

## Sex determination mechanisms and sex control approaches in aquaculture animals

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Aquaculture is one of the most efficient modes of animal protein production and plays an important role in global food security. Aquaculture animals exhibit extraordinarily diverse sexual phenotypes and underlying mechanisms, providing an ideal system to perform sex determination research, one of the important areas in life science. Moreover, sex is also one of the most valuable traits because sexual dimorphism in growth, size, and other economic characteristics commonly exist in aquaculture animals. Here, we synthesize current knowledge of sex determination mechanisms, sex chromosome evolution, reproduction strategies, and sexual dimorphism, and also review several approaches for sex control in aquaculture animals, including artificial gynogenesis, application of sex-specific or sex chromosome-linked markers, artificial sex reversal, as well as gene editing. We anticipate that better understanding of sex determination mechanisms and innovation of sex control approaches will facilitate sustainable development of aquaculture.

**sex determination, sex control approach, sexual dimorphism, sex chromosome, sex change, reproduction strategy, sex reversal**

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### Introduction

Sexual reproduction refers to the presence of meiosis followed by nuclear fusion, which is a key driving force for the diversity of life (Bachtrog et al., 2014; Capel, 2017; Lahr et al., 2011; Li and Gui, 2018; Spiegel, 2011). Increasing evidence supports the hypothesis that sex arises along with the origin of eukaryotes and is a synapomorphy for eukaryotic life (Hofstatter and Lahr, 2019; Lahr et al., 2011; Maciver, 2016; Speijer et al., 2015). Compared with unisexual re-

production lacking meiosis, sexual reproduction can combine beneficial alleles in the next generation more effectively and purge deleterious mutations, which speed the adaptation to changing environments (Avisé, 2015; Burke and Bonduriansky, 2017; McDonald et al., 2016; Speijer et al., 2015). This may be the reason why sex is pervasive throughout nature despite substantial mating costs.

Sex is one of the most complex traits in aquaculture animals and shows diverse phenotypes, including simultaneous hermaphroditism (one individual contains both female and male sexual organs at the same time), sequential hermaphroditism (one individual changes sex at some developmental stage during their life cycle), gonochorism (animals) and

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dioecy (plants) (Bachtrog et al., 2014; Mei and Gui, 2015; Ortega-Recalde et al., 2020). Different reproductive strategies, such as unisexual reproduction, sexual reproduction, and facultative reproductive systems (where individuals can switch between unisexual and sexual reproduction), exist in aquaculture animals (Burke and Bonduriansky, 2017; Gui and Zhou, 2010; Speijer et al., 2015) (Figure 1).

Sex is also one of the most valuable traits in fish and other aquatic animals because significant sexual dimorphisms commonly exist, including growth, size, shape, color, behavior, and ornament (Horne et al., 2020; Martínez et al., 2014; Mei and Gui, 2015). Therefore, many countries and institutions have initiated appropriate genetic breeding programs to attempt to create monosex (all-male or all-female) populations or to produce a high ratio of males or a high ratio of females with higher aquaculture value (Gui, 2007; Gui et al., 2018; Gui and Hughes, 2022; Martínez et al., 2014; Zhou and Gui, 2018). Recently, Sex Control in Aquaculture, a comprehensive book edited by Wang et al. (2018), has provided numerous detailed information on genetic rationales and technical protocols of sex control in some major aquaculture species of the world. However, rapid advances and expanding trends from basic studies to practical applications have greatly developed. Here, we review recent advances in sex determination mechanisms, sex chromosome evolution, reproduction strategies, sexual dimorphism, and sex control approaches in aquaculture animals.

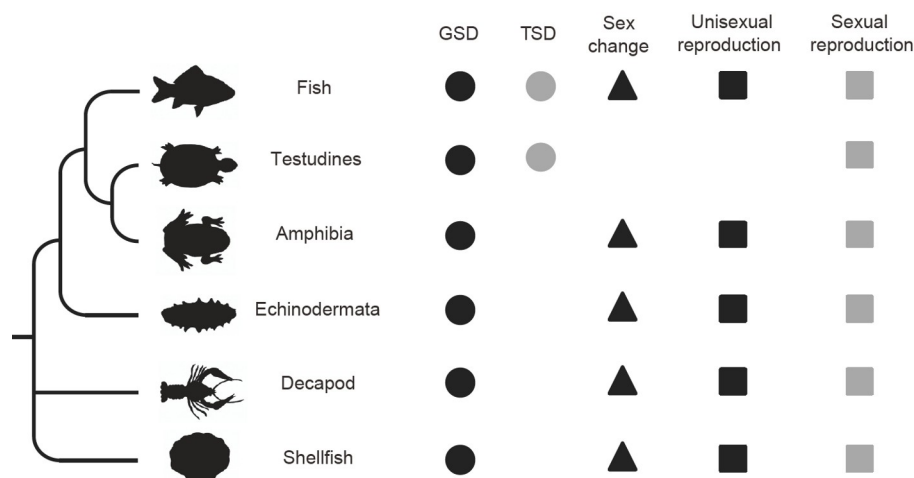
## Sex determination mechanisms

Unlike the most-studied mammals and birds with conserved sex chromosomal systems, aquaculture animals have a wide range of sex determination mechanisms, encompassing three broad strategies: genotypic sex determination (GSD), en-

vironmental sex determination (ESD), and genotypic sex determination with environmental effect (GSD+ESD) (Bachtrog et al., 2014; Capel, 2017; Li and Gui, 2018; Mei and Gui, 2015; Sarre et al., 2004) (Figure 1). The sexual fate of a GSD individual is driven by genetic elements with sex differences, while the sex of an ESD individual is determined by the effects of environmental factors during development (Bachtrog et al., 2014; Capel, 2017; Li and Gui, 2018; Mei and Gui, 2015; Meisel, 2020; Ortega-Recalde et al., 2020). These two seemingly distinct sex determination strategies are not mutually exclusive and coexistence of them (GSD+ESD) has been widely observed in aquaculture animals. Besides, transitions between different sex determination systems occur frequently (Holleley et al., 2015; Pennell et al., 2018; Vicoso, 2019; Xiong et al., 2020b).

## Genotypic sex determination

GSD can be divided into male heterogametic system and female heterogametic system, where male and female individuals are heterozygous for the sex-determining locus, respectively (Bachtrog et al., 2014). The most common male heterogametic system is the XX/XY system, in which males contain two different sex chromosomes (XY), while females contain two of the same sex chromosomes (XX). *Dmy* (*DM-domain gene on the Y chromosome*) of medaka fish (*Oryzias latipes*), also named *dmrt1Y* or *dmrt1bY*, is the first identified sex-determining gene in non-mammalian vertebrates (Matsuda et al., 2002). The most common female heterogametic system is the ZZ/ZW system, in which females contain two different sex chromosomes (ZW), while males contain two of the same sex chromosomes (ZZ). *Dm-w* (*W-linked DM-domain gene*) of clawed frog (*Xenopus laevis*) is the first identified sex-determining gene on W chromosomes that participates in primary ovary



**Figure 1** Sex determination, sex change, and reproduction strategy in aquaculture animals. GSD, genotypic sex determination; TSD, temperature-dependent sex determination.

development (Yoshimoto et al., 2008).

GSD with XX/XY or ZZ/ZW system has been widely identified in fish species, such as common carp (*Cyprinus carpio*) (Wu et al., 1990), yellow catfish (*Pelteobagrus fulvidraco*) (Liu et al., 2013b; Wang et al., 2009a), Ussuri catfish (*Pseudobagrus ussuriensis*) (Pan et al., 2015), Lanzhou catfish (*Silurus lanzhouensis*) (Wang et al., 2021), Nile tilapia (*Oreochromis niloticus*) (Li et al., 2015), Northern pike (*Esox lucius*) (Pan et al., 2019), half-smooth tongue sole (*Cynoglossus semilaevis*) (Chen et al., 2014; Cui et al., 2017), oyster pompano (*Trachinotus anak*) (Fan et al., 2021), and Amur sturgeon (*Acipenser schrenckii*) (Ruan et al., 2021) (Figure 1 and Table 1). GSD has also been detected in other aquaculture animals, including turtles (Montiel et al., 2016), frogs (Flament, 2016; Wallace et al., 1999; Yoshimoto et al., 2008), sea urchins (Lipani et al., 1996), sea cucumbers (Wei et al., 2021), crabs (Fang et al., 2020; Shi et al., 2018; Waiho et al., 2019), lobsters (Chandler et al., 2017), and shellfish (Guo and Allen, 1994; Luo et al., 2021) (Figure 1 and Table 1).

In addition, numerous variants of the XX/XY system and ZZ/ZW system have evolved in some fish species. The karyotype of the neotropical fish (*Hoplias malabaricus*) exhibits remarkable diversity, and  $X_1X_1X_2X_2/X_1X_2Y$  and  $XX/XY_1Y_2$  sex chromosome systems have been reported in some strains (Bertollo et al., 2000; Cioffi and Bertollo, 2010). In rock bream (*Oplegnathus fasciatus*), chromosome numbers are different between females ( $2n=48$ ) and males ( $2n=47$ ) because of the presence of an  $X_1X_1X_2X_2/X_1X_2Y$  sex chromosome system (Xu et al., 2013; Xu et al., 2015). Multiple systems have also been found in catfishes of the genus *Ancistrus* including  $XX/XO$ ,  $XX/XY_1Y_2$ , and  $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$  sex chromosome systems (Alves et al., 2006; de Oliveira et al., 2007) (Table 1).

Supernumerary B chromosomes (supernumerary chromosomes, B chromosomes, or extra chromosomes) are non-essential karyotypic components in addition to standard A chromosomes (autosomes and sex chromosomes), which occur in approximately 15% of eukaryotic species (Ahmad and Martins, 2019; Houben et al., 2014). In many species, the presence of supernumerary chromosomes is closely associated with sex-ratio distortion (Beladjal et al., 2002; Camacho et al., 2011; Clark and Kocher, 2019). In addition, the supernumerary or extra chromosomes are supposed to have a female determination or male determination function in Lake Victoria cichlid fish (*Lithochromis rubripinnis*) (Yoshida et al., 2010) and gynogenetic gibel carp (*Carassius gibelio*) (Ding et al., 2021; Li et al., 2016), respectively. Importantly, a potential sex-determining gene *gdf6b* (growth differentiation factor 6b) has been identified in the B chromosome, triggering male sex determination in Pachon cavefish (*Asytanax mexicanus*) (Imarazene et al., 2021). These findings indicate that supernumerary or extra chromosomes can play

important roles in GSD.

### Environmental sex determination

Environmental factors of ESD exhibit an extraordinary diversity, such as temperature, social factors, hypoxia, and photoperiod (Li and Gui, 2018). Temperature-dependent sex determination (TSD) is the most common form of ESD and exists in many testudines and some fish species: such as gynogenetic gibel carp (*C. gibelio*) (Li et al., 2018), Atlantic silverside (*Menidia menidia*), pejerrey (*Odontesthes bonariensis*), and topminnow (*Poeciliopsis lucida*) (Ospina-Alvarez and Piferrer, 2008).

Turtles with TSD are highly variable in their patterns of temperature sensitivity. For many turtle species, low incubation temperatures during development lead to male embryos (male-producing temperature or MPT), whereas higher incubation temperatures lead to females (female-producing temperature or FPT). In contrast, some turtle species same as crocodiles exhibit a different mode of TSD in which extremely cool and warm temperatures produce females, while intermediate temperatures lead to males (Bull and Vogt, 1979). The mechanism by which temperature is transduced into a sex determination molecular signal remains elusive in turtles. Importantly, isolated gonads of red-eared slider turtle (*Trachemys scripta*) cultured in a whole organ culture system were shown to be directly receptive to thermal stimuli, which suggests that the initial reception of environmental cues might be triggered through an endogenous sensory mechanism (Shoemaker-Daly et al., 2010). It is possible that the presence of an environmental sensor-like element is responsible for the initiation of a sex-determining cascade. Several candidate factors have been investigated in TSD species, such as heat shock proteins (Kohno et al., 2010), cold-inducible RNA binding protein (Rhen and Schroeder, 2010), and transient receptor potential cation channels (Czerwinski et al., 2016; Yatsu et al., 2015; Yuan et al., 2021). These findings provide new insight into TSD and suggest that further studies examining the role of these factors during gonadal development should be needed.

Socially controlled sex determination has been well studied in bluehead wrasse (*Thalassoma bifasciatum*) and orange-spotted grouper (*Epinephelus coioides*). Bluehead wrasses are small reef fish, whose natural social group commonly contains one territorial terminal-phase male, several lower-ranking females, and some initial-phase males (Warner and Swearer, 1991). All females and initial-phase males share the same color pattern, while the terminal-phase male with a larger size displays a distinct phenotype (Liu et al., 2017a). Initial-phase males mimic females in appearance and behavior to gain fertilization (Todd et al., 2017). When the dominant terminal-phase male disappears, commonly one of the large females changes to a dominant terminal-

**Table 1** Sex-specific or sex chromosome-linked markers and their characterization identified from aquaculture species

Species	Sex dimorphism and other traits of economic importance and breeding program	Sex determination system	Identified method	Marker characterization	Linked gene	References
Fish						
Medaka ( <i>Oryzias latipes</i> )	As genetic model, the cross between female (X'X') and heterozygous orange-red male (X'Y <sup>R</sup> ) produces white females (X'X') and orange-red males (X'Y <sup>R</sup> ) in equal numbers. The <i>r</i> and <i>R</i> can be used as markers for identification of the genotypic sex, XX and XY, respectively.	XY	Gene seq and genome comparison between males and females	Y-linked	<i>dmy</i>	Matsuda et al., 2002
Large yellow croaker ( <i>Larimichthys crocea</i> )*	Females grow faster than males, and no morphological differences are observed in the early stages.	XY	Genomic re-seq	Male-specific	<i>dmr1</i>	Lin et al., 2017a
Yellow drum ( <i>Nibea albiflora</i> )*	Females grow much faster than males, and no morphological differences are observed in the early stages.	XY	Genomic re-seq	Male- and female-specific	<i>dmr1</i>	Sun et al., 2018
Luzon ricefish ( <i>Oryzias luzonensis</i> )	It have lost <i>dmy</i> and utilized another sex-determining gene <i>gsdf</i> .	XY	Gene seq	Y-linked	<i>gsdfy</i>	Myosho et al., 2012
Gopher rockfish ( <i>Sebastes carnatus</i> )	Females and males are not easily distinguished by external morphology.	XY	ddRAD-seq	Male-specific	<i>gsdf</i>	Fowler and Buonaccorsi, 2016
Black-and-yellow rockfish ( <i>Sebastes chrysomelas</i> )	Females and males are not easily distinguished by external morphology.	XY	ddRAD-seq	Male-specific	<i>gsdf</i>	Fowler and Buonaccorsi, 2016
Nile tilapia ( <i>Oreochromis niloticus</i> )*	Males have faster growth rate than females, because males have better food conversion efficiency with higher metabolic capacity.	XY	AFLP AFLP QTL mapping QTL mapping SSR Screen XY genomic library ddRAD-seq	Sex-specific Sex chromosome-linked Male-specific Male-specific Sex-linked Y-linked Sex-linked microsatellite	<i>amh4-y</i>	Ezaz et al., 2004 Lee et al., 2011 Palaiokostas et al., 2013b Palaiokostas et al., 2015 Li et al., 2015 Sultana et al., 2020
Patagonian pejerrey ( <i>Odontesthes hatcheri</i> )	Low (13–15°C) and high (25°C) temperatures can produce an excess of females and males, and the molecular mechanism studies are limited by the difficulty in discerning the genotypic sex of embryos and larvae prior to phenotypic gonad differentiation.	XY	AFLP Gene seq	Y-linked Y-linked	<i>amly</i>	Koshimizu et al., 2010 Hattori et al., 2012
Black rockfish ( <i>Sebastes schlegelii</i> )*	Females growing about 25% faster than males.	XY	Genomic re-seq and sex-determining locus mapping	Y-linked	<i>amly</i>	Song et al., 2021
Northern pike ( <i>Esox lucius</i> )		XY	Genomic seq and RAD-seq	Y-specific	<i>amhby</i>	Pan et al., 2019
Tiger pufferfish ( <i>Takifugu rubripes</i> )*	Their testes possess delicate taste.	XY	Linkage mapping	Sex-linked SNP	<i>amhr2</i>	Kamiya et al., 2012; Matsunaga et al., 2014
Obscure pufferfish ( <i>Takifugu obscurus</i> )*	Their testes possess delicate taste.	XY	Gene sequence comparison and HRM	Sex-linked SNP	<i>amhr2</i>	Gao et al., 2020
Yellow perch ( <i>Perca flavescens</i> )		XY	Genomic seq	Y-specific markers	<i>amhr2by</i>	Feron et al., 2020
Ayu ( <i>Plecoglossus altivelis</i> )*	Female ayu are more valuable than males as food, because mature fish with eggs are considered delicious.	XY	AFLP 2b-RAD-seq	Male-specific Male-specific	<i>amhr2by</i>	Watanabe et al., 2004 Nakamoto et al., 2021 Li et al., 2021b
Common seadragon ( <i>Phyllopteryx taeniolatus</i> )		XY	Genomic seq	Male-specific	<i>amhr2y</i>	Qu et al., 2021
Alligator pipefish ( <i>Syngnathoides biaculeatus</i> )		XY	Genomic seq	Male-specific	<i>amhr2y</i>	Qu et al., 2021

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Species	Sex dimorphism and other traits of economic importance and breeding program	Sex determination system	Identified method	Marker characterization	Linked gene	References
Atlantic herring ( <i>Clupea harengus</i> )		XY	Genome-wide association analysis	Y-specific	<i>bmpr1bby</i>	Rafati et al., 2020
Turquoise killifish ( <i>Nothobranchius furzeri</i> )		XY	Genomic seq	Y-specific	<i>gdff6y</i>	Reichwald et al., 2015
Indian ricefish ( <i>Oryzias latipes</i> )	Sex of the medaka-related species is genetically determined by a single locus on the homomorphic XY chromosomes.	XY	Gene seq and positional cloning	Y-linked	<i>sox3y</i>	Takehana et al., 2014
New Zealand trevally ( <i>Pseudocaranx georgianus</i> )	Sexually monomorphic externally.	XY	Genomic seq and re-seq	male-specific	<i>cyp19ala-like</i>	Catanach et al., 2021
Rainbow trout ( <i>Oncorhynchus mykiss</i> )*	Females grow faster than males, and females usually mature one year later than males.	XY	SSR AFLP	Sex-linked Male-specific Y-linked	<i>sdY gh-ψ</i>	Sakamoto et al., 2000 Felip et al., 2005 Yano et al., 2012 AkbariMajough et al., 2020
Chinook salmon ( <i>Oncorhynchus tshawytscha</i> )*	Females grow faster than males, and females usually mature one year later than males.	XY	AFLP	Y-linked Male-specific	<i>gh-ψsdY</i>	Devlin et al., 1991 Du et al., 1993 Devlin et al., 1998 Yano et al., 2013
Coho salmon ( <i>Oncorhynchus kisutch</i> )*	Females have larger body size than males, and die after spawning.	XY	Allele sequence comparison	Y-linked	<i>gh-ψ</i>	Forbes et al., 1994
Masu salmon ( <i>Oncorhynchus masou</i> )*	Salmon exhibit much greater sexual dimorphism than trout.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Atlantic salmon ( <i>Salmo salar</i> )*	Salmon exhibit much greater sexual dimorphism than trout.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Brown trout ( <i>Salmo trutta</i> )*	Females grow faster than males, and females usually mature one year later than males.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Brook trout ( <i>Salvelinus fontinalis</i> )*	Females grow faster than males, and females usually mature one year later than males.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Grayling ( <i>Thymallus thymallus</i> )	To inform genetic sex prior to maturation.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Dolly Varden trout ( <i>Salvelinus malma</i> )*	Females grow faster than males, and females usually mature one year later than males.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Lake char ( <i>Salvelinus namaycush</i> )	To inform genetic sex prior to maturation.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Arctic char ( <i>Salvelinus alpinus</i> )	To inform genetic sex prior to maturation.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Huchen ( <i>Hucho hucho</i> )	To inform genetic sex prior to maturation.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Sakhalin taimen ( <i>Parahucho perryi</i> )	To inform genetic sex prior to maturation.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Sheefish ( <i>Stenodus leucichthys</i> )	To inform genetic sex prior to maturation.	XY	Gene sequence comparison	Male-specific	<i>sdY</i>	Yano et al., 2013
Platyfish ( <i>Xiphophorus maculatus</i> )	Different male size classes show courtship versus sneak mating behavioral differences, and females prefer large over small males.	XY	Gene seq	Y-linked	<i>mc4r</i>	Lampert et al., 2010

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Species	Sex dimorphism and other traits of economic importance and breeding program	Sex determination system	Identified method	Marker characterization	Linked gene	References
Yellow catfish ( <i>Pelteobagrus fulvistraco</i> )*	Males grow faster than females with over 100%.	XY	AFLP Genomic walking, SSR	X-linked, Y-linked	<i>pfpdz1</i>	Wang et al., 2009a Dan et al., 2013 Chen et al., 2015 Dan et al., 2018
Channel catfish ( <i>Ictalurus punctatus</i> )*	Males grow faster than females with 37%.	XY	Genomic seq QTL fine mapping of sex Genomic seq of YY individual	Male-specific Sex-linked SSR markers Male-specific	<i>bcar1</i>	Nimwichian et al., 2012 Zhang et al., 2019b Bao et al., 2019
Bighead catfish ( <i>Clarias macrocephalus</i> ) Ussuri catfish ( <i>Pseudobagrus ussuriensis</i> )*	Crossbreeding between female bighead catfish and male African catfish is used to produce hybrids with vigorous phenotypes. Males grow faster than females with over 300%.	XY	DARtseq	Sex-specific	<i>gtsf11</i>	Nguyen et al., 2021
Lanzhou catfish ( <i>Silurus lanzhouensis</i> )* Red-tail catfish ( <i>Hemibagrus or Mystus wyckioide</i> )* Southern catfish ( <i>Silurus meridionalis</i> )* African catfish ( <i>Clarias gariepinus</i> )* Longsnout catfish ( <i>Leiocassis longirostris</i> )* Common carp ( <i>Cyprinus carpio</i> )*	Males grow faster than females, and long juvenility period of 3 years is difficult to distinguish females and males. Males grow faster than females, and long juvenility period of 3–5 years is difficult to distinguish females and males. Females grow faster than males, and males attain maturity at an age of 2–4 years while females at 3–5 years. There is a distinct growth difference between males and females. Males are generally larger than females. Females grow faster than males with 30%.	XY	AFLP SSR 2b-RAD-seq 2b-RAD-seq 2b-RAD-seq Genomic seq and re-seq RAPD 2b-RAD-seq RAPD SSH Genomic re-seq	X-linked, Y-linked Male-specific Male-specific X-linked, Y-linked X-linked, Y-linked Sex-linked Male-specific X-specific, Y-specific Male-specific Male-specific Y-linked		Pan et al., 2015 Zhu et al., 2018a Zhu et al., 2021 Wang et al., 2021 Zhou et al., 2019 Zheng et al., 2020 Kovács et al., 2000 Dai et al., 2021b Chen et al., 2009 Chen et al., 2010 Zhang et al., 2017a
Grass carp ( <i>Ctenopharyngodon idellus</i> ) Silver carp ( <i>Hypophthalmichthys molitrix</i> ) Bighead carp ( <i>Hypophthalmichthys nobilis</i> )	Long juvenility period of 5 years is difficult to distinguish females and males. Long juvenility period of 4–5 years is difficult to distinguish females and males. Long juvenility period of 5 years is difficult to distinguish females and males.	XY	Genomic seq and re-seq QTL mapping Genomic seq and re-seq QTL mapping	Male-specific Sex-specific Male-specific Sex-specific		Liu et al., 2018 Zhou et al., 2020 Liu et al., 2018 Zhou et al., 2020
Amazon Tambaqui ( <i>Colossoma macropomum</i> )* Largemouth bass ( <i>Micropterus salmoides</i> L.)*	Females reaching larger sizes. Females live longer and reach larger sizes than males.	XY	ddRAD-seq	Sex-linked		Varela et al., 2021 Du et al., 2021
Three-spined stickleback ( <i>Gasterosteus aculeatus</i> )	Females and males are not easily distinguished by external morphology, thus a diagnostic molecule tool for sex identification is required.	XY	Re-seq AFLP	Male-specific Y-linked		Griffiths et al., 2000

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Species	Sex dimorphism and other traits of economic importance and breeding program	Sex determination system	Identified method	Marker characterization	Linked gene	References
Nine-spined stickleback ( <i>Pungitius pungitius</i> )	Females and males are not easily distinguished by external morphology, thus a diagnostic molecule tool for sex identification is required.	XY	SSR	Sex-linked		Shikano et al., 2011
Fathead minnow ( <i>Pimephales promelas</i> )	As common ecotoxicological model, the markers can be used to genotype sex.	XY	AFLP	Sex-specific		Olmstead et al., 2011
Hāpuku ( <i>Polyprion oxygenetos</i> )	To take over 5 years to reach sexual maturity and are not externally sexually dimorphic.	XY	ddRAD-seq	Male-specific		Brown et al., 2016
Atlantic halibut ( <i>Hippoglossus hippoglossus</i> *)	Females mature later one year than males, and grow faster than males.	XY	RAD-seq	Sex-specific SNP markers		Palaiokostas et al., 2013a
Topmouth culter ( <i>Erythroculter ilishaeformis</i> )	Females have faster growth rate and larger body weight than males.	XY	AFLP	Male-specific		Liu et al., 2017d
Mandarin fish ( <i>Siniperca chuatsi</i> *)	Females have 10%–20% growth superiority over males.	XY	Genomic seq and re-seq	Sex-specific		Han et al., 2020
Zig-zag eel ( <i>Mastacembelus armatus</i> *)	Males have 62.3% growth superiority over females	XY	2b-RAD Genomic seq and re-seq	Sex-specific	<i>hmg16</i>	Xue et al., 2020 Xue et al., 2021
Northern snakehead ( <i>Channa argus</i> *)	Males grow faster than females, resulting in a twofold size difference after 2–3 years of culture.	XY	NGS-based and HRM typing system QTL mapping QTL mapping	Y-linked Sex-specific Sex-specific SNP marker		Ou et al., 2017 Wang et al., 2019 Liu et al., 2020
Blotched snakehead ( <i>Channa maculata</i> *)	Males grow faster than females.	XY	NGS-based and HRM typing system NGS	Sex-specific X-linked, Y-linked		Yang et al., 2020 Han et al., 2021
Mozambique tilapia ( <i>Oreochromis mossambicus</i> *)	Males have faster growth rate than females.	XY	Sex-determining loci mapping	Sex-linked microsatellite		Liu et al., 2013a
Blackchin tilapia ( <i>Sarotherodon melanocheirus</i> *)	Males have faster growth rate than females.	XY	Genomic seq	Sex-linked SNP		Gammerdinger et al., 2016
Half-smooth tongue sole ( <i>Cynoglossus semitaenia</i> *)	Females have 2–4 times of growth rate faster than males.	ZW	AFLP Genomic seq Genomic re-seq	Female-specific AFLP Z-linked Female-specific	<i>dmr1</i>	Chen et al., 2007 Chen et al., 2014 Zhang et al., 2019a
Amberjack ( <i>Seriola dumerili</i> )		ZW	Gene seq	Sex-linked SNP	<i>hsd17b1</i>	Koyama et al., 2019
Oyster pompano ( <i>Trachinotus anakt</i> *)	Females grow faster than males.	ZW	Genomic seq	Z-linked and W-linked	<i>hsd17b1</i>	Fan et al., 2021
Blue tilapia ( <i>Oreochromis aureus</i> *)	Males have faster growth rate than females.	ZW	SSR RAPD NGS, TGS, and comparative genomics	Sex-linked microsatellite Female-specific W-specific, Z-specific		Lee et al., 2004 Meng et al., 2013 Wu et al., 2021
Amur sturgeon ( <i>Acipenser schrenckii</i> )	Sex cannot be directly distinguished by the appearance.	ZW	comparative genomics with high-throughput sequencing	Female-specific		Ruan et al., 2021

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Species	Sex dimorphism and other traits of economic importance and breeding program	Sex determination system	Identified method	Marker characterization	Linked gene	References
Sterlet ( <i>Acipenser ruthenus</i> )*	Females are commercially attractive for caviar-producing.	ZW	chromosome-scale assemblies and pool-sequencing	Female-specific		Kuhl et al., 2021
Western mosquitofish ( <i>Gambusia affinis</i> )	Diagnosis of phenotypic sex in embryos or juveniles is often impossible, thus, a sex-specific marker is required to identify sex at early life-history stages.	ZW	RNA-seq	Female-specific		Lamatsch et al., 2015
Yellowtail ( <i>Seriola quinqueradiata</i> )	No external morphological characters are observed at early and even adult stages.	ZW	SSR	Female-specific		Fuji et al., 2010
Pacific halibut ( <i>Hippoglossus stenolepis</i> )*	Females grow much faster and larger than the males, making difficult to determine sex from length-at-age patterns.	ZW	SSR	Female-specific		Galindo et al., 2011
Turbot ( <i>Scophthalmus maximus</i> )*	Females largely outgrow males.	ZW	RAPD RAPD	Sex-associated RAPD Female-specific		Viñas et al., 2012 Vale et al., 2014
Spotted halibut ( <i>Verasper variegatus</i> )*	Females grow much faster and larger than the males.	ZW	AFLP	Female-specific AFLP		Ma et al., 2010
Sockeye Salmon ( <i>Oncorhynchus nerka</i> )	To know sex-specific survival during various stages and to inform genetic sex prior to maturation.	X <sub>1</sub> X <sub>2</sub> Y	Using genotyping-by-sequencing and comparison	Male-specific	<i>sdv</i>	Larson et al., 2016
Rock bream ( <i>Oplegnathus fasciatus</i> )	Chromosome number difference between males (2n=47) and females (2n=48) is due to the presence of a X <sub>1</sub> X <sub>1</sub> X <sub>2</sub> X <sub>2</sub> /X <sub>1</sub> X <sub>2</sub> Y sex chromosome system.	X <sub>1</sub> X <sub>1</sub> X <sub>2</sub> X <sub>2</sub> /X <sub>1</sub> X <sub>2</sub> Y	AFLP SSR	Male-specific		Xu et al., 2013 Xu et al., 2015
Spinyhead croaker ( <i>Collichthys lucidus</i> )	To help to understand the evolution of sex determination mechanism.	X <sub>1</sub> X <sub>1</sub> X <sub>2</sub> X <sub>2</sub> /X <sub>1</sub> X <sub>2</sub> Y	Genomic sequencing	Male- and female-specific		Xiao et al., 2020
Spotted knifejaw ( <i>Oplegnathus punctatus</i> )	Long juvenility period of 3–4 years is difficult to distinguish females and males.	X <sub>1</sub> X <sub>1</sub> X <sub>2</sub> X <sub>2</sub> /X <sub>1</sub> X <sub>2</sub> Y	Comparative analysis of female and male genomes	Male-specific		Li et al., 2020a
Pachon cavefish ( <i>Astyanax mexicanus</i> )	Females grow faster than males with over 20%.	B-sex chromosome	Genomic sequencing	Male-specific	<i>gf16b</i>	Imarazene et al., 2021
Gibel carp ( <i>Carassius gibelio</i> )*		Male-specific microchromosomes	AFLP	Male-specific		Wang et al., 2009b Li et al., 2016 Li et al., 2016 Ding et al., 2021
Flathead grey mullet ( <i>Mugil cephalus</i> )	Mullet roe is obtained from the gonads of at least 4 to 5-year-old females.		Mapping and analysis of Pool-Seq data against the draft genomes		<i>fshr</i>	Ferrareso et al., 2021
Matrincha ( <i>Brycon amazonicus</i> )	Successive hatchery generations led to an extremely different proportion between male and female births, as almost 92% of the individuals stand for males.		RAPD	Female-specific	<i>pigw</i>	da Silva et al., 2012
Roach ( <i>Rutilus rutilus</i> )	As a species for studies into endocrine disruption, the feminised phenotypes are required to develop genetic sex diagnostic tool.		RAD-seq	Sex-specific		Lange et al., 2020
Testudines						
Soft-shelled turtle ( <i>Pelodiscus sinensis</i> )*	Males have larger body size, faster growth, thicker and wider calipash, and less body fat than females.	ZW	RAD-seq	Female-specific Z-specific 18S rRNA gene		Liang et al., 2019 Zuo et al., 2020

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Species	Sex dimorphism and other traits of economic importance and breeding program	Sex determination system	Identified method	Marker characterization	Linked gene	References
Amphibia						
Clawed frog ( <i>Xenopus laevis</i> )	A model organism of developmental biology.	ZW	Southern blot analyses	Female-specific	<i>dhn-w</i>	Yoshimoto et al., 2008
Giant salamander ( <i>Andrias davidianus</i> )	To develop genetic sex identification at early stage.	ZW	RAD-seq	Sex-specific		Hu et al., 2019
Echinodermata						
Sea cucumber ( <i>Apostichopus japonicus</i> )	The immunocompetence of male sea cucumbers might have an advantage over that of female individuals.	XY	2b-RAD-seq	Male-specific		Wei et al., 2021
Sea urchin ( <i>Mesocentrotus nudus</i> )*	Gonads are the sole edible parts for people; growth rate and immunocompetence differ by sex.	ZW	2b-RAD-seq	Sex-specific		Cui et al., 2021
Decapod						
Eastern spiny lobster ( <i>Sagmariasus verreauxi</i> )			Transcriptome sequencing	Sex-specific	<i>idmy</i>	Chandler et al., 2017
Pacific white shrimp ( <i>Litopenaeus vannamei</i> )*	Females are bigger than males after sexual maturity.	ZW	Sex-determining locus mapping putative sex-linked sequence comparison	Sex-specific Sex-specific SNP Sex-linked genes		Yu et al., 2017 Perez-Enriquez et al., 2020 Wang et al., 2020
Kuruma shrimp ( <i>Penaeus japonicus</i> )*	Females grow faster and achieve a larger size than males.	ZW	Transcriptome sequencing Construct genetic linkage map, QTL mapping	Sex-specific SNP		Zhang et al., 2021 Mohanakumaran Nair et al., 2006
Giant prawn ( <i>Macrobrachium rosenbergii</i> )*	Males grow faster than females, in which adult males are 2–3 times bigger and heavier than females, and all-male culture can increase yield and income by 60%.	ZW	AFLP ZW-derived BAC sequencing	Female-specific Z- and W-linked Sex-specific		Ventura et al., 2011 Jiang and Qiu, 2013 Ma et al., 2019 Shen et al., 2020
Swimming crab ( <i>Portunus trituberculatus</i> )*	Females exhibit higher growth rate, and more profitable value due to the accumulation of vitellogenin in the ovary.	XY	QTL mapping of sex 2b-RAD-seq	Sex-specific Sex-specific		Lv et al., 2018 Li et al., 2020b
Crucifix crab ( <i>Charybdis feriatus</i> )	To develop genetic sex identification technique before observing obvious sexual phenotype.	XY	RAD-seq, SNP	Male-specific SNP		Fang et al., 2020
Mud crabs ( <i>Scylla paramamosain</i> , <i>Scylla tranquebarica</i> , and <i>Scylla serrata</i> )*	Females have higher growth rate and economic value.	ZW	RAD-seq	Female-specific		Shi et al., 2018 Waiho et al., 2019
Shellfish						
Pacific abalone ( <i>Haliotis discus hannai</i> Ino)		XY	Re-seq, GWAS	Male-specific		Luo et al., 2021

\*, Asterisk indicates aquaculture species with significant sexual dimorphism.

phase male. The initial-phase males also have the potential to transform into terminal-phase males which is reported to occur at very low frequency (Godwin, 2009; Warner and Swearer, 1991). In the orange-spotted grouper, social factors also regulate sex change. When the dominant males are removed away, the most dominant female in the social group initiates sex change. And physical interaction among individuals has been revealed to be crucial for the initiation and completion of sex change in social groups (Chen et al., 2021).

Hypoxia has been revealed to affect sex determination and differentiation in zebrafish (Shang et al., 2006), whose sex is determined via multiple mechanisms (Anderson et al., 2012; Wilson et al., 2014). All individuals of zebrafish exhibit a juvenile ovary phase first before differentiating into the designated sex. Subsequently, the oocytes of some juveniles degenerate and the individuals become males, while the oocytes of other juveniles survive and these individuals become females (Uchida et al., 2002). Hypoxia can lead to a male-biased population in zebrafish lacking sex chromosomes (74.4%±1.7% males in the hypoxic groups versus 61.9%±1.6% in the normoxic groups), which is associated with expression changes in various genes controlling sex hormones (Shang et al., 2006).

### Genotypic sex determination with environmental effect

Although the sex of many aquaculture species can be affected by the ambient environmental factors during development, these species usually have evidence for the presence of sex chromosomes, whose sex determination system can be defined as genotypic sex determination with environmental effect (GSD+ESD). Thus, the pure ESD in aquaculture species is far less widespread than usually believed (Li and Gui, 2018; Ospina-Alvarez and Piferrer, 2008; Santerre et al., 2013; Valenzuela et al., 2003).

Half-smooth tongue sole (*C. semilaevis*) has ZZ/ZW sex determination and the *dmrt1* gene on the Z chromosome has been thought to be the candidate sex-determining gene (Chen et al., 2014; Cui et al., 2017). Meanwhile, high-temperature treatment (28°C) can induce female to male sex reversal at a rate of ~73%, and methylation modification in these sex-reversed males (ZW males) is globally inherited in their ZW offspring. Interestingly, these ZW offspring can develop into males without high-temperature treatment (Shao et al., 2014), and a remarkable methylation contrast on the genes from the sex determination/differentiation pathway has been observed between normal ZW females versus sex-reversed males (ZW males) and normal males (ZZ males) (Shao et al., 2014).

In medaka with XX/XY sex determination system, the sex-determining gene *dmy* is derived from a duplicated copy of *dmrt1* (Nanda et al., 2002) and *dmy* initiates testis differ-

entiation and male development via directly activating *gsdf* (Chakraborty et al., 2016; Zhang et al., 2016b). Meanwhile, hypoxia can turn genotypic females into phenotypic males and also upregulate the sex-determining gene *dmy*, which leads to a male-dominant population (Cheung et al., 2014). Besides, environmental sex determination effect also has been observed in many other fish species with GSD, such as Nile tilapia (*O. niloticus*) (Wang et al., 2017), goldfish (*Carassius auratus*) (Wen et al., 2020), and common carp (*C. carpio*) (Wu et al., 2003).

### Molecular mechanisms of GSD

Master sex-determining genes of aquaculture animals exhibit an extraordinary diversity and are commonly derived from three types of genes: DM (doublesex and Mab-3) domain-containing genes, genes from the TGF- $\beta$  (transforming growth factor beta) signaling pathway (Bachtrog et al., 2014; Li and Gui, 2018), and *sox* (*Sry-related HMG box*) family genes (Li and Gui, 2018). Since *dmy* was revealed in medaka (*O. latipes*) (Matsuda et al., 2002), some other DM domain-containing genes have also been identified as sex-determining genes or candidate genes, including *dmrt1* (*dsx- and mab-3-related transcription factor 1*) in half-smooth tongue sole (*C. semilaevis*) (Chen et al., 2014; Cui et al., 2017), *dm-w* (*W-linked DM-domain gene*) in clawed frog (*X. laevis*) (Yoshimoto et al., 2008), and *idmy* (*Y-linked, truncated paralog of the autosomal idmrt1*) in the Eastern spiny lobster (*Sagmariasus verreauxi*) (Chandler et al., 2017). Most identified sex-determining genes and candidate genes are from the TGF- $\beta$  signaling pathway, such as *amhy* (*Y-linked anti-Müllerian hormone duplication*) in Patagonian pejerrey (*Odontesthes hatcheri*) (Hattori et al., 2012; Hattori et al., 2019), *amhr2* (*anti-Müllerian hormone receptor type II*) in tiger pufferfish (*Takifugu rubripes*) (Kamiya et al., 2012), *bmpr1bby* (*Y-linked BMP1B receptor*) in Atlantic herring (*Clupea harengus*) (Rafati et al., 2020), *gsdfy* (*gonadal soma derived growth factor on the Y chromosome*) in medaka (*Oryzias luzonensis*) (Myosho et al., 2012), *gdf6y* (*growth differentiation factor 6 on Y chromosome*) in turquoise killifish (*Nothobranchius furzeri*) (Reichwald et al., 2015), and so on. Other members of the sexual regulatory genetic network also evolve to master sex-determining genes or candidate genes, such as *sox3y* (*sox3 on the Y chromosome*) in Indian ricefish (*Oryzias dancena*) (Takehana et al., 2014), *hsd17b1* (*17 $\beta$ -hydroxysteroid dehydrogenase 1*) in amberjack (*Seriola dumerili*) (Koyama et al., 2019), and *fshr* (*follicle-stimulating hormone receptor*) in flathead grey mullet (*Mugil cephalus*) (Ferrareso et al., 2021). However, some other genes, which are not derived from the members of the sexual regulatory network, can also evolve to master sex-determining switches, such as *sdY* (*sex-determining gene on the Y chromosome*) in rainbow trout (*Oncorhynchus*

*mykiss*) that is evolved from an immune-related gene *irf9* (*interferon regulatory factor 9*) (Yano et al., 2012), *pfpdz1* (*PDZ domain-containing gene*) in yellow catfish (*P. fulviventris*) that is a novel PDZ domain-containing gene (Dan et al., 2018), and *bcar1* (*breast cancer anti-resistance 1*) in channel catfish (*Ictalurus punctatus*) that can increase c-Src kinase activity and modulates estrogen in breast cancer cells (Bao et al., 2019) (Table 1).

In contrast with the diversity of master sex-determining signals, the downstream genetic cascades of sex differentiation and the basic pattern of gonad development are relatively conserved (Matson and Zarkower, 2012; Morrish and Sinclair, 2002). Despite differences in the sex determination systems and sex-determining genes, the male pathway usually contains genes such as *dmrt1*, *amh*, and *gsdf*, while the female pathway contains genes such as *foxl2* (*forkhead box L2*), *cyp19a1a* (*cytochrome P450, family 19, subfamily A, polypeptide 1a*) (Capel, 2017; Dai et al., 2021a; Wu et al., 2020; Yang et al., 2017) (Figure 2).

In domesticated zebrafish without sex chromosomes, the complex polygenic nature of sex determination has been verified by recent knockout studies. Loss of *dmrt1* results in a female-biased sex ratio and severe testicular developmental defects, which indicates that *dmrt1* is necessary for male differentiation (Lin et al., 2017b; Webster et al., 2017). Knockout of *amh* leads to a female-biased sex ratio and gonadal hypertrophy in both sexes (Lin et al., 2017b; Zhang et al., 2020), and *amh* has been revealed to control gonadal homeostasis and gametogenesis through Bmpr2a (Zhang et al., 2020). *Cyp19a1a* mutant zebrafish display an all-male phenotype, whereas disruption of *dmrt1* rescues the all-male phenotype of *cyp19a1a* deficiency (Lau et al., 2016; Wu et al., 2020). In addition, *foxl2a* and *foxl2b* cooperate to regulate ovary development and maintenance in zebrafish (Yang et al., 2017) (Figure 2A).

In the commercial fish Nile tilapia (*O. niloticus*) with the XX/XY sex determination system, *amhy* is identified as a candidate sex-determining gene, and *amhy/amhr II* signal is suggested to be essential for male determination (Li et al., 2015). Gain- and loss-of-function analyses suggest that *gsdf* plays an important role in male differentiation in tilapia (Jiang et al., 2016; Kaneko et al., 2015). The expression of *gsdf* is also activated by the male differentiation gene *dmrt1* in the presence of *sfl* (*splicing factor 1*) (Jiang et al., 2016). And *dmrt1* also directly regulates *sox9b* (*SRY-box transcription factor 9b*) by binding to a *cis*-regulatory element in the *sox9b* promoter (Wei et al., 2019). In addition, both mutant lines of *foxl2* and *cyp19a1a* display female-to-male sex reversal (Zhang et al., 2017c) (Figure 2A).

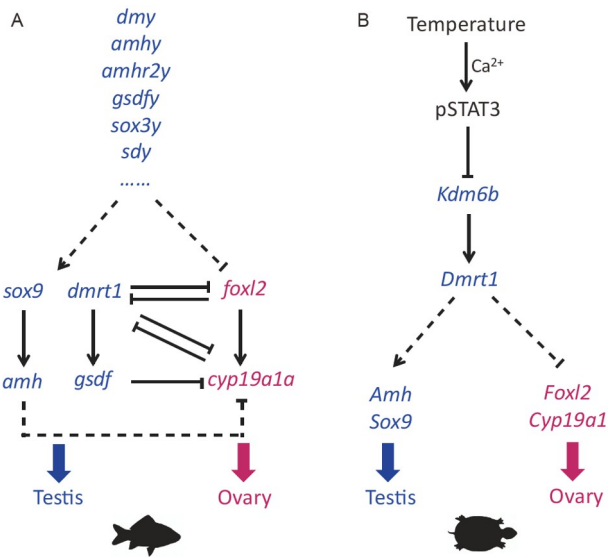
In hexaploid gibel carp (*C. gibelio*) with the ability of gynogenesis, three divergent *foxl2* homeologs (*foxl2a-B*, *foxl2b-A*, and *foxl2b-B*) are identified, and each of them contains three highly conserved alleles. Disruption of *foxl2a-*

*B* results in the arrest of ovary development or sex reversal, while deficiency of *foxl2b-A* and *foxl2b-B* leads to the depletion of germ cells (Gan et al., 2021). Recently, two *gsdf* homeologs *gsdf-A* and *gsdf-B* have been revealed to synergistically induce male differentiation by inhibiting *cyp19a1a* transcription. Dysfunction of *gsdf-A* or/and *gsdf-B* result in partial/complete male-to-female sex reversal, which could be rescued by an aromatase inhibitor. *In vitro*, the presence of Gsdf-A and Gsdf-B reduces Nco5 (nuclear receptor coactivator 5) interaction with Rora (retinoic acid-related orphan receptor- $\alpha$ ), leading to downregulation of Rora/Nco5-induced activation of *cyp19a1a* (unpublished data) (Figure 2A).

In the Chinese soft-shelled turtle (*Pelodiscus sinensis*), a species known as GSD with ZZ/ZW sex determination system, *Dmrt1* exhibits early male-specific embryonic expression, preceding the onset of gonadal sex differentiation. Importantly, the *Dmrt1* protein is expressed in the nuclei of precursor Sertoli cells. *Dmrt1* knockdown in ZZ embryos by RNAi results in male-to-female sex reversal with significant downregulation of testicular markers *Amh* and *Sox9*, and remarkable upregulation of ovarian regulators *Cyp19a1* and *Foxl2*. Conversely, ectopic expression of *Dmrt1* lead to largely masculinized genotypic females with production of *Amh* and *Sox9*, and a decline expression of *Cyp19a1* and *Foxl2*. These findings suggest that *Dmrt1* exerts a key upstream regulator role in primary male sexual differentiation of *P. sinensis*. However, it is not certain whether a Z-linked gene exists upstream of *Dmrt1*, acting as the male sex-determining gene. It cannot be ruled out that a W-linked gene functions as the female sex-determining gene in this species (Sun et al., 2017).

In the African clawed frog (*X. laevis*), *dm-w* is expressed transiently in the primordial gonads. Transgene of *dm-w* expression vector driven by its promoter and cytomegalovirus promoter both trigger the formation of ovarian cavities and primary oocytes in some gonads of ZZ tadpoles, which suggests that *dm-w* is the candidate sex-determining gene in African clawed frog (Yoshimoto et al., 2008). In females with ZW chromosomes, *dm-w* induces ovarian differentiation by antagonizing *dmrt1*, while in males with ZZ chromosomes, *dmrt1* activates the testicle developmental pathway (Yoshimoto et al., 2010; Yoshimoto and Ito, 2011).

It has long been believed that the sexual fate of germ cells is determined by the factors derived from somatic cells, as many sex-determining genes are expressed in somatic cell specifically, such as *Sry* in mammals (Koopman et al., 1991; Sinclair et al., 1990), *dmy* in medaka (Matsuda et al., 2002; Nanda et al., 2002), and *amhy* in Nile tilapia (Li et al., 2015). However, this thought is challenged by recent studies about *foxl3* (*forkhead-box protein L3*) in medaka (*O. latipes*). *Foxl3* is expressed in germ cells specifically and dysfunction of *foxl3* leads to spermatogenesis in a female gonadal



**Figure 2** Gene network of sexual fate in GSD fish (A) and TSD turtle (B). Genes in feminizing and masculinizing pathway are indicated in red and blue color, respectively.

environment in medaka, indicating that *foxl3* is a switch gene involved in the sexual fate of germ cells (Nishimura and Tanaka, 2016). And *foxl3* initiates oogenesis via two independent molecular pathways involving *fbxo47* (*F-box protein 47*) and *rec8a* (*REC8 meiotic recombination protein a*) respectively in medaka (Kikuchi et al., 2020). Recently, antagonistic interaction of *dmrt1* and *foxl3* has been proposed to determine the sexual fate of germline in tilapia (Dai et al., 2021a). In male tilapia with XY genotype, *dmrt1* expression in somatic cells inhibits female differentiation genes *foxl2* and *cyp19a1a*, leading to Sertoli cell development. Meanwhile, *dmrt1* expression in germ cells represses *foxl3* to ensure spermatogenesis. In females with XX genotype, upregulation of *foxl2* activates *cyp19a1a* to produce estrogen, resulting in downregulation of *dmrt1* in somatic cells and upregulation of *foxl3* in germ cells to ensure oogenesis (Dai et al., 2021a).

### Molecular mechanisms of TSD

In turtles with TSD, *Dmrt1* is expressed early in the thermosensitive period at MPT with sexually dimorphic expression, suggesting that *Dmrt1* plays an important role in determining the fate of the bipotential gonad in TSD turtles (Czerwinski et al., 2016; Kettlewell et al., 2000; Murdock and Wibbels, 2006). In red-eared slider turtle (*T. scripta*), knockdown and overexpression analyses provided strong evidence that *Dmrt1* is both necessary and sufficient for testicular differentiation. Upregulation of both *Amh* and *Sox9* by *Dmrt1* overexpression, together with *Dmrt1* expression preceding the expression of *Amh* and *Sox9* at MPT, implies that *Dmrt1* lies upstream of *Amh* and *Sox9* (Ge et al., 2017).

It has generally been shown that *Sox9* and *Amh* are highly expressed in reptile gonads at MPT. In *T. scripta*, *Sox9* expression is restricted to medullary cord cells. At the MPT, *Sox9* is highly expressed during bipotential stages. At stages 21–26, the pre-Sertoli cells lining the inside of the testis cords maintain strong *Sox9* expression (Barske and Capel, 2010). Interestingly, treatment with estradiol at MPT caused premature suppression of *Sox9* with the dissolution of cord structures in the medulla, while aromatase inhibition at FPT maintained the *Sox9* expression and testis cords, and resulted in ovotestis development (Barske and Capel, 2010). It is not clear from previous studies whether *Sox9* is expressed before *Amh* or vice versa among reptilian species. In Chinese pond turtle (*Mauremys reevesii*) (Tang et al., 2017), as in chicken, *Amh* expression precedes *Sox9* expression. In *T. scripta*, differential expression of *Amh* occurs primarily by upregulation at MPT, whereas *Sox9* obtains MPT enrichment by downregulation at FPT (Czerwinski et al., 2016).

In several reptile species with TSD, regardless of the TSD pattern, eggs incubated at MPT and treated exogenously with estradiol produce all females, whereas administration of an aromatase inhibitor to eggs incubated at FPT produces all males (Guillette and Iguchi, 2003; Lance, 2009; Pieau and Dorizzi, 2004). *Cyp19a1* expression level is higher in embryonic gonads of reptiles at FPT and is almost undetectable at MPT. These results are consistent with a critical role of aromatase and subsequent estrogen production in ovarian differentiation in TSD reptiles. Interestingly, *Foxl2* expression starts to increase just before the initial increase in *Cyp19a1* expression in *M. reevesii* gonads at FPT (Tang et al., 2017), suggesting that the regulation of *Cyp19a1* expression may be through that of *Foxl2*. In *T. scripta*, *Rspo1* (*R-spondin 1*) expression is higher in embryos incubated at the FPT, and the expression decreases in embryos shifted from the FPT to MPT. In both *T. scripta* and *M. reevesii*, *Rspo1* is more highly expressed in gonads incubated at FPT, during the middle of the temperature-sensitive period and before the onset of gonadal sex differentiation. *Rspo1* may therefore lie upstream in the genetic cascade leading to female development in species with TSD (Smith et al., 2008; Tang et al., 2017).

Mounting evidence supports that epigenetic factors regulate the processes of sex determination and gonadogenesis in ESD species (Garcia-Moreno et al., 2018; Piferrer, 2013). The critical role of epigenetic modification (DNA hypomethylation and H3K4me3 modification) in the regulation of *Cyp19a1* has been suggested to be a primary mechanism that releases a transcriptional block of *Cyp19a1* to initiate a cascade of ovarian differentiation in red-eared slider turtle (Matsumoto et al., 2013; Matsumoto et al., 2016). However, DNA methylation profiles of the *Cyp19a1* promoter in chemically (polychlorinated biphenyls) treated gonads of red-eared slider turtles did not support the typical methyla-

tion pattern observed in embryos incubated at FPT. Rather, the MPT typical methylation profiles were retained despite the induced ovarian formation. Importantly, these studies suggest that exogenous chemicals cannot singularly reverse epigenetic marks in differentiating gonads despite their well-established effects on transcriptional and phenotypic changes (Matsumoto et al., 2014).

Recently, Ge et al. showed that the DNA methylation status of the *Dmrt1* promoter in gonads of *T. scripta* displayed significant temperature-dependent dimorphism and responded rapidly to temperature shifts, suggesting that DNA methylation of the *Dmrt1* promoter might act as a crucial mediator in the regulation of *Dmrt1* by temperature (Ge et al., 2017). This suggestion has been further demonstrated by the evidence that the epigenetic regulator *Kdm6b* (*Lysine demethylase 6B*) plays a causal role in male sex determination by demethylating H3K37me3 in the promoter of *Dmrt1* (Ge et al., 2018). Knockdown of *Kdm6b* at MPT triggers male-to-female sex reversal, and KDM6B directly promotes the transcription of *Dmrt1* by eliminating the trimethylation of H3K27 near its promoter. Overexpression of *Dmrt1* is sufficient to rescue the sex reversal induced by disruption of *Kdm6b*. Further analysis by Weber et al. (2020) has shown that a temperature-sensitive  $\text{Ca}^{2+}$  influx promotes phosphorylation of STAT3 (signal transducer and activator of transcription 3). Phosphorylated STAT3 binds the *Kdm6b* locus and represses *Kdm6b* transcription, thus blocking the male pathway (testis development) (Figure 2B). These findings provide a direct genetic link between epigenetic mechanisms and TSD in turtle species. It will be of interest to determine how  $\text{Ca}^{2+}$  influx is regulated at different temperatures (Ge et al., 2018; Georges and Holleley, 2018).

### ***Molecular mechanisms of sex change***

Sexual phenotype is not a static feature resulting from embryonic sex determination, but is a dynamic trait influenced by genetic and/or environmental factors. In some fish, amphibians, echinoderms, decapods, and shellfish, individuals develop as one sex and then change sex sometime later as a usual part of their life cycle (Ortega-Recalde et al., 2020) (Figure 1). Three strategies of sex change are observed in sequential hermaphrodites, including protogyny, protandry, and serial bidirectional sex change (Avisé and Mank, 2009). An individual from a protogynous species starts reproductive life as female and later switches to a male, while an individual from a protandrous species begins reproductive life as male and later changes to a female. An individual with a serial bidirectional sex change can switch back and forth between functional female and male (Avisé and Mank, 2009).

Sequential hermaphroditism in vertebrates was first reported in rice field eel (*Monopterus albus*) by the famous

Chinese biologist Jian-Kang Liu, the pioneer of sex determination studies in 1944 (Liu, 1944), which has opened a fresh field for research on sex determination in lower vertebrates (Bullough, 1947; Zhou and Gui, 2016). The rice field eel starts its sexual life as an egg-producing female and then experiences a sex change to a sperm-producing male. Subsequently, approximately 2% of teleost species across more than 20 taxonomic families of 9 orders have been revealed to have sequential hermaphroditism (Avisé and Mank, 2009), and the sex change rice field eel has become a model fish for decoding genome recombination and sex reversal (Cheng et al., 2021; Cheng and Zhou, 2022). In Pacific oyster (*C. gigas*), bidirectional sex change is revealed as a characteristic feature based on sex observations of the same individuals in 130 oysters. Among these samples, 96 individuals exhibit female phenotype at the first sexual maturation and 20.8% change to males subsequently, while 34 individuals exhibit male phenotype at the first sexual maturation and 52.9% change to females subsequently (Yue et al., 2020).

Conserved sex differentiation genes (female pathway including *cyp19a1a*, *foxl2*, *rspo1*, etc. and male pathway including *dmrt1*, *amh*, *sox9*, etc.) are commonly involved in the process of sex change (Ortega-Recalde et al., 2020; Todd et al., 2019). For instance, *cyp19a1a* encodes aromatase that is responsible for the conversion of androgens to estrogens, which is fundamental for the maintenance of ovarian function. In protogynous rice field eel (*M. albus*) and red-spotted grouper (*Epinephelus akaara*), the expression of *cyp19a1a* is downregulated at the initiation of female-to-male sex change. Downregulation of *cyp19a1a* expression is thought to be the trigger of female-to-male gonadal sex change, as collapsing estrogen interrupts the expression of feminizing genes and the suppression of masculinizing genes. *Dmrt1* is essential for male development and testis differentiation (Smith et al., 2009), which may play a prominent role in male-to-female sex change in protandrous species, such as protandrous black porgy (*Acanthopagrus schlegelii*) (Wu et al., 2012) and the Red Sea clownfish (*Amphiprion bicinctus*) (Casas et al., 2016). In addition, some genes that are not involved in sex determination and differentiation are also revealed to be potential key components of sex change, such as *sox8* (*SRY-box transcription factor 8*) (Casas et al., 2016) and *cyp26b1* (*cytochrome P450, family 26, subfamily b, polypeptide 1*) (Liu et al., 2015).

Epigenetic modification is suggested to play a key role in the switching of sex pathways in sex-changing or environment-sensitive species. In the half-smooth tongue sole with both the ZW GSD system and temperature effects, substantial DNA methylation modifications are changed during female to male sex reversal via exposure to high temperatures. Interestingly, the methylation pattern in pseudomales (ZW males) is globally transmitted to the offspring, which

can naturally produce pseudomales without temperature treatment (Shao et al., 2014). In addition, DNA methylation and demethylation of the *cyp19a1a* promoter are involved in sex changes in many other temperature-sensitive or hermaphroditic fish species, such as European sea bass (*Dicentrarchus labrax*) (Navarro-Martín et al., 2011), Nile tilapia (*O. niloticus*) (Wang et al., 2017), and rice field eel (*M. albus*) (Zhang et al., 2013).

### Sex determination system transition

The extensive diversity of sex determination mechanisms in each clade indicates that transitions between different sex determination systems frequently occur in aquaculture animals (Capel, 2017; Ma and Veltsos, 2021; Pennell et al., 2018). ESD is thought to be favored when the environment differentially influences the fitness of females and males (Charnov and Bull, 1977; Warner and Shine, 2008). The evolution of GSD is favored in environments with low variability or unpredictable environments (Pennell et al., 2018). In fish, transitions from ESD to GSD occur at higher rates than transitions from GSD to ESD, which suggests that the GSD is more stable than ESD (Pennell et al., 2018). The transition between ESD and GSD and the underlying driving forces have been revealed in gibel carp (*C. gibelio*) (Li and Gui, 2018; Li et al., 2018) and yellow catfish (*P. fulvidraco*) (Xiong et al., 2020b).

Hexaploid gibel carp (*C. gibelio*) is able to reproduce via unisexual gynogenesis but contains rare proportions of males in wild populations, which are determined via GSD (genotypic male) or TSD (temperature-dependent male) (Li et al., 2018). When the maternal individual is mated with the temperature-dependent males or the males of host sexual species, typical gynogenesis is initiated in which all the offspring show the same genetic information as the maternal individual (Zhao et al., 2021; Zhu et al., 2018b), and the phenotypic sex of offspring is determined via ambient temperature during larval development (Li et al., 2018). When the maternal individual is mated with genotypic males, a variant of gynogenesis is triggered along with male occurrence and creation of genetic diversity in the offspring (Zhao et al., 2021; Zhu et al., 2018b), whose sex is probably driven by male-specific supernumerary microchromosomes (B chromosomes) (Ding et al., 2021; Li et al., 2016). These findings reveal that sex determination system transition is closely associated with the reproduction mode transition in gibel carp (Li and Gui, 2018). Moreover, a group of artificially synthesized octoploids (more than 200 chromosomes) contain the whole genome (more than 150 chromosomes) of gynogenetic hexaploid gibel carp (*C. gibelio*) ( $6n > 150$  chromosomes) and half of the genome (50 chromosomes) of sexual tetraploid crucian carp (*C. auratus*) ( $4n = 100$  chromosomes) (Lu et al., 2021). Intriguingly, the X and Y

chromosomes of sexual tetraploid crucian carp are transferred into the synthesized octoploids, and the sex ratio of this group of octoploids is 1:1 (Lu et al., 2021). Thus, the XY sex determination system of tetraploid crucian carp can be transferred into hexaploid gibel carp with TSD via artificial polyploidy.

In the yellow catfish (*P. fulvidraco*) with the XY sex determination system (Dan et al., 2013; Dan et al., 2018; Wang et al., 2009a), female to male sex reversal individuals (XX males) can be induced by an aromatase inhibitor (Xiong et al., 2020b). The offspring of XX maternal individuals mating with the XX paternal individual are all females with the XX genotype at normal temperature (26–28°C), but XX male offspring are induced at high rearing temperature (Xiong et al., 2020b). The ratio of XX male offspring increases with increasing larval rearing temperature (Xiong et al., 2020b). These findings indicate that sex reversal leads to the transition from GSD to TSD in yellow catfish (XX temperature-sensitive pattern), which is a similar result found in the Australian bearded dragon (*Pogona vitticeps*) (ZZ temperature-sensitive pattern) (Holleley et al., 2015).

Transitions of sex determination can also occur between different GSD systems (Li and Gui, 2018; Pennell et al., 2018). Close related species of tilapia (genus *Oreochromis*) have different sex determination systems, such as *O. niloticus* with the XX/XY system and *O. aureus* with the ZZ/ZW system. Cross-combination of *O. niloticus* (♀ XX) with *O. niloticus* (♂ YY), *O. aureus* (♀ ZW) with *O. niloticus* (♂ YY), *O. aureus* (♀ ZZ) with *O. niloticus* (♂ XX), and *O. aureus* (♀ ZZ) with *O. aureus* (♂ ZZ) generate all-male offspring (XY, ZY, WY, ZX, and ZZ), while cross-combination of *O. aureus* (♀ WW) with *O. niloticus* (♂ XX) generates all-female offspring. These various cross-combinations indicate that these two sex determination systems can coexist in tilapia and the dominant relationship of sex chromosomes is  $Y > W > Z > X$  (Chen et al., 2018). Moreover, both male heterogamety and female heterogamety sex determination systems have also been observed in the clawed frog (*Xenopus tropicalis*), which contains three kinds of males (ZY, WY, and ZZ) and two kinds of females (ZW and WW) (Roco et al., 2015).

### Sex chromosome evolution

Sex chromosomes were first discovered in 1905 by Nettie Stevens in mealworms, in which males contain one chromosome smaller than the other chromosomes, while all the chromosomes in females are of equal size (Stevens, 1905). Sex chromosomes have evolved independently numerous times and exhibit remarkable diversity among different species. Via chromosomal morphology, sex chromosomes can be divided into homomorphic and heteromorphic

chromosomes. Classic theory predicts that homomorphic proto sex chromosomes evolved from ordinary autosomes by acquiring sex-determining gene/genes. Sex-determining gene/genes can be derived from gene mutation (Kamiya et al., 2012; Koyama et al., 2019), gene duplication (Li et al., 2015), and gene translocation (Nakamoto et al., 2021). Subsequently, sexually antagonistic alleles (genes with different fitness in males and females) accumulate around sex-determining gene/genes and spread along the sex chromosomes. It is hypothesized that along with the sexually antagonistic alleles gathering on sex chromosomes, the evolution of reduced recombination is promoted between sex chromosomes. In addition, recombination suppression can also be caused by chromosome inversion, transposable elements, recombination modifiers, and epigenetic changes (Furman et al., 2020). Owing to lack of recombination, loss of gene function and degeneration usually occur on sex chromosomes (Y or W chromosomes), which lead to heteromorphic sex chromosomes (Charlesworth and Charlesworth, 2000).

### Origin of sex chromosomes

Commonly, sex chromosomes originate from autosome pairs. The mosquitofish *Gambusia affinis* and *G. holbrooki* are sister species, whose sex determination mechanisms have been identified to be ZZ/ZW and XX/XY systems. The ZW chromosomes of *G. affinis* and XY chromosomes of *G. holbrooki* correspond to different linkage groups, which indicates that these two sex determination systems of sister species might evolve from separate autosomes independently (Kottler et al., 2020). In zig-zag eel (*Mastacembelus armatus*) with the XX/XY system (Xue et al., 2020), the sex-linked region containing a candidate sex-determining gene *hmgn6* is in a pericentromeric region that has similar sequence compositions between the X and Y chromosomes. And the pericentromeric regions with low recombination can give rise to sex-linked region in the absence of structural variations (Xue et al., 2021).

In some available cryptodiran turtles (*Staurotypus triporcatus*, *Glyptemys insculpta*, *Siebenrockiella crassicollis*, *Apalone spinifera*, and *Pelodiscus sinensis*), the evolution of XY and ZW chromosomes has been revealed to be occurred by the co-option of different ancestral reptilian autosomes (Montiel et al., 2016). However, these turtle sex chromosomes may share a deeper homology with blocks of a more ancient proto sex chromosome (Montiel et al., 2016). The ZW of softshell turtles (e.g., *A. spinifera* and *P. sinensis*), which are derived from a single origin in the common ancestor of the softshell family *Trionychidae* (Badenhorst et al., 2013; Rovatsos et al., 2017), are homologous to each other, to the chromosome 15 of chicken *Gallus gallus*, and to the X chromosome of lizard *Anolis carolinensis* lizard, and share

partial homology with a second block of chromosome 1 of frog *Xenopus tropicalis* (Montiel et al., 2016).

Interestingly, the two turtle lineages *G. insculpta* and *S. crassicollis* are known to have recruited the same pair of ancestral autosomes independently (Montiel et al., 2016). Indeed, the XY chromosomes in these two species are both homologous to chromosome 5 of *G. gallus* and *X. tropicalis*, which contains the male development gene *Wtl* (*Wilm's tumor suppressor gene 1*) (Montiel et al., 2016; Montiel et al., 2017). The notion that these two XY systems represent convergent evolution and follow independent trajectories is also supported by a secondary homology shared between the short arm of XY chromosomes of *G. insculpta* (but not *S. crassicollis*) and chromosome 26 of *G. gallus*, which surprisingly is homologous to a block of *X. tropicalis* chromosome 1 (Montiel et al., 2016; Montiel et al., 2017). Thus, the origin of these independently derived sex chromosomes appears to be nonrandom. Like other reptiles, turtle sex chromosomes vary in the degree of heteromorphism, and some of them carry the genes of the nucleolar organizing region (Montiel et al., 2016).

In addition, there is increasing evidence that sex chromosomes can originate from supernumerary B chromosomes instead of autosomes. Supernumerary B chromosomes commonly originate from A chromosomes along with the accumulation of mitochondrial DNA and repetitive sequences (Hanlon et al., 2018; Serrano-Freitas et al., 2020). The Y chromosome in *Drosophila* species (Bernardo Carvalho et al., 2009; Hackstein et al., 1996) and the W chromosome in Lepidoptera (Fraïsse et al., 2017) are both suggested to be the evolved B chromosomes. And the giant sex chromosome in some cichlid fish species is hypothesized to be a fusion chromosome of an autosome and a B chromosome (Conte et al., 2021).

### Degeneration of sex chromosomes

Sex chromosomes are degenerated in many species, and the current theory of “selective interference (also known as the Hill-Robertson effect)” is commonly used to explain sex chromosome degeneration. Selective interference refers to the inefficacy of natural selection on genomic regions lacking recombination, which can result in the accumulation of deleterious mutations (background selection, Muller's ratchet, and genetic hitchhiking) and a lower rate of adaptation on Y/W-linked genes relative to homologous X/Z-linked genes (ruby in the rubbish) (Bachtrog, 2006; Bachtrog, 2013). Subsequently, dosage compensation has evolved to conquer the dosage differences of X-linked or Z-linked genes caused by the gene decay of the Y or W chromosomes. “Dosage compensation” was first discovered by Muller in *Drosophila* (Muller, 1950). Different organisms have dramatically diverse dosage compensation mechanisms

(Graves, 2016; Lucchesi, 1978). For examples, female mammals inactivate one of two X chromosomes (Carrel and Willard, 2005; Nguyen and Distèche, 2006), male *Drosophila* approximately increase X transcription twofold (Baker et al., 1994), and hermaphrodite *Caenorhabditis elegans* with XX genotype halves the expression from each X chromosome (Meyer, 2000). Unlike these global dosage compensations, *Gallus gallus* and *Taeniopygia guttata* have partial or gene-by-gene dosage compensation, in which expressions of many genes differ between sexes (Ellegren et al., 2007; Itoh et al., 2007; Mank, 2013).

In the background selection model, the Y/W chromosomes with strongly deleterious mutations will be eliminated from a large and non-recombining population, and only Y/W chromosomes free of strongly deleterious mutations will contribute to future generations. The effective population size of the Y/W will be sharply reduced without recombination, and the intensity of selection will be less efficient accordingly, which increases the rate of fixation of weakly deleterious mutations on Y/W chromosomes (Charlesworth, 1994). In Muller's ratchet model, the Y/W chromosomes with the fewest number of deleterious mutations will be lost stochastically in the absence of recombination and back mutation. These irreversible steps will lead to the fixation of deleterious mutations on the Y/W chromosomes (Charlesworth, 1978; Charlesworth and Charlesworth, 1997; Muller, 1964). In the genetic hitchhiking model, selection of strongly beneficial mutations can cause the fixation of any deleterious mutations closed linked, when the selection coefficients for beneficial mutants are larger than those for deleterious mutants (Rice, 1987). Although Y/W chromosomes reflect adaptation at some loci, deleterious mutations also accumulate in most other genes on Y/W chromosomes. In the model of ruby in the rubbish, beneficial mutations can be uncoupled from linked deleterious mutations via recombination on the X/Z chromosomes and fixed in the population. However, these beneficial mutations will be eliminated on Y/W chromosomes without recombination by purifying selection (Peck, 1994). X/Z-linked genes will continue to adapt and incorporate beneficial mutations, while Y/W-linked genes will fail to do so. Eventually, it can be advantageous to upregulate well-adapted X/Z-linked genes and silence or eliminate maladapted homologs on Y/W chromosomes (Orr and Kim, 1998). The first three models all assume that purifying selection against deleterious mutations is reduced on the Y/W chromosomes, while the last model assumes that positive selection for beneficial mutations on Y/W chromosomes is less efficient (Bachtrog, 2006).

Recently, a new "degeneration by regulatory evolution" (DRE) theory was proposed to explain sex chromosome degeneration. This theory does not require selective interference and is based on the instability of *cis*-regulatory sequences in non-recombining regions on sex chromosomes.

These *cis*-regulatory sequences are selectively haploidized to mask deleterious mutations on coding sequences, and this haploidization is asymmetric between X/Z and Y/W chromosomes (preventing no gene expression on the X/Z) (Lenormand et al., 2020). This process causes rapid Y/W degeneration and triggers the evolution of dosage compensation simultaneously (Charlesworth and Charlesworth, 2020; Lenormand et al., 2020).

Although differentiation and degeneration are suggested to be the default path of sex chromosome evolution, recent genomic analyses refute the claim that the Y-chromosomes will be extinct via a constant rate of gene loss (Aitken and Marshall Graves, 2002; Graves, 2006). The non-recombining Y/W chromosomes degenerate rapidly at the initial stage, but gene decay slows down over evolutionary time and ultimately halts on a gene-poor chromosome after reaching the threshold (Bachtrog, 2013). And the old Y/W chromosome can be a stable component of the genome under an equilibrium status with gene gain and loss (Bachtrog et al., 2014).

### **Sex chromosome turnover**

Along with the availability of genome sequences or detection of sex-linked sequences of many non-model species with differentiated and undifferentiated sex chromosomes (Palmer et al., 2019), two contrasting patterns of sex chromosome evolution have been highlighted, including sex chromosome conservation and sex chromosome turnover. In mammals and birds, the same pair of well-differentiated sex chromosomes have been maintained for over 100 million years (Graves, 2016; Zhou et al., 2014), while sex chromosome turnovers have occurred frequently in some fish (Kitano et al., 2009; Kitano and Peichel, 2012), amphibians (Jeffries et al., 2018), and reptiles (Gamble et al., 2015). Sex chromosome turnover commonly refers to the process that the ancestral sex chromosomes revert to autosomes and are replaced by a new set of sex chromosomes (Vicoso, 2019). In addition, there is an alternative paradigm of sex chromosome turnover in which a new master sex determiner replaces the ancestral sex-determining locus on the same chromosome (Meisel, 2020).

Sex chromosome turnover frequently occurred in ricefish within approximately 60 million years ago (Mya) (genus *Oryzias*). Linkage group 1 with sex-determining gene *dmy* is Y chromosome in *O. latipes* (Matsuda et al., 2002), while linkage group 12 with sex-determining gene *gsdfy* and linkage group with sex-determining gene *sox3y* are demonstrated to be Y chromosome in *O. luzonensis* (Myosho et al., 2012) and *O. dancena* (Takehana et al., 2014), respectively. Moreover, linkage groups 2, 5, 8, and 16 have also been revealed to be sex-linked chromosomes in *O. mekongensis*, *O. hubbsi*, *O. minutillus*, and *O. javanicus*, respectively (Gammerdinger and Kocher, 2018). The diversity of sex



chromosomes has also been detected in tilapia (genus *Oreochromis*). Sex determination markers are mapped to linkage group 1 or linkage group 23 in *O. niloticus* (Eshel et al., 2011; Lee et al., 2003), and the sex-determining gene *amhy* on linkage group 23 has been identified via loss of function (Li et al., 2015). The XY sex determination system of linkage group 1 is also found in *O. mossambicus* and *O. aureus* (Cnaani et al., 2004), while the ZW system on linkage group 3 has been demonstrated in *O. karongae*, *O. tanganyicae*, and *O. aureus* (Gammerdinger and Kocher, 2018).

In the true frogs (Ranidae), at least 13 turnover events have been detected among 28 species within approximate 55 Mya, which results in the homomorphy of sex chromosomes. These species display an extremely fast rate of sex chromosome turnover, however, transitions are not random and some chromosomes are significantly more likely to be recruited for sex determination than other chromosomes. Chromosomes 1, 3, and 5 have been recruited for sex chromosomes multiple times independently, especially chromosome 5, which has been recruited five times (Jeffries et al., 2018).

### Neo-sex chromosome

The Y or W chromosomes in most studied species especially regular mammals have originated over 100 million years, thus most of genes have lost and repetitive elements have accumulated in these sex chromosomes, which is hard to trace their evolutionary trajectory. However, neo-sex chromosomes in the newly formed sex determination systems, which commonly originates from sex chromosome-autosome fusions, provide an opportunity to conquer these obstacles. Neo-sex chromosomes have also been widely studied in some aquaculture and non-aquaculture animals, such as spotted knifejaw (*Oplegnathus punctatus*), Japanese three-spine stickleback (*Gasterosteus aculeatus*), black muntjac (*Muntiacus crinifrons*), and drosophilid flies (*Drosophila miranda*), which are ideal models to understand the processes of sex chromosome evolution and dosage compensation mechanism.

In the spotted knifejaw (*O. punctatus*) with  $X_1X_2Y$  sex determination system, the evolutionary history of neo-Y chromosome has been revealed via full assembly and annotation of all the three sex chromosomes. The ancestral X ( $X_1$ ) and Y chromosomes evolved from a pair of autosomes over 60 Mya, and recombination was further suppressed by an inversion approximately 55 Mya. Finally, the Y chromosome was fused to the  $X_2$  chromosome via Robertsonian translocation at 48 Mya, leading to neo-Y sex chromosome (Li et al., 2021a). Compared with the ancient time of neo-Y chromosome formation in spotted knifejaw, the neo-sex chromosome occurred much more recent in Japanese three-spine stickleback fish (*G. aculeatus*), which were estimated

to 1.5–2 Mya. The Japanese threespine stickleback fish contains a sympatric pair of the Pacific Ocean and the Japan Sea species, which have XY and  $X_1X_2Y$  sex chromosome systems, respectively. The chromosomal fusion between an autosome (LG9) and an ancestral Y chromosome (LG19) in the Pacific Ocean stickleback fish results in the neo-Y chromosome in the Japan Sea stickleback, in which the ancestral X chromosome (LG19) is  $X_1$  and the neo-X chromosome (LG9) is  $X_2$ . Intriguingly, the loci for male courtship display traits are distributed in the neo-X chromosome, contributing to reproductive isolation between the Japanese stickleback species pair (Kitano et al., 2009; Yoshida et al., 2014).

## Reproductive strategies

### Sexual reproduction

Sexual reproduction refers to organismal procreation via meiosis followed by nuclear fusion (Bachtrog et al., 2014; Capel, 2017; Lahr et al., 2011; Li and Gui, 2018; Spiegel, 2011). In sexual species, it takes time and energy to find a mate and some phenotypes/behaviors during courtship are dangerous for prey, and mating between females and males increases the risk of sexually transmitted diseases. The reproductive output of sexual species is half of that for unisexual lineages, as the unit of reproduction in sexual reproduction is the couple while it is the individual in unisexual reproduction. In addition, it is risky to randomly mix genes with those of another individual (Otto and Lenormand, 2002). Although there are many substantial costs, sex reproduction is ubiquitous in eukaryotic life, which is known as the paradox of sex (Otto and Lenormand, 2002; Speijer et al., 2015). The longstanding theory argues that sexual reproduction is beneficial, as sex can increase the rate of adaptation by separating deleterious mutations from advantageous backgrounds and combining beneficial mutations. That is, sex reproduction can make natural selection more efficient at sorting deleterious and beneficial mutations (McDonald et al., 2016).

### Unisexual reproduction

Unisexual reproduction generally refers to reproduction modes without meiosis and meiotic recombination, which are mainly divided into three modes: parthenogenesis, gynogenesis, and hybridogenesis (Avisé, 2015; Neaves and Baumann, 2011). In the parthenogenetic biotype, females produce unreduced eggs with the same genomic composition as somatic cells, and these unreduced eggs spontaneously develop into individuals without fertilization (Lutes et al., 2010). Consistent parthenogenesis is widespread in invertebrates and Squamata of vertebrates (Avisé, 2015; van

der Kooi and Schwander, 2015). In aquaculture animals, marbled crayfish (*Procambarus virginalis*) has been detected to reproduce via parthenogenesis (Scholtz et al., 2003). Genome anatomy reveals that the triploid marbled crayfish (AA'B) originates from an autopolyploid *P. fallax* gamete (AA') fertilizing with another gamete from a distant related *P. fallax* (Gutekunst et al., 2018). Triploidy, heterozygosity, and parthenogenesis provide a significant evolutionary advantage in a short term, which makes the marbled crayfish an emerging invasive taxon and has formed wild populations in diverse habitats (Gutekunst et al., 2018).

Gynogenesis refers to a unisexual reproduction mode and the gynogenetic lineages commonly consist of all-female individuals. Similar to parthenogenesis, females also produce unreduced eggs, but sperm is required to activate the eggs to initiate embryogenesis using only maternal chromosome complement (Gui and Zhou, 2010; Schlupp, 2005). The Amazon molly (*Poecilia formosa*), which is an interspecific hybrid derived from an Atlantic molly (*P. mexicana*) mating with a Sailfin molly (*P. latipinna*), is the first described vertebrate with unisexual gynogenesis. Hexaploid gibel carp (*C. gibelio*) with wide distributions throughout the Eurasian continent (Gao et al., 2012; Liu et al., 2017b; Liu et al., 2017c) is able to reproduce via gynogenesis (Gui and Zhou, 2010). Hexaploid gibel carp is an unique amphitriploid (AAABBB) (Gan et al., 2021; Mou et al., 2021) that originated from sympatric ancestral amphidiploid (*C. auratus*) (AABB) via autotriploidy approximately 0.5 million years ago (Li et al., 2014). Unlike other gynogenetic taxa, variable proportions of males have been observed in wild populations of gibel carp, and these males have been revealed to be determined via GSD and ESD (Li et al., 2018). In addition, the genotypic males can trigger the creation of genetic diversity in their offspring (Zhao et al., 2021), which contributes to environmental adaptations of gynogenetic gibel carp (Jiang et al., 2013).

Hybridogenesis is an unusual form of unisexual reproduction and is found in hybrids between different taxa. These hybridogenetic offspring contain both maternal and paternal haploid chromosomes, but only one of the parental genomes is selected for transmission to the next generation (Lavanchy and Schwander, 2019). The *Poeciliopsis* fish with all-female composition from northwestern Mexican streams were first revealed to reproduce via hybridogenesis (Miller and Schultz, 1959). All the females were discovered to be the result of ancient hybridization between the species *P. monacha* and *P. lucida*. During gametogenesis of these *P. nomacha-lucida* females, only the maternal *P. monacha* genome is transmitted to their eggs, while the entire *P. lucida* genome is eliminated in the process. These *P. nomacha-lucida* females are systematically mated with the sexual species *P. lucida* to reproduce; thus, the offspring can maintain the F1 hybrid configuration (Lavanchy and

Schwander, 2019; Moore et al., 1970). Moreover, hybridogenesis is widely studied in the frog *Pelophylax esculentus*, which is an extremely complicated genetic system. *P. esculentus* arose from hybridization between the sexual species *P. ridibundus* and *P. lessonae*. In sharp contrast with other unisexual taxa, some of these hybridogenetic frogs display a high incidence of males and even an all-male composition (Doležalková-Kaštánková et al., 2018). These hybridogenetic males can mate with females of host sexual species and transmit one genome via sperm (Avise, 2008).

### **Facultative reproduction**

There is a growing body of evidence that some taxa feature facultative reproduction modes. A famous example is the captive hammerhead shark (*Sphyrna tiburo*) with sexual reproduction mode. A hammerhead shark was caught in the Florida Keys as an immature individual less than 1-year-old, and gave birth to a female offspring 3 years later in the absence of males. Subsequently, this pup was demonstrated to be the offspring of automictic parthenogenesis via molecular examination (Chapman et al., 2007). Moreover, genetic evidence of facultative parthenogenesis was provided in other shark species including the blacktip (*Carcharhinus limbatus*) (Chapman et al., 2008) and white-spotted bamboo shark (*Chiloscyllium plagiosum*) (Feldheim et al., 2010). Polyploid gibel carp have been demonstrated to reproduce via multiple modes. When the maternal individual of gibel carp is mated with males from other sexual species or temperature-dependent males of gibel carp, typical gynogenesis is triggered that all the offspring are females with the same genetic information as the maternal individual. When the maternal individual of gibel carp is mated with genotypic males of gibel carp, a variant of gynogenesis or sexual reproduction is initiated along with male occurrence and creation of genetic diversity in the offspring (Zhao et al., 2021).

### **Evolutionary mechanisms of unisexual reproduction**

Unisexual reproduction without meiosis and meiotic recombination cannot purge deleterious mutations (Muller's ratchet) (Muller, 1964) and create genetic diversity (Red queen hypothesis) (Van Valen, 1973), which is considered an evolutionarily dead-end (Avise, 2015). However, some unisexual lineages have exhibited wide ecological distribution and outlived their predicted time of extinction, such as bdelloid rotifers (Mark Welch and Meselson, 2000), amoebae (Maciver, 2016), salamanders (Bi and Bogart, 2010; Bogart, 2019), Amazon molly (Loewe and Lamatsch, 2008; Warren et al., 2018), and gibel carp (Liu et al., 2017b; Liu et al., 2017c).

There are several ways to counter Muller's ratchet in

unisexual taxa. First, introgression of sexual host DNA has been reported in Amazon molly and gibel carp, which partially explains the substantial polymorphism (Lu et al., 2021; Warren et al., 2018; Yi et al., 2003; Zhu and Gui, 2007). Second, gene conversion, a form of homologous recombination, is used for the reversal of spontaneous mutations in some polyploids with unisexual reproduction, such as amoebae (Maciver, 2016). Third, facultative sexual reproduction allows recombination and provides fit offspring for selection (Burke and Bonduriansky, 2017). Fourth, a large population size of microorganisms allows the existence of few non-debilitated individuals (Maciver, 2016). Moreover, a special mechanism in gynogenetic gibel carp has also been revealed, in which genotypic males can trigger a variant of gynogenesis to cause the creation of genetic diversity (Zhao et al., 2021). Thus, studies on these unisexual taxa that have conquered Muller's ratchet will shed light on the evolution of unisexual reproduction.

## Sexual dimorphism

### *Diversity of sexual dimorphism*

Sexual dimorphism, a common phenomenon describing the systematic difference between male and female individuals in the same species, has been frequently observed in many cultured fishes and other aquatic species through empirical observations and extensive aquaculture practices. A wide range of sexually dimorphic traits have been detected in aquaculture animals, such as body size, color, shape, physiology, and behavior (Gui et al., 2021; Mei and Gui, 2015). Sexual size dimorphism is the most common form of sexual dimorphism, which leads to faster growth in males than in females or in females than in males. The former includes most cultured species of tilapias, catfishes, and snakehead fishes, and the latter comprises many species in cyprinids, salmonids, flounders, perches, halibuts, soles, etc. (Mei and Gui, 2015; Wang et al., 2018; Zhou and Gui, 2018). In several ornamental fishes, such as platyfish (*Xiphophorus maculatus*), swordtail (*Xiphophorus helleri*), guppy (*Poecilia reticulata*), Siamese fighting fish (*Betta splendens*), and bitterling fish (*Rhodeus ocellatus*), sexual color or shape dimorphism can increase ornamental value more in males than in females because of better body shape and color pattern, and males with dominant color or shape tend to attract females (Casalini et al., 2009; Oliveira et al., 2021; Rosenthal and Evans, 1998; Turan et al., 2006).

### *Genetic basis of sexual dimorphism*

The genetic basis of sexual dimorphisms is an intriguing puzzle in evolutionary genetics. As a result of natural selection and sexual selection during the evolutionary process

(Berns, 2013; Bonduriansky and Chenoweth, 2009; Laporte et al., 2018), sexual dimorphism is the consequence of sex-biased gene expression not only on the sex chromosomes but also on the autosomes (Dean and Mank, 2014; Grath and Parsch, 2016; Mank, 2017; Parsch and Ellegren, 2013; Snell and Turner, 2018; Williams and Carroll, 2009). A great range of sex dimorphisms have been studied in vertebrates, such as metabolism (Hedrington and Davis, 2015; Palmer and Clegg, 2015), immunity (Caballero-Huertas et al., 2020; Rubtsova et al., 2015), and social behaviors (Bayless and Shah, 2016).

In fish species, the genetic mechanisms of sexual size dimorphism have been extensively studied, which is generally caused by different rates of somatic growth and nutritional intake. Growth hormone/insulin-like growth factor (GH/IGF) axis genes, key endocrine modulators of growth and metabolism (Dai et al., 2015; Li and Lin, 2010), show higher expression in fast-growing male yellow catfish and tilapia than in slow-growing females (Ma et al., 2016; Riley et al., 2002), but lower expression in slow-growing male European eels (*Anguilla anguilla*) than in fast-growing females (Degani et al., 2003), and their expressions are regulated by sex hormones. Appetite, nutritional intake, and energy balance are usually regulated by peripheral hormonal signals such as Ghrelin, Leptin, and Melanocortin receptors (MCRs) (Dai et al., 2015). In yellow catfish, sex-biased expression of *ghrelin* and its receptor *ghsr* is associated with sexual size dimorphism (Zhang et al., 2016a), while miR-200a/-200b targets *leptin* to regulate male-biased sexual size dimorphism (Zhang et al., 2017b). *Pomc* (*pro-opiomelanocortin*) contributes to the sexual size dimorphism in tilapia (Wan et al., 2021). The expression of *mc4r* (*melanocortin 4 receptor*), a signaling system regulating appetite and energy, determines the body size and mating behavior of male swordtails (Lampert et al., 2010).

*Stat5b* (signal transducer and activator of transcription 5b), a key downstream mediator of GH signaling for somatic growth, has been shown to regulate sexual size dimorphism in mice (Udy et al., 1997) and zebrafish (Xiong et al., 2017). The body weight of female zebrafish is heavier than that of males at adult stages, while loss of *stat5b* function not only reduces the sexual size dimorphism, but also attenuates the sex-biased gene expression in the liver (Huang et al., 2018; Xiong et al., 2017). The zebrafish microRNA-200 cluster on chromosome 23 (chr23-miR-200s) is predicted to target *stat5b*, and the females with deletion of chr23-miR-200s show significantly larger body sizes than wild type females at adult stages, whereas it has no obvious effect on the body size of male zebrafish (Xiong et al., 2018; Xiong et al., 2020a). Loss of *dmrt1* function promotes the body growth of male zebrafish, but does not affect the growth of female zebrafish (Lin et al., 2017b). The complex mechanism of sexual size dimorphism needs to be further studied.

In addition, the genetic mechanisms of sexual dimorphism,

including color, body shape, physiology, and behavior have also been investigated. For example, the sexual color dimorphism in Lake Malawi cichlid fishes is controlled by *pax7* (*paired box 7*) gene (Roberts et al., 2009), and the neo-X chromosome contributes to the physiology and reproductive behavior of male sticklebacks (Kitano et al., 2009). Natural allelic variations of Cyp11b1 (cytochrome P450, family 1, subfamily B, polypeptide 1) are associated with geographical differences in sexual dimorphism in the anal fin morphology of medaka (Katsumura et al., 2014). Neuropeptide B is specifically expressed in the brain of female medaka and mediates female sexual receptivity (Hiraki-Kajiyama et al., 2019).

### ***Aquaculture species with significant sexual dimorphisms***

The traits of sexual dimorphism are important for aquaculture. The application of sexual dimorphism and the development of monosex aquaculture species could improve aquaculture production or value (Mei and Gui, 2015). Especially in sturgeons and pufferfishes, the gonads or gametes themselves have direct commercial value. Sturgeons are famous among fishes, as female pre-ovulation eggs can be made into the world known caviar, so that the all-female populations of caviar-producing sturgeons have been expected to enhance the industry viability (Du et al., 2020; Keyvanshokoo and Gharaei, 2010). Pufferfishes are celebrities because their testes possess delicate taste, and therefore, all-male population production has been attempted to elevate the aquaculture benefits (Gao et al., 2020; Kamiya et al., 2012; Matsunaga et al., 2014). Moreover, flesh quality can be improved by culturing all-female populations in common carp and some salmons, because it may be affected by the endocrine changes along with their maturation, and as a result, farmers are more interested in developing all-female production because their females mature later than males (Baroiller and D’Cotta, 2016; Mei and Gui, 2015). Altogether, the sex-biased benefits produced by monosex (all-male or all-female) populations have strongly stimulated research interests. Therefore, the first key step for sex control breeding is to determine whether the target species exhibits significant sexual dimorphism, in which the larger of the difference is, the greater the benefits. Table 1 summarizes the known aquaculture species with significant sexual dimorphism, including 42, 1, 1, and 7 species in fish, testudines, echinodermata, and decapods, respectively.

### **Approaches of sex control breeding**

Most vertebrates are believed to have lost sexual plasticity after the terminal differentiation of their gonads and remain the same sex throughout their life spans. In fish and other

aquaculture animals, sexual phenotype plasticity is ubiquitous. As mentioned above, the sexual fate of an individual may be determined by sex chromosomes and sex-determining genes or temperature and other environmental cues, or by a combination of the two, but how the genetic signals and the environmental cues interact to provoke sexual phenotype reversal remains unclear (Li and Gui, 2018). Significantly, sex reversibility may be used to understand sex determination and differentiation mechanisms, and also to perform sex control for aquaculture directly or indirectly (Baroiller and D’Cotta, 2016; Ortega-Recalde et al., 2020). A number of aquatic animals display diverse traits of sexual dimorphisms, such as body size, color, and shape. Manipulation of sexual dimorphism and production of mono-sex populations have great effects on the growth rate, feed conversion efficiency, and product quality of aquatic animals. In this section, we summarize the major approaches for controlling sex in aquatic animals, such as artificial gynogenesis, application of sex-specific or sex chromosome-linked markers, artificial sex reversal, and gene editing.

### ***Artificial gynogenesis***

Gynogenesis and androgenesis are the established techniques to obtain specimens from female and male gametes (Purdom, 1969). Artificial gynogenesis techniques have been developed and applied in many aquatic animals to obtain mono-sex populations, such as fishes, shrimp, and mollusks (Manan et al., 2022). Gynogenesis is a natural mode of reproduction in some fishes, such as gibel carp, in which the all-female population can be massively produced by gynogenesis using the sperm of other species (Gui and Zhou, 2010; Zhou and Gui, 2017). However, in other aquatic animals without natural gynogenesis, the production of all-females at commercial scales for aquaculture is still a challenge, because massive embryonic and larval mortality are caused by gynogenesis induction with cold shock, heat shock, and hydrostatic pressure (Luo et al., 2011).

### ***Identification and application of sex-specific or sex chromosome-linked markers***

Sex-specific or sex chromosome-linked genetic markers have been extensively exploited and developed in most aquaculture species with significant differences between males and females, as the genetic markers enable the determination of genetic sex at early developmental stages when phenotypic differences are not identifiable, which are able to shorten the breeding time and backcross testing time, particularly in aquaculture species with relatively long generation intervals (Mei and Gui, 2015; Ventura et al., 2011; Wang et al., 2009b; Zhou and Gui, 2018). For example, the age of first maturation normally takes 4–5 years for bighead

carp and approximately 4 years for silver carp in central and northern China, and the inability to determine sex before sex maturation has brought many troubles for broodstock management in breeding programs (Zhou et al., 2020). In addition, backcross testing of YY super-males or other important neomales requires killing the males to obtain sperm in some catfish. Therefore, the identification of sex-specific or sex chromosome-linked genetic markers is a crucial key for sex control breeding.

Along with rapid advances of molecular genetics and genomic sequencing, numerous sex-specific or sex chromosome-linked DNA markers have been successfully screened and identified from a wide range of more than 100 aquatic species including fish, testudines, amphibia, echinodermata, decapod, and shellfish (Table 1) by different genetic and genomic methods, such as restriction fragment length polymorphism (RFLP) (Devlin et al., 1991), random amplified polymorphic DNA (RAPD) (Chen et al., 2009), suppression subtractive hybridization (SSH) (Chen et al., 2010), microsatellite or simple sequence repeats (SSR) (Sakamoto et al., 2000), amplified fragment length polymorphism (AFLP) (Dan et al., 2013; Ezaz et al., 2004; Griffiths et al., 2000; Ma et al., 2010; Olmstead et al., 2011; Pan et al., 2015; Wang et al., 2009a; Wang et al., 2009b), fine and linkage mapping of sex quantitative trait locus (QTL) or sex determination (SD) locus (Kamiya et al., 2012; Liu et al., 2020; Sun et al., 2014; Viñas et al., 2012; Yu et al., 2017; Zhang et al., 2019b), putative sex-linked and sex-related gene sequence comparison or next-generation sequencing (NGS)-based and high-resolution melting (HRM) typing system (Gao et al., 2020; Ou et al., 2017; Perez-Enriquez et al., 2020; Yang et al., 2020), genome sequencing and re-sequencing (Han et al., 2020; Lin et al., 2017a; Lin et al., 2018; Liu et al., 2018; Xiao et al., 2020; Zhang et al., 2017a), comparative transcriptome and RNA sequencing (RNA-seq) (Lamatsch et al., 2015; Sun et al., 2018), restriction-site associated DNA sequencing (RAD-seq) (Fang et al., 2020; Lange et al., 2020; Liang et al., 2019; Palaio-kostas et al., 2013a; Palaio-kostas et al., 2013b), double digestion restriction-site associated DNA sequencing (ddRAD-seq) (Brown et al., 2016; Fowler and Buonaccorsi, 2016; Palaio-kostas et al., 2015), and type IIB restriction endonuclease-produced sequencing (2b-RAD-seq) (Wang et al., 2012).

To overcome analytical barriers and limitations preventing precise identification of sex-specific genomic sequences, Feron et al. (2021) recently developed a RAD-Seq computational analysis workflow, RADSex, to identify sex-specific genomic sequences and to study the genetic basis of sex determination. Using the RADSex workflow, they identified sex-specific markers and XY sex determination system from six species of ray-finned fish, such as common carp (*C. carpio*), banded knifefish (*Gymnotus carapo*), ayu (*Ple-*

*coglossus altivelis*), tench (*Tinca tinca*), Atlantic cod (*Gadus morhua*), and common molly (*Poecilia sphenops*), and only one (common carp) of them had known sex-markers prior to the study.

The sex-specific DNA markers developed in various fish species were mainly based on insertion/deletion and single nucleotide polymorphism (SNP) mutations. With insertion/deletion mutations (indels), dominant and co-dominant DNA markers were developed and applied to determine the genetic sex of aquatic species by using PCR and agarose gel electrophoresis (Xiao et al., 2020; Zhang et al., 2017a; Zheng et al., 2020). Sex-linked SNP markers can be detected by allele-specific PCR assays. In recent years, some high-throughput sex identification methods based on sex-specific SNP markers have been developed by using an amplification refractory mutation system (Gao et al., 2020; Zhang et al., 2019a). Overall, universal approaches of molecular sexing could easily identify the genetic sex of the offspring of gynogenesis/androgenesis and hormonal sex reversal at early developmental stages, with no need to wait for the sexually mature stage (Kovács et al., 2000).

### Artificial sex reversal

Sex reversal has been carried out extensively in fish. Half a century ago, Yamamoto (1969) completed pioneering work in ornamental species such as medaka, in which he produced viable and fertile YY males through estrogen feminization of XY individuals and mating feminized XY individuals with normal XY males (Yamamoto, 1969). Since then, the direct technique of endocrine sex control, involving masculinization or feminization by androgen or estrogen treatment during early fry stage with undifferentiated gonads, has been studied on a large range of cultured species (Donaldson, 1996; Piferrer, 2001). Actually, an efficient sex reversal procedure should include three main steps: (i) characterizing the labile period in which the gonad is most susceptible to endocrine induction, (ii) selecting the optimal natural or synthetic androgen or estrogen, and (iii) deciding the minimal effective dosage. In tilapia, all-male populations have already been produced by androgen masculinization and applied to aquaculture in the last century (Guerrero, 1975), but direct endocrine techniques have been prohibited because of potential hormone residues and environmental hazards.

Following a better understanding of sex determination mechanisms and extensive identification of sex-linked genetic markers, YY “super-males”, sex-reversed XX neomales, WW “super-females”, sex-reversed ZW neomales, and sex-reversed ZZ neofemales have been proposed and developed either through the exogenous hormone administration procedure coupled with progeny backcross testing or through other approaches including androgenesis, gynogen-

esis of sex-reversed females, or sex chromosome-linked DNA markers-directed technique in some important aquaculture fish species (Gui, 2007; Gui and Zhu, 2012; Xiong et al., 2020b). Especially in Nile tilapia and yellow catfish, the YY “super-males” have been generated and successfully used to mate with normal XX females to produce large scales of all-male populations for commercial aquaculture (Baroiller and D’Cotta, 2016; Liu et al., 2013b; Mei and Gui, 2015; Zhou and Gui, 2018). Similarly, in common carp and rainbow trout, mass production of all-female monosex populations have been obtained by crossing sex-reversed XX neomales and normal XX females (Cnaani and Levavi-Sivan, 2009; Donaldson, 1996; Piferrer, 2001; Wu et al., 1990). In a well-known aquaculture species giant freshwater prawn (*Macrobrachium rosenbergii*) with ZW sex determination system, all-male ZZ populations have also been produced through mating sex-reversed ZZ neofemales with normal ZZ males, and significant economic benefits have resulted from the all-male monosex culture (Aflalo et al., 2006; Ventura et al., 2011).

Even though monosex population production has succeeded in several important fish and prawns by utilizing their sexual plasticity and reversibility (Li et al., 2019; Mei and Gui, 2015), the key YY “super-male” individuals or WW “super-female” individuals for producing all-males or all-females have been reported to have only low survival rates or even to be inviable in some fish species (Baroiller and D’Cotta, 2016). Especially in the species with late sexual maturity, the reversal process and backcross testing generally require 5 years or more time, such that monosex population production of many species has not yet been developed to a commercial level despite numerous attempts.

### Gene editing

The sex of aquaculture animals is usually determined by sex-determining related genes, loss-of or gain-of which functions lead to sex reversal. Sex reversed XX neomales, XY neofemales, ZW neomales, and ZZ neofemales could be produced by knockout or ectopic expression of male or female sex-determining related genes. Neomale common carps have been created by knockout of the *cyp17a1* (cytochrome P450, family 17, subfamily A, polypeptide 1) gene with CRISPR/Cas9 technology, and the all-female population is produced by crossing XX neomale and XX female carps (Zhai et al., 2022). XY neofemales have been successively created in a number of aquatic animals by knockout of the sex-determining genes. The primordial germ cells could be targeted by Cre/loxP and Gal4/UAS systems in zebrafish, and all-male zebrafish have been produced by ablation of the PGCs, which can also be applied in other aquaculture animals (Xiong et al., 2013).

### Perspectives

Aquaculture animals display extremely diverse sexual phenotypes and underlying mechanisms, providing ideal models for sex-related fundamental research. Recently, the rapid developments of sequencing techniques and wide applications of gene editing technology in aquaculture animals have triggered burgeoning illustration of sex determination mechanisms. However, the sex differences in the genomics of aquaculture animals have been ignored in the genetic analysis of complex traits, thus it is urging to construct precise sex-control technology system by digging out the key genes and regulatory elements of excellent breeding traits in both the female and male populations, and developing sex-specific excellent trait-associated markers. After deeply investigating the regulation and transition mechanism between GSD and ESD, environmentally friendly methods of sex reversal and directional sex reassignment technology will be established and developed in aquaculture animals. The innovation and application of sex control approaches have facilitated yield increases and quality improvements in aquaculture. Aquaculture animals with high nutrition and low carbon footprint, also known as “blue foods” (Gephart et al., 2021; Golden et al., 2021), are playing an increasingly important role in global food security (Gui et al., 2022; Naylor et al., 2021). Thus, further exploitation of sex determination mechanisms and sex control approaches in aquaculture animals will still be the main driving force for sustainable aquaculture and global food supply (Gui and Hughes, 2022).

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