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Review

Sex chromosome turnover and biodiversity in fishes

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ABSTRACT

The impact of sex chromosomes and their turnover in speciation remains a subject of ongoing debate in the field of evolutionary biology. Fishes are the largest group of vertebrates, and they exhibit unparalleled sexual plasticity, as well as diverse sex-determining (SD) genes, sex chromosomes, and sex-determination mechanisms. This diversity is hypothesized to be associated with the frequent turnover of sex chromosomes in fishes. Although it is evident that *amh* and *amhr2* are repeatedly and independently recruited as SD genes, their relationship with the rapid turnover of sex chromosomes and the biodiversity of fishes remains unknown. We summarize the canonical models of sex chromosome turnover and highlight the vital roles of gene mutation and hybridization with empirical evidence. We revisit Haldane's rule and the large X-effect and propose the hypothesis that sex chromosomes accelerate speciation by multiplying genotypes via hybridization. By integrating recent findings on the turnover of SD genes, sex chromosomes, and sex-determination systems in fish species, this review provides insights into the relationship between sex chromosome evolution and biodiversity in fishes.

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Introduction

The vast majority of vertebrates are sexually reproducing organisms in which sex is usually determined by a polymorphism at a single locus, the sex-determining (SD) gene (Herpin and Schartl, 2015). The SD gene helps to direct gonad differentiation towards either a testis or ovary phenotype. Sex chromosomes are highly conserved in some clades (e.g. birds and mammals) but evolve rapidly in others (e.g., reptiles and fishes) (Bachtrog et al., 2014). Sex chromosomes have evolved independently many times in fishes, accompanied by switching between different SD genes, sex chromosomes, and modes of sex determination (Devlin and Nagahama, 2002; Mank et al., 2006; Martínez et al., 2014; Sember et al., 2021; Kitano et al., 2024). In contrast to the heteromorphic and highly differentiated sex chromosomes in mammals and birds, sex chromosomes of most fishes are homomorphic and poorly differentiated (Charlesworth et al., 2005).

The "fountain-of-youth" theory has been suggested to explain why the sex chromosomes of most amphibian species are homomorphic (Perrin, 2009; Rodrigues et al., 2018). It suggests that occasional recombination between sex chromosomes in sex-reversed females can prevent sex chromosome degeneration. Examples supporting this theory have also been found in both animals and plants with homomorphic sex chromosomes, including fishes (Bergero et al., 2019; Long et al., 2023; Smith et al., 2023; Zhu et al., 2024). Heterochiasmy (differences of recombination rates between sexes) (Bergero et al., 2019), a key component of the "fountain-of-youth" theory, together with tolerance to sex reversal, is critical to prevent the evolution of heteromorphic sex chromosomes, as it accelerates sex chromosome turnover cycles (Berset-Brändli et al., 2008; Jeffries et al., 2018). Therefore, heterochiasmy has the potential to promote speciation, as postulated in stickleback and halibut (Kitano et al., 2009; Edvardsen et al., 2022). Sex chromosomes are one of the important drivers for speciation, and their role in promoting biodiversity has been widely discussed in plants, insects, birds, and mammals (Presgraves, 2008; Graves, 2016; Bracewell et al., 2017; Beaudry et al., 2020), but has received less attention in fish species.

Making up more than half of extant vertebrate biodiversity, fishes are in fact the most diverse vertebrate group (Nelson et al., 2016). Interestingly, the most rapidly speciating fishes, the East African cichlids, also show the highest rates of sex chromosome turnover (El Taher et al., 2021), suggesting that the evolution of sex

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chromosomes may contribute to speciation and biodiversity in this group. In this review, we summarize the diversity of sex determination, the repeated recruitment of *amh* and *amhr2* as SD genes, and the role of sex chromosome turnover in speciation. We highlight the role of mutation and hybridization in sex chromosome turnover. Finally, we propose future research directions using the latest technologies for genome sequencing and gene editing. In brief, we integrate findings from genetic, developmental, and evolutionary studies of fish sex determination and try to establish the relationship between sex chromosome turnovers and species diversity in fishes.

Diversity of sex determination and sex chromosome turnover in fishes

Prevalence of genetic sex determination (GSD)

Mammals, birds, and most amphibians adopt GSD (Ezaz et al., 2006). In contrast, fishes and reptiles have evolved different SD mechanisms, including GSD, environmental sex determination (ESD), and a combination of both (Kobayashi et al., 2013) (Fig. 1). GSD is far more prevalent than ESD in fishes (Bachtrog et al., 2014). This could be explained by: (1) GSD is stable and heritable across generations, ensuring consistent sex determination. (2) GSD maintains a stable sex ratio and is less susceptible to environmental fluctuations, enabling better adaptation to specific ecological niches (Van Dooren and Leimar, 2003; Schwanz and Proulx, 2008). While genetic factors primarily determine sex, environmental factors like temperature can still influence the outcome, creating a continuum rather than distinct categories (Ospina-Alvarez and Piferrer, 2008; Heule et al., 2014).

XY, ZW system, and sex chromosome turnover

Eutherian mammals have primarily heterogametic males (XY), while birds have heterogametic females (ZW). The sex chromosome and SD gene in these two lineages are mostly stable and highly conserved (Marshall Graves, 2008). In contrast, fishes, amphibians, and reptiles show a remarkable evolutionary lability of sex-determination systems. We updated the dataset from a previous study (Sember et al., 2021) and identified a total of 355 XY and 156 ZW systems among the fishes with GSD (Table S1), confirming that more species segregate an XY sex-determination system. The use of XY or ZW systems might be favored by two factors: differences in the frequencies of masculinizing and feminizing mutations or the establishment of these two systems after their appearance by mutation (Bachtrog et al., 2011). Turnovers toward XY systems might be favored in fish because dominant masculinizing mutations on a Y chromosome can protect against female sex-ratio biases caused by cytoplasmic sex ratio distorters (Bachtrog et al., 2011). However, dominant masculinizing mutations are likely part of a more complex interplay of genetic, environmental, and other factors driving sex chromosome turnovers in fish. Interestingly, statistical analysis of the updated dataset (Table S1), evaluated with Fisher's exact test ($P = 1.23 \times 10^{-14}$), supported the conclusion that species with XY system exhibit more homomorphic sex chromosomes, while species with ZW system have more heteromorphic sex chromosomes (Sember et al., 2021).

Polygenic systems and sex chromosome turnover

Polygenic sex determination, comprehensively reviewed recently (Schartl et al., 2023), manifests in three forms: i) multiple loci on autosomes cumulatively determining sex, ii) two sex chromosome systems in a transitory stage of sex chromosome turnover when the old system still segregates with the new system, and iii) cryptic autosomal modifier which can modulate the action of sex chromosomes. Polygenic sex determination has been reported in fishes like *Dicentrarchus labrax*, *Danio rerio*, *Xiphophorus multilineatus*, and *X. nigrensis* (Kallman, 1984; Vandeputte and Piferrer, 2018; Valdivieso et al., 2022). A variety of polygenic systems have also been identified in cichlid fishes such as *Astatotilapia burtoni*, *Metriaclima pyrsonotus*, and *M. mbenjii* (Ser et al., 2010; Moore et al., 2022; Lichilín et al., 2023). The latter two species segregate both an XY system on chromosome 7 and a ZW system on chromosome 5. Polygenic systems are considered to be rare, either being a transient state between XY and ZW or arising because of hybridization among populations with different systems (Nguyen et al., 2022; Schartl et al., 2023).

Multiple sex chromosome systems and sex chromosome turnover

Besides the standard sex chromosome constitutions (♀XX/♂XY; ♂ZZ/♀ZW), the sex chromosome karyotypes ♀XX/♂XO and ♂ZZ/♀ZO derived from the loss of the Y or W, as well as multiple sex chromosome systems ($♀X_1X_1X_2X_2/♂X_1X_2Y$, $♀XX/♂XY_1Y_2$, $♀X_1X_1X_2X_2/♂X_1Y_1X_2Y_2$, $♂ZZ/♀ZW_1W_2$ and $♂Z_1Z_1Z_2Z_2/♀Z_1W_1Z_2W_2$), have also been reported in fish (de Oliveira et al., 2008; Kitano and Peichel, 2012;

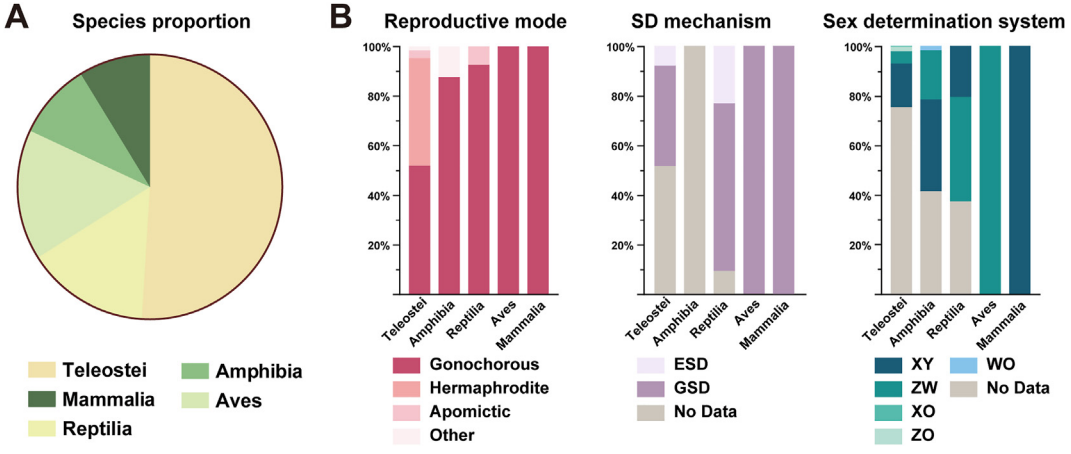

Fig. 1. Schematic representation of diversity of species and sex determination systems in vertebrates. **A**: Proportion of species. **B**: Reproductive mode, SD mechanism, and sex determination system in different groups of vertebrates. The graphs are drawn based on the Tree of Sex Database (Ashman et al., 2014).

Ferreira et al., 2016; Araya-Jaime et al., 2017). These systems have evolved independently in different lineages and have distinct patterns of inheritance and mechanisms of sex determination (Sember et al., 2021). It should be noted that multiple sex chromosome systems are not commonly found in fish, being reported in only about 100 species. To date, most cases of multiple sex chromosomes have been reported in the African annual killifishes of the genus *Nothobranchius*, Perciformes, and Amazonian catfishes of the genus *Harttia* (Siluriformes) (Deon et al., 2020; Sassi et al., 2020; Sember et al., 2021). Many studies have shown that the multiple neo-sex chromosomes in fish, originating through structural rearrangements, do not undergo heterochromatinization (Natri et al., 2013; Bitencourt et al., 2017; Sember et al., 2021). Multiple sex chromosomes in fishes are thought to be derived from sex chromosome-autosome fusions and hybridization between populations with different sex chromosome systems (Chen and Reisman, 1970; Caputo et al., 2001; Pennell et al., 2015; Araya-Jaime et al., 2017; Sember et al., 2021; Kitano et al., 2024). Species with multiple sex chromosomes may be rare if these systems are not conducive to sex chromosome turnover.

Extraordinary diversity of SD gene in fishes

Sry was the first SD gene identified in vertebrates (Sinclair et al., 1990), and it has been shown to be the master controller of male sex determination in most eutherian mammals (Waters et al., 2007). *DMRT1*, located on the Z chromosome, is the SD gene in the majority of birds (Smith et al., 2009; Ioannidis et al., 2021; Mazzoleni et al., 2021). The first fish SD gene (*dmy/dmrt1by*) was identified in the Japanese medaka (*Oryzias latipes*) (Matsuda et al., 2002; Nanda et al., 2002). In contrast to mammals and birds, 19 SD genes have already been identified in 140 fish species (Table S2). Sixty percent (84/140) of the species recruited genes in the TGF-β signaling pathway, while the others employed the genes encoding transcription factors or steroidogenic enzymes. Two mechanisms, gene duplication and allelic diversification, are involved in the origin of new SD genes (Fig. 2) (Guiguen et al., 2018). Some genes, such as *amh*, *amhr2*, and *dmrt1*, have been repeatedly recruited as SD genes in different fish lineages (Curzon et al., 2023), supporting the "limited options" hypothesis (Marshall Graves et al., 2010). In contrast, other genes, such as *sdY*, *bcar1*, and *figla-like*, have been found to be the SD genes in certain lineages. Although 2078 fish genomes have been sequenced and deposited in NCBI, SD genes have been reported for only 140 species. The identification of 19 SD genes in 140 fish species demonstrates the extraordinary diversity of SD genes in fishes and rejects the idea of "limited options", even before new SD genes are identified in the remaining species. Moreover, as demonstrated by *irf9/sdY* in salmonids, novel SD genes can be recruited if they tightly interact with the conserved downstream sex-determination pathway (Yano et al., 2012). The diversity of SD genes in fish may promote rapid turnover of sex chromosomes and sex-determination systems. A possible scenario is described by the "birth-and-death" model, in which a new SD gene replaces or coexists with existing SD genes, leading to the emergence of novel sex chromosomes and a possible transition among sex-determination systems. As reported in medaka, the recruitment of new SD genes promotes the repeated turnover of sex chromosomes (Takehana, 2011).

The repetitive recruitment of *amh* and *amhr2* as SD genes and sex chromosome turnover in fishes

A small number of genes from the TGF-β pathway make up a significant portion of the SD gene list (Guiguen et al., 2018; Pan et al., 2021a, 2021b; Kitano et al., 2024). We specifically focus on the *amh/amhr2* genes here. Interestingly, *amh* and its receptor *amhr2* have been repeatedly recruited as SD genes in fishes (75/142, accounting for 52.82% of the species with identified SD gene), which has raised an intriguing question of why these genes take over the role of sex determination so frequently. The *amh/amhr2* genes first appear in cartilaginous fishes, in parallel with the emergence of the Mullerian duct (Adolfi et al., 2021). The function of Amh in female vertebrates is conserved and is mainly involved in inhibiting primordial follicle activation. Disruption of *amh* results in expedited recruitment of ovarian follicles, leading to early exhaustion of the oocyte reserve in mammals (Durlinger et al., 1999) and ovarian hypertrophy with significantly increased primary follicles in fishes (Liu et al., 2020). In male tetrapods, the main function of Amh is to induce the regression of Müllerian duct. Unlike tetrapods, teleost fishes do not have Müllerian ducts, but they do possess *amh* and *amhr2* genes. Oviducts in teleosts are derived from the extension of the gonad wall (Braungart, 1951). Mutation of *amhr2* in fish results in male-to-female sex reversal (Morinaga et al., 2007; Li et al., 2015; Zheng et al., 2022), rather than a female reproductive tract superimposed onto a male reproductive tract as it is in tetrapods (Behringer et al., 1994). It might be the disconnection of *amh/amhr2* and reproductive ducts that endows teleosts with tolerance to sex reversal. Therefore, recruiting *amh/amhr2* as SD genes can be a reuse of genes that are functionally redundant in male fish (Adolfi et al., 2019; Jeffries et al., 2022).

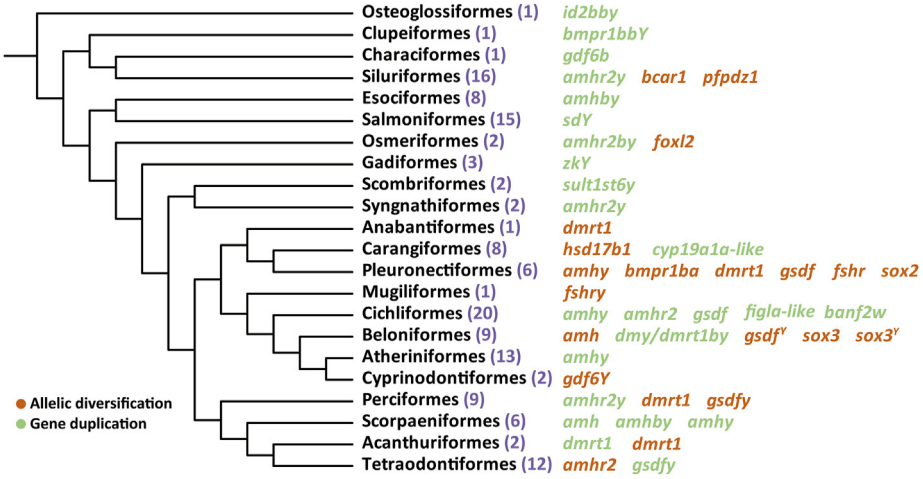

Fig. 2. SD genes mapped to a phylogenetic tree of teleost fishes. The SD genes and the mechanisms involved for their origin (highlighted in orange and green) were mapped on the tree adapted from The Fish Tree of Life (https://fishtreeoflife.org/downloads/).

Both *amh* and its receptor *amhr2* gene are widely expressed in the brain−pituitary−gonad axis (BPG axis) (Poonlaphdecha et al., 2011; Silva and Giacobini, 2021; Tumurbaatar et al., 2021), which is critical for regulating reproduction. The enormous coordinating ability demonstrated by the *amh/amhr2* signaling pathway, together with the ability shown by the BPG axis, provides new insights into the sex plasticity of fishes. In medaka, mutation of the *amhr2* gene results in excessive germ cell proliferation and male-to-female sex reversal (Morinaga et al., 2007). One possible mechanism by which *amhy/amhr2y* determines sex would be to reduce the number of germ cells, thereby prompting gonad to adopt testis fate, similar to *dmy* (Matsuda et al., 2007). Another possible mechanism is that *amhy/amhr2y* regulates the expression of aromatase, the key enzyme for estrogen synthesis in vertebrates. *Amhy* has been shown to inhibit the transcription of *cyp19a1a* through Amhr2/Smads signaling in XY Nile tilapia (Liu et al., 2022). The two mechanisms are not mutually exclusive. Although it is evident that *amh* and *amhr2* are repeatedly and independently recruited as SD genes, the detailed molecular mechanisms of *amh/amhr2* in sex determination and their relationship to the rapid turnover of sex chromosomes in fishes remain to be elucidated.

Models for sex chromosome turnover in fishes

Canonical models of sex chromosome turnover

Within the standard sex-determination systems, sex chromosome turnovers can be classified by whether the pattern of heterogamety is maintained (homogeneous transitions XY → XY or ZW → ZW) or not (heterogeneous transitions XY → ZW or ZW → XY). Also, the new SD allele can arise on the same chromosome pair (*cis*) or on a different chromosome pair (*trans*) (Vicoso, 2019; Meisel, 2020; Tao et al., 2021). Turnover of sex chromosomes can occur in at least four ways (Graves, 2016). (1) Turnover can occur if a new mutation usurps control of a sex-determination pathway from the ancestral SD gene (Meisel, 2020; Holborn et al., 2022). (2) Turnovers can be stimulated by hybridization between closely related populations with different sex chromosome systems (discussed below). (3) Turnover can arise by translocation of a pre-existing SD gene (Faber-Hammond et al., 2015; Bertho et al., 2021; Kabir et al., 2022). In three salmonid species, an RNA-mediated (retrotransposon) mechanism has been proposed for the translocation of the *sdY* locus (Faber-Hammond et al., 2015). In three species of the *Takifugu* genus, CACTA transposable elements (TEs) were reported to mediate the transposition of a *gsdfY* supergene, resulting in the turnover of sex chromosomes (Kabir et al., 2022). Comparative genomic analysis reveals that *amhr2y* on chromosome 24, the common SD gene of *Silurus* species including *S. meridionalis*, originates from a copy and paste of *amhr2* on chromosome 8, and transposition to chromosome 5 in *S. asotus* (Zheng et al., 2022, 2023; Wang et al., 2024). (4) Turnover of sex chromosomes can occur via chromosome-autosome fusions (Y-A, X-A, W-A, Z-A fusion) (Yoshida and Kitano, 2012; Bracewell and Bachtrog, 2020; Sember et al., 2021). Notably, Y-A fusion is predominant in the order Perciformes with multiple sex chromosomes. In *Gasterosteus wheatlandi* and *G. nipponicus*, the X1X2Y system evolved independently via Y-A fusion (Natri et al., 2013; Sardell et al., 2021). An X-A fusion has been observed in one karyotypic form of the erythrinid wolf fish *Hoplias malabaricus* and the knifefish *Gymnotus bahianus* (Almeida et al., 2015; de Oliveira et al., 2018).

Experimental models of sex chromosome turnover

Gene mutation

The bottom-up hypothesis posits that new sex-determination systems arise through the addition of new SD genes on top of the existing conserved downstream key actors in the sex-determination pathway (Wilkins, 1995; Herpin and Schartl, 2015; Adolfi et al., 2021). In an SD cascade evolving by the bottom-up process, new SD genes may also arise through duplication or modification of a downstream key actor (Schartl, 2004). The introduction of certain mutations in a downstream gene, for example, mutations in the *cis*-regulatory element in *gsdfY* of *O. luzonensis* (Myosho et al., 2012), a duplicated copy with insertion in the 3rd intron in *amhy* of *Odontesthes hatcheri* (Hattori et al., 2012), and a missense SNP in *amh2y* of *T. rubripes* (Kamiya et al., 2012), can be utilized to establish trimmed-down models.

Few discussions have been focused on the relationship between the trimmed-down model and gene mutation, especially the loss-of-function mutation of key downstream actors. Recently, functional studies in Nile tilapia have provided a new perspective for exploring the relationship between gene mutation and the rapid turnover of sex chromosomes and even the transition of sex-determination systems. For example, in Nile tilapia with the SD gene *amhy* on LG23, disruption of either *dmrt1* (LG12), *gsdf* (LG7), or *amhr2* (LG20) in XY fish leads to male-to-female sex reversal (Li et al., 2013; Jiang et al., 2016). Taking one of the genes as an example, continuous mating of $gsdf^{-/-}$ females with $gsdf^{+/-}$ males leads to the change of the SD gene from *amhy* (LG23) to *gsdf* (LG7) (XY→XY; Fig. 3A). In contrast, disruption of either *foxl2* (LG14) or *cyp19a1a* (LG1) in XX fish leads to female-to-male sex reversal (Zhang et al., 2017). Continuous mating of $cyp19a1a^{-/-}$ males with $cyp19a1a^{+/-}$ females leads to the transition of SD gene from *amhy* (LG23) to *cyp19a1a* (LG1) (XY→ZW; Fig. 3B). Disruption of *foxl2* or *cyp19a1a* in ZW fish leads to the change of SD gene from *banf2w* (LG3) to *foxl2* (LG14) or *cyp19a1a* (LG1) (ZW→ZW, Fig. 3C). Moreover, disruption of *dmrt1*, *gsdf*, or *amhr2* in ZZ fish leads to the change of SD gene from *banf2w* (LG3) to *dmrt1* (LG12), *gsdf* (LG7), or *amhr2* (LG20) (ZW→XY, Fig. 3D). Taken together, in XY systems, mutation of male pathway genes leads only to the turnover of SD genes and sex chromosome, while mutation of female pathway genes leads to the turnover of SD genes and sex chromosomes, as well as the transition of sex-determination system. In contrast, in ZW systems, mutation of male pathway genes leads to the turnover of SD genes and sex chromosomes, as well as the transition of sex-determination system, while mutation of female pathway genes only leads to the turnover of SD genes and sex chromosomes. Our mutational analysis has reconstructed an explicit transitional process of the SD gene, sex chromosome, and sex-determination system based on the SD cascade. Consistent with our experimental results, it has been hypothesized that an XY sex-determination system exists in Carangidae fishes and that an X-linked mutation in the ancestral *hsd17b1* gene creates a new female determiner (or W-linked allele) and leads to turnover from an XY to ZW system (Fan et al., 2021). The frequent *in situ* turnovers between sex chromosomes in the carangid fishes are consistent with our empirical evidence that mutation of key downstream actors leads to the rapid turnover of sex chromosomes and even the transition of sex-determination systems. Under natural conditions, the formation of SD genes must have been achieved via gene mutation. The turnover of sex-determination genes, sex chromosomes, and even sex-determination systems demonstrated by gene mutation in experimental condition mimics, to some extent, the evolution of sex determination in nature, but with different time scales.

Hybridization

An intriguing scenario is that the hybridization of species with different sex chromosomes or sex-determination systems leads to the emergence of a neo-sex chromosome. In the Japanese frog *Glandirana rugosa*, two different heteromorphic sex chromosome systems, XY (Chr.7) and ZW (Chr.7), are adopted in different geographic populations. These two systems arose via hybridization

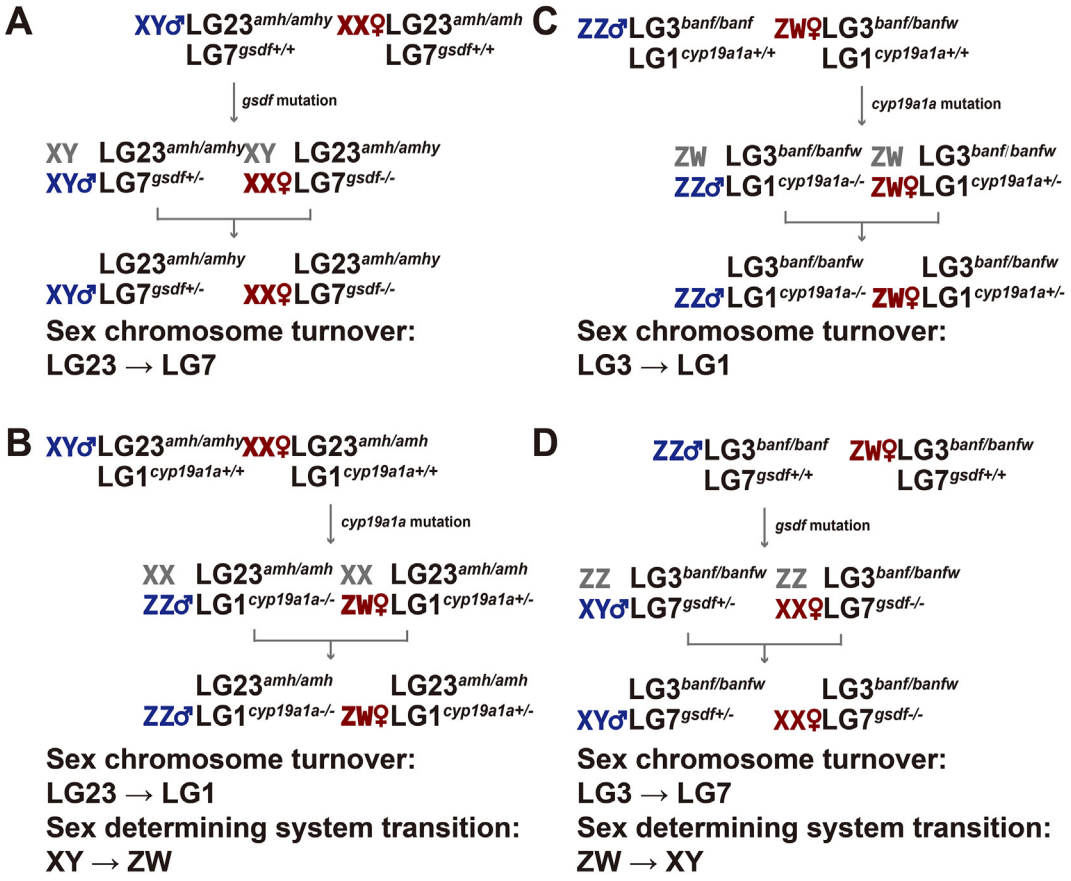

Fig. 3. Sex determination system and sex chromosome turnovers driven by gene mutation. **A**: Disruption of *gsdf* in *O. niloticus* leads to the transition of sex chromosome from LG23 to LG7. **B**: Disruption of *cyp19a1a* in *O. niloticus* leads to the transition of sex chromosome from LG23 to LG1, and the sex determination system from XY to ZW. **C**: Disruption of *cyp19a1a* in *O. aureus* leads to the transition of sex chromosome from LG3 to LG1. **D**: Disruption of *gsdf* in *O. aureus* leads to the transition of sex chromosome from LG3 to LG7, and the sex determination system from ZW to XY.

between two ancestral lineages of West Japan (XY, Chr.1) and East Japan (XY, Chr.3) populations, indicating that cosegregation of the two ancestral sex chromosomes induced turnover to a new one in their hybrids (Miura et al., 2022). A potentially ongoing sex chromosome turnover has been suggested in admixed marine populations of nine-spined sticklebacks (*Pungitius pungitius*), where the evolutionarily younger and homomorphic LG3 replaces the older and heteromorphic sex chromosome LG12 (Yi et al., 2024). Frequent turnover of sex chromosomes can also be achieved experimentally via the hybridization of fish species. Nile tilapia (XY, with *amhy* as SD gene on LG23) and blue tilapia (ZW, with *banfw* as SD gene on LG3) are two closely related species with different sex-determination systems. Interestingly, *figla-like* on LG1 has been identified as the Y-linked SD gene of several tilapia species (Curzon et al., 2022). It is also detected on LG1 of both ZZ and ZW blue tilapia, but absent on LG1 of both XX and XY Nile tilapia. Epistasis in sex determination has been observed in the order of LG23Y > LG3W > LG1Y > X, which is typically correlated to the hierarchy of SD genes of *amhy*, *banfw*, and *figla-like* in hybridization. In the hybrid fish and backcross progeny, all fish with the male SD gene *figla-like* on LG1 are males, indicating that this male locus takes over the role of sex determination in ZX hybrids (Fig. 4A). Because the W locus on LG3 and Y locus on LG23 are not included in this hybrid, epistatic interaction from W and Y is absent, and the role of *figla-like* in sex determination could be observed. Similarly, hybridization and backcross between a female *X. maculatus* (XX, LG21) and a male *X. hellerii* (ZZ, LG21) demonstrates the evolution of a neo-W chromosome (LG2) in hybrid offspring (Fig. 4B) (Franchini et al., 2018). These experiments mimic the evolutionary scenario that has potentially led to the transition of the sex-determination system, sex chromosome, and SD gene.

Four possible mechanisms have been proposed to explain why hybridization could lead to sex chromosome turnover. (1) Hybridization frequently activates TEs, which could lead to translocation of the sex determiner. Interspecific hybridization-induced TE reactivation plays a recurring role in enabling the rapid copying and pasting of *cis*-regulatory sequences (Michalak, 2009; Chuong et al., 2017). (2) Hybridization destabilizes the existing sex-determination systems and promotes sex chromosome turnover (Sember et al., 2021). For example, during the hybridization of *O. curvinotus* and *O. latipes*, skewed sex ratios have been observed in F1 hybrids by the interference of different SD genes of these two species (Shinomiya et al., 2006). (3) Hybridization promotes the evolution of unstable polygenic sex determination, a transient state during the turnover of sex-determination systems from one monogenic system to another after hybridization. For example, the co-existence of male- and female-associated loci mapped to different LGs has been reported in hybrids of *Clarias microcephalus* and *C. gariepinus* (Nguyen et al., 2022). (4) Hybridization promotes the introgression of sex chromosomes from other species. The young Y chromosome in *P. pungitius* has been demonstrated to be established by introgression from the Amur stickleback, *P. sinensis*, because a large shared region is identified on the Y chromosome of *P. pungitius* and the homologous chromosome in *P. sinensis* (Dixon et al., 2019).

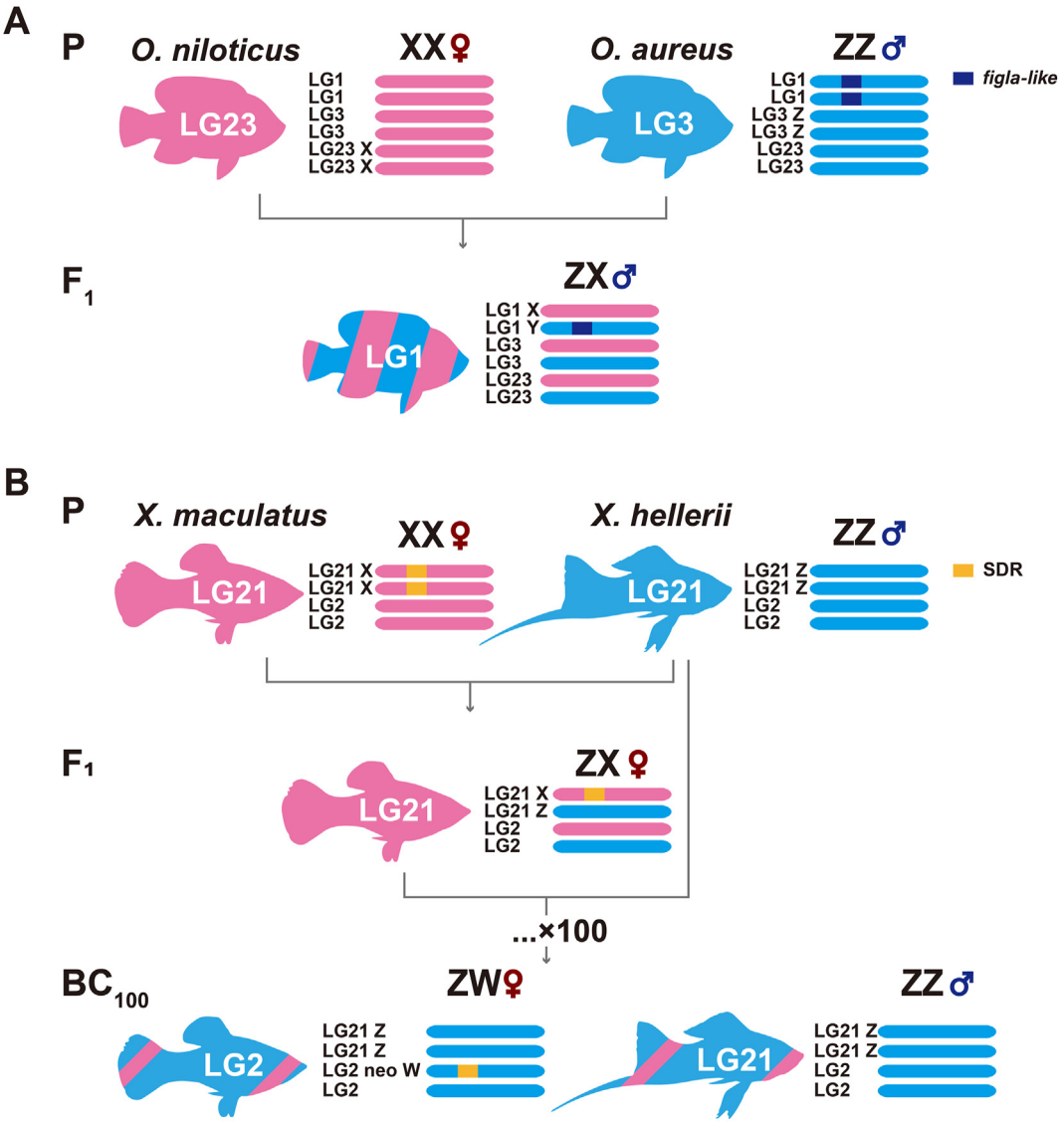

Fig. 4. Sex-determination system and sex chromosome turnovers driven by hybridization. **A**: Sex chromosome turnovers occur in the hybridization and backcross between female *O. niloticus* and male *O. aureus*. Female *O. niloticus* (XX) uses LG23 as sex chromosome and male *O. aureus* (ZZ) uses LG3 as sex chromosome. The F1 hybrids (ZX) are males with LG1 as sex chromosome. Continuous backcross of ZX male with XX female results in the sex chromosome turnover from LG23 and LG3 to LG1. **B**: Sex chromosome turnovers occur in the hybridization and backcross between male *X. hellerii* and female *X. maculatus*. A translocation event of a genomic region on X chromosome of *X. maculatus* has contributed to the turnover of the sex chromosome from LG21 to LG2 and transition of sex-determination systems in the hybrids during the backcross experiment (Franchini et al., 2018).

Sex chromosomes and speciation

Canonical theories on the role of sex chromosomes in speciation

The central role of sex chromosomes in the process of speciation has been widely accepted for organisms with differentiated sex chromosomes. Most work in this area has centered on "two rules of speciation," namely the Haldane's rule (Haldane, 1922) and the large X-effect (Coyne and Orr, 2004). Haldane's rule states the preferential sterility or inviability of hybrids of the heterogametic sex rather than the homogametic sex due to a genetic imbalance. The large-X effect rule states the hemizygous nature of the X chromosome, leading to exposure of recessive hybrid incompatibilities in the heterogametic sex, in which male genes are supposed to evolve faster due to sexual selection, resulting in male sterility in male heterogametic taxa (Wu and Davis, 1993). These two rules imply a special role of sex chromosomes in species with highly differentiated sex chromosomes. Moreover, when new sex chromosome systems replace old ones, it sometimes results in temporary incompatibilities that inhibit rather than promote hybridization (Xue et al., 2024). However, species with poorly differentiated sex chromosomes have also provided support for Haldane's rule and the large X effect rule. F_2-hybrids from females of *Pundamilia pundamilia* and *P. nyererei* crossed with males of *Neochromis omnicaeruleus* showed a female-biased sex ratio (Svensson et al., 2016). In medaka, *O. latipes* and *O. curvinotus* share the same XY system and SD gene *dmy/dmrt1bY*, but their XY male hybrids are sterile due to meiosis arrest without producing sperm (Martinez-Bengochea et al., 2022). In threespine stickleback (*G. aculeatus*), the testes of F_1 hybrid males from a cross between a female from the Japan Sea lineage and a male from the Pacific/Atlantic Ocean lineage lack mature sperm (Kitano et al., 2009). These cases are probably due to intrinsic postzygotic incompatibilities in hybrids. Nevertheless, both heteromorphic and homomorphic sex chromosomes may accelerate speciation through reproductive isolation caused by reduced fertility or impaired development.

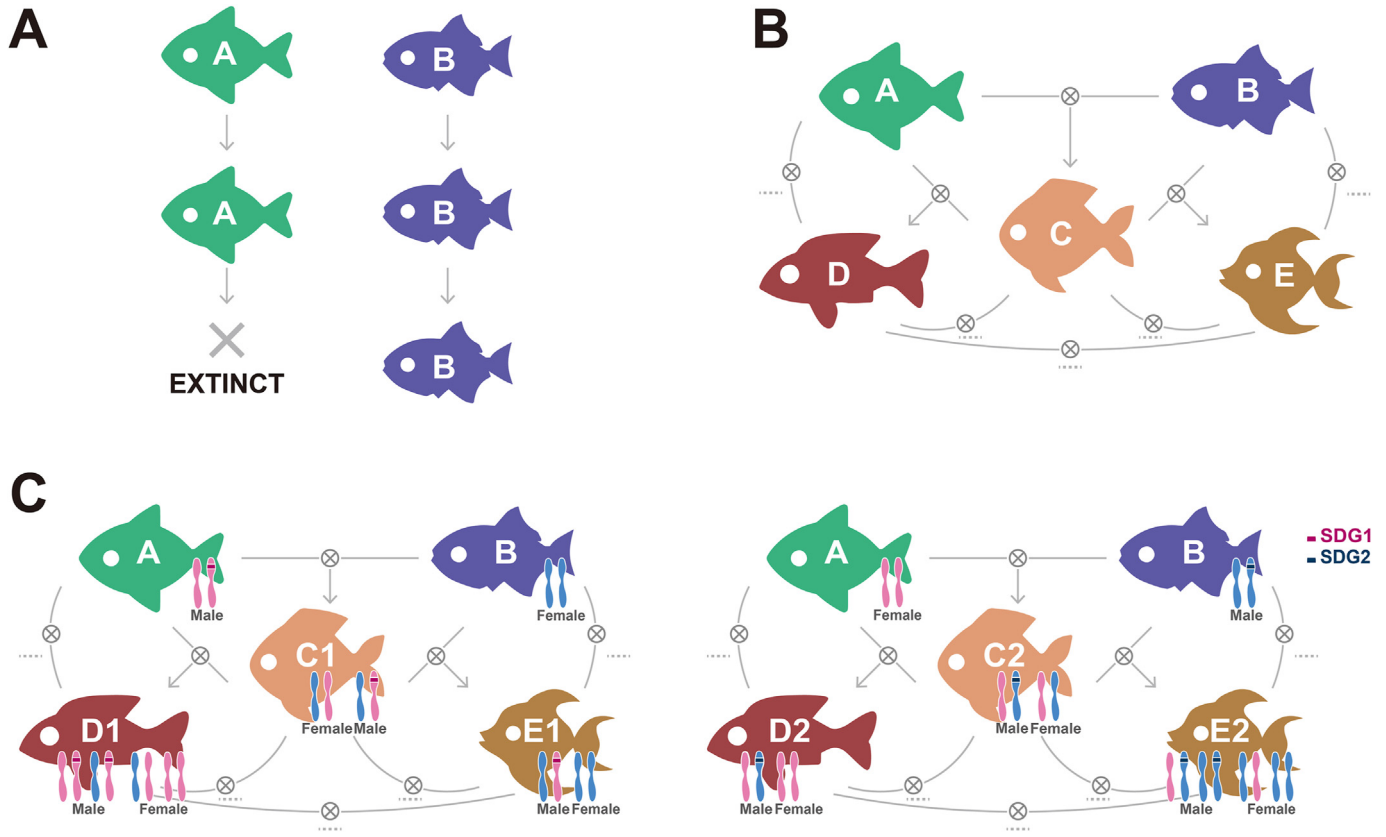

Fig. 5. Sex chromosomes facilitate speciation via interspecific hybridization. **A**: Clades lacking the ability to engage in interspecific hybridization experience a decrease in diversity. **B**: Clades with the ability to hybridize exhibited an increase in biodiversity. **C**: Sex chromosomes multiply biodiversity by randomly combining with autosomes. When two different sex chromosomes are present, the hybridization results in a doubling effect of (**B**) as illustrated in left and right panel of (**C**). Colors of fishes and chromosomes represent autosome constitutions and different sex chromosomes, respectively. Capital letters inside the fish images represent species.

Sex chromosomes may accelerate speciation by multiplying genotypes via hybridization in fishes

Sex chromosomes evolve by inversions, mutation accumulation, and chromosome rearrangements similar to the genetic changes that accumulate during speciation, but the relationship between sex chromosomes and speciation is unclear in fishes. Cichlids, with their extraordinary diversity of sex chromosomes (El Taher et al., 2021; Tao et al., 2021) and biodiversity in a variety of features (size, color, body shape, behavior, etc.), are an ideal model to study the role of sex chromosome turnover and speciation (Sabbah et al., 2010; Koblmüller et al., 2019). Intriguingly, during the rapid adaptive radiation, cichlids from Lake Tanganyika and Victoria show the highest rates of sex chromosome turnover known to date in fishes (El Taher et al., 2021; Kocher et al., 2022). At least 12 sex chromosomes have been reported among populations of 79 species. Some of these may represent ancestral polymorphisms, while others may have been shared among species or strains due to hybridization. For example, hybridization of ancient lineages is believed to create the diverse gene pool from which the Lake Victoria cichlid radiation is then selected (Meier et al., 2017, 2023). Repeated hybridization has promoted the divergence of *Oryzias* ricefishes on Sulawesi (Mandagi et al., 2021). At the macro level, clades with more species are strongly driven by hybridization to produce new species more quickly. Hybridization is frequently observed in fishes that rapidly diversify from a single ancestral species into many descendant lineages that occupy different ecological niches (Hubbs, 1955; Kocher, 2004; Svardal et al., 2020). At the micro level, in clades with a variety of sex chromosomes, the combination of sex chromosomes and autosomes will be more diverse during interspecific hybridization, which multiplies biodiversity (Fig. 5). Interspecific hybridization facilitates the creation of new combinations of sex chromosomes and autosomes, multiplying the number of genotypes and creating new genome variations.

Future directions and perspectives

Although increasing evidence suggests that sex chromosomes have evolved independently many times throughout fish evolution, our understanding of the processes driving the turnover of sex chromosomes and their relationship to speciation is still limited. In contrast to mammals and birds, SD genes in fishes are diverse. How many genes could become SD genes? How do these SD genes crosstalk with the canonical male and female pathway genes? Why are *amh* and *amhr2* widely recruited as SD genes in more than half of the fish species with identified SD genes? The undifferentiated sex chromosomes and rapid sex chromosome turnover in most fishes represent an interesting deviation from the classical pattern of sex chromosome differentiation in mammals and birds. Are there any new theoretical models that can better explain the evolution of fish sex chromosomes? What is the exact role of sex chromosomes in fish speciation? Fortunately, the integration of multi-omics data and gene editing makes it possible to untangle these questions in the near future. The affordability and accessibility of new sequencing technologies have allowed the assembly of many fish genomes (Ramos and Antunes, 2022), contributing to a more comprehensive understanding of sex chromosome evolution. The gene editing techniques that have been established in an increasing number of fishes are

helping to decipher the function and regulation of SD genes. Functional and evolutionary studies at the population level are required to answer the question of to what extent our experimental models mimic the evolutionary trajectories of sex chromosome turnover induced by gene mutation or hybridization. Adaptation, which serves as a stepping stone to ecological speciation, plays a crucial role in shaping species diversity and facilitating range expansion. Consequently, if improved fitness is observed at the population level associated with the turnover of SD genes, sex chromosomes, and sex-determination systems, these advantageous mutations are more likely to be fixed in the population rather than the neutral expectation. Addressing these questions will truly take us towards a comprehensive and holistic understanding of sex chromosome turnover and biodiversity in fishes.

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Supplementary data

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