Review

The Cause-Effect Model of Master Sex Determination Gene Acquisition and Evolution of Sex Chromosomes

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Article

The Cause-Effect Model of Master Sex Determination Gene Acquisition and Evolution of Sex Chromosomes

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Abstract: The canonical model of vertebrate sex chromosome evolution predicts one way of trend toward degradation. However, most sex chromosomes in lower vertebrates are homomorphic. Recent progress in studies of sex determination resulted in the discovery of more than 30 master sex determination (MSD) genes, most of which were from teleost fish. Analysis of MSD gene acquisition, recombination suppression, and sex chromosome-specific sequences revealed correlation of the modes of MSD gene acquisition and evolution of sex chromosomes: Sex chromosomes remain homomorphic with MSD genes acquired by simple mutations, gene duplications, allelic variations or neofunctionalization; in contrast, they become heteromorphic with MSD genes acquired by chromosomal inversions, fusions and fissions. There is no recombination suppression with sex chromosomes carrying MSD genes gained through simple mutations. In contrast, there is extensive recombination suppression with sex chromosomes carrying MSD genes gained through chromosome inversion. There is limited recombination suppression with sex chromosomes carrying MSD genes gained through transpositions or translocations. We proposed the cause-effect model that predicts sex chromosomes evolution being consequential of the acquisition modes of MSD genes, which explains evolution of sex chromosomes in various vertebrates. A key factor determining the trend of sex chromosome evolution is if non-homologous regions are created during the acquisition of MSD genes. Chromosome inversion creates inversely homologous but directly non-homologous sequences which lead to recombination suppression but remain recombination potential. Over time, recurrent recombination in the inverted regions causes degradation of sex chromosomes. Depending on the nature of deletions in the inverted regions, sex chromosomes may evolve with dosage compensation or mechanisms to retain haploinsufficient genes.

Keywords: sex determination; sex differentiation; master sex determination gene; recombination suppression; chromosome inversion; sex chromosome evolution

1. Introduction

The mechanism of sex determination in vertebrates is enormously diverse, especially with teleost fish [1,2], ranging from unisexuals, hermaphroditism, environmental sex determination, to genetic sex-determination [3]. With genetic sex determination, 30 distinct MSD genes have been identified from vertebrates under various sex determination (SD) systems, XY, ZW, and multiple sex chromosomes [1,4]. Despite such diversities, the molecular pathways and downstream players are generally conserved (Table 1). The basic operation of the sex determination network is through the dynamics of the opposing male and female pathways to ensure 1:1 male and female ratio [5], thus demanding the SD gene to be expressed in a temporal, spatial, dose- and temperature-sensitive fashion. The gene products in the male pathway and female pathway are antagonistic, and their expression is negatively regulated against each other (Figure 1).

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As with the MSD genes, sex chromosomes in vertebrates have evolved with various pace to various degrees, ranging from entirely homomorphic in many species of lower vertebrates to highly degraded heteromorphic in higher vertebrates. The canonical model of sex chromosome evolution predicts one way of trend toward degeneration and degradation of the sex chromosome carrying the MSD gene [6–9]. However, empirical evidence in support of this theory does not go beyond mammals and birds. The sex chromosomes in reptiles, amphibians, and teleost fish can be homomorphic or heteromorphic, and most of them are homomorphic. A popular explanation is that sex chromosomes in lower vertebrates are young, but both homomorphic and heteromorphic sex chromosomes are present across all lower vertebrates ranging from Cartilaginous fish, teleost fish, amphibians, and reptiles; some homomorphic sex chromosomes are older than some highly differentiated heteromorphic sex chromosomes, directly challenging the canonical theory of sex chromosome evolution. The availability of genome sequences and known MSD genes from various vertebrate species, especially from lower vertebrates, made it possible to determine: 1) How MSD genes were acquired; 2) How sex chromosomes evolve in relation to the modes of MSD gene acquisition; 3) How MSD genes affect the evolution of sex determination systems. Here we present the cause-effect model that states the mode of MSD acquisition determines the evolution of sex chromosomes. This model explains for evolution of vertebrate sex chromosomes in relation to the MSD genes and their chromosome karyotypes.

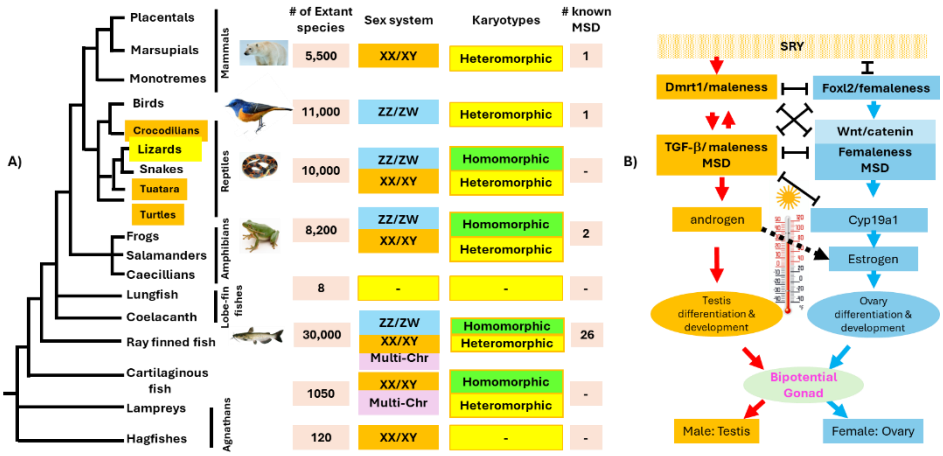

Figure 1. Schematic presentation of sex determination systems. Sex chromosomes and master sex determination (MSD) genes and their association with temperature (**A**). Note a single sex determination system and a single master sex determination gene in homeothermic mammals and birds (upper part), but multiple sex determination systems and master sex determination genes in ectothermic vertebrates (lower part). The groups whose sex are determined by temperature are colored in orange (crocodilians, tuatara, and turtles), and the group in which some species have temperature sex determination while others are controlled by genetic determinants are shown in yellow (lizards). (**B**) Male and female pathway of sex determination, starting with the master sex determination gene, e.g., Sry in mammals, dmrt1 in birds, and various other genes in lower vertebrates. The male and female pathways are antagonistic each other, but this process is regulated by temperature, which affect sex differentiation through expression of aromatase. Note that SRY transcription factor is an upstream of all other MSD genes.

Only one MSD gene and one SD system has evolved from homeothermic mammals and birds, respectively, but multiple MSD genes and SD systems have evolved from poikilothermic vertebrates (Figure 1). In mammals, Sry is both necessary and sufficient for sex determination [10]. In birds, dmrt1 works in a dose-sensitive fashion, where two copies of dmrt1 make a male, while one copy makes a female [11]. Apparently, the two-fold difference in dmrt1 expression is sufficient to reach the threshold for the sex phenotype in birds. With reptiles, ZW system was believed to be the only genetic SD system for over 50 years until the recent demonstration of the XY SD system in boa and python snakes [12]. Similarly, with amphibians, DM-W in African clawed frog, *Xenopus laevis*, was the only

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MSD gene known from 8,470 amphibian species until the recent discovery of MSD gene *bot1l* in the European green toad, *Bufo viridis*, with an XY system [13].

Table 1. Examples of genes involved in male or female pathway of sex determination, which are antagonistic each other.

Genes in the maleness camp			Genes in the femaleness camp		
SRY	Sex-determining region on the Y chromosome	HMG-box transcription factor	Foxl2	Forkhead Box L2	Transcription factor
sox9	*SRY*-like, HMG-box-containing gene family, member 9	HMG-box transcription factor	RUNX1	RUNX Family Transcription Factor 1	Works with FOXL2 for fetal granulosa cell identity
wt1	Wilms' Tumor 1	Transcription factor	*RSPO1*	R-spondin-1	Secreted intercellular signal
dmrt1	Doublesex and mab-3 related transcription factor 1	Transcription factor	*WNT4*	Wnt Family Member 4	Secreted intercellular signal
sox3	*SRY*-like, HMG-box-containing gene family, member 3	HMG-box transcription factor	Cyp19a1	Aromatase/estrogen synthetase	Convert androgens to estrogens
Fgf9	fibroblast growth factor9	Secreted intercellular signal	Hsd17b1	17β-Hydroxysteroid dehydrogenase 1	
Amh & other TGF-β	TGF-b superfamily	TGF-signaling	β-catenin	Cell adhesion	Key player for Wnt pathway
			MAP3K1	Mitogen-activated protein kinase kinase kinase 1	Activate Wnt4/β-catenin/FOXL2 pathway
			Dax-1	Dosage-sensitive sex reversal	Regulated by Wnt4
Newcomers	zkY, sdY, pfpdz1, hydin, cyce3, RIN3, FIGLA, znrf3	To be defined	Newcomers	paics, banf2	

As poikilotherms living in the aquatic environments, teleost fish must delicately respond to broad temperature variations in the SD process. In correlation, 28 distinct MSD genes have evolved in teleost fish (Figure 1), including transcriptional factors, TGF-β cytokines, steroidogenesis genes, and many "newcomers", all are downstream players in the SD pathways as compared to the MSD gene Sry in mammals. While Sry functions as a decisive MSD gene, most other MSD genes are quantitative in response to dose and temperature sensitivity.

2. Molecular Mechanisms for the Acquisition of MSD Genes

The first step of sex chromosome evolution is the emergence of MSD genes. To serve as a sex chromosome, one gene on one of the autosome pairs must initially gain new functions or new expression patterns in favor of male or female pathway, thereby becoming a master switch turning on the genes for either the male or female development. As shown in Figure 2, different mechanisms of MSD gene acquisition determine the pathway of sex chromosome evolution. In the context of sex chromosome evolution, we classify the mechanism of MSD gene acquisition into four categories: 1) Simple mutations, such as allelic variations (including base substitutions, neofunctionalization and subfunctionalization), gene duplications, and small deletions or insertions [14]. This category accounts for the largest numbers of known MSD genes in vertebrates (Table 2). Sex chromosomes in this category is characterized by not harboring any non-homologous sequences and, therefore, they stay homomorphic. Examples of this category include missense mutations in the coding regions, mutations in the regulatory sequences such as promoters, enhancers, silencers, and splicing junctions, as well as gene duplications; 2) Translocations or transpositions of DNA carrying the MSD gene, or small-sized inversions. Sex chromosomes of this category carry a limited region of non-homologous sequences that are not recombining, but they stay homomorphic. Examples of this category include MSD gene DM-W in African clawed frog, sdY in salmonids, and amhr2 among silurid catfishes; 3) Large chromosomal inversions. An MSD gene is activated due to juxtaposition of the MSD gene to a new regulatory context that lead to changes in expression profiles, and or temporal or spatial

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regulation in favor of male or female pathway. Sex chromosomes of this category carry large non-homologous regions that are inverted between the sex chromosomes, which reduce crossovers, but retain the recombination potential upon formation of the recombination loop [15]. Reduced recombination leads to sequence degeneration, but any recombination and recurrent recombination in the inverted region would lead to duplication in one and deletion in the other chromosome [16,17], and most likely requires double crossovers to survive. As a result, sex chromosomes of this category become heteromorphic; 4) Chromosome fusions or fissions. Chromosome fusions or fissions lead to non-homologous regions of large sizes, and often the multi-chromosome SD system.

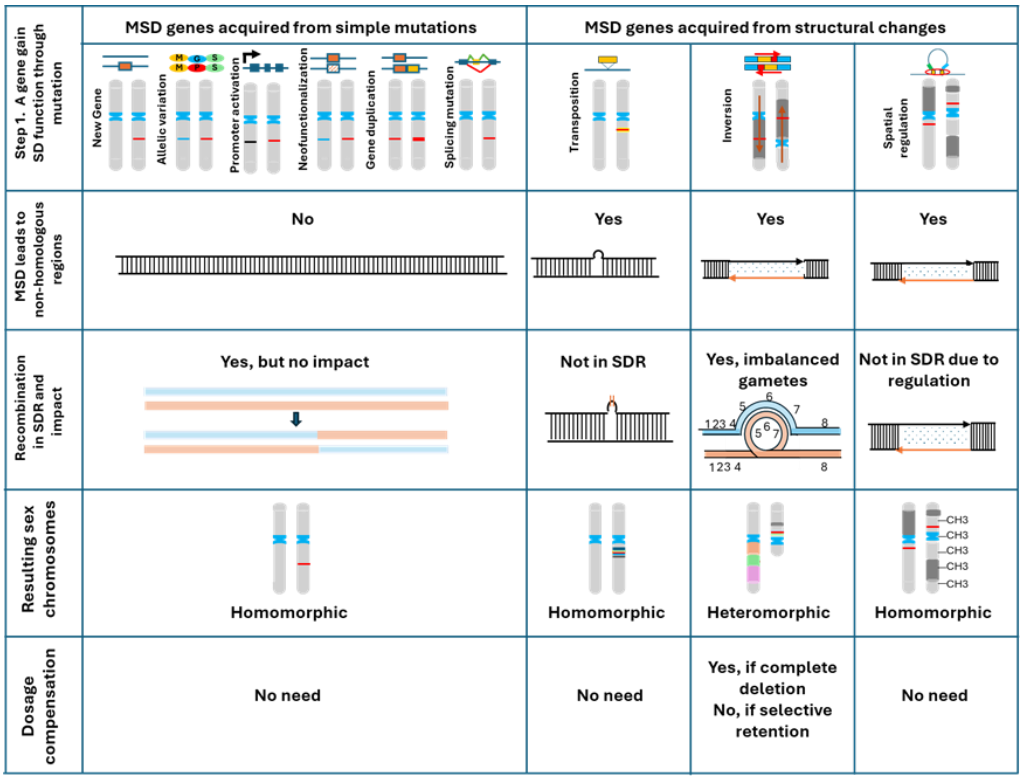

Figure 2. Schematic presentation of the cause-effect model and predicted paths of sex chromosome evolution. The pathway MSD genes were acquired (the cause) determines the way sex chromosomes evolve (the effect). The way MSD genes were acquired are categorized into two major categories: 1) Simple mutations (left) such as gaining of a new gene, base substitutions, mutations in the promoter region, neofunctionalization, subfunctionalization, tandem gene duplication, and splicing junction mutations, and 2) Structural changes (right) such as large insertions, chromosomal inversion, and fusion and fission. A key element is if non-homologous regions are created during MSD acquisition. With simple mutations, non-homologous regions are not involved, and therefore, no recombination suppression, leading to homomorphic sex chromosomes. Non-homologous sequences reduce recombination, and hence, involve recombination suppression, leading to hoteromorphic sex chromosomes. In the case of major structural change, regions of non-homologous sequences are involved, e.g., in the inserted segment, which lead to recombination suppression. Although recombination suppression causes sequence degeneration and accumulation of transposable elements, recurrent recombination in the reverted regions over time leads to deletions and rearrangements.

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Table 2. Diversity of sex determination (SD) genes and SD systems in vertebrates. ZW sex system is heighted in red. Question mark (?) indicates unknown master sex determination (MSD) genes, but their karyotypes are indicated. *indicates that inversion is likely involved because whole chromosome is non-recombining except a small PAR. The size of sex determination region (SDR) is indicated for those that are well characterized.

MSD gene	Order or major groups	Common name	Species	MSD gene acquisition	Karyotype	Functional validation	Sex system	References
Teleost fish								
Dmrt1	Beloniformes	Japanese medaka	*Oryzias latipes*	Allelic	Ho	Natural mutation	XY	[17]
Dmrt1	Beloniformes	Hainan medaka	*Oryzias curvinotus*	Allelic	Ho	-	XY	[70]
Dmrt1	Beloniformes	Northern medaka	*Oryzias Sakaizumii*	Allelic	Ho	-	XY	[71]
Dmrt1	Beloniformes	Hubbs's medaka	*Oryzias hubbsi*	Inversion	Hetero	-	ZW	[38]
Dmrt1	Beloniformes	Javanese ricefish	*Oryzias javanicus*	Inversion	Hetero	-	ZW	[38]
Dmrt1	Perciformes	Spotted scat	*Scatophagus argus*	Allelic	Ho	-	XY	[72]
Dmrt1	Perciformes	Spotbanded scat	*Selenotoca multifasciata*	Allelic	Ho	-	XY	[73]
Dmrt1	Perciformes	Siamese fighting fish	*Betta splendens*	Allelic	Ho	-	XY	[74]
Dmrt1	Perciformes	Yellow drum	*Nibea albiflora*	Allelic	Ho 10 Mb SDR	-	XY	[75]
Dmrt1	Perciformes	Bighead croaker	*Collichthys lucidus*	Chromosome fusion generated Y (male 2n=47) female 2n=48	Hetero	-	$X_1X_1X_2X_2/X_1X_2Y$	[76]
Dmrt1	Pleuronectiformes	Chinese tongue sole	*Cynoglossus semilaevis*	*Whole chromosome non-recombining but a small PAR	Hetero W larger	knockout	ZW	[31]
Dmrt1	Pleuronectiformes	Genko tongue sole	*Cynoglossus interruptus*	Allelic	Ho	-	ZW	[77]
Dmrt1	Acanthuriformes	Yellow croaker	*Larimichthys crocea*	Allelic	Ho	-	XY	[78]
sox2	Pleuronectiformes	Turbot	*Scophthalmus maximus*	Allelic	Ho	-	ZW	[79]
sox3Y	Beloniformes	Dwarf medaka	*Oryzias minutillus*	Allelic	Ho	Transgenic, knockout	XY	[80]
sox3Y	Beloniformes	Marmorated ricefish	*Oryzias marmoratus*	Allelic	Ho	Transgenic, knockout	XY	[80]
sox3Y	Beloniformes	Yellow finned medaka	*Oryzias profundicola*	Allelic	Ho	Transgenic, knockout	XY	[80]
sox3Y	Beloniformes	Indian ricefish	*Oryzias dancena*	Allelic	Ho	Transgenic, knockout	XY	[80]
Sox7	Beloniformes	Celebes ricefish	*Oryzias celebensis*	Allelic	Ho	-	XY	[71]
Sox7	Beloniformes	Matano ricefish	*Oryzias matanensis*	Allelic	Ho	-	XY	[71]
Sox7	Beloniformes	Wolasi ricefish	*Oryzias wolasi*	Allelic	Ho	-	XY	[71]
Sox7	Beloniformes	Daisy's ricefish	*Oryzias woworae*	Allelic	Ho	-	XY	[71]
FIGLA	Cichliformes	Tilapia	*Oreochromis niloticus LG1*	Allelic	Ho	-	XY	[20]

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ptfa1	Siluiriformes	Chinese longsnout catfish	*Leiocassis longirostris*	Allelic	Ho	-	XY	[21]
amhby	Esociformes	Norther pike	*Esox Lucius*	Tandem duplication	Ho	-	XY	[81,127]
amhby	Esociformes	Southern pike	*E. cisalpinus*	Tandem duplication	Ho	-	XY	[81]
amhby	Esociformes	Amur Pike	*E. reichertii*	Tandem duplication	Ho	-	XY	[81]
amhby	Esociformes	Muskellunge	*E. masquinongy*	Tandem duplication	Ho	-	XY	[81]
amhby	Esociformes	Chain pickerel	*E. niger*	Tandem duplication	Ho	-	XY	[81]
amhby	Esociformes	Olympic mudminnow	*Novumbra hubbsi*	Tandem duplication	Ho	-	XY	[81]
amhby	*Pleuronectiformes*	Olive flounder	*Paralichthys olivaceus*	Duplication and transposition	Ho	-	XY	[82]
amhY	Atheriniformes	Patagonian pejerrey	*Odontesthes hatcheri*	Duplication and transposition	Ho	Knockdown	XY	[83]
amhY	Atheriniformes	Silversides	*Odontesthes argentinensis*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes nigricans*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes piquava*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes incisa*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes smitti*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes humensis*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes regia*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes mauleanum*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Silversides	*Odontesthes perugiae*	Duplication and transposition	Ho	-	XY	[84]
amhY	Atheriniformes	Pejerrey	*Odontesthes bonariensis*	Duplication and transposition	Ho		XY	[85]
amhY	Atheriniformes	Cobaltcap silverside	*Hypoatherina tsurugae*	Truncated duplication on Y	Ho	-	XY	[86]
amhY	Cichliformes	Nile tilapia	*Oreochromis niloticus*	Tandem duplication	Ho	-	XY Chr23	[87–89]
amhY	Beloniformes	Sulawesian meedaka	*Oryzias eversi*	Allelic	Ho	-		[90]
amhY	Perciformes	Black rockfish	*Sebastes schlegelii*	Duplication and transposition	Ho	Overexpression	XY	[91]

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amhY	Perciformes	Korean rockfish	*Sebastes koreanus*	Duplication and transposition	Ho	-	XY	[91]
amhY	Perciformes	Rockfish	*Sebastes pachycephalus*	Duplication and transposition	Ho	-	XY	[91]
amhY	Perciformes	Threespine stickleback	*Gasterosteus aculeatus*	Inversion	Hetero	-	XY	[32]
amhY	Perciformes	Japan Sea stickleback	*Gasterosteus nipponicus*	Inversion followed by chromosome fusion	Hetero	-	X1X2Y	[92]
amhY	Perciformes	Blackspotted stickleback	*Gasterosteus wheatlandi*	Inversion followed by chromosome fusion	Hetero	-	X1X2Y	[92]
amhY	Perciformes	Brook stickleback	*Culaea inconstans*	Duplication on Y	Ho	-	XY	[93]
amhY	Perciformes	Common lumpfish	*Cyclopterus lumpus*	Duplication on Y	Ho	-	XY	[94]
amhY	Perciformes	Lingcod	*Ophiodon elongatus*	Duplication on Y	Ho	-	XY	[95]
amhY	Siluriformes	Southern catfish	*Silurus meridionalis*	Transposition	Ho 2.38 Mb SDR	-	XY	[40]
Amhr2Y	Siluriformes	Amur catfish	*Silurus asotus*	Transposition	Ho 400 Kb SDR	-	XY	[41]
amhr2y	Siluriformes	Lanzhou catfish	*Silurus lanzhouensis*	Transposition	Ho 400 Kb SDR	-	XY	[42]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasianodon hypophthalmus*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius djambal*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasianodon gigas*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius bocourti*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius conchophilus*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius elongatus*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius siamensis*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius macronema*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius larnaudii*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius mekongensis*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius krempfi*	Transposition	Ho 320 Kb SDR	-	XY	[39]
Amhr2Y	Siluriformes	Pangasiidae catfishes	*Pangasius sanitwongsei*	Transposition	Ho	-	XY	[39]

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					320 Kb SDR			
amhr2Y	Osmeriformes	Ayu	*Plecoglossus altivelis*	Duplication and translocation	Ho 2.03 Mb SDR	Knockout	XY	[96]
amhr2Y	Syngnathiformes	Common seadragon	*Phyllopteryx taeniolatus*	Truncated duplication and transposition	Ho	-	XY	[97]
amhr2Y	Syngnathiformes	Alligator pipefish	*Syngnathoides biaculeatus*	Truncated duplication and transposition	Ho	-	XY	[97]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus amarillo*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus astorquii*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus chancbo*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus citrinellus*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus flaveolus*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus globosus*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus labiatus*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus sagittae*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus tolteca*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus viridis*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus xiloaensis*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Cichliformes	Midas Cichlids	*Amphilophus zaliosus*	Duplication and transposition	Ho	-	XY	[98]
amhr2Y	Perciformes	Yellow perch	*Perca flavescens*	Allelic	Ho	-	XY	[99]
amhr2Y	Perciformes	Balkhash perch	*Perca schrenkii*	Allelic	Ho	-	XY	[100]
amhr2Y	Perciformes	Walleye	*Sander vitreus*	Allelic	Ho	-	XY	[100]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu rubripes*	Allelic	Ho	-	XY	[101]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu obscurus*	Allelic	Ho	-	XY	[102]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu ocellatus*	Allelic	Ho	-	XY	[102]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu xanthopterus*	Allelic	Ho	-	XY	[102]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu stictonotus*	Allelic	Ho	-	XY	[102]

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amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu porphyreus*	Allelic	Ho	-	XY	[102]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu poecilonotus*	Allelic	Ho	-	XY	[102]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu chrysops*	Allelic	Ho	-	XY	[102]
amhr2Y	Tetraodontiformes	Pufferfish	*Takifugu pardalis*	Allelic	Ho	-	XY	[102]
amhr2Y	Characiformes	blind cave fish	*Astyanax mexicanus*	B chromosomes	B chr	Knockout	B chr	[103]
gdf6bB	Cyprinodontiformes	African killifish	*Nothobranchius furzeri*	Allelic	-	Knockout	XY	[104]
gdf6bY	Beloniformes	Philippine Medaka	*Oryzias luzonensis*	Allelic	Ho	Transgenic	XY	[105]
gsdfY	Cichliformes	Tilapia	*Oreochromis niloticus*	Allelic	Ho	Transgenic	XY	[106]
gsdfY	Perciformes	Sablefish	*Anoplopoma fimbria*	Allelic	Ho	-	XY	[107]
gsdfY	Pleuronectiformes	Atlantic halibut	*Hippoglossus hippoglossus*	Allelic	Ho 11.6 Mb SDR	-	XY Chr12	[108]
gsdfY	Tetraodontiformes	Pufferfish	*Takifugu niphobles*	Transposition	Ho		XY	[102]
gsdfY	Tetraodontiformes	Pufferfish	*Takifugu snyderi*	Transposition	Ho		XY	[102]
gsdfY	Tetraodontiformes	Pufferfish	*Takifugu vermicularis*	Transposition	Ho		XY	[102]
bmpr1ba	Pleuronectiformes	Pacific halibut	*Hippoglossus stenolepis*	Inversion compared to Chr9 of Atlantic halibut	Ho 12 Mb SDR	-	ZW Chr9	[109]
bmpr1bbY	Clupeiformes	Atlantic herring	*Clupea harengus*	Allelic	Ho	-	XY Chr8	[110]
fshrY	Mugiliformes	Flathead grey mullet	*Mugil cephalus*	Allelic	Ho	-	XY	[111]
fshrY	Pleuronectiformes	Senegalese sole	*Solea senegalensis*	Allelic	Ho	-	XY	[112]
Hsd17b1	Carangiformes	Yellowtail amberjack	*Seriola lalandi*	Allelic	Ho	-	ZW	[113]
Hsd17b1	Carangiformes	Greater amberjack	*Seriola dumerili*	Allelic	Ho	-	ZW	[113]
Hsd17b1	Carangiformes	Japanese yellowtail	*Seriola quinqueradiata*	Allelic	Ho	-	ZW	[113]
Hsd17b1	Carangiformes	California yellowtail	*Seriola dorsalis*	Allelic	Ho	-	ZW	[114]
Hsd17b1	Carangiformes	Oyster pompano	*Trachinotus anak*	Allelic	Ho	-	ZW	[115]
Cyp19a1	Carangiformes	Silver trevally	*Pseudocaranx georgianus*	Allelic	Ho	-	XY	[116]
Hsdl1 or *Tbc1d32*	Perciformes	European perch	*Perca fluviatilis*	Allelic	Ho	-	XY	[100]
sult1st6y	Scombriformes	Southern bluefin tuna	*Thunnus maccoyii*	Allelic	Ho	-	XY	[117]
sult1st6y	Scombriformes	Pacific bluefin tuna	*Thunnus orientalis*	Allelic	Ho	-	XY	[117]
cephx1Y	Carangiformes	Cobia	*Rachycentron canadum*	Allelic	Ho 4.04 Mb SDR	-	XY	[118]
Paics/ banf2W	Cichliformes	Blue tilapia	*Oreochromis aureus LG3*	Duplication	Ho	-	ZW	[119,120]
Paics/ banf2W	Cichliformes	Tanganyika tilapia	*Oreochromis tanganicae*	Duplication	Ho	-	ZW	[119,120]
Paics/ banf2W	Cichliformes	Wami tilapia	*Oreochromis hornorum*	Duplication	Ho	-	ZW	[119,120]

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Paics/ banf2W	Cichliformes	Spotted tilapia	*Pelmatolapia mariae*	Duplication	Ho	-	ZW	[119,120]
RIN3	Cichliformes	-	*Chromidotilapia guntheri*	Allelic coding region	Ho	-	XY	[121]
zkY	Gadiformes	Atlantic cod	*Gadus morhua*	Allelic	Ho	-	XY	[122]
zkY	Gadiformes	Arctic cod	*Arctogadus glacialis*	Allelic	Ho	-	XY	[122]
zkY	Gadiformes	Pacific cod	*Gadus macrocephalus*	Allelic	Ho	-	XY	[122]
sdY	Salmoniformes	Salmonids	*13 species Salmonids*	Transposition / translocation	-	Transgenic and knockout	XY	[24,25]
pfpdz1	Siluiriformes	Yellow catfish	*Pelteobagrus fulvidraco*	Chromatin architecture, epigenetic regulation	Ho	Overexpression Knockout	XY	[44,123]
Hydin	Siluiriformes	Channel catfish	*Ictalurus punctatus*	Epigenetic regulation	Ho 8.9 Mb SDR	Methylation blocker	XY	[59,60]
? (new comer)	Siluiriformes	Ussuri catfish	*Pseudobagrus ussuriensis*	Epigenetic regulation	Ho 16.83 Mb SDR	-	XY	[124]
HMGN6/ CYCE3	Synbranchiformes	The zig-zag eel	*Mastacembelus armatus*	Allelic	Ho 7.0 Mb SDR	-	XY	[125]
Gipc PDZ1Y?	Cyprinodontiformes	Eastern mosquitofish	*Gambusia holbrooki*	-	Ho	-	XY	[126]
?	Cyprinodontiformes	Western mosquitofish	*Gambusia affinis*	W chr larger, fusion?	Hetero	-	ZW	[126]
Mammals								
Sry	Mammals	All mammals		Inversion*	Hetero	Knockout Over expression	XY	[10]
Birds								
Dmrt1	Birds	All birds		Inversion*	Hetero	Allelic knockout	ZW	[11]
Reptiles								
?		Python and Boa snakes	-	-	Hetero	-	ZW	[131]
?		Amazonian puffing snakes and viper snakes	-	-	Ho	-	XY	[12]
Amphibians								
DM-W		African clawed frog	*Xenopus laevis*	Allelic	Ho	Transgenic	ZW	[66]
Bod1l		European green toad	*Bufo viridis*	Allelic	Ho		XY	[13]

3. The Diversity of Master Sex Determination Genes in Vertebrates

Since the discovery of SRY gene in humans [10], it took 12 years to find the second vertebrate MSD gene, dmY, from medaka [18]. However, recent advances in sequencing technologies drastically accelerated the pace. Now 30 distinct MSD genes have been identified from vertebrates (Table 2), with the vast majority of these being identified from teleost fish. Only two MSD genes have been identified from amphibians, DM-W from African clawed frog and *bod1l* from the European green toad [13]. Although both XY and ZW SD systems have been found in reptiles, no MSD genes have yet been identified.

In teleost fish, 28 distinct MSD genes have been identified, including transcriptional factors, TGF-β cytokines, genes involved in steroidogenesis, and many "newcomers" (Table 2). Of the

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transcriptional factors, dmrt1 is the most popular although sox family of transcription factors including sox2, sox3, and sox7 also serve as MSD genes. In addition, transcriptional factors FIGLA-like and ptf1a were identified to be the MSD genes. Of these, dmrt1 and sox genes were well known as the "usual suspects" [19], but FIGLA-like and ptf1a are newcomers. FIGLA-like gene is the MSD gene for Nile tilapia [20]; it encodes a protein of 99 amino acids including a 45-amino-acid basic helix-loop-helix domain and specifically expressed in testis. While the autosomal FIGLA gene is a femaleness gene promoting ovary formation, FIGLA-like gene on the Y interferes with the functions of the autosomal FIGLA gene, leading to testis development [20]. Similarly, a truncated form of Ptf1a (pancreas transcriptional factor 1 alpha), named ptf1aY, was identified as MSD gene in Chinese longsnout catfish [21]. Five TGF-β genes including amh, amhr2, gdf6, gsdf, and bmpr1b have been identified as MSD genes in teleost fish. In approximately a dozen species, genes involved in steroidogenesis have been identified as MSD genes, including hsd17b1, cyp19a1, hsdl1, sult1st6y, and fshrY. Of these, the functions of hsd17b1 and cyp19a1 are well known: 17β-Hydroxysteroid dehydrogenase 1 oxidizes or reduces the C17 hydroxy/keto group of androgens and estrogens and, hence, regulates the potency of these sex steroids, while cyp19a1 is a temperature-sensitive aromatase that convert androgens into estrogens. Therefore, these are generally regarded as femaleness genes, and they serve as MSD genes mostly in ZW systems (Table 2). In addition to these three groups, over a dozen of newcomers have been identified as MSD genes (Table 2). These include sdY, cephx1Y, paics, banf2, RIN3, zkY, pfpdz1, hydin, and gipc1 (a pdz domain-containing gene). The pathways for sex determination of these genes are unknown, with exception of sdY that is a truncated form of interferon regulatory factor 9; sdY functions as sex determination gene in salmonids as a dominant negative regulator through its interaction with FOXL2 [22].

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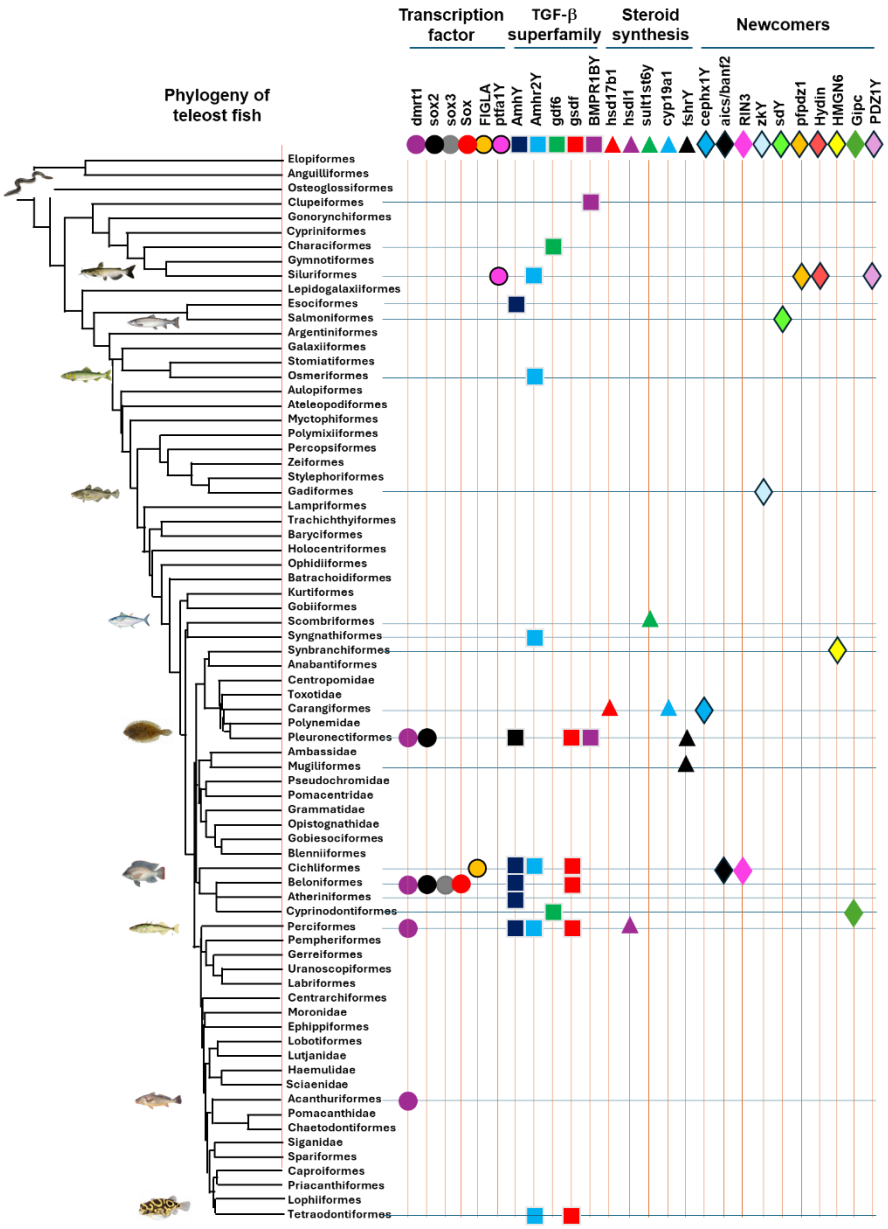

Figure 3. Divergent evolution of master sex determination (MSD) genes across the teleost fish (Actinopterygii). Shown on the left is the phylogeny of teleost fish, adopted from Hughes et al. [69]. Shown on the right are MSD genes in various categories such as transcription factors (circles), TGF-b (squares), genes involved in steroid synthesis (triangles), and "newcomers" (stars), in various colors and patterns, each representing a specific MSD gene. The symbols are positioned to where they each serve as MSD genes. .

4. Convergent and Divergent Evolution of MSD Genes

Despite convergent evolution of MSD genes in mammals and birds, most known MSD genes from lower vertebrates evolve independently. The identification of 30 distinct MSD genes indicated unlimited options of MSD genes, and the options go far beyond the usual suspects, with a trend of moving from upstream "master" to downstream players. Limited information is available from amphibians and reptiles, but MSD genes evolve mostly independently in lower vertebrates, with some local convergence. As shown in Figure 3, many orders involve more than one MSD genes, reflecting divergent evolution of MSD genes. Even if a specific MSD gene is found in multiple orders, the molecular mechanisms of their acquisition were different (Table 2). Of all teleost orders, Cichliformes has the largest known number of seven MSD genes, involving transcription factors (banf2 and FIGLA), TGF-β members (amh, amhr2, gsdf), and the newcomers category of unknown

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pathways (paics and RIN3), followed by Beloniformes and Pleuronectiformes, each with six known MSD genes, and then by Perciformes and Siluriformes, each with five known MSD genes. Convergent evolution, mostly through shared ancestry [23], does exist locally, mostly within the orders. The most dramatic is the conservation of sdY as the MSD gene across the entire salmonids [24,25]. The common GTF-β factors could be shared by several genera, or families, but none goes beyond the scope of the order.

5. The Canonical Model of Sex Chromosome Evolution

The canonical model of sex-chromosome evolution includes four consecutive phases: 1) First, a MSD gene acquires sex-determining function by mutation from a pair of autosomes; 2) Recombination suppression between the sex chromosomes; 3) Degeneration of the sex chromosome with accumulation of deleterious mutations and TE in non-recombinational regions of the sex chromosomes (Y or W); 4) Deletions of the degenerated sex chromosome lead to the decay of the sex chromosome, and evolution of dosage compensation [6–9]. The diversity and evolutionary lability of sex chromosomes across the tree of life [26] have challenged the canonical model: The vast majority of known sex chromosomes in lower vertebrates are homomorphic; recombination suppression is involved only in a small fraction of species with known sex chromosomes; recombination suppression does not always cause degradation of sex chromosomes; sex chromosome degeneration and degradation have been demonstrated only in a small fraction of cases in lower vertebrates [27]; sex antagonistic genes have not been widely identified, and even in a few cases of examples, linkage of genes and their related phenotypes cannot automatically interpreted as causations [8,28]; the remarkable turnover of sex chromosomes in many systems, especially in teleost fish, does not support inevitable linearity of sex chromosome evolution [28–30]; and dosage compensation was observed in some but not in all species with heteromorphic sex chromosomes. Instead, in some teleost fish species, "selective retention of haploinsufficient genes is apparently an alternative strategy for coping with the imbalanced expression of X- or Z-linked genes [31,32]. All these situations differ from those of human [33] and birds [34] and thus inspires for the proposal of new theories of sex chromosome evolution.

6. The Proposed Cause-Effect Model for the Evolution of Sex Chromosomes

Based on analysis of mechanisms of MSD gene acquisition, we propose a cause-effect model of sex chromosome evolution, which is depicted in Figure 2: How sex chromosomes evolve depends on how the MSD genes were acquired. Sex chromosomes carrying MSD genes acquired from simple mutations such as allelic variations, neofunctionalization, and gene duplications stay homomorphic, without recombination suppression nor degeneration; Sex chromosomes carrying MSD genes acquired from translocations and transpositions carry a small non-homologous region between the sex chromosomes, and therefore, they develop recombination suppression within the transposed segments, but they remain homomorphic; Sex chromosomes carrying MSD genes acquired from chromosomal inversions carry inverted regions between the sex chromosomes. Recombination is reduced in the inverted region, but single crossover in the inverted region causes deletions or duplications, which may be lethal to the gametes, and thus requires double crossovers to survive (Figure 4). Over time, such sex chromosomes degenerate and decay, especially with large inversions. A key factor for the evolution of sex chromosomes is if non-homologous sequences are being created during acquisition of MSD genes. Sex chromosomes with MSD genes acquired through simple mutations do not create non-homologous regions. In contrast, sex chromosomes with MSD genes acquired through transpositions, inversions, fusions and fissions all create non-homologous regions between the sex chromosome pairs, leading to recombination suppression. Key differences of this model from the canonical sex conflict model include: 1) Sex chromosome evolution is to resolve the structural problems created during acquisition of MSD genes, such as the presence of inverted regions on the sex chromosome pairs; 2) Recombination suppression is resultant of inter-

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chromosomal non-homologous regions between the sex chromosome pairs, not of intrachromosomal linkage of antagonistic alleles; 3) In spite of the reduced recombination due to inversions, recurrent recombination in inverted regions over time leads to deletions on the sex chromosomes. Sequence degeneration and accumulation of transposable elements over time result in evolutionary strata.

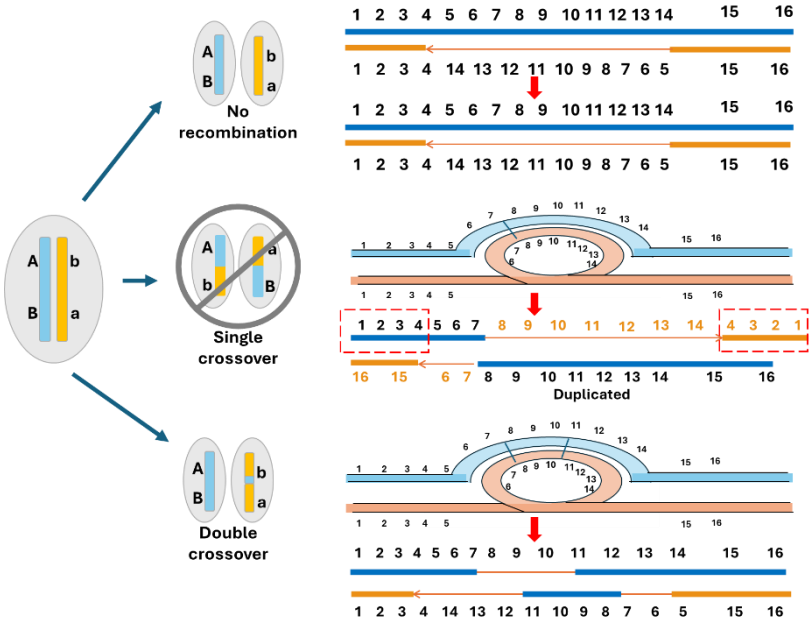

Figure 4. Recombination involving chromosome inversions. Single crossover of the sex chromosome with a major inversion produces gametes that are not viable because one would contain duplication of one side of the chromosome while the other would contain the other half of the chromosome. Double crossovers produce viable gametes but causing deletions and chromosomal rearrangements. Inversions, therefore, are the driving force of sex chromosome evolution.

6.1. Homomorphic Sex Chromosomes Without Recombination Suppression

The cause-effect model of sex chromosome evolution predicts that sex chromosomes harboring MSD genes acquired from simple mutations are homomorphic, with no recombination suppression or sequence degeneration (Figure 2). These sex chromosomes became sex chromosomes because of a gene they carry had become the MSD gene. Allelic and/or neofunctionalization/subfunctionalization accounted for the largest number of teleost fish species with known MSD genes to date. Teleost fish went through a third round of whole genome duplication [35], and neofunctionalization and subfunctionalization of ohnolog genes is a part of rediploidization processes [14,36]. Similarly, in teleost species with known MSD genes, tandem gene duplications were involved in many species (Table 2). Tandem duplications occur frequently in teleost fish species, especially with cytokines and chemokines [37].

One may argue that these sex chromosomes have not evolved much simply because they are young. However, sex chromosomes included in this category included taxa from cartilaginous fish, teleost fish, amphibians, and reptiles. Among teleost fish, a broad spectrum of teleost orders is involved, ranging from the base of teleost order such as Clupeiformes to the most advanced order of Tetraodontiformes, covering approximately 300 million years of evolutionary time. It is difficult to imagine that all these sex chromosomes are at the very beginning of their evolution. In contrast, in various medaka, both homomorphic and heteromorphic chromosomes were found, where allelic variations were found with homomorphic sex chromosomes (e.g., in *O. latipes*), but chromosomal inversion was found with heteromorphic sex chromosomes (e.g., *O. javanicus*), even though their evolutionary time is similar [38].

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6.2. Homomorphic Sex Chromosomes with Limited Region of Recombination Suppression

The cause-effect model of sex chromosome evolution predicts that sex chromosomes harboring MSD genes acquired with insertions, translocations or transpositions may develop a sex chromosome-specific region (often referred to as MSY, for male-specific Y under an XY sex system) related to the insertion. In the inserted segments, recombination suppression may be present; sequence degeneration may occur, but the region of recombination suppression or the degenerated sequences may be limited to the insertional segments or slightly larger, while the bordering homologous sequences should have continued homologous recombination. As a result, the sex chromosome-specific region may carry fixed haplotypes, but the sex chromosomes stay homomorphic. As listed in Table 2 and illustrated in Figure 2, good examples of this category are sex chromosomes in species of *Silurus*, *Pangasianodon*, and *Pangasius* genera in the order of Siluriformes. A common insertion was found to have occurred at the *Pangasidae* base, which carried the MSD gene amhr2 [39–42]. After acquiring the MSD gene, the sex chromosomes evolve to an extent that sequence degeneration and recombination suppression were observed within the insertions but was limited to the sex chromosome-specific insertion. It should be noted that here the MSD gene is ancient, estimated to have emerged over 100 million years ago [43], directly against the interpretation that limited evolution of the sex chromosomes is because the evolution time has been short. Similarly, sdY gene in all salmonids has evolved approximately 60 million years, but sex chromosomes continue to be homomorphic [25].

6.3. Homomorphic Sex Chromosomes with Extended Recombination Suppression

The cause-effect model of sex chromosome evolution predicts that sex chromosomes involving large chromosomal inversions develop recombination suppression in the region of inversion. If the sex chromosome is young, they are observed as homomorphic sex chromosomes. In several cases in teleost fish, large SDRs were observed (Table 2). Interestingly, levels of sequence degeneration within the SDRs are low in such cases. It is likely that such sex chromosomes are still young; it is also possible that such sex chromosomes stay homomorphic because of other mechanisms of regulation that slow down the evolution of sex chromosomes (see below).

6.4. Heteromorphic Sex Chromosomes

In contrast to the situations of homomorphic sex chromosomes, heteromorphic sex chromosomes evolved from major structural changes of the sex chromosomes. Chromosome inversion is probably the most frequent cause for the evolution of heteromorphic sex chromosomes. With chromosomal inversion, a promoter or enhancer may be directly juxtaposed to a gene close to the inversional junction. If such a gene is a maleness or femaleness gene, the chromosomal inversion may have activated a gene as an MSD gene. In addition to direct juxtaposition of regulatory elements to bordering genes near the inversion junction, changes in chromatin structure and architecture may also have the capacity for activation of inactivation of genes within or near the inversion segment. While genomic and epigenomic regulation of the multi-dimensional chromatin architecture is not well understood, it is increasingly recognized that such architecture and spatial regulation is important for sex chromosome evolution [44]. If inversion is involved in the acquisition of the MSD gene, the “new” chromosome carrying the inversion initially still has the DNA contents, but the major change is the creation of non-homologous sequences between the pair of sex chromosomes in the inverted region. As demonstrated in ninespine stickleback, inversions may have a role in both the evolution of sex determination systems and the differentiation of sex chromosomes [45].

Accurate chromosome segregation during meiosis relies on homology between the maternal and paternal chromosomes. Yet, by definition, heterogametic sex involving heteromorphic sex chromosomes lacks a homologous partner [46]. The presence of chromosomal inversions reduces recombination; however, recombination does occur in inverted regions, which causes unequal

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crossovers, leading to segmental duplications or deletions in gametes. Once the recombination is suppressed, over time, sequences degenerate, TEs accumulate, and if no regulation, deleterious mutations accumulate and eventually, upon any recombination or rearrangements, deletions occur, leading to decay of sex chromosomes. The sex chromosomes eventually become stable when large, inverted regions are eliminated or are repositioned within heterochromatin regions such as being close to the centromere where there is no recombination.

While such processes have been well documented in the canonical model of sex chromosome evolution, as with those in mammals and birds, the best studied teleost fish under this category is perhaps the sex chromosomes of threespine stickleback (*Gasterosteus aculeatus*), with a large SDR of 17.5 Mb [32]. The MSD gene amhY is located in the oldest region of the stickleback Y chromosome close to the original inversion junction (stratum one), adjacent to the pseudoautosomal region. The three evolutionary strata suggested additional inversions and rearrangements on the Y chromosome, and such inversions were confirmed by genetic mapping [47]. The Y chromosome is less than 26 million years old, its sequence is degenerated, and it lost the majority of the genes that are present on chromosome X, retaining just 44.1% of the genes [32]. However, gene loss may not be random as many haploinsufficient genes were retained on the Y chromosome [32].

6.5. Multiple and Unequal Sex Chromosome SD Systems

In teleost fish, multiple sex chromosome SD system has been adopted in a sizable number of species. The actual number must be larger, but 75 multiple sex chromosome systems with 60 estimated independent origins have been documented to date [48]. Multiple sex chromosome systems can be viewed as special cases of heteromorphic sex chromosomes. In this context, the cause-effect model of sex chromosome evolution predicts that sex chromosomes derived from chromosome fusion or fission would create heteromorphic sex chromosomes; the extent of recombination suppression depends on the size of fusion or fission segments, as well as on size of additional inversion. Here we present one example to show the cause-effect model also fits the multiple sex chromosome systems. In the spotted Knifejaw (*Oplegnathus punctatus*), the X1X2X1X2/X1X2Y multiple chromosome SD system is operating. Genome sequencing revealed large genomic regions of recombination suppression, 29.3 Mb of X1 (from 0 to 29.30 Mb) and 17.58 Mb of X2 (from the centromere to 17.58 Mb) were associated with sex, of which a large inversion on X1, and the centromere on X2, as well as the SD locus, accounted for the observed recombination suppression. Sequence degeneration and gene loss were also observed [49].

7. Dosage Compensation and Epigenetic Regulation of Sex Chromosome Evolution

Dosage compensation equalizes the level of expression of X- or W-linked genes between the sexes. While dosage compensation is well understood for mammals [50,51], and somewhat understood for avian species [52,53], it is not well studied with lower vertebrates. Studies with tongue sole and threespine stickleback indicated the lack of dosage compensation, but selective retention of dosage sensitive genes [31,54]. In contrast, complete dosage compensation was observed with guppy species, *Poecilia parae* and *P. picta* [55,56]. This appears to be conflicting but may represent alternative strategies of coping with imbalanced expression of sex chromosomes in species with heteromorphic sex chromosomes. In cases where large deletions had occurred from the Y chromosome such as the guppies, complete dosage compensation was needed, and it was so observed. This may be an effective way to balance expression of the lost genes, especially those that are dosage sensitive. Alternatively, with selective retention of dosage sensitive genes, there is no need to develop dosage compensation, as observed with less degenerated sex chromosomes in threespine stickleback and tongue sole [28,29].

Epigenetic regulation is involved in growth, reproduction, disease resistance and stress responses of various fish species [57]. Several isolated studies suggested epigenetic regulation of sex

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determination and sex chromosome evolution. In channel catfish, an epigenetically marked locus of 8.9 Mb was well aligned with the SDR where recombination is completely suppressed [58,59]. The X chromosome was hypermethylated, leading to silencing of the X-borne hydin gene. In contrast, the Y chromosome was hypomethylated, and the Y-borne hydin gene is expressed, serving as MSD gene [60,65]. Similarly, in threespine stickleback, the sex chromosomes had the majority (65%) of differentially methylated CpG sites (DMS) of the genome, with hypermethylation in females and hypomethylated in males. Most interestingly, the DMS were predominantly located in the SDR, especially in strata 2 and 1, where recombination is suppressed [61]. Similar work was also conducted in two guppies, where differential methylation was observed in testis, with hypermethylation in females and hypomethylation in males. Again, the DMS were found mostly in the SDR, particularly in stratum 2 and stratum 1 where MSD gene is located [62]. These examples demonstrated differential methylation of the sex chromosomes, especially within the SDR. In addition to regulation of gene expression, one possibility is that hypermethylation in the SDR could block recombination, thereby protecting the inverted region from being deleted, possibly allow selective retention of dosage sensitive genes. Inhibition of recombination during meiosis by hypermethylation is well documented in plants [63,64]. If DNA methylation plays a similar role within the SDR, especially with chromosomal inversion, hypermethylation in the SDR would de facto slow down degeneration of sex chromosomes, and perhaps such a mechanism could be used to selectively retain haploinsufficient genes.

8. Conclusions and Perspectives

Recent advances in sequencing technologies allowed rapid discoveries of the enormously diverse MSD genes in vertebrates and the mechanisms of their acquisition. It is apparent that the trajectories of sex chromosomes evolution are resultant of the acquisition modes of MSD genes: sex chromosomes with MSD genes acquired through simple mutations do not involve recombination suppression, nor degeneration; in contrast, sex chromosomes with MSD genes acquired through chromosomal inversions involve recombination suppression that leads to accumulation of transposable elements and sequence degeneration; and when the inversions are large in size, they may lead to the decay of the sex chromosomes. A key factor determining the evolution of sex chromosomes is if inverted regions are being created between the sex chromosome pairs for the acquisition of MSD genes. The creation of long inverted regions between the sex chromosome pairs provided driving force for sex chromosome evolution [17]. Depending on the sizes of the inversions and the nature of the deletions during sex chromosome evolution, dosage compensation evolves when essential or haploinsufficient genes are deleted. In contrast, regulatory mechanisms may have evolved from lower vertebrates with selective retention of haploinsufficient genes [32].

A correct model of sex chromosome evolution is important for future work. For example, large non-recombining SDR likely is predictive of a large inversion. Although allelic variations are the predominant way through which MSD genes were acquired in lower vertebrates, chromosomal inversions are a common way for the acquisition of MSD genes among vertebrates. However, identification of inversion junctions from tens of millions of base pairs are still difficult. Future focus should be given to structural analysis around the borders of SDR, between X and Y or W and Z sex chromosomes and given to epigenetic modification and spatial architecture that may regulate not only the acquisition of MSD genes, but also the evolution of sex chromosomes. Additional efforts with amphibians and reptiles, as well as many taxa of teleost fish, will make up the knowledge gaps for full understanding of the diversity of MSD genes and their chromosome evolution.

Gene knockout is well accepted as the functional analysis of genes. However, with the sex trait, pure reliance on gene knockout could lead to incorrect conclusions. This is because knockout of MSD genes is expected to cause sex reversal, but sex reversal can be achieved with many genes involved in the sex determination pathways (Table 1), not just with the MSD gene under study. For example, knockout of bcar1 gene in channel catfish led to sex reversal of genetic males to neofemales [58], but bcar1 was later demonstrated not to be the MSD gene for channel catfish [60,65]. Similarly, with

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Japanese strain of Nile tilapia (*Oreochromis niloticus*), knockouts of maleness genes amhy, gsdf or dmrt1, or femaleness genes foxl2 or cyp19a1a all led to sex reversal [66–68]. Thus, caution must be exercised when working with certain candidate MSD genes, especially with transcriptional factors and TGF-β cytokines. Fst mapping, when coupled to demonstrated expression profiles at critical time points of sex differentiation, should provide strong positional and expression evidence for the determination of MSD genes. When the size of SDR is very large, there can still be multiple differentially expressed genes (DEGs) in the mapped SDR. Large differences of DEGs within the SDR, even between closely related species such as channel catfish and blue catfish, are suggestive of distinct MSD genes [128]. Detailed analysis of spatial and temporal expression, as well as epigenetic regulation, is required for the identification of MSD genes.

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