [3,4]. Moreover, fish and other aquatic products as safer food sources have been well identified with most of the people [5]. At the same time, China’s aquaculture is in a crucial change stage from traditional farming to modern industry, in which the severely lagged basic research has become the outstanding issue for restricting aquaculture development. Especially, the improved varieties were very few, and major aquaculture fish and shellfish were still de-

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pendent on the wild species. The frequent incidences of infectious diseases led to serious economic losses of about 15 billion RMB annually, and the unreasonable responses and non-standard medication to the diseases further resulted in drug residues, which affected quality and food safety of aquatic products, and in turn restricted the sustainable development of aquaculture [6]. In order to solve the above problems, the National Key Basic Research Program of China (973 Program) has successively supported 7 projects since 1999, such as “Fundamental research on the disease occurrence and disease resistance of the commercially important organisms in mariculture”, “Studies on genetic and developmental biological basis for improvement of important aquaculture fishes”, “Studies on the disease outbreak and immunological control of commercially important organisms in mariculture”, “Investigation of crucial scientific issues in intensive aquaculture of freshwater ponds in China”, “Basic studies on functional genomics and molecular design breeding in important aquaculture fishes”, “The molecular basis for the important economic traits of the mollusks and the study on the molecular design breeding”, and “Basic studies on outbreak mechanism and immunological control of main virus diseases in marine aquaculture animals”, which have promoted significant advances in basic studies on genetic improvement and disease control of aquaculture animals. Especially in basic studies on genetic improvement in the aquaculture performance, the molecular basis of several economically important traits including reproduction, sex, growth, disease resistance, cold tolerance, and hypoxia tolerance have begun to elucidate, although these traits are highly complex and problematic. Here, we will attempt to retrace and review some significant breakthrough progress in the field for the recent 10 years, especially the major achievements obtained by Chinese scientists, and attempt to forecast developmental prospects for the future.

## 1 Genetic improvement technologies of aquaculture animals

### 1.1 Aquaculture animals in which complete genomes have been sequenced

As complete genomes have been sequenced and released from human, model animals and plants, as well as several model fish including zebrafish (*Danio rerio*), fugu (*Fugu rubripes*), Tetraodon (*Tetraodon nigroviridis*), medaka (*Oryzias latipes*), and three-spined stickleback (*Gasterosteus aculeatus*) since this century, the genomes of some important aquaculture fish, such as channel catfish (*Ictalurus punctatus*), Nile tilapia (*Oreochromis niloticus*), Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), and Atlantic cod (*Gadus morhua*), have been also sequenced [7,8]. From 2010, the

complete genomes of some main aquaculture animals including tongue sole (*Cynoglossus semilaevis*), Pacific oyster (*Crassostrea gigas*), large yellow croaker (*Pseudosciaena crocea*), orange-spotted grouper (*Epinephelus coioides*), common carp (*Cyprinus carpio*) and Japanese flounder (*Paralichthys olivaceus*) have been announced to be sequenced by Chinese fisheries-related institutes (Table 1). In 2011, Institute of Hydrobiology, Chinese Academy of Sciences and the collaborators have also started to sequence the complete genomes of grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) of the “four important domestic fish”, and small RNA transcriptomes of bighead and silver carp have been characterized [9]. Therefore, we are convinced that the complete genome bioinformatics and the detailed molecule anatomy will provide significantly referent and even directive knowledge for their trait improvement and disease control of these major aquaculture animals.

### 1.2 Genome technologies of aquaculture animals

In 2007, Prof. Zhanjiang Liu at Auburn University of USA, together with more than 40 aquaculture genomics experts in the world, edited a highly technical and comprehensive book *Aquaculture Genome Technologies* [10]. According to the book, the major aquaculture genome technologies include DNA marker technologies, such as amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphism, novel DNA sequencing technologies, such as the SOLiD sequencing platform, the Solexa sequencing platform and the 454 sequencing platform, gene discovery technologies of expressed sequence tags and *de novo* sequencing of whole transcriptomes, genome mapping technologies, such as genetic linkage mapping, QTL mapping, radiation hybrid mapping and BAC-based physical mapping, and genome expression analysis technologies, such as microarray technology and sequence tag-based technology.

Recently, numerous polymorphic DNA markers for aquaculture animals have been developed and significant advances have been made in China. In polyploid gibel carp (*Carassius auratus gibelio*), several significant molecular markers including transferrin alleles [11], SCAR [12], microsatellites [13], AFLP [14] and mitochondria DNA sequences [15,16] were identified, and dual modes of unisexual gynogenesis and sexual reproduction were revealed by these markers [17,18]. Significantly, the unique dual reproduction mode, as commented by Avise (2008), was the first documented instance in vertebrates [19], and the discovery firstly explained reproduction mechanism of high genetic diversity and an evolutionarily long-lived timescale of unisexual species [18]. In common carp (*Cyprinus carpio*), along with advances of polymorphic markers and genome sequencing, various density linkage maps were constructed

**Table 1** Aquaculture animals that have been announced to be completely sequenced in China

Species	Genome size	Predicted gene number	Mainly announced institute	Announced time
Tongue sole ( <i>Cynoglossus semilaevis</i> )	520 Mb	More than 20000	Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences	August 2010
Pacific oyster ( <i>Crassostrea gigas</i> )	8 Gb	More than 20000	Institute of Oceanology, Chinese Academy of Sciences	August 2010
Large yellow croaker ( <i>Pseudosciaena crocea</i> )	750 Mb	Data not given	Zhejiang Ocean University	January 2011
Orange-spotted grouper ( <i>Epinephelus coioides</i> )	1.1 Gb	More than 20000	Sun Yat-Sen University	March 2011
Common carp ( <i>Cyprinus carpio</i> )	1.7 Gb	Data not given	The Centre for Applied Aquatic Genomics, Chinese Academy of Fishery Sciences	May 2011
Japanese flounder ( <i>Paralichthys olivaceus</i> )	620 Mb	About 23000	Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences	December 2011

[20–23], and used to locate cold tolerance [20] and muscle fiber-related [22] QTLs. A physical map was constructed from a BAC library containing a total of 92160 BAC clones with an average insert size of 141 kb [24,25]. In addition, a consensus linkage map was also constructed in grass carp [26]. As a required approach for positional gene cloning, physical mapping, and genome sequencing, several BAC libraries have been developed and characterized in other important aquaculture animals of China, such as gibel carp [27], zhikong scallop (*Chlamys farreri*) [28], pacific white shrimp (*Litopenaeus vannamei*) [29], grass carp [30], and half-smooth tongue sole (*Cynoglossus semilaevis*) [31].

### 1.3 Somatic cell nuclear transfer and stem cell technologies in fish

Nuclear transfer has been successfully applied to fish genetic breeding for about half a century in China [32], and their developmental potentiality and reprogramming have been observed from the transplanted nuclei [33,34]. During this century, the cloned zebrafish were obtained by nuclear transfer from long-term-cultured cells [35], and the cytoplasmic impact was analyzed in cross-genus cloned fish derived from transgenic common carp (*Cyprinus carpio*) nuclei and goldfish (*Carassius auratus*) enucleated eggs [36]. Moreover, some differentially expressed genes were identified from the cloned zebrafish embryos at the dome stage [37], and the critical developmental stages for high integration efficiency were determined in zebrafish [38,39].

Stem cell culture and application have been rapidly developed in model fishes of medaka and zebrafish [40,41], and some stem cell technologies, such as gene targeting, germ cell transplantation and semi-cloning by nuclear transfer, have been successfully formed because of specific potentials of fish germ stem cells and embryonic stem cells [42,43]. Especially, Yi et al. [44] used the semi-cloning technology to create the semi-cloned female medaka which shows normal fertility and germline transmission over three generations. This approach represents a significant development for germline transmission of cultured cells, and will be a potential reproduction technology to combat infertility

in vertebrates including human. Additionally, primordial germ cells (PGCs) and spermatogonial stem cells could be transplanted between two different salmonid species, and resulted in production of donor-derived offspring [45–47]. In combination with natural and artificial polyploid fishes with special reproduction modes [18,48], the surrogate approach might have significant values for producing polyploidy stocks in aquaculture or for restoring an endangered species in wild life conservation.

## 2 Molecular bases for economic traits of aquaculture animals

### 2.1 Reproduction trait and candidate reproduction-related genes

In comparison with mammals, fish reproduction modes and strategies exhibit abundant diversities and extreme particularities [49,50]. Owing to the diversities and particularities, much attention has begun to be paid to some important fish with special reproduction modes. Gibel carp, because of the polyploid background and dual reproduction modes of gynogenesis and sexuality, has become a promising fish for screening and isolating reproduction-related genes that are involved in primordial germ cell formation, oocyte maturation and egg fertilization. Using suppression subtractive hybridization and protein purification [51,52], some important genes, such as germ plasm markers *vasa* [53] and *Dazl* [54], oocyte maturation-related factors *cyclin A2* [55], *C-type lectin* [52], *Clq-like* [56–58], histone H2A variant *h2af1o* [59], and maternal-effect factor *spindlin* [60], have been identified and functionally characterized from gibel carp. For example, Sun et al. [61] have observed oocyte-specific expression pattern and dynamic distribution of Spindlin during oocyte maturation and egg fertilization, demonstrated its association and interaction with  $\beta$ -tubulin and spindle, and found that its elimination destroys spindle assembly, and thereby disturbs the oocyte-to-embryo transition and first cleavage, which provided the first direct evidence for the critical oocyte-to-embryo transition function of Spindlin in vertebrates.

It is well known that fish reproduction is regulated and controlled by a cascade of hormones along the hypothalamus-pituitary-gonad (HPG) axis. In this cascade, gonadotropin-releasing hormones (GnRHs) produced by hypothalamus are found to be the primary neuropeptides, and gonadotropic hormones (GtHs) synthesized and released by pituitary are found to lead ovaries or testicles to produce mature eggs or sperms, whereas dopamine (DA) produced by pituitary has been found to exhibit an inhibiting effect on the functions of GnRHs [62–64]. Multiple GtHs have been reported by Chinese scientists in groupers [65] and Chinese sturgeon (*Acipenser sinensis*) [66]. In common carp, two GnRH variants have been isolated [67]. Significantly, when common carp sGnRH expression was inhibited by sGnRH antisense, the plasma GtH-II level was significantly reduced, and the gonad development was severely diminished or hampered [68]. Moreover, Xu et al. [69] used the gonad hampered transgenic fish as a model for defining global gene expression changes of the hypothalamic-pituitary-gonadal axis, and revealed 9, 28, and 212 differentially expressed genes respectively in hypothalamus, pituitary, and ovary, of which 87 genes were novel. Obviously, this study not only indicated the progressive scaling-up effect of hypothalamic sGnRH antisense acting on the pituitary and ovary, but also provided new insights with respect to the identification of reproduction-related genes and the understanding of the molecular mechanisms and regulative pathways in fish reproduction system. Recently, Zhou et al. (unpublished data) have also conducted a detailed exploration and functional annotation of hypothalamus and pituitary transcriptomes of mature gibel carp by Roche 454 sequencing. By this way, 878430 high quality sequences (average sequence length of  $(480\pm 50)$  bp) of hypothalamus and pituitary were assembled into 51111 contigs and 113046 singletons, resulting in 23012 unigenes. Moreover, RNA-Seq approach was applied to analyze the expression level difference of the 23012 unigenes between mature female and male hypothalamus and pituitary. The detailed report will be soon coming.

## 2.2 Sex determination and candidate sex determination genes

Fishes are an attractive group of organisms for studies on vertebrate sex determination, because they include various types of sexuality from hermaphroditism to gonochorism and from environmental to genetic sex determination. Fishes can provide important insight into the plasticity of the sex determination process in vertebrates, and can be used to explore the cascade regulatory pathways and their evolutionary conservation of upstream or downstream function of sex determination genes [70,71]. In 2002, the *DMY* gene, a recent duplicate of *DMRT1* (*Drosophila* doublesex and *Caenorhabditis* mab-3-related transcription factor 1) in the Japanese medaka, was identified as the first sex-determining

gene among non-mammalian vertebrates [72], but it was quickly demonstrated that *DMY* is not the universal primary sex determining gene in fish [73]. Significantly, *DMRT1*, the first conserved zinc finger-like DNA binding domain (DM domain) gene identified in vertebrates, has also been shown to be intimately linked to male gonadogenesis and further differentiation among almost all studied fishes including gonochoristic and hermaphroditic species [74]. As a group of protogynous hermaphrodite fishes that the gonad development undergoes transition from ovary to intersexual gonad and then to testis, and cells at different stages during oogenesis and spermatogenesis can be synchronously observed in the transitional gonads, groupers have been used as a good model for mining sex determination or sex differentiation-related genes. Xia et al. [75] have found that grouper *DMRT1* is specifically expressed in spermatogenic cells during female-to-male sex reversal, and the spermatogenic cell-specific and dynamic expression pattern was revealed to be basically similar in several sex reversal fishes including protogynous wrasse (*Halichoeres tenuispinis*) [76] and rice field eel (*Monopterus albus*) [77] as well as protandrous black porgy (*Acanthopagrus schlegelii*) [78,79] and gilthead seabream (*Sparus aurata*) [80].

*SOX3* has been suggested to play significant roles in sex determination and differentiation in vertebrates, but the evidence is insufficient and controversial [81]. In grouper gonads, some special cysts synchronously contain primordial germ cells, oogonia and spermatogonia, and *SOX3* is expressed very early and can be detected in the nuclei of some primordial germ cells along the germinal epithelium, which provide an excellent chance to examine the functional roles of *SOX3*. The detailed observation and analysis have indicated that when the *SOX3*-positive primordial germ cells develop toward oogonia and then oocytes, *SOX3* expression continues to increase greatly, whereas, as the *SOX3*-positive primordial germ cells develop toward spermatogonia, *SOX3* expression is stopped and expelled from the nuclei of spermatogonia. The data confirm that *SOX3* is involved in oogonium differentiation and oocyte development [82].

In hermaphrodite red-spotted grouper and rice field eel [83,84], the gonad-specific aromatase (*cyp19a1a*) had been also demonstrated to be expressed by follicular cells of follicular layer around oocytes. The promoter activity analysis showed a number of potential binding sites for some transcriptional factors, such as *SOX5*, *GATA* gene family, *CREB*, *API*, *FOXL1*, *C/EBP*, *ARE* and *SF-1*, and most of the motifs are conserved among the studied fish species. Recently, a hypothesis proposes that the gonadal aromatase upregulation is necessary not only for triggering but also for maintaining ovarian differentiation, and the down-regulation is the only necessary step for inducing a testicular differentiation pathway [85].

Actually, several important genes, such as *Fushi Tarazu* factor-1 (*FTZ-F1*), *Sry*-related HMG-box gene 9 (*SOX9*),

Wilms tumor gene 1 (*WT1*), anti-mullerian hormone (*AMH*), *DMRT1* and the zinc finger transcription factor GATA binding proteins 4 (*GATA4*), have been supposed as candidate sex determination genes, and a hypothetical regulation hierarchy for sex determination and differentiation has been suggested in the hermaphroditic groupers [86]. In mammals, primary sex determination genes have been divided into 4 classes, in which class I comprises Y genes that have a male-specific effect required for spermatogenesis, class II are X genes that are expressed at a higher level in females than in males by virtue of the 2:1 ratio, class III are X genes that receive a parental imprint, and class IV are sex-specific regions of heterochromatin that exert effects on the epigenetic status of the rest of the genome [87]. Of course, sex determination mechanisms are also intricate in fishes, because fishes, like other aquatic animals of amphibians and reptiles, include male or female heterogametic genetic sex determination with or without strongly differentiated sex chromosomes [88,89]. A recent report indicates that sex-chromosome homomorphy in European tree frogs does not result from a recent turnover but is maintained over evolutionary timescales by occasional X-Y recombination, implicating that seemingly ever-young sex chromosomes may thus carry old-established sex-determining genes [90]. Moreover, B chromosomes in addition to standard chromosomes are also found to have a functional effect on sex determination in one cichlid species (*Lithochromis rubripinnis*) from Lake Victoria [91], which implies that a portion of sex chromosomes has been derived from B chromosomes. Along with the successive revelation of complete genome sequences in some main aquaculture animals and the quick development of next-generation sequencing techniques, more sex determination genes and the underlying mechanisms will be identified and clarified as performed previously in human and chimpanzee [92].

### 2.3 Growth trait and candidate growth-related genes

Growth trait is one of the most valuable traits of interest in genetic improvement of aquaculture animals, because growth rate increase can directly reduce aquaculture costs and lead to the benefit increase. Since growth hormone (GH) gene was identified, many important growth-related genes have been demonstrated to play critical roles in growth regulation of vertebrates. Numerous candidate genes are correlated with growth, including main growth-related genes within the somatotrophic axis, such as GH, growth hormone receptor (GHR), insulin-like growth factors (IGF-I and -II), growth hormone-releasing hormone (GHRH), Leptin, and growth hormone inhibiting hormone (GHIH or somatostatin), important transforming growth factor genes expressed in muscle tissues, such as myostatin (MSTN) and myogenic regulatory factors (MRFs), and other possible candidate genes have been recommended in fish through reviewing the livestock and fish literatures [93], and the

regulative mechanisms of muscle growth have been explored by introducing different growth models including compensatory growth, genetic manipulation and *in vitro* tissue culture [94].

Recently, several important growth-related genes have been investigated by Chinese fish endocrinologists. For example, multiple somatostatin and somatostatin receptor genes were characterized in the Chinese sturgeon (*Acipenser sinensis*) [95] and in groupers [96–99]. Orange-spotted grouper orexin was found to be involved in feeding and orexin-A was a stimulator of NPY mRNA expression *in vivo* and *in vitro* [100]. Zebrafish ghrelin was demonstrated to play an essential role in GH expression during adenohypophysis development [101]. Follistatin 1 (*Fst1*) overexpression was revealed to promote muscle growth in the transgenic zebrafish [102].

### 2.4 Disease resistance trait and candidate disease resistance-related genes

Along with rapidly growing and highly intensifying in the past 30 years, many aquaculture species have been threatened by some serious diseases caused by viruses, bacteria, fungi, parasites or other undiagnosed and emerging pathogens. Especially in infectious virus diseases, a lot of pathogenic viruses were identified from freshwater and marine culture animals [103], and the complete genomes of several viruses, such as grass carp hemorrhage virus (GCHV) [104], shrimp white spot syndrome virus (WSSV) [105], mandarin fish infectious spleen and kidney necrosis iridovirus (ISKNV) [106], and flounder lymphocystis disease virus isolated in China (LCDV-C) [107], were sequenced in China. Moreover, their structure proteins were characterized [108–111], and some of them were functionally analyzed [112,113].

In order to identify innate antiviral genes that protect fish from some virus infections, suppressive subtractive hybridization technology has been used to isolate virally induced genes from UV-inactivated GCHV-infected CAB cells [114]. Through this model system, a series of interferon (IFN) induced genes have been cloned and characterized, and great progress has been made in identifying fish anti-disease genes involved in IFN antiviral response [115–120]. Significantly, some novel interferon-inducible genes have been found [121], and their functional roles and signaling pathways have been well understood in fish [122–124]. Additionally, several candidate genes, such as *PKR* [125], *PKR-like* (or called *PKZ*) [126],  $\beta$ -Defensin [127], *TRBP* [128], *C-type lectin* [129], *Enzyme E2* [130], *MDA5* and *LGP2* [131], have been revealed to play crucial roles in the antiviral defense response of fish and shrimp.

### 2.5 Cold tolerance trait and candidate cold tolerance-related genes

Cold tolerance is an especially attractive trait in fish, and a

recent experiment evidence suggests that some fishes might carry sufficient genetic variation to adapt to temperature and the cold tolerance should be under strong selection [132]. To identify candidate cold tolerance-related and cold change-related genes, a large-scale EST sequencing of multi-tissues including brain, liver, head kidney, and ovary had been performed in Antarctic notothenioid (*Dissostichus mawsoni*), and some important cold-specific expressed genes had been identified by comparative transcriptome analyses between *D. mawsoni* and non-notothenioid warm-water teleost fishes [133,134]. Moreover, the molecular origination of antifreeze protein III (AFP III) was demonstrated to come from a duplicate copy of sialic acid synthase (SAS) gene, supporting the evolutionary mechanism by neofunctionalization under escape from adaptive conflict [135].

## 2.6 Hypoxia tolerance trait and candidate hypoxia tolerance-related genes

Variable hypoxia tolerance has been observed in aquatic animals including reptiles, amphibians, and fishes [136,137], but its molecular mechanisms remain largely unknown. Since *Carassius* species have been found to survive many days or even several months of anoxia at low temperature, *Carassius auratus* blastulae embryonic cells have been used to screen gene expression changes responding to hypoxia stress by suppression subtractive hybridization (SSH). Through this approach, a total of 211 differentially expressed genes are identified, in which most of them are reported for the first time from hypoxia-treated teleosts [138].

Moreover, several potential hypoxia tolerance-related genes have been characterized and functionally analyzed in fish. For example, heme oxygenase-1 (*HO1*) has been demonstrated to have protective role in fish under hypoxic stress [139]. Grass carp insulin-like growth factor-binding protein 1 (*IGFBP1*) has been revealed to play a key role in mediating the hypoxia-induced embryonic growth retardation and developmental delay [140]. A novel function of p53 that directly suppresses *BNIP3* expression to protect against hypoxia-induced cell death has been found in model fish zebrafish [141]. Also in zebrafish, a stable transgenic fish has been created by expressing the *Vitreoscilla* hemoglobin (vhb) gene driven by common carp beta-actin promoter, and the expressed VHb can partially alleviate the hypoxia stress response to improve the survival rate has been confirmed by comparing the expression profiles of the vhb transgenic zebrafish [142].

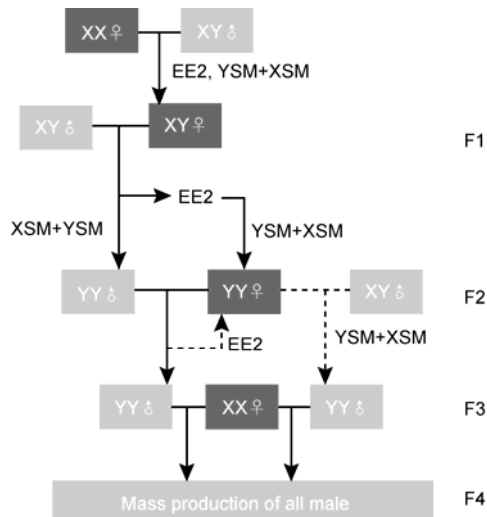
## 3 Application of genetic improvement in aquaculture animals

Genome technologies, especially molecular markers, have been designed to apply to breeding program and genetic

improvement of main aquaculture animals including rainbow trout, Atlantic salmon, tilapia, channel catfish and ornamental fishes in the world, and their potential values have been evaluated by aquaculture geneticists [49,143–145].

In China, several nuclear genome markers, such as microsatellite patterns, AFLP profiles and transferrin alleles, and one cytoplasmic marker, the whole mitochondrial genome sequence, have been used to identify genetic organization of a novel clone A<sup>+</sup> created by sexual mating between clone D female and clone A male in polyploid gibel carp [18], which have demonstrated that the novel clone is a nucleo-cytoplasmic hybrid between the known clones A and D, because it contains an entire nuclear genome from the paternal clone A and a mtDNA genome (cytoplasm) from the maternal clone D [146]. Therefore, it is suggested to be formed via androgenesis of clone A sperm in clone D ooplasm. Significantly, the selected nucleo-cytoplasmic hybrid female still maintains its gynogenetic ability, and thereby produces a novel clone variety “CAS III” that has been approved by National Certification Committee for Aquatic Varieties. Owing to the significant growth superiority, the novel clone variety has been propagated by gynogenesis in more than 10 hatcheries, and their offspring have been cultured throughout China [146]. Moreover, the finding will be of great significance for exploiting the genetic breeding approaches in gibel carp [18], because the nucleo-cytoplasmic hybrid clone escapes the genetic and developmental destruction caused by drastic treatments of irradiation and physical shocks in induced androgenesis and nuclear transplantation [146].

Additionally, sex-specific molecule markers have been developed and applied in China. Based on artificial propagation families, and gynogenetic XX, XY and YY individuals obtained previously [147], two pairs of Y chromosome- and X chromosome-specific markers were isolated from yellow catfish (*Pelteobagrus fulvidraco*) by AFLP screening, which thereby develops a Y- and X-specific allele marker-assisted sex control technique for mass production of all-male populations [148]. Using this technique, all-male yellow catfish has been approved as a novel variety “yellow catfish all-male No. 1” by National Certification Committee for Aquatic Varieties. Because yellow catfish males grow faster than females, and result in an ultimate size difference of about two to threefold, the all-male yellow catfish has been widely used for commercial production. Recently, all-female flounder has been also recognized as a novel variety for aquaculture in China. According to the developments, we have proposed an integration sex control technique for mass production of all-male populations in aquaculture fish [148]. As shown in Figure 1, the XY physiological females are selected by the Y chromosome-specific marker (YSM) and X chromosome-specific marker (XSM) from sex reversal progeny through 17 $\alpha$ -ethinyloestradiol (EE2) treatment, and about 25% YY super-males can be obtained from the mating progeny of the



**Figure 1** A schematic diagram of mass production of all-male populations through an integration sex control technique of female hormone 17 $\alpha$ -ethinyloestradiol (EE2) treatment and genetic identification of Y chromosome-specific marker (YSM) and X chromosome-specific marker (XSM) in aquaculture fish.

reversed XY female and normal XY male by YSM and XSM identification. Then, the YY physiological females can be induced from the EE2 treated YY fingerlings, and YY super-males can be produced continually from the mating of the YY physiological females and YY super-males. Subsequently, the YY super-males can be used to mate with XX females for commercial mass production of all-XY males.

#### 4 Conclusion and perspective

Along with complete genome sequence anatomy of some main aquaculture animals, ongoing development of genome technologies, somatic cell nuclear transfer and stem cell technologies, as well as identification and function analysis of candidate genes relative to economically important traits including reproduction, sex, growth, disease resistance, cold tolerance and hypoxia tolerance, genetic improvement research in aquaculture animals has begun to enter a new era for molecular design breeding. The future issues are mainly aimed at understanding gene regulation and control networks that are related to reproduction, growth, disease-resistance, cold tolerance and hypoxia tolerance, at identifying the relative functional genes and molecular markers, and at creating novel breeding materials and technologies, which can be used to develop the theoretical strategies and feasible approaches for molecular design breeding in aquaculture animals. It will establish the theoretical and technical foundation for breeding new varieties with high yield and quality, and will make greater contribution for sustainable fisheries and biotechnological innovation.

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