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JB Review

Sex chromosome cycle as a mechanism of stable sex determination

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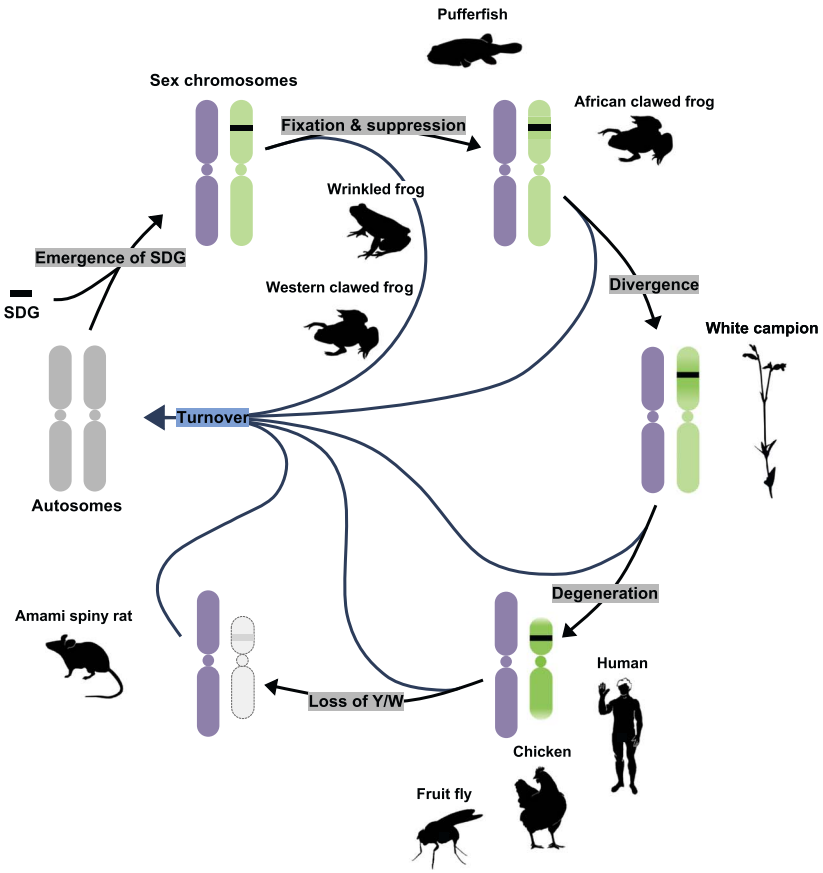
Recent advances in DNA sequencing technology have enabled the precise decoding of genomes in non-model organisms, providing a basis for unraveling the patterns and mechanisms of sex chromosome evolution. Studies of different species have yielded conflicting results regarding the traditional theory that sex chromosomes evolve from autosomes via the accumulation of deleterious mutations and degeneration of the Y (or W) chromosome. The concept of the 'sex chromosome cycle,' emerging from this context, posits that at any stage of the cycle (i.e., differentiation, degeneration, or loss), sex chromosome turnover can occur while maintaining stable sex determination. Thus, understanding the mechanisms that drive both the persistence and turnover of sex chromosomes at each stage of the cycle is crucial. In this review, we integrate recent findings on the mechanisms underlying maintenance and turnover, with a special focus on several organisms having

Graphical Abstract



unique sex chromosomes. Our review suggests that the diversity of sex chromosomes in the maintenance of stable sex determination is underappreciated and emphasizes the need for more research on the sex chromosome cycle.

Keywords: recombination suppression, sex chromosome loss, sex chromosome turnover, sex determination, transposable elements.

Abbreviations: MSY, male-specific region of the Y; Mya, million years ago; PAR, pseudoautosomal region; SDGs, sex-determining genes; TEs, transposable elements.

Sex chromosomes are one of the most successful systems for sex determination and have emerged in many lineages independently *(1)*. In this system, the ratio of females to males can be stably maintained at approximately 1:1, unless segregation distortion occurs at meiosis; this is a potential advantage over other mechanisms of sex determination. However, once sex chromosomes emerge from a pair of autosomes, the Y (and W) chromosome (Y and W, hereafter, respectively) starts to degenerate and lose genes due to the suppression of recombination with the X (and Z) chromosome (X and Z, hereafter, respectively) *(2)*. If this process continues, the Y (and W) may eventually disappear, which could disrupt sex determination and result in species extinction. Therefore, using sex chromosomes for sex determination is a kind of double-edged sword. However, the successful diversification of species with sex chromosomes suggests that there are mechanisms to avoid this risk of extinction.

In this context, the concept of the 'sex chromosome cycle' has recently been proposed (Fig. 1) *(3)*. In this concept, sex chromosomes can be reverted to autosomes at any stage in chromosome evolution, and new sex chromosomes take over the function of sex determination. In addition, a variety of mechanisms have evolved to avoid the loss of sexes with the continuous utilization of sex chromosomes at each stage of the cycle: (i) emergence of a sex-determining gene (SDG), (ii) establishment of recombination suppression, (iii) massive loss of Y-linked genes, (iv) loss of the Y and (v) sex chromosome turnover.

In this review, we summarize recent findings and discuss the molecular and genetic bases of stable sex determination using sex chromosomes. We also discuss the evolutionary trajectories of sex chromosomes by which organisms avoid the loss of sex determination.

How Are New Sex-Determining Genes and Sex Chromosomes Established?

In most species, genetic sex is determined by a master SDG on a sex chromosome. In evolution, SDGs have been replaced with new ones on different chromosomes in different lineages. A new sex chromosome can occur through the translocation of a preexisting SDG or sex-determining region to an autosome (Fig. 2a, b). This process has been reported in salmonid fish, *Takifugu*, octoploid strawberries, poplar plants and houseflies; however, reports are limited owing to the narrow range of taxa for which SDG information is available *(4–8)*. Alternatively, a new sex chromosome can form through the emergence of a novel SDG, either on the sex chromosome or another chromosome. This process has been documented in various taxa, including mammals, amphibians, fishes and angiosperms.

The establishment of novel SDGs generally falls into two modes: gene duplication and allelic diversification (Fig. 2b). While exceptions exist, the establishment of SDGs through these two modes generally involves sex-related genes that function in the sex determination or sexual differentiation cascades. For these sex-related genes to transform into master sex determinants, changes in protein functions and/or gene expression are required. In *Takifugu rubripes*, *Amhr2* (anti-Mullerian hormone receptor type 2) underwent a functional change due to a single nucleotide mutation within a kinase domain, leading to the establishment of a sex-determining allele *(9)*. Remarkably, many recently discovered SDGs in fishes arose from changes in spatiotemporal patterns of gene expression. Changes in gene expression are less likely to be deleterious than functional alterations involving amino-acid substitutions and therefore might be more likely to contribute to the emergence of new SDGs. It should be mentioned that gene duplication itself can affect gene dosage and, consequently, gene expression levels.

Regarding the emergence and evolution of sex chromosomes and SDGs, transposable elements (TEs) have recently been recognized as key factors (Fig. 2c). For instance, TEs can mediate the translocation of preexisting SDGs to autosomes and the duplication of sex-related genes *(5–7)*. Additionally, TE insertion near sex-related genes may establish a sex-specific cascade of gene expression. Indeed, TEs likely influence the expression of the candidate SDG *gsdfY* (gonadal soma-derived factor on the Y) of sablefish *(10)*. In fighting fish, TE-induced epigenetic silencing affects the expression of *dmrt1* (doublesex and mab-3 related transcription factor 1) on the X *(11)*. In mice, a DNA fragment derived from an insertion of the L3 retrotransposon became a second exon in a well-known male-determining gene, *Sry* (sex-determining region Y). This alteration enables the formation of a degron (degradation)-free variant of the *Sry-T* transcript, which is essential for male determination *(12)*. In this case, the insertion of the L3 retrotransposon likely contributed to the maintenance of sex determination, rather than the generation of an SDG. As an additional example, we here illustrate the contribution of TEs to the establishment of the SDG *dm-W* in African clawed frog.

Chimeric SDG, dm-W, driven by TEs in the allotetraploid ancestor of the genus Xenopus

The W-linked SDG *dm-W* in *Xenopus laevis* acts as an anti-masculinizing factor, inducing ovarian development in ZW gonads *(13, 14)*. Approximately 17–18 million years ago (Mya), the subgenus *Xenopus* experienced hybridization, leading to allotetraploidization and two subgenomes, L and S, derived from each diploid ancestor *(15)*. The *dm-W* on the L subgenome evolved from *dmrt1* (*dmrt1.S*) on the S subgenome. *dm-W* originated after allotetraploidization but before the diversification of extant allotetraploid *Xenopus* species *(16)*. *dm-W* contains four exons, including *dmrt1.S*-derived exons 2 and 3 as well as exons 1 and 4 with unknown origin.

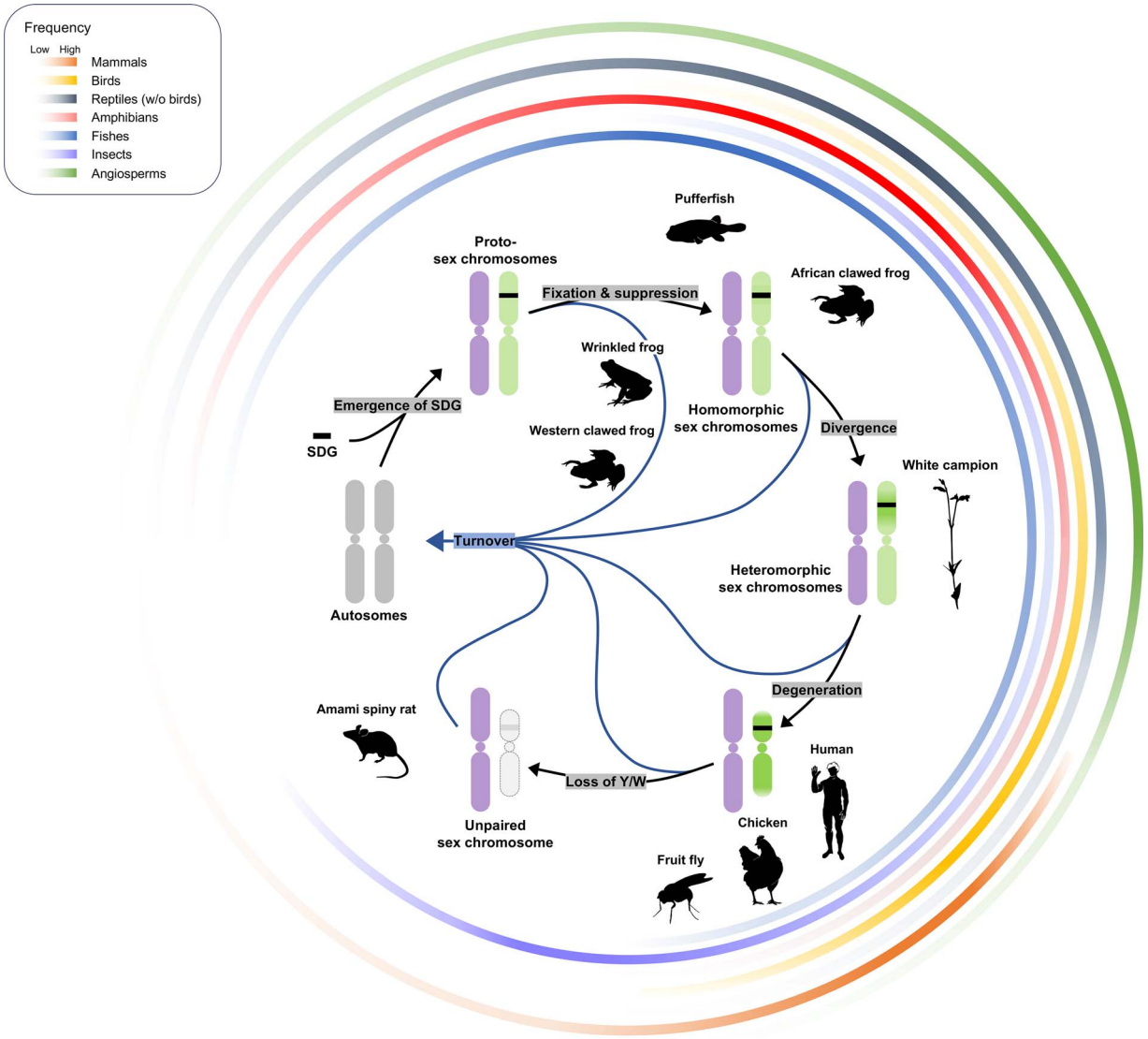


Fig. 1. Sex chromosome cycle (inside) and rough frequency of species in seven groups of organisms at each stage of the cycle (outside). The sex chromosome cycle illustrates the evolutionary process where new sex chromosomes emerge, differentiate, degenerate and eventually disappear (circumferential black arrows). In the cycle, sex chromosome turnover can occur at any stage while maintaining stable sex determination (inside blue arrows). Colors corresponding to each taxonomic group are shown in the top left. Color intensity roughly corresponds to the relative frequency of species at the stage of the sex chromosome cycle in each group. The illustration was originally inspired by Furman et al. *(3)*.

Recently, exon 4 of *dm-W* was reported to originate from the DNA transposon *hAT-10* family *(17)*. Intriguingly, a noncoding portion of *hAT-10* was transformed into the functional coding sequence of exon 4, encoding the C-terminal region of DM-W. This exon 4-derived domain likely enhanced the DNA-binding ability of the DM domain encoded by exons 2 and 3. This molecular evolution from a noncoding to a coding sequence was critical in the emergence of *dm-W* as an SDG, with the *hAT-10*-derived sequence contributing significantly to the neofunctionalization of ancestral *dm-W*. Additionally, noncoding exon 1 and its TATA-type promoter were newly formed in the common ancestor of allotetraploid *Xenopus* species *(18)*. TEs account for approximately 87% of this surrounding region, suggesting that they were involved in the generation of promoter and/or enhancer regions.

Behind the emergence of the chimeric SDG *dm-W*, the TEs that were activated due to hybridization likely triggered three events: a partial duplication of *dmrt1*, promoter/enhancer generation, and neofunctionalization *(17–19)*. Furthermore, TEs that accumulated in the W- and Z-specific regions during the early differentiation of sex chromosomes contributed to the expansion of regions of recombination suppression *(16)*. These findings clearly illustrate that TEs contribute to the establishment and maintenance of SDGs by generating duplicated sex-related genes, providing *cis*-elements, and/or generating partial protein-coding sequences (Fig. 2c). The contribution of TEs to the generation of SDGs and evolution of sex chromosomes is likely underestimated owing to their fast rate of evolution and difficulty in sequencing.

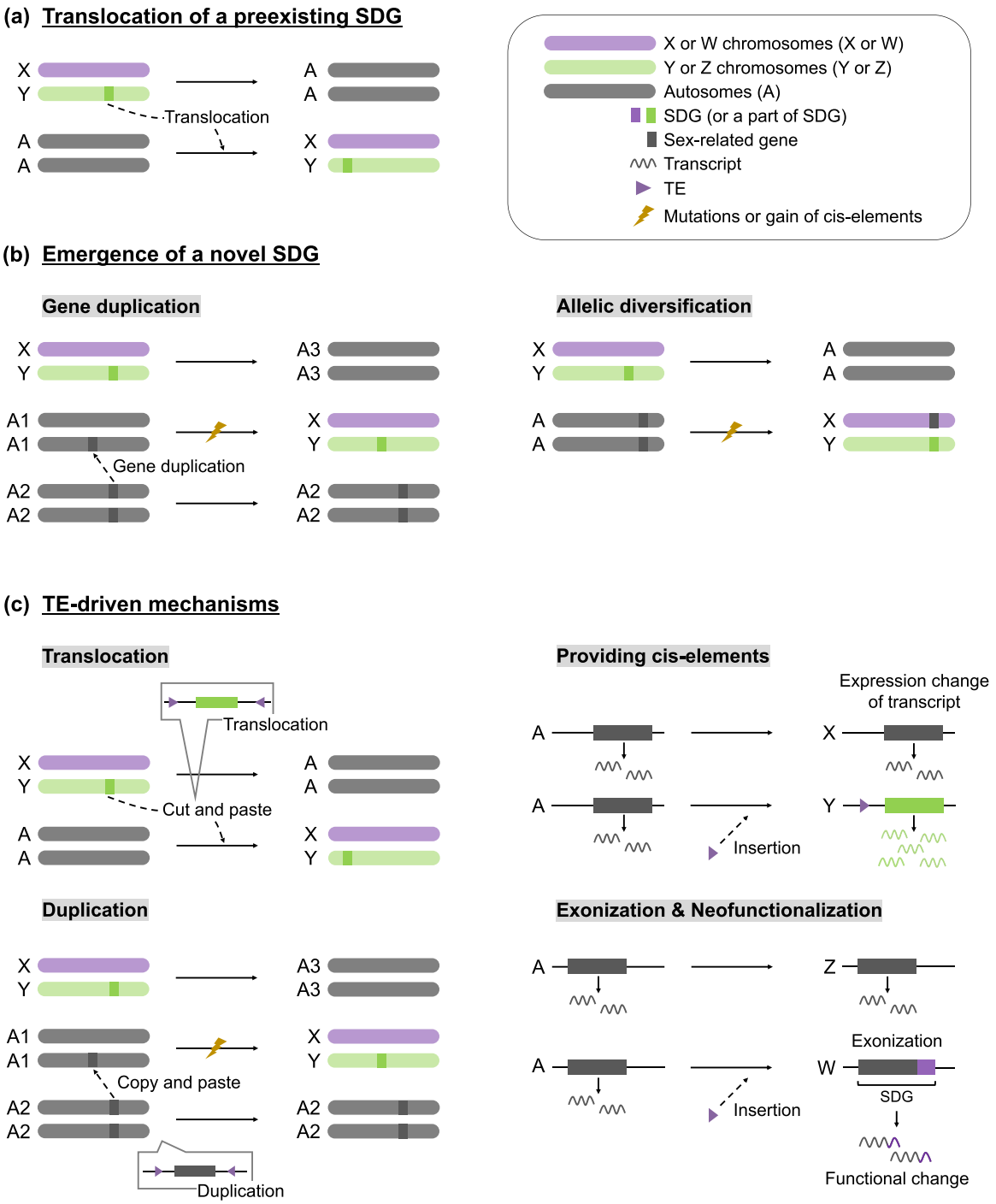


Fig. 2. Mechanisms underlying the emergence of new SDGs and sex chromosomes. (a, b) Overview of the general processes of sex chromosome replacement and the establishment of SDGs. (c) Mechanisms underlying new SDG emergence driven by TEs. Left: TE insertions near an preexisting SDG or a sex-related gene triggering translocation or gene duplication through its transposition mechanisms. Top right: TE insertion into an allele of a sex-related gene creates sex differences in gene expression. Bottom right: Gene duplication followed by TE insertion and exonization lead to the emergence of an SDG with a new function.

How Have Sex Chromosomes Differentiated?

Many organisms possess sex chromosomes that exhibit varying degrees of differentiation, ranging from homomorphic to highly divergent. For instance, homomorphic sex chromosomes with a small sex-determining region have been observed in several unrelated fish species *(5, 20)* and in certain plant species *(21–24)*. On the other hand, birds, mammals and some dioecious plants have large heteromorphic sex chromosomes *(25–27)*. It is important to note that the variation in the extent of sex chromosome divergence does not merely reflect the history or time course of sex chromosome evolution (Fig. 3). For example, young heteromorphic systems and old homomorphic systems have been reported in the *Drosophila (54)* and the *Python (57)*

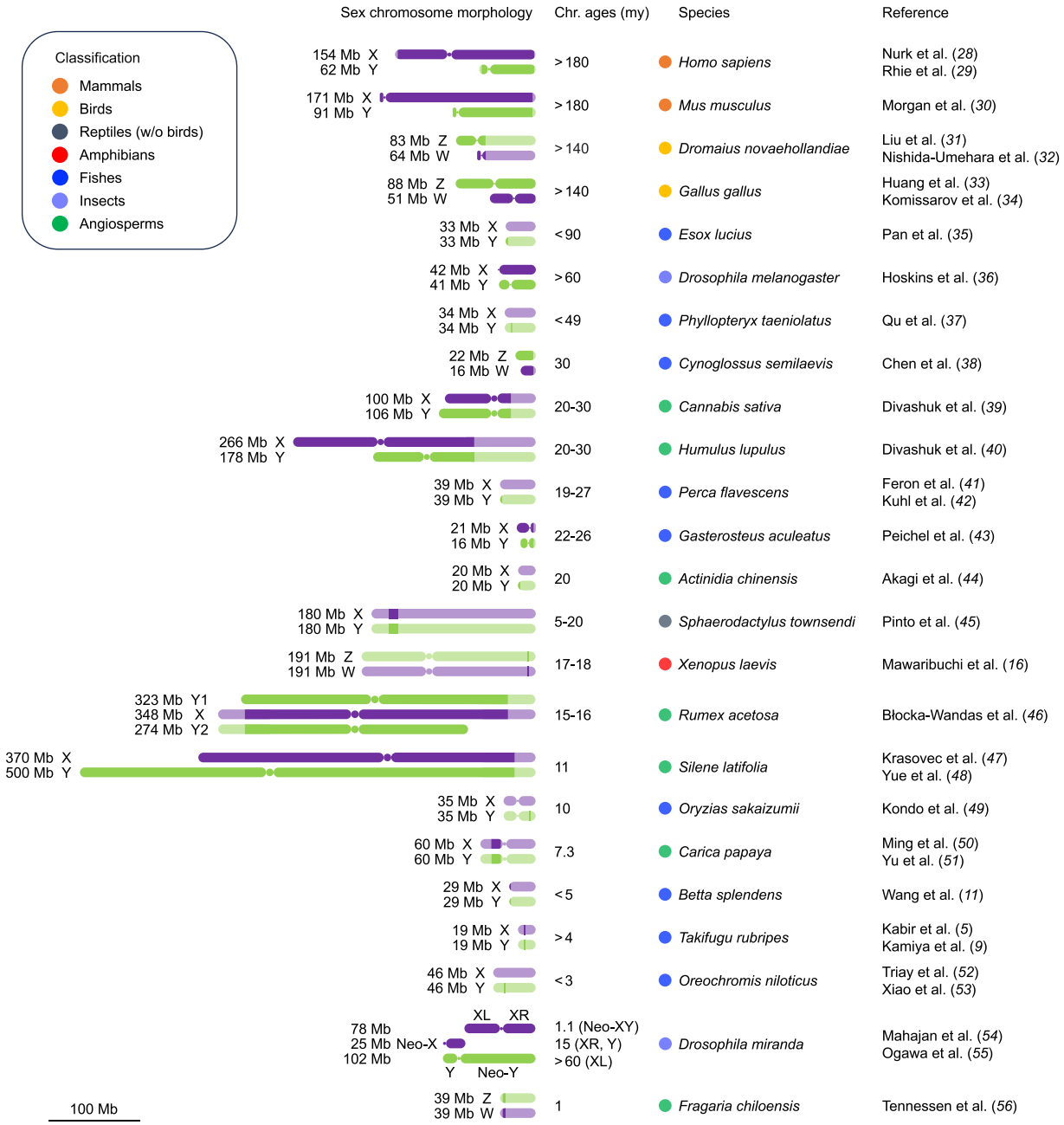


Fig. 3. Ages and divergence of sex chromosomes in 24 species. The figure displays sex chromosome information of various species in order of their ages, from the oldest to the youngest ones, demonstrating that the degree of sex chromosome differentiation does not solely depend on time after emergence. Dark and light colors represent the recombination suppression regions and the PARs, respectively. Colors of filled circles corresponding to each taxonomic group are shown in the top left. Chromosome sizes were estimated based on cytological or sequencing data in the individual references. Chr, chromosome; my, million years.

species, respectively. Nevertheless, the differentiation of sex chromosomes is an inevitable process.

Variation in rates of differentiation can be attributed to several factors *(58, 59)*. The age of sex chromosomes is a straightforward determinant of the extent of differentiation. Additionally, the relative length of the haploid phase may influence the rate of sex chromosome divergence. For instance, plants with a longer and more intricate haploid phase experience strong haplotype-specific purifying selection for Y- or W-linked genes. The dioecious plant *Silene latifolia* exhibits a slower rate of differentiation per generation compared with those of certain animal species *(47)*. Divergence between the X and Y (or Z and W) can be explained by various factors. Sexual conflict is often considered a driver of sex chromosome differentiation *(60–62)*. In this model, the increment of linkage disequilibrium between sexually antagonistic loci and sex-determining loci generates genetic disequilibrium, leading to the resolution of intragenomic conflict and expansion of the non-recombining region. However, the role of sexual conflict in the expansion of the non-recombining region remains largely unknown owing to the difficulty

of directly identifying sexually antagonistic genes. Several alternative models have been proposed. For example, the incomplete evolution of dosage compensation can subject Y- and W-linked dosage-sensitive genes to purifying selection, slowing down sex chromosome divergence *(63, 64)*. Another model involves neutral evolution, where the balance among the mutation frequency, recombination rate, and effective population size results in the expansion of the non-recombining region independent of purifying selection *(65)*. Another hypothesis suggests that linkage to the sex-determining region prevents new deleterious mutations from becoming homozygous in the Y in males (or the W in females). Furthermore, sex reversal can impede sex chromosome divergence. In some species, there is discordance between phenotypic and genotypic sex, wherein recombination occurs throughout the sex chromosomes, inhibiting sex chromosome divergence *(66–69)*. These factors collectively contribute to the differentiation of certain sex chromosomes. Here, we introduce the process of sex chromosome differentiation, focusing on a dioecious plant, *S. latifolia*, which has been a focus of recent research.

Evolutionary traces of differentiated chromosomes in S. latifolia

The sex chromosomes in the dioecious plant *S. latifolia* are heteromorphic at the differentiated stage with recombination suppression. These sex chromosomes exhibit size variation, with the Y (approximately 570 Mb in size) being 1.4 times larger than the X *(70, 71)*. The Y is predominantly composed of a non-recombining region *(72)*. A recent study of the female genome revealed that recombination cessation was triggered by an inversion in a distal region on the proto-X, leading to the formation of stratum 1 *(48)*. Note that evolutionary strata are defined as chromosome regions, each of which shows a similar level of sequence divergence between the X and Y, reflecting the times after recombination suppression. Intriguingly, the majority of pericentromeric regions of the X and autosomes (∼90%) exhibit minimal recombination between homologous chromosomes, likely contributing to the establishment of stratum 2 *(48)*. Furthermore, the recently identified stratum 3 appears to have developed gradually *(72)*. These findings suggest that sexually antagonistic selection may not be the primary cause for the expansion of this extensive non-recombining region on the Y.

Recent insight into the evolution of the sex determination system in *S. latifolia* was based on the identification of a SDG, *GSFY*, located in the extensive non-recombining region *(73)*. *GSFY* is one of two SDGs in this plant: a gynoecium suppression factor and stamen promotion factor. It is an ortholog of the *Arabidopsis CLAVATA 3* gene, which suppresses gynoecium development *(73–75)*. The X carries *GSFX*, a gametolog of *GSFY* that has undergone non-functionalization, suggesting that the non-functionalization of the X copy played a role in establishing the sex determination system. Notably, *SlWUS1*, an ortholog of *Arabidopsis WUSCHEL,* which promotes the enlargement of the gynoecium, is in stratum 1 of the X but is absent from the Y *(48, 76)*. This observation indicates that the X functions in sex determination and female enlargement, suggesting that X chromosome evolution led to improved female fitness. Furthermore, even if *GSFY* were to become non-functional, sex determination could still be achieved by altering the activity of *SlWUS1*, which may be an excellent example of a mechanism to avoid the loss of female fitness *(76)*.

Do Important Genes on the Y and W Chromosome Prevent the Loss of the Chromosomes?

The differentiation of sex chromosomes eventually results in the degeneration of the Y and W, although the extent and rate of degeneration vary considerably among taxa (Fig. 3). Indeed, most sex chromosomes that emerged more than 100 Mya are fully differentiated, with extensive gene loss on the Y and W. Mammalian Ys and avian Ws are typical examples of degenerated sex chromosomes *(77)*. Although species in Palaeognathae, such as emu, are exceptions *(31, 78)*, many mammals and birds have lost a majority of genes on the Y and W, respectively. In species with a degenerated Y and W, the functions of these chromosomes have become specialized for sex determination. Although it has been predicted that some mammalian species will lose their Y in the future *(79)*, the loss of Y is rare *(80, 81)*, and there are no reports of the loss of the W in birds, suggesting that the complete loss of the Y and W is quite unusual. In many species, the rate of degeneration of the Y or W is thought to be slowing down. An example of the decreasing rate of degeneration can be seen in a comparison of the human and rhesus macaque Y. Genes on the human Y were rapidly lost just after its birth about 180 Mya. However, only one gene was lost in the last 25 million years in the human lineage after the divergence from the lineage leading to rhesus macaque, indicating a significant decrease in the rate of degeneration in terms of gene number *(82)*. This means that unnecessary genes on the Y and W were lost before 25 Mya, while functionally important genes have been retained. In this section, we discuss the features and fate of the degenerated Y and W by focusing on the remaining genes in human and chicken. For simplicity, we define important genes as those that are essential for the survival of cultured human cells. Essential genes in human cultured cells have been roughly identified by screening with CRISPR-Cas9 gRNA libraries *(83–85)*. In the cases of KBM7 and HAP1 cells, which are nearly haploid with a single X but lacking a Y, 2,054 out of 17,465 genes (11.8%) and 2,181 out of 17,465 genes (12.5%) were identified as essential for viability, respectively.

Genes on the human Y chromosome

The human Y contains ∼70 genes; however, they include multi-copy genes (such as the *TSPY* or *RBMY* gene family), newly acquired genes resulting from the human-specific X-transposed region, and genes in the pseudoautosomal region (PAR), which has not been affected by Y degeneration *(86)*. After clustering multi-copy genes and excluding newly acquired and PAR genes, only 17 genes remain as gametologs in the MSY (male-specific region of the Y) (Supplementary Table S1). Since the Y is lost in nearly haploid cells, viability depends on gametologs on the X, which may result in synthetic lethality if they are lost concurrently. Among 17 gametologs, four (*RPS4X*, *USP9X*,

Table 1. Sex chromosome types in vertebrates, insects and angiosperms

Taxonomic group	Number of species					Ratio	
	XY	XO	ZW	ZO	Other[1]	XO/XY	ZO/ZW
Vertebrates	722	15	480	3	254	0.02	0.01
Insects	4415	1857	37	25	156	0.42	0.68
Angiosperms	23	0	1	0	19	0.00	0.00
Total	5160	1872	518	28	429	0.36	0.05

Data are based on the Tree of Sex database downloaded on Nov. 16, 2023.

[1]Other includes species with complex sex chromosomes (e.g., more than one X or Z) and homomorphic sex chromosomes.

DDX3X and *EIF1AX*) (23.5%) are essential for the survival of both KBM7 and HAP1 cells. This percentage is about twice as high as that of essential genes in the total gene population. Additionally, the simultaneous loss of *ZFX/ZFY* greatly suppresses human cell proliferation *(87)*, and *UTX/UTY* double-knockout mice show embryonic lethality *(88)*. These facts suggest that many genes remaining on the human Y are important for cell growth and organismal development. Furthermore, seven genes on the human Y (*SRY*, *RBMY*, *TSPY*, *DDX3Y*, *UTY*, *USP9Y* and *ZFY*) have been retained not only in apes but also in most placentals (i.e., they are ∼100 million years old) *(89)*. Therefore, although their losses may not cause immediate lethality, they likely have deleterious effects and cannot be fixed in a population.

Genes on the chicken W chromosome

The chicken W is one of the most highly degenerated Ws in birds, with only 29 genes compared with nearly 1,000 genes on the Z *(33, 90)*. The chicken W and human Y share no genes due to their different origins. Unlike the human Y, all genes on the chicken W have gametologs on the Z, and 28 genes, except for *MYO5B,* are located in female-specific regions of the W. Since all 28 genes have homologs in humans, we investigated whether they are essential in human cells. We identified nine (32%) and eleven (39%) essential genes in KBM7 and in HAP1, respectively (Supplementary Table S2). These include genes encoding proteins that are universally required for cell proliferation, such as the cohesin loader NIBPL, the AAA-type molecular chaperone VCP, the ribosomal protein RPL17, and the heteronuclear ribonucleoprotein HNRNPK. Although it is not clear whether these genes are essential in chicken cells, the high rate of chicken W genes essential in human cells is noteworthy.

What Evolutionary Trajectories Make the Loss of the Y or W Chromosome Possible?

As indicated in the previous section, Y degeneration stops or slows as the number of genes decreases. However, there are many species in which the Y or W is absent in males or females, respectively.

What are major factors contributing to the loss of the Y or W? Based on data obtained from the Tree of Sex (The Tree of Sex Consortium 2014, https://coleoguy.github.io/tos/data, *1*), we classified all species for which karyotypes have been identified into sex chromosome types (Table 1). In vertebrates, the proportion of species without a Y or W is very small (Table 1, Supplementary Table S3). However, many insect species lack the Y or W. Although taxon sampling bias should be considered, the Y is clearly dispensable in many species.

In vertebrates, the Y usually possesses a male-determining gene (e.g., *SRY* in mammals) *(91)* as well as male-beneficial (or sexually antagonistic) genes *(1, 92)*. In this case, the Y is indispensable and cannot be lost unless all of these genes are translocated or duplicated to different chromosomes, while retaining male-specific functions, or a novel SDG emerges with the same function in the sex determination cascade. A well-known example of Y loss in mammals is Amami spiny rat. Unlike other mammals in which *Sry* functions as an SDG, in this species, both females and males are XO (2n = 25) and *Sry* is absent in the genome *(93)*. Kuroiwa and colleagues *(80)* recently discovered that only males have the duplication of a *Sox9* (SRY-box transcription factor 9) enhancer on chromosome 3 as heterozygotes, whereas in females, the corresponding genomic region is homozygous without a duplication. Although the transcription factor that binds to this enhancer has not been identified, chromosome 3 must be a novel sex chromosome on which sex-specific differentiation is in an early stage and the X returned to an autosome, by definition. This is why the 'old' Y could be lost in this species.

Several insect orders likely adopt the X dose for sex determination. For example, the majority of species belonging to Blattodea and Odonata do not have a Y (Supplementary Table S4). The ancestor of each order likely had the XO type. On the other hand, the ancestor of Mecoptera was likely the XY type even though many species in this order are the XO type at present *(94)*. However, the Y is not necessarily dispensable even in species that use the X dose for sex determination. In *Drosophila*, for example, the Y is considered to have been derived from the B chromosome *(95, 96)*; however, there are ∼10 genes on the Y in *D. melanogaster* and at least some of these genes that have been retained on the Y for more than 40 million years *(97)* are essential for male fertility *(98)*. Therefore, the Y loss must require the integration of these genes on other chromosomes. Indeed, in many species belonging to the *D. montium* group, such integration occurred *(99)*, potentially making the Y dispensable. It should be mentioned that such integration also occurred independently in the lineage of *D. pseudoobscura* and its closely related species *(100)*; however, XO males of *D. pseudoobscura* are sterile *(101)*.

Since there are no homologous genes on the Y between *D. melanogaster* and *D. pseudoobscura*, the Y in *D. pseudoobscura* acquired different Y-linked genes that are essential for male fertility.

Bacteria and viruses have also contributed to the loss of the Y and W, particularly in insects. For example, the bacterial genus *Wolbachia* infects a variety of insects and control their reproduction *(102)*. In the *Eurema* butterfly, the W has the female-determining function and is thus essential. However, *Wolbachia*-infected individuals become females even without the W (i.e., ZO females), indicating that *Wolbachia* confers a female-determining function and compensates for the loss of the W *(103(*. A similar phenomenon was also reported in the *Ostrinia* moth *(104)*. Although the contribution of bacteria and viruses to the loss of the Y and W in general is not clear, particularly for non-insect organisms, the effects of symbionts and parasites on the evolution of sex chromosomes may be greater than is currently recognized.

Only 5–6% of flowering plants are dioecious *(105)*. The proportion of dioecious plants with sex chromosomes remains unknown; however, flowering plants are unlikely to have evolved fully environmental sex determination *(106)*. All known dioecious plants with sex chromosomes have either a Y or W (Table 1, Supplementary Table S5). This observation implies that the Y and W are essential in these plants, although available information is still limited compared with that for animals. It should be mentioned, however, that some plant species, such as sorrels (genus *Rumex*), use the X dose for sex determination *(107)*. These species might theoretically be able to lose the Y in the future, although the Y may still be indispensable if it harbors the genes essential for male fertility.

How Are Sex Chromosomes Reverted to Autosomes?

The evolution of sex chromosomes, particularly sex chromosome turnover, is a complex and fascinating area of research in genetics and evolutionary biology. Sex chromosome turnover is defined as '*trans*-heterogamety turnover' if the sex chromosome system changes from XY to ZW or vice versa, and as '*cis*-heterogamety turnover' if the system changes from XY to XY or from ZW to ZW, but with different evolutionary origins *(108)*. It is still unclear how sex chromosomes are replaced by new chromosomes and what factors trigger turnover events. A *trans*-heterogamety turnover is proposed to have occurred in the common ancestor of mammals *(109)*. Multiple XYs in monotremes are homologous to chicken ZW and not to therian XY, providing evidence for the transition from ZW to XY *(109)*. In addition, as discussed in the previous section, *cis*-heterogamety turnover likely occurred in Amami spiny rat *(80)*. Yet, the turnover rate seems very low in mammals. In addition, there are no reports of sex chromosome turnover in birds. By contrast, such turnover likely occurred at a higher rate in amphibians and fishes *(1)*. Extreme examples are the Japanese wrinkled frog (*Glandirana rugosa*) and western clawed frog (*Xenopus tropicalis*), in which the *trans*-heterogamety turnover is ongoing within each species. In this section, we focus on the progress in our understanding of the evolution of sex chromosomes in these two species, with an emphasis on the complexity.

Convergent evolution of sex chromosomes in G. rugosa

The Japanese wrinkled frog, *G. rugosa*, is unique in having both XX-XY and ZZ-ZW sex chromosomes within a species *(110, 111)*. *G. rugosa* has unique genome characteristics, including a high CG dinucleotide content due to transposons and a large genome size (∼7 Gb) *(112)*. However, it is not clear whether these genomic features are directly related to the sex chromosome turnover in this species.

G. rugosa is distributed throughout most of Japan and consists of at least six geographic populations: East (homomorphic XY: currently defined as a new species, *G. reliquial*), West (homomorphic XY), North-Western (heteromorphic ZW), Eastern Central (heteromorphic XY), Western Central (heteromorphic ZW, named Neo-ZW) and Neo-West (homomorphic XY) (Fig. 4) *(113–116)*. The XY in the West and East populations are homomorphic but the origin of sex chromosomes differs, i.e., chromosomes 1 and 3, respectively *(114)*. The North-Western and Western Central populations have the heteromorphic ZW whereas the Eastern Central population has the heteromorphic XY. Yet, these three populations commonly use chromosome 7 as the sex chromosomes *(116, 117)*. In addition, Ogata et al. *(118)* proposed the Western Central population as Neo-ZW, because this population was likely derived from hybridization between the West and Eastern Central populations, both of which are the XY. Furthermore, the western and south-western edge populations in the Western Central population were later found to have homomorphic XY *(119)*. Therefore, Ogata et al. *(119)* defined them as the Neo-West population although their sex chromosomes are not clarified yet (Fig. 4). In addition, new hybrids are found at the boundary area of the Western Central population with Neo-ZW and the Eastern Central population with XY, in which XY and ZW cannot be distinguished (Fig. 4) *(113)*. These observations suggest that *trans*-heterogamety turnover is ongoing in these populations. Yet, the number of populations in Japan and the sex chromosome types are still controversial.

It remains unclear when and how many sex chromosome turnover events have occurred in this species. *G. rugosa* possesses 13 chromosomes (2n = 26); chromosome 7, the sex chromosome in North-Western, Western Central, and Eastern Central populations, includes three candidate SDGs (sex-related genes, *Sox3, Sf1/Ad4BP*, and *Ar*) *(111, 117)*. It should be mentioned that *Sox3* is an X-linked gametolog of *SRY* in therian mammals and a candidate SDG in the African bullfrog, *Pyxicephalus adspersus (111, 120, 121)*. On chromosomes 1 and 3, the sex chromosomes in the West and East populations, respectively, sex-linked SNPs were also detected in the genes that have been identified as SDGs in other vertebrates or the sex-related genes (*Dmrt1*, *Amh, Irf9, Hsd17β1, Foxl2,* etc.) involved in sex differentiation *(114)*. This tendency suggests convergent evolution of sex chromosomes, i.e., chromosomes that harbor sex-related genes have independently become sex chromosomes in this species.

Coexistence of three sex chromosomes in X. tropicalis

During sex chromosome turnover, new sex chromosomes coexist with the ancestral sex chromosomes within a population. The amphibian model species *X. tropicalis*, which

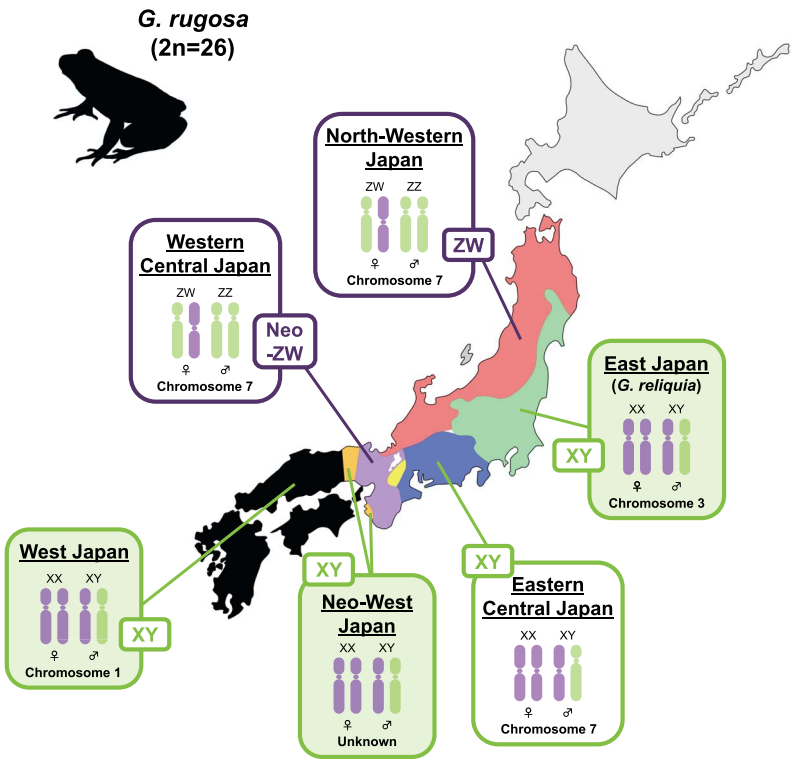


Fig. 4. Six geographic populations of *Glandirana rugosa* in Japan with their sex chromosome types. Each color in the map shows the distribution of each population. Filled and open square with rounded corners in the margin represent the homomorphic and heteromorphic sex chromosomes, respectively. Male and female heterogamety are depicted by green and purple of each square with rounded corners, respectively. The yellow area in the map represents the intermingling population between the Western Central Japan population (Neo-ZW) and the Eastern Central Japan population (XY).

has homomorphic sex chromosomes, exemplifies this scenario. In the case of the ancestral ZW sex chromosomes, the feminizing factor on the W would degenerate and the masculinizing factor on a new Y would emerge from the ancestral Z. This would result in the coexistence of three sex chromosomes, Z, W and Y, in the same population. The coexistence of the Y alongside the Z and W was reported in laboratory stocks through crossing experiments of sex-reversed individuals *(122)*. This finding has been corroborated in natural populations, where the Y bears a male-sex factor in a region almost identical to that of the W *(123)*. Moreover, the sex-linked regions of these W and Y are notably small, with most of both chromosomes comprising PARs that still undergo recombination, similar to autosomes *(123, 124)*. This suggests that the sex chromosomes of *X. tropicalis* have undergone recent turnover and have been maintained for long periods within each natural population.

Consequently, *X. tropicalis* serves as a model for elucidating the mechanisms underlying the maintenance of multiple sex chromosomes and/or frequent sex chromosome turnover, as observed in *G. rugosa (110, 111)*. The closely related species *X. laevis* is the only amphibian whose SDG (*dm-W*) has been determined *(13, 17)*. The SDGs on the multiple sex chromosomes of *X. tropicalis* remain unidentified, even though sex-associated regions were reported *(123–125)*. The difficulty lies in the fact that the emergence of SDGs can be achieved by small mutations, such as single nucleotide substitutions (e.g., *9*), and most *X. tropicalis* are not inbred stocks and retain nucleotide polymorphisms *(126)*. Therefore, further studies using inbred strains are needed to identify SDGs, which will enable to understand the sex chromosome turnover initiated by the new SDGs at the molecular level.

Concluding Remarks

Substantial variation in sex chromosomes has been reported, in terms of not only size and shape but also functional status and evolutionary processes. This review compiles these findings, with a focus on mechanisms underlying the maintenance of stable sex determination using sex chromosomes at each stage of the sex chromosome cycle.

The emergence of sex chromosomes is typically accompanied by the birth of a novel SDG. In this review, we emphasized the potential contributions of TEs to the generation and maintenance of SDGs. We also introduced a mechanism in which both the X and Y function in sex determination in *S. latifolia*, likely strengthening the stability of sex determination in this species. The human Y and chicken W show substantial degeneration and contain few genes. Yet, the rate of gene loss has slowed considerably by the selective maintenance of functionally important genes. However, the loss of the Y and W is possible when a novel SDG emerges on a different chromosome and acquires the function of the extant SDG, as observed in Amami spiny rat. We also emphasized the potential importance of symbionts and parasites that function as sex determiners, enabling the loss of the Y and W, particularly in insects. Furthermore, chromosomes containing

sex-related genes became sex chromosomes repeatedly in *G. rugosa*.

Our understanding of the sex chromosome cycle is still based on a limited number of organisms and thus is far from complete. More examples from a diverse range of 'unusual' organisms are necessary. More importantly, experimental approaches are needed to evaluate the necessity and relative contributions of the factors discussed in this review to sex chromosome turnover. With such deeper knowledge, we may be able to predict the fate and future of our own sex chromosomes.

Supplementary Data

Supplementary data are available at JB Online.

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