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Diverse and variable sex determination mechanisms in vertebrates

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Sex is prevalent in nature and sex determination is one of the most fundamental biological processes, while the way of initiating female and male development exhibits remarkable diversity and variability across vertebrates. The knowledge on why and how sex determination mechanisms evolve unusual plasticity remains limited. Here, we summarize sex determination systems, master sex-determining genes and gene-regulatory networks among vertebrates. Recent research advancements on sex determination system transition are also introduced and discussed in some non-model animals with multiple sex determination mechanisms. This review will provide insights into the origin, transition and evolutionary adaption of different sex determination strategies in vertebrates, as well as clues for future perspectives in this field.

sex determination, sex-determining gene, sex chromosome, sex transition, sex differentiation, sex control breeding

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Most vertebrates are gonochoristic (two sexes present in different individuals) (Herpin and Schartl, 2015; Mei and Gui, 2015), and females and males are determined via two broad strategies including genotypic sex determination (GSD) and environmental sex determination (ESD) (Bachtrog et al., 2014; Capel, 2017). GSD refers to sex determination that is driven by genotypic elements with sex difference, and the primary sex of an individual with GSD is usually determined at the moment of fertilization. Diverse mechanisms of GSD can be generally divided into male heterogametic system of XY sex chromosomes and female heterogametic system of ZW sex chromosomes, which have been identified in mammals (Koopman et al., 1991), birds (Smith et al., 2009) and most reptiles (Gamble et al., 2015), amphibians (Yoshimoto et al., 2008), and fishes (Matsuda et al., 2002). ESD refers to sex determination that is triggered by the effects of environmental factors during development. Diverse stimuli for ESD have been revealed to include temperature (Holleley et al., 2015), photoperiod (Brown et al., 2014), social factors (Warner et al., 1996), and so on. GSD and ESD actually are not mutually exclusive, and transitions between these two seemingly distinct sex determination strategies have been unveiled in many species (Holleley et al., 2015; Li et al., 2018; Pennell et al., 2018).

Here, we summarize diverse sex determination systems, variable master sex-determining genes and plastic gene-regulatory networks across vertebrates. We also introduce the recent advancements on transitions between different sex determination systems in some ectotherms, which are at special evolutionary stages and have multiple sex determination mechanisms co-existed. Thus the present review will help us in further understanding the origin, transition and evolutionary adaption of different sex determination mechanisms.

DIVERSE SEX DETERMINATION SYSTEMS AMONG VERTEBRATES

XX/XY system and XX/XY variants

Male heterogametic XX/XY sex determination system is

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prevalent in mammals, and sex-determining region Y (*Sry*) located in mammalian Y chromosome is the first sex-determining gene identified in vertebrates (Koopman et al., 1991). *Sry* encodes a transcription factor with a high mobility group (HMG) box, which is transiently expressed and initiates testis differentiation and male development via triggering differentiation of Sertoli cells (Koopman et al., 1991). XX/XY sex determination system also have been observed in reptiles, amphibians and fishes (Figure 1), but sex chromosomes with limited Y chromosome degeneration are morphologically indistinguishable in many species of these classes (Gamble et al., 2015). Till now, no sex-determining gene(s) have been identified on the XY sex chromosomes without *Sry* gene in reptiles and amphibians. By contrast, numerous sex-determining genes have been revealed in many fish species with XX/XY systems, such as *dmy* in medaka (*Oryzias latipes*) (Matsuda et al., 2002), *sox3y* in Indian ricefish (*Oryzias dancena*) (Takehana et al., 2014), *amhy* in perjerry (*Odontesthes hatcheri*) and Nile tilapia (*Oreochromis niloticus*) (Hattori et al., 2012; Li et al., 2015), and *gsdfy* in Luzon ricefish (*Oryzias luzonensis*) (Myosho et al., 2012).

Although most mammals depend on XY system with *Sry* at the top of the sex-determining cascade, variants of XX/XY system have evolved in some mammalian species. In the spiny rats (*Tokudaia osimensis osimensis* and *Tokudaia osimensis* spp.) (Sutou et al., 2001), Y chromosomes and *Sry* gene have been lost, and both females and males have an identical XO karyotype. Two vole species (*Ellobius lutescens* and *Ellobius tancrei*) have no Y chromosome and no *Sry* gene either (Just et al., 1995). Although male differentiation pathway is conserved in the XO mammalian species (Otake and Kuroiwa, 2016), it is still unknown that which gene(s) determine sex in these mammals without *Sry*. Sex determination systems in monotremes are different to XX/XY systems in marsupials and placentals. Female platypuses (*Ornithorhynchus anatinus*) have five pairs of X chromosomes ($X_1X_2X_3X_4X_5$), and males have the five X chromosomes ($X_1X_2X_3X_4X_5$) and five Y chromosomes ($Y_1Y_2Y_3Y_4Y_5$) (Rens et al., 2004). The short-beaked echidna (*Tachyglossus aculeatus*) also has similar sex chromosome system with five X chromosomes ($X_1X_2X_3X_4X_5$) and four Y chromosomes ($Y_1Y_2Y_3Y_4$) that are not completely homologous to those of platypus (Rens et al., 2007). However, these two monotreme mammals have no *Sry* gene (Wallis et al., 2008), and the most probable sex-determining gene is *Amh* gene on Y_5 chromosome (Cortez et al., 2014). Moreover, variants of XX/XY system have also been identified in non-mammalian vertebrates such as $X_1Y_1Y_2$ system in neotropical fish (*Hoplias malabaricus*) (Cioffi and Bertollo, 2010) and X_1X_2Y system in leaf litter frog (*Eleutherodactylus maussi*) (Schmid et al., 2002).

ZZ/ZW system and ZZ/ZW variants

Unlike mammals, all studied birds have female heterogametic ZZ/ZW sex chromosomal system, and their sex is determined via the dosage of doublesex and mab-3 related transcription factor 1 (*Dmrt1*) on Z chromosome or an unknown female-determining gene on the W chromosome (Smith et al., 2009). *Dmrt1* gene encodes a transcription factor with a conserved DNA-binding domain, which is highly expressed in male gonads in fishes, reptiles, birds and mammals (Ge et al., 2017; Li et al., 2014a; Smith et al., 1999). In birds, knockdown of *Dmrt1* expression via RNA interference (RNAi) in the early chicken embryos leads to feminization of the gonads in genotypic males (ZZ) (Smith et al., 2009), while overexpression of *Dmrt1* induces male pathway and antagonizes female pathway embryonic gonads (Lambeth et al., 2014). Snakes also have ZZ/ZW sex determination system, and sex chromosomes in advanced snakes have been demonstrated to exhibit significant evolutionary stability (Rovatsos et al., 2015). Snake Z chromosome is homologous to the chicken autosome rather than to the chicken Z chromosomes, which indicates that sex chromosomes in snakes and birds were derived from different autosomal pairs of the common ancestor (Matsubara et al., 2006). ZZ/ZW system also have been identified in some species of amphibians and fishes (Figure 1), and the corresponding sex-determining genes have also been illustrated, including *Dm-w* on the W chromosome of the clawed frog (*Xenopus laevis*) (Yoshimoto et al., 2008) and *dmrt1* on Z chromosome of the half-smooth tongue sole (*Cynoglossus semilaevis*) (Chen et al., 2014; Cui et al., 2017).

Variants of ZZ/ZW system have evolved in some amphibian and fish species. In the New Zealand frog (*Leiopelma hochstetteri*), Z chromosome has been lost and sex is determined via univalent W chromosome in female (Green, 1988), showing a unique OW female and OO male sex determination system (Ferro et al., 2018). Besides, multiple sex chromosome systems also have been identified in the catfish (*Ancistrus sp.2*) with Z1Z2W1W2 female and Z1Z1Z2Z2 male sex determination system (de Oliveira et al., 2007). ZW1W2 female and ZZ male system have been uncovered in the lizardfish (*Trachinocephalus myops*) (Kitano and Peichel, 2012).

Extra chromosomes for GSD

Extra chromosomes, also known as B chromosomes or supernumerary chromosomes, are dispensable for the normal life cycle of host individuals (Houben et al., 2014). Compared with standard A chromosomes including autosomes and sex chromosomes, extra chromosomes do not follow the rules of Mendelian inheritance, as they do not pair with A chromosomes (Martis et al., 2012). Recently, active genes

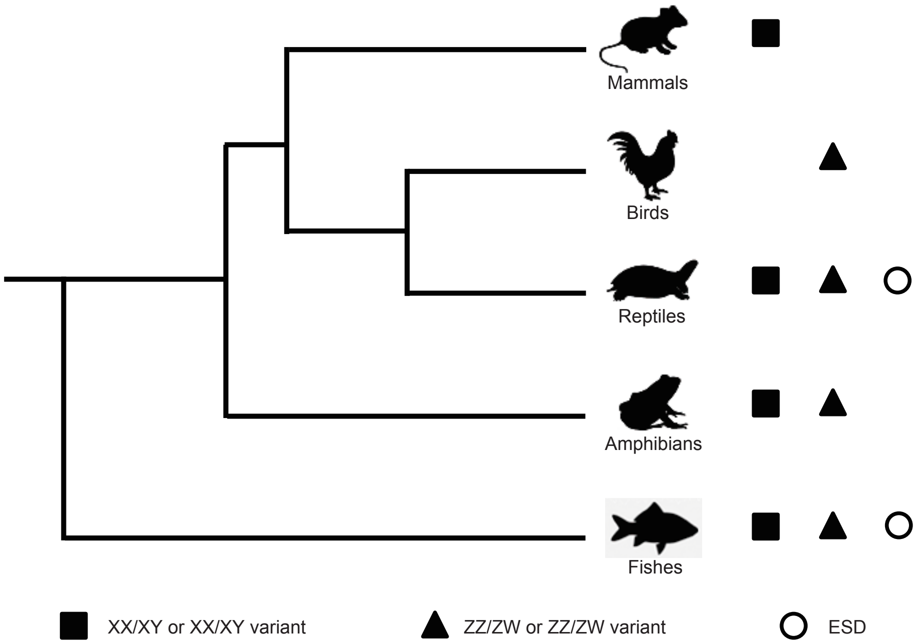

Figure 1 Diverse sex determination systems among vertebrate clades. "Variant" indicates absence of a sex chromosome or presence of multiple sex chromosomes. ESD, environmental sex determination.

have been identified on the extra chromosomes, which have functional effect on the host phenotypes especially the sex (Camacho et al., 2011; Ma et al., 2017). In the Lake Victoria cichlid fish (*Lithochromis rubripinnis*), B chromosomes restricted to females have been found to have functional effect on female sex determination (Yoshida et al., 2010). In polyploid gibel carp (*Carassius gibelio*), 3–4 extra microchromosomes with repetitive sequences and transposable elements have been identified in male individuals. Moreover, sex-reversed females (genotypic males) induced by estradiol hormone treatment have been used to establish gynogenetic families, and the proportions of males in these gynogenetic families have been revealed to be closely with the extra microchromosome number of the sex-reversed females. Thus, these findings suggest that the extra microchromosomes in males resembling common feature of sex chromosomes play male determination role in gibel carp (Li et al., 2016). Although extra chromosomes have been revealed to be associated with sex determination in many vertebrates (Camacho et al., 2011) and contain functional gene related with testis development and spermatogenesis (Li et al., 2017), the master sex-determining gene(s) on the extra chromosomes and the corresponding mechanism remain unknown.

ESD in ectotherms

ESD widely exists in ectotherms including reptiles and fishes, but have not been identified in amphibians although sex reversal induced by environmental factors has been revealed both in the wild populations and laboratory strains of common frog *Rana temporaria* (Alho et al., 2010; Flament, 2016). The most common form of ESD is temperature-dependent sex determination (TSD). TSD in reptiles has been studied most extensively, and sex ratios of reptiles with TSD exhibit three main patterns. The first pattern is that females develop at high incubation temperature and males develop at low temperature, such as the red-eared slider turtle (*Trachemys scripta*) (Ge et al., 2018). The second pattern is that high incubation temperature leads to male occurrence and low incubation temperature leads to female occurrence, such as the tuatara (*Sphenodon guntheri*) (Mitchell et al., 2006). The third pattern is that almost all females are produced at high and low temperature, while males (or both sexes) are produced at intermediate temperature, such as the common snapping turtle (*Chelydra serpentina*) (Schroeder et al., 2016). Some species in fish also have TSD, whereby the ambient temperature during development determines the sex of offspring, including gynogenetic gibel carp (*C. gibelio*) (Li et al., 2018) and topminnow (*Poeciliopsis lucida*) (Ospina-Alvarez and Piferrer, 2008).

Although TSD has been identified in rainbow agama (*Agama agama*) for half a century, the molecular mechanism for how temperature determines sex has only been unveiled recently in red-eared slider turtle (*T. scripta*). The epigenetic regulatory switch *Kdm6b* is revealed to play a causal male-determining role via eliminating H3K27 trimethylation at the promoter of *Dmrt1* (Ge et al., 2018), which was demonstrated to be a strong candidate gene for male sex determination (Ge et al., 2017). Therefore, these studies provide insights into the evolutionary trajectories and consequences of plastic sex determination strategies in vertebrates (Li and

Gui, 2018).

Moreover, photoperiod, social factors, dissolved oxygen, external hormones, and nutrients also can be the environmental factors for regulating sex determination (Baroiller et al., 2009; Capel, 2017). In the California grunion (*Leuresthes tenuis*) with photoperiod-dependent sex determination, longer day lengths lead to more females in the offspring, while more males are produced at shorter day lengths (Brown et al., 2014). Some fishes can change sex via social interaction with conspecifics. In the gobiid fish (*Trimma okinawae*), the largest female sex reversal to male upon the removal of the dominant male from a breeding group, but the resultant males retaining both ovarian and testis can change sex to females again in the presence of larger male in the group (Sunobe and Nakazono, 1993).

VARIABLE MASTER SEX-DETERMINING GENES

Sox family genes

Sry-related HMG box (*Sox*) genes have been vastly involved in vertebrate sex determination and differentiation (Table 1). *Sry* gene is almost the universal master male sex-determining gene in therian mammals, and gain- and loss-of-function studies have shown that *Sry* is sufficient and necessary for triggering male development. The transcription factor *Sry* is expressed transiently and initiates its target gene *Sox9*, which is critical for differentiation of Sertoli cells and testicle development (Barrionuevo et al., 2006; Chaboissier et al., 2004; Vidal et al., 2001). *Sry* gene is proposed to be the allelic diversification of *Sox3* gene, as the HMG box of Sry and Sox3 share high level of homology (Sato et al., 2010). Although *Sox3* gene has no primary sex-determining function in mammals *per se* (Rizzoti et al., 2004), ectopic expression of *Sox3* during gonadal development in the XX mice leads to complete sex reversal from XX females to males (Sutton et al., 2011).

Ortholog of *sox3* gene on the Y chromosome has been recruited for male determination in the Indian ricefish (*Oryzias dancena*) (Takehana et al., 2014). It has been found that the male-specific *cis*-regulatory element on the Y chromosome upregulates neighbouring *sox3y* expression during gonad development. *Sox3y* transgenic fish induces sex reversal of XX female, and *sox3y* loss-of-function in XY male leads to female development, which confirms the master male sex-determining role of *sox3y*. Recently, *Sox5* gene has been revealed to be involved in sex determination in medaka (*O. latipes*), which has the master sex-determining gene *dmy* (Schartl et al., 2018). *Sox5* downregulates the activity of *dmy* via binding to the *dmy* promoter, and *sox5* mutants have complete female-to-male sex reversal (Schartl et al., 2018).

Dmrt1 and its paralog genes

Doublesex and Mab-3 (DM) domain containing genes are involved in sex determination and sexual development among vast phylogenetic taxa including mammals, birds, reptiles, amphibians, fishes, flies, worms and corals (Bachtrog et al., 2014; Matson and Zarkower, 2012). *Dmrt1* gene has been identified to play an essential role for male pathway among vertebrates (Ge et al., 2017; Herpin and Schartl, 2015; Webster et al., 2017), and *Dmrt1* or its paralogs has held a conserved position as the master sex-determining gene triggering the gonadal sex differentiation in many species (Table 1). In birds (Smith et al., 2009) and half-smooth tongue sole (*C. semilaevis*) (Chen et al., 2014; Cui et al., 2017), *Dmrt1* gene on the Z chromosome has been illustrated as sex-determining gene *per se*. In medaka (*O. latipes*), the sex-determining switch *dmy* has been confirmed as a duplicated copy of *dmrt1* on the Y chromosome (Matsuda, 2005). In African clawed frog (*X. laevis*), the female sex-determining gene *Dm-w* is proposed to be a duplicate of *Dmrt1* on the W chromosome (Yoshimoto et al., 2008).

In the red-eared slider turtle (*T. scripta*) with TSD, *Dmrt1* exhibits a temperature-dependent sexually dimorphic expression prior to sexual differentiation. Loss- and gain-of-function analyses revealed that *Dmrt1* is necessary and sufficient for testicular differentiation, and DNA methylation of the *Dmrt1* promoter is tightly linked to temperature shifts. These findings demonstrate that *Dmrt1* is a strong candidate for master male sex determination in this TSD turtle (Ge et al., 2017).

TGF-β signaling pathway

Members of the transforming growth factor beta (TGF-β) signaling pathway also have been recruited to be sex-determining genes in vertebrates especially in fishes (Table 1). The anti-Müllerian hormone (*amh*), a growth factor from TGF-β family, controls the degeneration of the female primordial duct in mammals, which plays an essential role in male development among vertebrates. In platypus (*Ornithorhynchus anatinus*), *Amhy* on the Y_5 chromosomes has been proposed to be the prime candidate for sex-determining gene (Cortez et al., 2014). In the perjerrey (*Odontesthes hatcheri*), *amhy*, the duplicated copy of *amh* on Y chromosome, has become the male sex-determining gene (Hattori et al., 2012). In Nile tilapia (*Oreochromis niloticus*), a Y-specific duplicate of *amh* also plays an essential role for male sex determination (Li et al., 2015). A SNP that changes an amino acid (His/Asp384) in *amhr2* is associated with sex determination in the fugu (*Takifugu rubripes*) (Kamiya et al., 2012).

Gonadal soma derived factor (*gsdf*), a growth factor from TGF-β family, has been identified only in fish and assumed

Table 1 Master sex-determining genes or candidate genes in vertebrates

Master sex-determining genes	Species	Sex-determining systems	Gene ancestors	References
Sry	Therian mammals	XY	*Sox3*	(Koopman et al., 1991)
sox3y	Indian ricefish (*Oryzias dancena*)	XY	*sox3*	(Takehana et al., 2014)
Dmrt1	Birds	ZW	–	(Smith et al., 2009)
dmrt1	Half-smooth tongue sole (*Cynoglossus semilaevis*)	ZW	–	(Chen et al., 2014; Cui et al., 2017)
Dmrt1	Red-eared slider turtle (*Trachemys scripta*)	TSD	–	(Ge et al., 2017)
dmy	Medaka (*Oryzias latipes*)	XY	*dmrt1*	(Matsuda et al., 2002)
Dm-w	African clawed frog (*Xenopus laevis*)	ZW	*Dmrt1*	(Yoshimoto et al., 2008)
Amhy	Platypus (*Ornithorhynchus anatinus*)	$X_1X_2X_3X_4X_5Y_1Y_2Y_3Y_4Y_5$	*Amh*	(Cortez et al., 2014)
amhy	Perjerrey (*Odontesthes hatcheri*)	XY	*amh*	(Hattori et al., 2012)
amhy	Nile tilapia (*Oreochromis niloticus*)	XY	*amh*	(Li et al., 2015)
amhr2	Fugu (Takifugu rubripes)	XY	*amhr*	(Kamiya et al., 2012)
gsdfy	Luzon ricefish (*Oryzias luzonensis*)	XY	*gsdf*	(Myosho et al., 2012)
gdf6y	Turquoise killifish (*Nothobranchius furzeri*)	XY	*gdf6*	(Reichwald et al., 2015)
sdy	Rainbow trout (*Oncorhynchus mykiss*)	XY	*irf9*	(Yano et al., 2012)

to play an important role in testis development. In Luzon ricefish (*Oryzias luzonensis*), a sister species of medaka (*O. latipes*), *gsdfy* has replaced *dmy* as the master sex-determining gene (Myosho et al., 2012). In the sablefish (*Anoplopoma fimbria*), sex-specific insertions immediately upstream the sex-determining related gene *gsdf* implicates *gsdf* as an excellent candidate for master sex determination (Rondeau et al., 2013).

Growth differentiation factor 6 on Y chromosome (*gdf6y*), a member of the TGF-β family, is transiently expressed shortly after hatching and proposed to be the master sex-determining gene in the short-lived killifish (*Nothobranchius furzeri*) (Reichwald et al., 2015).

Other gene recruited for master sex determination

In rainbow trout (*Oncorhynchus mykiss*), *sdy* gene, the duplicate of the autosomal interferon regulatory factor 9 (*irf9*), encodes a protein that has lost the DNA-binding domain but preserved its protein interaction domain. Overexpression of *sdy* induces testicular differentiation, and targeted inactivation of *sdy* leads to ovarian differentiation. These results demonstrate that *sdy* is the sex-determining gene in *O. mykiss*; however, how *sdy* triggers male gonad development remains unclear (Yano et al., 2012).

Extraordinary diversity of sex-determining switches among vertebrates indicates that master sex-determining genes arise repeatedly and independently not only from the members of sexual regulatory network, but also from the *de novo* evolution of other genes.

GENE REGULATING NETWORK OF SEX DETERMINATION

In mice, expression of *Sry* in the somatic supporting cells of males with Y chromosome activates its direct downstream target *Sox9*, which is critical for testis determination (Barrionuevo et al., 2006; Chaboissier et al., 2004; Gonen et al., 2018; Vidal et al., 2001). Activation of *Sox9* leads to upregulation of *Fgf9* and other male differentiation related genes, which triggers male development of individuals with a Y chromosome. *Fgf9* plays repression role of *Wnt4* and the female pathway, and loss of *Fgf9* causes XY sex reversal to female (Kim et al., 2006). In individuals without Y chromosome, female development is induced by activation of *Wnt4* and other female differentiation related genes such as *β-catenin*, *Foxl2*, *Fst*, *Rspo1*, *Ctnnb1* and so on (Capel, 2017). Moreover, relationship between epigenetic regulation and mammalian sex determination also has been gradually illustrated. Mice lacking the H3K9 demethylase Jmjd1a (also called Tsga/Jhdm2a/Kdm3a) show male-to-female sex reversal, and Jmjd1a has been demonstrated to directly and positively control the *Sry* expression by regulating H3K9me2 levels (Kuroki et al., 2013). Most recently, histone acetyltransferases p300/CBP has been reported to be crucial for mammalian sex determination via regulating histone acetylation at the *Sry* locus, and loss of p300/CBP causes XY gonadal sex reversal (Carré et al., 2018) (Figure 2A).

In the chicken with ZZ chromosomes, *Dmrt1* is sufficient to activate *Hemgn*, *Sox9* and *Amh* (Lambeth et al., 2014), and *Hemgen* and *Sox9* also participate in the upregulation of

Dmrt1 (Nakata et al., 2013). As *Hemgen* expression starts to disappear after day 8.5, it is assumed that *Sox9* expression represses *Hemgen* and maintains *Dmrt1* expression at a high functional level which blocks the expression of *Foxl2* (Nakata et al., 2013; Sánchez and Chaouiya, 2018). Thus permanent activation of *Dmrt1* during gonadal development leads to male development. In the chicken ZW gonad, *Dmrt1* expression is too low to activate *Hemgn* and *Sox9*, then the expression of *Foxl2* reaches its functional level. *Foxl2* can activate the Aromatase, which is the terminal enzyme for Oestrogen (Govoroun et al., 2004). Thus repression of *Dmrt1* and *Sox9* (Lambeth et al., 2013) and activation of *Foxl2*, Aromatase and Oestrogen lead to female development (Sánchez and Chaouiya, 2018) (Figure 2B).

In the red-eared slider turtle (*T. scripta*) with TSD, *Dmrt1* exhibits a temperature-dependent sexually dimorphic expression and is a strong candidate sex-determining gene (Ge et al., 2017). Recently, the histone H3 lysine 27 demethylase Kdm6b has been revealed to promote the transcription of *Dmrt1* via eliminating the trimethylation of H3K27 near its

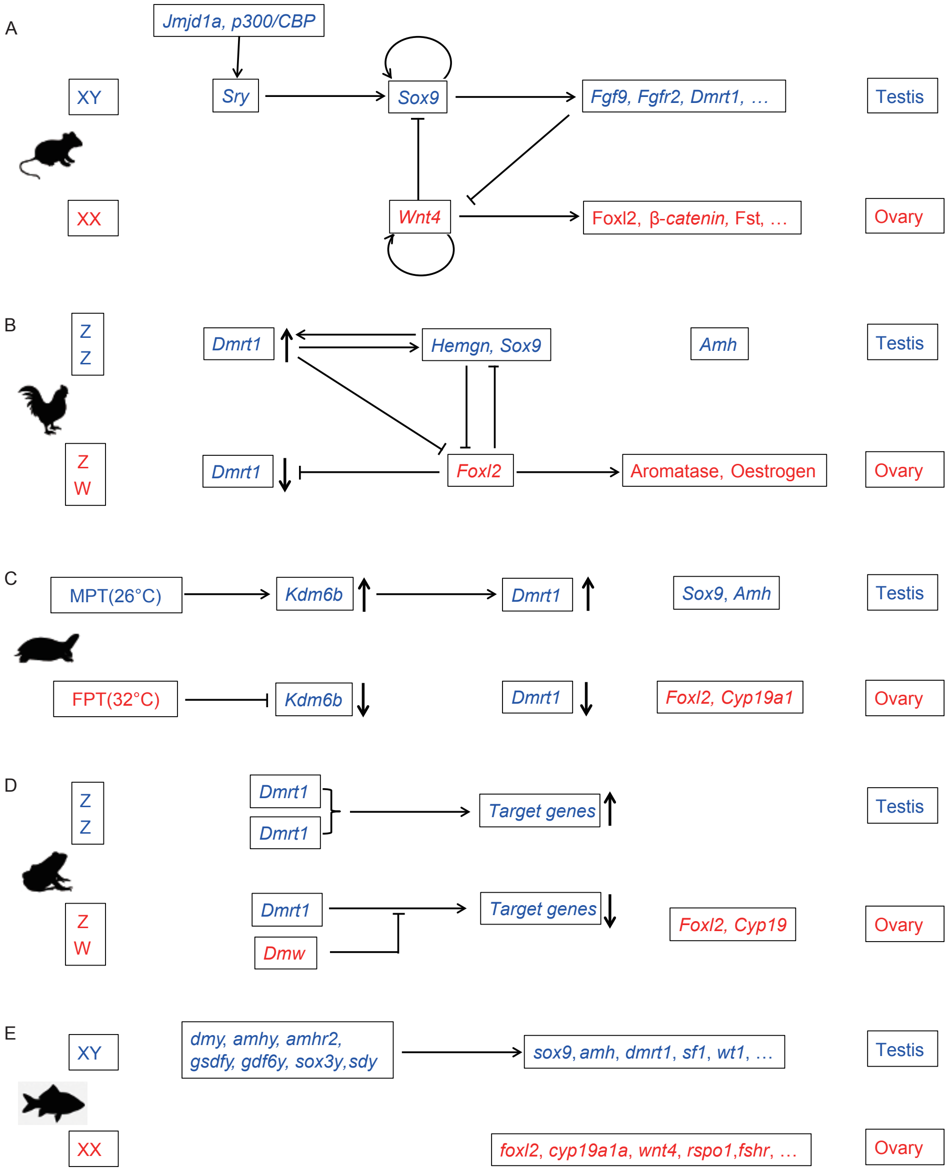

Figure 2 Gene-regulatory network of sex determination and gonadal differentiation. A, In mice, *Sry*, whose expression is modulated by *Jmjd1a* and *p300/CBP*, upregulates *Sox9* to initiate activation of the male pathway. B, In chickens, high amount of *Dmrt1* expression in the individual with ZZ chromosomes activates *Hemgn*, *Sox9* and *Amh*, and leads to testicle development. Low amount of *Dmrt1* expression in the individual with ZW chromosomes is unable to activate *Hemgn* and *Sox9*, meanwhile upregulation of *Foxl2*, Aromatase and Oestrogen leads to ovarian development. C, In the red-eared slider turtle (*T. scripta*), the epigenetic switch Kdm6b regulates the expression of sex-determining gene *Dmrt1* via eliminating H3K27 trimethylation at the promoter of *Dmrt1*. D, In the African clawed frog (*X. laevis*), *Dmrt1* activates testicle developmental pathway in males with ZZ chromosomes via stimulating target genes, while *Dm-w* induces ovarian differentiation via antagonizing *Dmrt1* in females with ZW chromosomes. E, In fishes with XY sex chromosomes, master sex-determining genes on Y chromosomes initiate male pathway, while in the individual with XX sex chromosomes, absence of master male sex-determining gene leads to activation of female pathway. Male pathway is indicated in blue and female pathway is showed in red.

promoter (Ge et al., 2018). At male-producing temperature (MPF 26°C), expression of *Kdm6b* reaches its functional level, and high amounts of Kdm6b activate *Dmrt1* expression and determine the male sex. At female-producing temperature (FPM 32°C), *Kdm6b* expression is down-regulated and the trimethylation of H3K27 on the *Dmrt1* promoter suppresses its expression, which leads to female development (Figure 2C).

Dm-w, the paralogue of *Dmrt1* on the W chromosome, is the female sex-determining gene in African clawed frog (*X. laevis*) (Yoshimoto et al., 2008). *Dm-w* is expressed transiently in the primordial gonads during the sex determination period, while expression of *Dmrt1* is still detected after the sex determination period. The DNA-binding domain of Dm-w shows high level of sequence identity with the corresponding region of Dmrt1; however, Dm-w has no significant sequence similarity with the Dmrt1's transactivation domain (Yoshimoto et al., 2010). Moreover, *Dm-w* is able to dose-dependently antagonize the transcriptional activity of *Dmrt1 in vitro*. Thus, these findings suggest *Dm-w* directs female sex determination via antagonizing *Dmrt1* (Yoshimoto and Ito, 2011) (Figure 2D).

In fishes with XY sex chromosomes, master sex-determining genes on Y chromosomes, including *dmy*, *amhy*, *amhr2*, *gsdfy*, *gdf6y*, *sox3y* and *sdy*, are expressed highly during sex determination period. High expression level of sex-determining switch is able to initiate downstream genetic network of male differentiation, which contains the essential genes for testicle development, such as *dmrt1*, *sox9*, *sf1* and *wt1*. While in the individual with XX sex chromosomes, absence of master male sex-determining gene leads to activation of the genetic network of female differentiation, which contains the essential genes for ovary development, such as *foxl2*, *cyp19a1a*, *wnt4* and *rspo1* (Figure 2E). For instance, divergent *foxl2a* and *foxl2b* cooperate to regulate ovarian development and prevent the ovary from differentiating as testis in the model organism zebrafish (*Danio rerio*) (Yang et al., 2017), and *dmrt1* and *amh* have been identified to play important roles in differentiation of male germ cells and testicle development (Lin et al., 2017; Webster et al., 2017). In Nile tilapia (*O. niloticus*) with the sex determiner *amhy* (Li et al., 2015), knockout of *gsdf* and *amhrII* induce in male-to-female sex reversal (Jiang et al., 2016; Li et al., 2015), while disruption of *foxl2* and *cyp19a1a* result in complete female-to-male sex reversal (Zhang et al., 2017). In yellow catfish (*Pelteobagrus fulvidraco*) with XY sex determination system (Dan et al., 2013; Wang et al., 2009), the Y chromosome linked gene *pfpdzl* has been revealed to be essential for male sex differentiation and maintenance through upregulating expression of *amh*, *dmrt1* and *sox9a1*, and downregulating expression of *cyp19a1*, *foxl2* and *wnt4* (Dan et al., 2018).

Although master sex-determining genes are variable among species and evolve independently (Table 1), the genes involved in the pathway of sex differentiation are relatively conserved (Figure 2) and the morphology of adult gonad is also similar across different vertebrate taxa (DeFalco and Capel, 2009; Herpin and Schartl, 2015). *Sox9*, *dmrt1* and *amh* are commonly involved in the pathway of testicle development and spermatogenesis, while *foxl2* and *cyp19a1a* are essential for ovarian development and oogenesis (Figure 2). *Sox9* is critical for testis determination, which was demonstrated by gain- and loss-of-function studies (Barrionuevo et al., 2006; Chaboissier et al., 2004; Vidal et al., 2001). Recent findings reveal that the deletion of a single distal enhancer of *Sox9* is able to induce XY sex reversal in mice (Gonen et al., 2018). *Dmrt1* has been identified as sex-determining switch in birds, half-smooth tongue sole (*C. semilaevis*) and red-eared slider turtle (*T. scripta*), and in the other vertebrates *dmrt1* also plays essential role for male development (Li et al., 2014a; Matson and Zarkower, 2012). Meanwhile, *amh* has been illustrated to be a key component of male sex determination and testicle differentiation across vertebrates (Herpin and Schartl, 2015; Lin et al., 2017). On the other hand, *foxl2* is a highly conserved gene for ovarian development and maintenance (Loffler et al., 2003), and *cyp19a1a* is a critical gene of steroidogenesis, which converts androgens to estrogens (Simpson et al., 1994; Xiong et al., 2015). Inhibition or deletion of *foxl2* or *cyp19a1a* induces female-to-male sex reversal in many species, and *foxl2* and *cyp19a1a* are believed to be involved in the process of ovarian differentiation in nearly all species studied (Kitano et al., 2000; Lau et al., 2016; Yang et al., 2017; Zhang et al., 2017).

TRANSITIONS BETWEEN SEX DETERMINATION SYSTEMS

Compared with relatively stable GSD systems in mammals and birds, sex determination systems exhibit contrasting diversity in ectotherms, including male heterogametic GSD, female heterogametic GSD, and ESD. Transitions between different sex determination strategies occur frequently in reptiles, amphibians and fishes (Capel, 2017; Pennell et al., 2018).

Comparative genomic mapping in reptiles has revealed the homology in sex chromosomes across reptile groups, meanwhile wide distribution of diverse sex determination systems has also been uncovered among reptiles. Thus, it is apparent that transitions between sex-determining systems occurred many times in reptiles, especially in geckos (Gamble et al., 2015; Sarre et al., 2011). Moreover, different sex-determining systems have been demonstrated to coexist in one species such as the Australian bearded dragon (*Pogona vitticeps*) (Quinn et al., 2007), in which sex reversal can induce the rapid transition from GSD to TSD (Holleley et al., 2015). The lizards (*P. vitticeps*) have female heterogametic sex chromosomes that females have a Z and a W chromosome while males have two Z chromosomes. However, laboratory experiments revealed that high incubation temperature induces ZZ offspring sex-reversed to females, and also nearly 20% of ZZ female individuals have been detected in wild populations (Holleley et al., 2015). The ZZ sex-reversed females mating with ZZ normal males produces fertile offspring without W chromosome, whose phenotypic sex is determined by incubation temperature solely. The novel transition pathway from GSD to TSD demonstrates that TSD can evolve rapidly in response to extreme ambient conditions such as high temperature, without sex-specific selective advantages (Holleley et al., 2015).

In amphibians, species studied till now all display GSD including XX/XY and ZZ/ZW systems, although temperature effect has been identified previously (Alho et al., 2010; Flament, 2016). The Japanese wrinkled frog (*Rana rugosa*) exhibits both male and female heterogamety, that the northern populations have ZZ/ZW system and the southern populations have XX/XY system (Miura, 2007; Ogata et al., 2003). Transition of sex-determining system from XY to ZW has been proposed, as part of ZW population belongs to the XY group but not to the main ZW group via phylogenetic analysis (Ogata et al., 2007). Recently, the simultaneous presence of three different sex chromosomes, W, Z, and Y, has been identified in the clawed frog (*Xenopus tropicalis*), containing three kinds of males (YZ, YW and ZZ) and two kinds of females (ZW and WW) (Roco et al., 2015). The existence of both male and female heterogametic individuals in one species provides the opportunity to seek emergence of neo sex-determining genes and transitions between sex-determining systems (Schartl, 2015).

Fishes exhibit the most diverse sex-determination mechanisms (Baroiller and D'Cotta, 2016; Mei and Gui, 2015), and coexistence of different sex-determining systems has also been found in the same species, such as zebrafish (*Danio rerio*) (Anderson et al., 2012; Wilson et al., 2014; Yang et al., 2017), pejerrey (*Odontesthes bonariensis*) (Yamamoto et al., 2014) and gibel carp (*C. gibelio*) (Li et al., 2018; Li et al., 2016). Gibel carp (*C. gibelio*) with two rounds of polyploidy origins is a hexaploid form of *Carassius* species complex (Li et al., 2014b; Zhou and Gui, 2017; Zhou et al., 2018), and has a wide geographic distribution among Eurasian continent and neighboring islands (Gui and Zhou, 2010; Liu et al., 2017a; Liu et al., 2017b). Compared with other unisexual vertebrates, rare but significant male incidences have been observed in natural habitats, and dual reproduction modes including unisexual gynogenesis and sexual reproduction have been illustrated (Gui and Zhou, 2010; Zhang et al., 2015). The maternal individual of *C. gibelio* mating with the male individual of another species *C. carpio* leads to gynogenesis, which produces offspring with nearly identical genetic background as mother's (Gui and Zhou, 2010), and the offspring's phenotypic sex is determined via ambient temperature during development (Li et al., 2018; Zhu et al., 2018). The maternal individual of *C. gibelio* mating with genotypic male of *C. gibelio* induces sexual reproduction, and the genetic male-specific marker is consistently linked to males, whose sex is determined via extra microchromosomes (Li et al., 2018; Li et al., 2016; Zhu et al., 2018) (Figure 3).

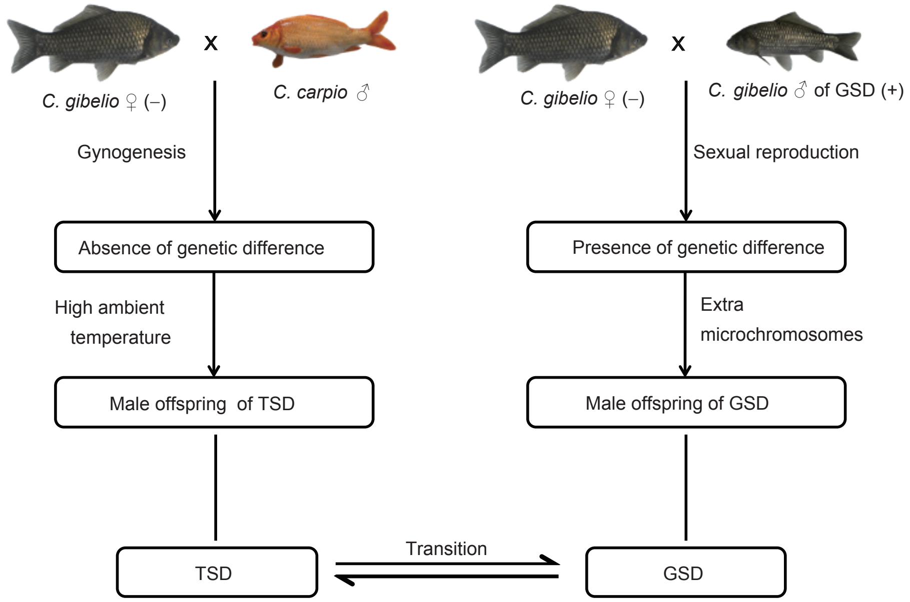

Figure 3 Sex determination system transition between GSD and TSD in *Carassius gibelio*. Offspring without genetic difference are produced via gynogenesis, and males of TSD are determined by high ambient temperature during development. Offspring with genetic difference are produced via sexual reproduction, and males of GSD are determined by extra microchromosomes. ♀, female; ♂, male; -, without the genetic male-specific marker; +, with the genetic male-specific marker; TSD, temperature-dependent sex determination; GSD, genotypic sex determination.

These findings have revealed a close association of sex-determining system transition between TSD and GSD and reproductive mode transition between unisexual gynogenesis and sexual reproduction (Li et al., 2018; Zhu et al., 2018).

CONCLUSION AND PERSPECTIVES

Along with whole genome sequencing (Reichwald et al., 2015), restriction site-associated DNA sequencing (Gamble et al., 2015), transcriptome sequencing (Rondeau et al., 2013), epigenetic modification (Shao et al., 2014; Shao et al., 2018) and molecular cytogenetic analysis (Li et al., 2016), diverse and variable sex-determining mechanisms have been revealed in vertebrates. However, knowledge on evolutionary trajectories and consequences of different sex determination systems remains limited. These ectothermic organisms with multiple sex determination strategies are supposed to be under the evolutionary process of sex determination mechanism transitions, such as the bearded dragon (*P. vitticeps*), wrinkled frog (*R. rugosa*) and gibel carp (*C. gibelio*). Thus, illustration of detailed knowledge on these species will accelerate to solve these enigmas, such as genomic and epigenetic modification, molecular regulatory cascades and networks of plastic sex determination systems, as well as environmental adaptation and ecological novelty during the evolutionary process.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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