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Review article

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# Evolution and regulation of animal sex chromosomes

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## Abstract

Animal sex chromosomes typically carry the upstream sex-determining gene that triggers testis or ovary development and, in some species, are regulated by global dosage compensation in response to functional decay of the Y chromosome. Despite the importance of these pathways, they exhibit striking differences across species, raising fundamental questions regarding the mechanisms underlying their evolutionary turnover. Recent studies of non-model organisms, including insects, reptiles and teleosts, have yielded a broad view of the diversity of sex chromosomes that challenges established theories. Moreover, continued studies in model organisms with recently developed technologies have characterized the dynamics of sex determination and dosage compensation in three-dimensional nuclear space and at single-cell resolution. Here, we synthesize recent insights into sex chromosomes from a variety of species to review their evolutionary dynamics with respect to the canonical model, as well as their diverse mechanisms of regulation.

## Sections



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## Introduction

The ubiquitous and fascinating differences between males and females of many animal species are the developmental outcomes of sex determination in early embryos. In most vertebrates, sex determination occurs when an upstream cue commits the initially bipotential gonads to start along the testis or ovary development pathway[1]. Subsequent sexual differentiation occurs first in gonad somatic cells (the male Sertoli and the female granulosa) and germline cells (the male sperm and the female egg), and conspicuous sexual differences in physiology, morphology or behaviour emerge later, usually influenced by gonadally produced hormones. The initial upstream cue can be either genetic or environmental. Only species with a genetic sex-determining cue contain sex chromosomes, whereas species with an environmental sex-determining cue do not exhibit genetic differences between sexes. Furthermore, the genetic cue can be either the copy number ratio of X chromosome versus autosomes (as in the fruitfly, *Drosophila melanogaster*) or an upstream sex-determining gene (USDG) on the sex chromosome, such as the *Sry* gene (for sex-determining region Y) found on the Y chromosome in placental mammals. Much less is known about the mechanisms of environmental (including temperature-induced) sex determination (but see refs. 2,3). There are two major types of sex chromosome in animals and plants including male heterogametic systems, whereby females have two X chromosomes (XX) and males have one X and one Y chromosome (XY), as in mammals and *Drosophila*. By contrast, in female heterogametic systems, females have different sex chromosomes (ZW), and males are homogametic (ZZ), as in birds, butterflies and moths. Sex-specific selection is expected to act differently in the XY and ZW systems; for example, male-specific selection favouring male displays[4] or male aggression acting on Y-linked factors[5], and female-specific selection favouring fecundity acting on the W[6]. Evolutionary biologists have long sought to understand the causes of the remarkable diversity in sex-determination mechanisms and sex chromosomes despite sexual reproduction being such an ancient and critical biological feature.

The canonical model stipulates that sex chromosomes, regardless of XY or ZW system, evolve in four consecutive phases (Fig. 1a). First, a USDG is born on a pair of ancestral autosomes that therefore become the proto-sex chromosomes. Second is the initiation and expansion of recombination suppression (starting in the sex-determining region). Third is Y (or W) chromosome degeneration, producing heteromorphism in size and structure relative to the X (or Z) chromosomes, which causes the evolution of dosage compensation. Fourth is the complete loss of the Y (or W) chromosome. However, most of our knowledge of sex-chromosome evolution comes from studies of a few genetic model organisms with heteromorphic sex chromosomes at later phases of evolution, which are far from being representative. Importantly, recent studies into a broad range of species have revealed the diverse evolutionary phases and regulatory mechanisms of sex chromosomes, many of which deviate from the canonical model.

Here, we first review the recent work demonstrating that instead of proceeding through the entire canonical evolutionary trajectory, sex chromosomes of many species have undergone turnovers at an early phase of evolution. Unlike the model organisms usually used in genetics research, many species (for example, pythons and some frogs and teleosts, Fig. 1a) have not evolved extensive or complete recombination suppression between sex chromosomes nor reached the seemingly inevitable (as proposed) complete degeneration of the Y chromosome. Next, after summarizing the typically transient expression pattern of USDGs, we describe a conceptualization of the organization of vertebrate sex-determination cascades that could explain the unexpectedly rapid turnovers of USDG and sex chromosomes. Last, we highlight how technological developments, such as those that measure interactions between genomic regions, have provided insights into how sex chromosome divergence affects their mode of regulation in terms of nuclear architecture and three-dimensional (3D) topology. In summary, we integrate the results from evolution, genetic and developmental studies mostly from animals (for plant sex determination and sex chromosomes, see reviews[7,8]), to comprehensively review the unique evolutionary and regulatory properties of sex chromosomes.

## Evolutionary turnover of sex chromosomes

After acquiring the sex-determining function on a proto-sex chromosome, a sexually antagonistic polymorphism (a genetic variant that is, for example, beneficial to males but detrimental to females) is hypothesized to become established near the USDG owing to their close linkage. This scenario might explain the progressive expansion of recombination suppression between the sex chromosomes[9]. The lack of recombination then leads to Y-chromosome degeneration and an imbalance of gene dosage between X chromosome versus autosomes in males, except in the recombining pseudoautosomal regions (PARs) at the chromosome ends[10]. This consequence selects for the evolution of global or incomplete dosage-compensation mechanisms, producing an equalized expression level of most or some X-linked genes between the sexes[11,12].

This canonical model accounts for the sex-chromosome evolution of mammals and birds that have gone through each of the four phases; however, many examples from non-model systems suggest this not always to be the case. Sex chromosomes can restart a new cycle of the four phases because of 'turnover' of a USDG or a sex chromosome – that is, sex-determination replacement by another gene, chromosome region or sex chromosome type (transition between XY and ZW systems)[13–16] (Fig. 1b). Thus, species with both homomorphic (younger) and heteromorphic (older) sex chromosomes that are recapitulating respective phases of sex chromosome evolution can inform their different chronological features, as well as different mechanisms that generate the sex chromosome diversity (Fig. 1a). Among vertebrates, probably the most extensively studied sex-determination cascades and sex chromosomes are those of mice and the Japanese rice fish (the medaka, *Oryzias latipes*), which provide paradigmatic examples of the late and early phases of sex-chromosome evolution, respectively.

### Homomorphic sex chromosomes

The medaka Y chromosome is only about 10 million years old[17] (for comparison, the eutherian Y chromosome is about 170 million years old) and still undergoes homologous recombination. It shows few sequence differences from the X chromosome outside the 258-kilobase-long male-determining region, which comprises less than 1% of the entire chromosome length (about 33 megabases long)[18]. Such cytogenetically indistinguishable or homomorphic sex chromosome pairs often represent early phases of sex-chromosome evolution. Most of their Y or W chromosome regions still undergo recombination and have not substantially degenerated. Therefore, homomorphic sex chromosomes are predicted to be more likely (compared with the older, heteromorphic chromosomes) to experience turnover of USDGs and sex chromosomes. They are thus important models for understanding the underlying molecular and evolutionary mechanisms of these turnovers.

Young and homomorphic sex chromosomes are widely distributed among 'lower vertebrates' (Fig. 2a), including boas and python[19]

(but not advanced snakes), many teleosts (for example, cichlids[20]) and frogs[21], and recently have also been reported in the vertebrate outgroup amphioxus[22]. These sex chromosomes could simply be too young to have evolved extensive pairwise divergence or degeneration. Another non-mutually exclusive explanation, as shown in seahorses[23], tree frogs[24] and amphioxus[22], is that their sex chromosomes have occasionally recombined in sexually reversed individuals and appear 'forever young' (known as the 'fountain of youth' scenario[25], Fig. 1b). In some other species (for example, in houseflies[26], salmonids[27] and fugu fish[28]), the young sex chromosomes did not have enough time to diverge before the USDG translocated onto another chromosome of the same species (a phenomenon known as the 'jumping gene' scenario). Alternatively, homomorphic sex chromosomes may in fact be old (known as ancient homomorphy) because they have not evolved large non-recombining regions (for example, in ratites[29]) or have been diverging very slowly (for example, in sturgeon[30]). It remains unclear why some sex-chromosome systems exhibit ancient homomorphy, but recent work in ratites[29] and scallops[31] has suggested that weak or reversible sexual selection may provide little opportunity for sexually antagonistic polymorphisms to be frequently established between sex chromosomes, hence potentially preventing further evolution of extensive recombination suppression and sequence divergence[31].

### Heteromorphic sex chromosomes

Heteromorphic sex chromosomes (more often the XY than the ZW[32]) can fuse or translocate to autosomes, which affects sex chromosome evolution differently compared with USDG turnover. The fusion between sex and autosomal chromosomes creates a 'neo-sex'-linked region that may be subjected to a similar trajectory of canonical sex chromosome evolution if recombination is suppressed (as would happen if, for example, an autosome fuses to the Y chromosome in *Drosophila*[33] because male *Drosophila* have achiasmatic meiosis; see below). Formation of a 'neo-sex' region has occurred during the evolution of eutherian sex chromosomes and also contributed to the large diversity of partially non-homologous sex chromosomes among insects[34,35], spiders[36], nematode worms[37], sticklebacks[38] and other teleosts[39], frogs[40] and birds[41,42]. Serial translocations can even produce unusual chromosome complexes, including multiple sex chromosomes that pair in a chain (in platypus[43]; Fig. 1b) or a ring shape (in the Taiwanese frog *Odorrana swinhoana*[44]) during meiosis.

Heteromorphic sex chromosomes can also inform whether and how Y chromosomes might become lost and lead to turnover of the sex-determination system at their advanced stage of evolution. Attrition of the PAR by recombination loss and degeneration of the Y chromosome is hypothesized to increase the likelihood of complete Y chromosome loss through formation of aneuploid gametes (the 'fragile Y' hypothesis[45]) (Fig. 1a). Conversely, PAR extension (potentially through translocations and fusions with recombining autosomes) could contribute to the preservation of the Y chromosome. In addition, the 'persistent Y' hypothesis[46] proposes that dosage-sensitive[47] or meiotic-executioner genes (that is, genes with critical functions in meiosis that would be lethal to germ cells if translocated from the Y to other chromosomes) could also contribute to Y chromosome preservation. However, few empirical studies have demonstrated how species can completely lose the Y chromosome and/or transition into a new (genetic or environmental) sex-determination system. Rodents include some promising models in this regard for understanding the 'future' of mammalian sex chromosomes (see review[48]). For example, mole voles have lost their Y chromosome and wood lemmings carry a polymorphic feminizing gene on the X chromosome that can outweigh the *Sry* function and produce XY females. Of particular interest, the Amami spiny rat has a single X chromosome in both sexes and has lost the Y chromosome (and *Sry*) but, as was recently discovered, has gained a male-specific duplication of a *Sox9* enhancer (Enh14) as the male-determining function[49].

## The sex-determining cascade

The birth of a new USDG is the first step in the canonical model of sex-chromosome evolution and a key step during the turnover into a new genetic sex-determination system (from either an environmental or another genetic system). Recent studies characterizing USDGs and their downstream targets suggest that their distinct molecular properties and the general organization of the sex-determining cascade have an important role in sex chromosome turnovers (also see recent reviews covering the evolutionary mechanisms for the turnovers[16,50]) .

### Origins of upstream sex-determining genes

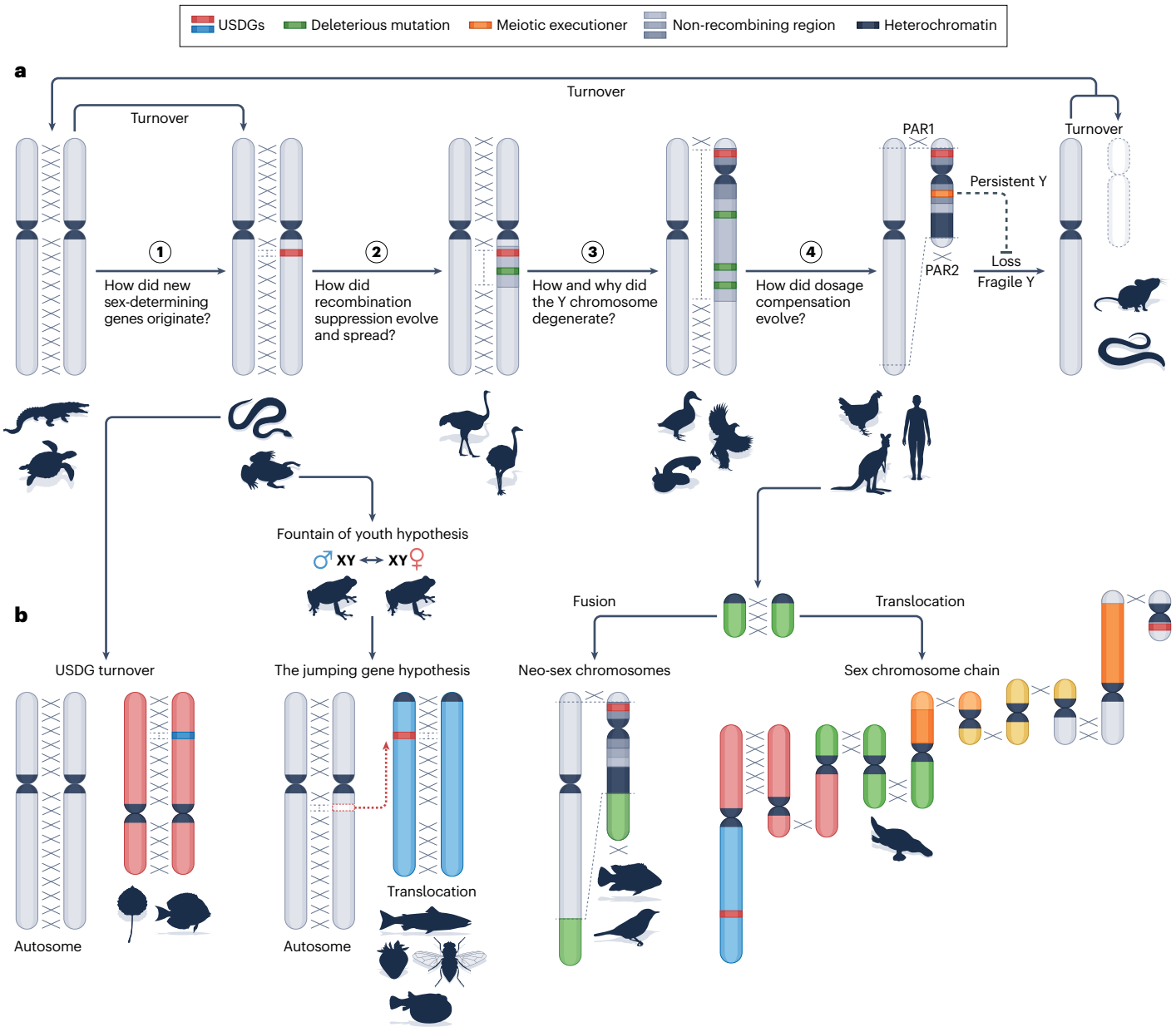
Origination of USDG can occur through allelic divergence in an ancestral gene often already involved in sex determination (as in mouse and human) or duplication of such a gene (as in the medaka)[51]. The dominant, Y-linked male-determiner in mice, *Sry*, is shared by other therian mammals and is inferred to have originated from mutations in its X-linked counterpart, *Sox3* (ref. 52). In medaka, a segmental duplication encompassing a conserved downstream vertebrate male-determining gene, *dmrt1a*, produced a Y-specific region now carrying only the USDG, *dmrt1bY*[53] or *dmy*[54]. Notably, almost all known USDGs in teleosts and amphibians originated from a limited number[51] of ancestral sex-determination genes (termed the 'usual suspects'[51,55,56]) that includes *dmrt1*, TGFβ family genes (for example, *amh*[57]) and the steroidogenesis pathway genes (Supplementary Table 1). The 'usual suspects' in insects, specifically the dominant male determiners, seem to often be related to splicing factors through either duplication (for example, *Mdmd* of houseflies[26]) or molecular interactions (for example, the highly conserved gene across insects, *doublesex*[58]).

### Short-lived upstream sex-determining genes

In the 1990s, the discovery of the dominant male-determiner *SRY* on the human Y chromosome[59] led to the widely used but somewhat misleading term 'master sex-determining gene'[60]. Later comparison of several orthologous genes between insects suggested that USDGs are much more diverse compared to downstream orthologous genes of the sex-determination cascade that are largely shared across species[61]. Overall, studies from various biological disciplines have argued against a 'master' role of USDGs.

First, evolutionarily stable USDGs, such as *Sry* in therians or the male-determining USDG *Dmrt1* in birds, are now known to be the exception rather than the rule. As discussed in the previous section, many vertebrate and invertebrate taxa with homomorphic sex chromosomes are prone to turnover, sometimes even between populations of the same species (for example, several frog species, see review[62]). Additionally, some species carry both XY and ZW chromosomes (for example, African clawed frogs[63]) or even both temperature and genetic sex-determination systems (for example, the snow skink[64]).

Second, several studies suggest that only a few regulatory mutations are needed for the seemingly 'master' function of *Sry* to be hijacked by the downstream genes, providing genetic evidence that the function of so-called 'master' genes can easily be compensated or replaced. For example, genetically engineered mice that lack the Y chromosome and *Sry* exhibit male infertility that can be partially rescued




by activating the downstream genes *Sox9* and *Eif2s3x*[65]. Another study showed that, in the presence of *Sry*, deleting a *Sox9* enhancer reduces *Sox9* expression to insufficient levels for normal testis development and produces XY females[66]. In the aforementioned case of Amami spiny rat without the *Sry*[49], the male-determining function is executed by a male-specific enhancer (potentially a new USDG) that activates *Sox9*. Together, these findings argue against the indispensability of the Y chromosome and the master role of *Sry* in mammalian sex determination and suggest that properly activating a conserved downstream gene, *Sox9*, seems to be more important than *Sry*.

Third, from a developmental perspective, most known USDGs have peak expression levels in very restricted time frames and cell types that usually trigger the downstream autoregulatory loop, involving a more conserved gene with longer-lasting expression during the sex-determination process (Table 1). For example, *Sry* is expressed in the supporting cells of the genital ridge of mice for 10.5 to 12.5 days post coitus[67]. This transient expression activates the *Sox9* positive feedback loop, which coordinates with downstream targets (for example, *Amh*) for testis development and, at the same time, suppresses the Wnt4-involved ovary development pathway and possibly quenches the *Sry* expression[68]. Female-determining genes may also suppress the testis-development pathway: deletion of the female-determining *Foxl2* leads to upregulation of *Sox9* and ovary-to-testis reversal[69]. Other downstream antagonistic regulatory mechanisms that have lasting effects on sex determination have also been reported in the teleost Patagonian pejerrey[70] (*amh* versus *foxl2*) and the nematode *Caenorhabditis elegans* (the male-determining *xol-1* represses the hermaphrodite-determining *sdc-2*, which further represses the male-determining *her-1* ref. 71).

**Fig. 1 | Canonical model of sex-chromosome evolution and sex-chromosome turnovers. a**, In the canonical model, sex chromosomes originate from a pair of autosomes and undergo four consecutive phases of evolution (pertinent research questions for each are indicated). First, an upstream sex-determining gene (USDG, red bar) acquires male- or female-determining function by mutation on one chromosome. This acquisition leads to the transition from environmental sex determination (as in crocodiles and turtles) or from another genetic sex-determination mechanism. Second, recombination suppression between sex chromosomes might evolve by accumulation of sexually antagonistic mutations – the segments that continue to recombine are called pseudoautosomal regions (PARs, indicated by crosses between chromosomes). Regions that lack recombination (grey) on the Y chromosome are expected to accumulate deleterious mutations (green). Some of the teleosts, amphibians, reptiles and palaeognathous birds have small sex-determining regions and homomorphic sex chromosomes. Third, the non-recombining region of the Y chromosome further expands and continues to accumulate more deleterious mutations (darker grey), and eventually become highly heterochromatic (black) (as in human and chicken) – although dosage-sensitive genes or genes with critical functions during meiosis (meiotic executioner, orange bar) can slow down this process. Fourth, the loss of Y-linked genes may select for the evolution of dosage compensation on the X chromosome. Extant species with sex chromosomes recapitulating one of the four phases are shown beneath each of the corresponding phases. **b**, The USDG can be replaced by a new gene in another chromosomal region, leading to a turnover event in the sex-determining region or in the sex chromosomes. Such turnovers may more frequently occur during the early phase of sex-chromosome evolution, when sex chromosomes are homomorphic. Some vertebrates have homomorphic sex chromosomes because they undergo occasional recombination between sex chromosomes mediated by sex reversals (the 'fountain of youth' hypothesis[25]). In houseflies[26], some teleosts (such as fugu[28]) and strawberries[167], USDG are translocated onto a different chromosomes mediated by transposable elements, leaving their sex chromosomes also homomorphic (the 'jumping gene' scenario). Highly divergent sex chromosomes of some other species may form 'neo-sex chromosomes' by incorporating autosomes into the ancient sex chromosomes through fusions or translocations. Serial translocations can create an unusual multiple-sex chromosome complex, such as that of platypus[43].

In mice, the antagonism between the male-determining *Dmrt1* and female-determining *Foxl2* even extends beyond the embryonic sex-determining stage. Disruption of either component leads to sex reversal of gonad cells in adults[69,72], suggesting that these downstream effectors are also important for the maintenance of sex.

In summary, USDGs seem to be more ephemeral or short-lived (relative to their downstream targets) in both developmental (Table 1) and evolutionary processes (Supplementary Table 1). The canalization towards one type of gonad, ensured by the ancient and more long-lasting downstream targets, is probably more essential to sex determination than the initiation process by USDGs. Indeed, species with environmental sex determination – such as turtles and crocodiles – and polygenic sex determination develop testes and ovaries without an USDG. Furthermore, even after the expression of USDGs, gonad development can still be interfered with or even reversed by temperature, chemical or other environmental factors.

### Parliamentary–monarchy organization

The current evidence suggests a parliamentary–monarchy model as an appropriate analogy for the relationship between USDGs and the downstream genes[1]. More specifically, USDGs are analogous to modern monarchs because they have little influence beyond tilting the gonad development decision towards either sex. By contrast, the developmental decision to become testes or ovaries is sustained and fulfilled by many downstream transcription factors and hormone regulators from opposing pathways promoting either male or female development, such as with members of a parliament of two parties.

Most of these downstream 'parliamentary' genes originated and gained their functions much longer ago than USDGs did theirs, which explains the conserved involvement of parliamentary genes in specification and proliferation of gonad cells (Fig. 3). For example, *Dmrt1* and *Amh* originated in the common vertebrate ancestor and are suggested to have had an ancestral function in regulating germ cell development and only subsequently acquired a masculinization function in somatic cells[73,74]. Additionally, *Foxl2* (shared by all metazoans) was recently found to have an ovary-biased expression among invertebrates including amphioxus[22], crab and molluscs[75], suggesting a deeply conserved female-determining function pre-dating the vertebrate ancestor. Nevertheless, downstream parliamentary genes can still undergo lineage-specific change-of-expression patterns, as indicated by a few comparative studies of spatiotemporal expressions of orthologous genes between turtles[76] and between medaka and mammals[77]. Some of these genes have even 'defected' to the opposite sex: a recent study reported that the conserved male-determining *Sox9* and *Amh* are surprisingly expressed at a high level in the developing ovaries of the central bearded dragon[78].

Looking ahead, although extensive efforts have been devoted to identifying and characterizing 'monarch' genes in animals (Fig. 2), we suggest that the parliamentary genes warrant more attention regarding their conservation and lineage-specific diversification and their antagonistic relationships. Such diversification can be gains or losses of genes (for example, the teleost male-determining gene, *Gsdf*, has been lost in amniotes; Fig. 3) or the abovementioned change-of-expression patterns. A particularly interesting hypothesis that remains to be tested is whether the 'tug-of-war' between male versus female gonad development by parliamentary genes contributes to the rapid turnover of monarch genes in some species. In this scenario, sexually antagonistic selection drives the recurrent fixation of mutations that enhance the functions of downstream male- or female-determining genes. These enhanced functions lead to the fixation of secondary mutations that correspondingly alter the gene expression in the determining pathway of the opposite sex. Indeed, such mutations could entail a parliamentary gene, even replacing[51] the former USDG to become a new monarch gene, such as in the Amami spiny rat in which duplication of an enhancer made *Sox9* replace the *Sry*.

## Recombination suppression and Y-chromosome degeneration

Once a USDG has originated, the canonical model predicts that the chromosome region may evolve suppressed recombination, which can sometimes recur and spread across almost the entire sex-chromosome pair. For example, the human XY-chromosome pair was inferred to have experienced at least four recombination loss events, possibly due to inversions[10,79]. The consequential stepwise sequence divergence pattern between sex chromosomes, termed 'evolutionary strata'[10], has now been widely reported in other vertebrates[43,80,81], worms[37] and plants[82] (see review[83]; Fig. 2a and Supplementary Table 2). Classic models of Y-chromosome evolution indicate that suppressed recombination will

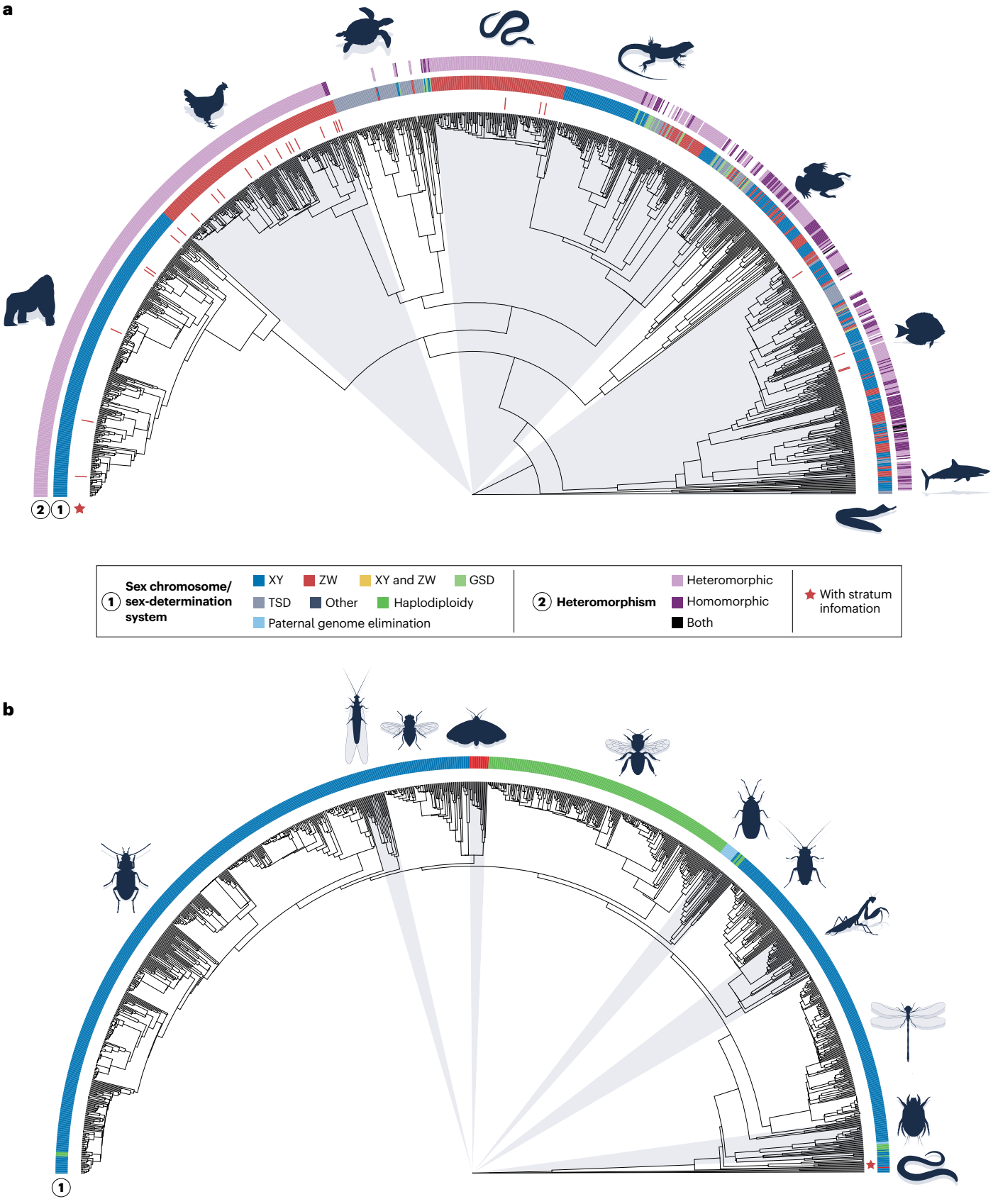

a
2
1
1 Sex chromosome/ sex-determination system
XY
ZW
XY and ZW
GSD
TSD
Other
Haplodiploidy
Paternal genome elimination
2 Heteromorphism
Heteromorphic
Homomorphic
Both
With stratum infomation
b
1

**Fig. 2 | Animal sex chromosomes and sex-determination systems.** **a**,**b**, Most of the known sex-chromosome types (for example, XY in blue; ZW in red), heteromorphism (for example, heteromorphic in light purple; homomorphic in dark purple) and sex-determination systems (for example, genetic and temperature sex-determination (GSD and TSD) in light green and grey, respectively) with the phylogenies for vertebrates (**a**) and invertebrates (**b**). Species with reported patterns of evolutionary strata in vertebrates are marked with red dashes. See Supplementary Table 1 for a list of the reported USDGs of animals. The tree structure is derived from taxonomic information provided by Timetree; sex-chromosome data are from Tree of Sex[168].

then lead to the eventual degeneration of non-recombining Y-linked regions (see review[84]) owing to the weakened ability of selection to purge deleterious mutations and fix adaptive mutations.

### Indirect evidence for the canonical model

The canonical model predicts that recombination suppression evolves in response to the accumulation of sexually antagonistic polymorphic alleles linked to the sex-determination genes[9]. Some population genetic signatures of such polymorphisms have been found in the partially sex-linked PARs of sticklebacks[38] (and the plant *Silene latifolia*[85]). Otherwise, there is little direct evidence for sexually antagonistic polymorphism within sex-linked regions, in part because complete sex-linkage precludes genetic studies that can detect them. In addition, several species of fungus that lack sexual dimorphism have been found to form extensive evolutionary strata between their mating-type chromosomes, suggesting that recombination suppression can evolve in the absence of sexually antagonistic selection[86,87]. Finally, extensive recombination suppression does not necessarily evolve between sex chromosomes[88]. Many teleost (for example, medaka) sex chromosomes only lack recombination at the small Y-specific region; in the extreme case of tiger fugu, the X and Y chromosomes differ by only a single nucleotide[89]. These findings have suggested many hypotheses that could account for the irreversible fixation of inversions between sex chromosomes.

### Alternative models

One model — the 'regulatory' model — takes gene regulatory processes into account during the evolution of recombination suppression between sex chromosomes. More specifically, this model explicitly considers regulatory mutations in a region of recombination suppression established by chance (caused by a 'lucky' inversion[90] or other mechanisms[91]) and assumes stabilizing selection on expression levels of genes on the proto-sex chromosomes[90] (Fig. 4a). Thanks to recombination suppression, Y-linked deleterious mutations are expected to accumulate in a *cis*-regulatory region and downregulate gene expression. This process facilitates further degeneration of coding regions[92] and increases selection for male-specific *trans*-regulatory mutations upregulating the Y-linked alleles as a form of early, incomplete dosage compensation. Selection may also actively favour reduced expression of Y-linked gene products with deleterious mutations in coding regions. Reversion of the initial 'lucky' inversion is now selected against because recombination is expected to generate mismatches between the *cis*- and *trans*-regulators of the proto-X and proto-Y chromosomes. This newly evolved state produces a sexually antagonistic relationship that can potentially fix successive recombination suppression events between sex chromosomes — a prediction that is now testable. Indeed, a few genes on the extremely young (only 90,000 to 150,000 years old) neo-Y chromosome of the fruitfly *D. albomicans* seem to have been downregulated before the degeneration in coding regions[93], although dosage compensation on the neo-X has not yet been identified. Another model — the 'sheltering' model — proposes that inversions, including those that affect supergenes[94] (that is, genes that are tightly linked and responsible for a variety of traits), can be favoured between sex chromosomes if they capture fewer recessive deleterious mutations than the population average and display a heterozygote advantage[95].

Both the regulatory and sheltering models require further population genetic simulations that address how the 'lucky' or sheltering inversions could become established. Additional empirical evidence for sex-linked regulatory feedback is also needed. Other models also require empirical tests, such as those that consider meiotic drivers[96] or neutral accumulation of sequence divergence[97] close to the sex-determining locus, thus causing recombination suppression. Looking ahead, many birds[80], particularly the palaeognaths (ostrich, emu and tinamous)[29] (Fig. 4b), may be useful systems for testing these models because independent evolutionary strata have recurrently formed

**Table 1 | Expression duration of USDGs**

| Species | USDGs to downstream genes | Peak expression window | Ref. |
|---|---|---|---|
| Human | *SRY* to *SOX9* | 40–60 dpc (peak 44) | 148 |
| Mouse | *Sry* to *Sox9* | 10.5–12.5 dpc (peak 11.5) | 149 |
| Rat | *Sry* to *Sox9* | 12–14 dpc (peak 13) | 150 |
| Cattle | *SRY* to *SOX9* | 37–44 dpc (peak 39) | 151 |
| Rabbit | *SRY* to *SOX9* | 14–18 dpc (peak 15/16) | 152 |
| Pig | *SRY* to *SOX9* | 21/23–26 dpc (peak 21/23) | 153 |
| Sheep | *SRY* to *SOX9* | 23–49 dpc (peak 27/44) | 154 |
| Dog | *SRY* to *SOX9* | 29–33 dpc (peak 33) | 155 |
| Goat | *SRY* to *SOX9* | 28–36 dpc (peak 36) | 156 |
| Clawed frog | *dm-W* | stages 48–52 (peak 50) | 157 |
| Japanese rice fish | *dmy/dmrt1bY* | stage 37 to adult | 158 |
| Turquoise killifish | *gdf6Y* to *foxl2* | 3 dph to adult | 159 |
| Rainbow trout | *sdY* to *foxl2* | 40–90 dpf | 160 |
| Patagonian pejerrey | *amhy* | 8 dpf to 140 dph | 70 |
| Philippine medaka | *gsdfY* to *cyp19a1* | 2 dph to adult | 161 |
| Pirarucu | *id2bbY* | juvenile to adult | 162 |
| *Drosophila* | *sisA* (XSE) to *Sxl* | 8–14 nuclear cycles (peak 12/13) | 163 |
| *Drosophila* | *sisB/sc* (XSE) to *Sxl* | 9–14 nuclear cycles (peak 12) | |
| Nematode | *fox-1* (XSE) to *xol-1* | 8/16–550 cells (peak 100) | 164 |
| Nematode | *sex-1* (XSE) to *xol-1* | 28/100–550 cells (peak 28/100) | 165 |
| Medfly | *MoY* to *tra* | 2/3–48 hpo (peak 15) | 166 |
| Silkworm | *Fem* to *Masc* | 15–18/21 hpo (peak 18/21) | 113 |

dpc, days post-conception; dph, days post-hatching; dpf, days post-fertilization; hpo, hours post-oviposition; XSE, X-linked signal element.

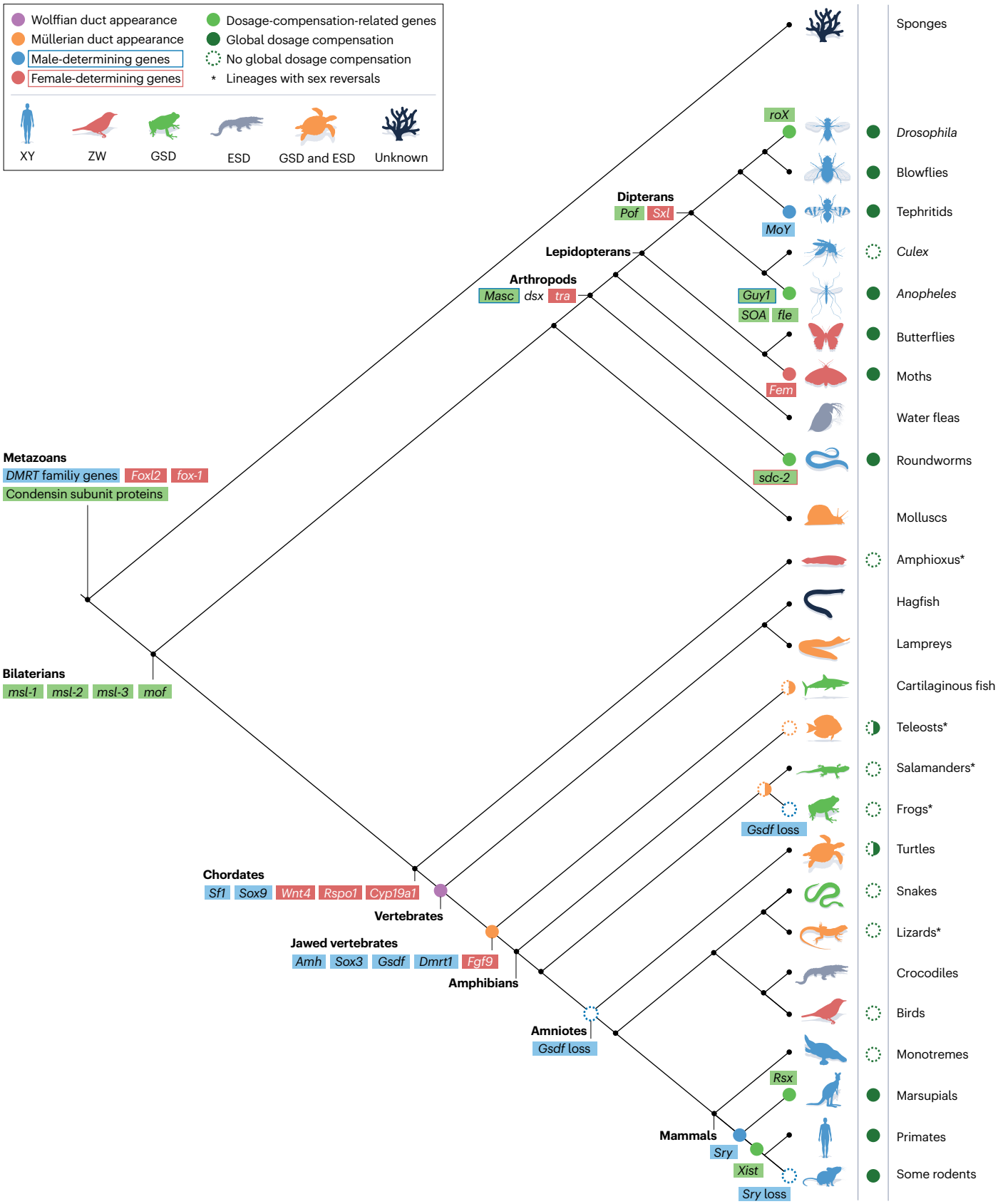

Wolffian duct appearance
Müllerian duct appearance
Male-determining genes
Female-determining genes
Dosage-compensation-related genes
Global dosage compensation
No global dosage compensation
* Lineages with sex reversals
XY
ZW
GSD
ESD
GSD and ESD
Unknown
Metazoans
DMRT familiy genes
Foxl2
fox-1
Condensin subunit proteins
Bilaterians
msl-1
msl-2
msl-3
mof
Chordates
Sf1
Sox9
Wnt4
Rspo1
Cyp19a1
Vertebrates
Jawed vertebrates
Amh
Sox3
Gsdf
Dmrt1
Fgf9
Amphibians
Amniotes
Gsdf loss
Mammals
Sry
Xist
Sry loss
Rsx
Arthropods
Masc
dsx
tra
Dipterans
Pof
Sxl
Lepidopterans
roX
MoY
Guy1
SOA
fle
Fem
sdc-2
Gsdf loss
Sponges
Drosophila
Blowflies
Tephritids
Culex
Anopheles
Butterflies
Moths
Water fleas
Roundworms
Molluscs
Amphioxus*
Hagfish
Lampreys
Cartilaginous fish
Teleosts*
Salamanders*
Frogs*
Turtles
Snakes
Lizards*
Crocodiles
Birds
Monotremes
Marsupials
Primates
Some rodents

**Fig. 3 | Evolution of sex-related genes and gonad tissues.** A phylogeny displaying the origination of some upstream sex-determining genes (USDGs) (blue for male-determining genes; red for female-determining genes) (references are in Supplementary Table 1) and genes that have important roles in global dosage compensation in certain species (green). We note that one gene may originate at an ancestral time point and acquire the function for sex determination or global dosage compensation subsequently within a certain lineage (for example, *msl-2*). Genes involved in global dosage compensation that also carry a sex-determining function are shown by blue (male) or red (female) frames (for example, *Guy1*). The presence or absence of global dosage compensation is represented with filled or hollow circles, respectively — some lineages, such as teleosts, have both states of dosage compensation reported. The evolution of vertebrate reproductive systems is also shown: the Wolffian duct (purple dot) originated in the ancestor of vertebrates and regresses in males of amniotes; Müllerian duct (orange dot) originated in the ancestor of jawed vertebrates and undergoes more complete regression in females of amniotes than those of cartilaginous fish and amphibians[74]. Different colours of species icons indicate different sex chromosome/sex-determination systems. ESD, environmental sex determination; GSD, genetic sex determination. Asterisks indicate lineages reported to have natural sex reversals[22,169–171].

during the species radiation, with some recent enough to have retained many genes on the W chromosomes.

### Molecular mechanisms of recombination suppression

The above models deal with the evolutionary mechanisms of recombination suppression. Regarding the molecular mechanisms, the discovery of evolutionary strata in humans suggested that successive chromosome inversions led to recombination suppression[10], supported by comparisons of the human X/Y[98] or avian Z/W[80] chromsosomes, which found sequence footprints indicative of inversions. However, completely sex-linked regions are not necessarily caused by recombination suppression related to sex chromosome evolution. First, inversions can be the consequence rather than the cause of recombination suppression[88]. Second, recombination suppression may predate sex chromosome evolution or have evolved for other reasons. For example, many arthropods and some rodents have achiasmatic meiosis, whereby both autosomes and sex chromosomes of usually the heterogametic sex (XY male or ZW female individuals) pair without recombination during meiosis[99]. The less extreme form of this phenomenon, known as heterochiasmy (a great difference of recombination rate between sexes), is ubiquitous[100]. This phenomenon can include extreme crossover localization to telomeric regions of all chromosomes, not specifically the sex chromosomes, as recently revealed by sex-specific genetic maps in guppies[101]. We note that heterochiasmy in guppies probably predates the origin of their sex chromosomes[101]. Similar heterochiasmy has been reported in seahorses[23] and some frogs[24] (as well as the plant *Rumex hastatulus*[102]), in which new USDGs/sex chromosomes and sexually antagonistic polymorphisms can emerge at any genomic region with ancestrally low recombination without the two loci needing to be close to each other. Neither the molecular mechanisms nor the evolutionary causes of heterochiasmy and achiasmy are as yet well understood[103]. Other mechanisms, such as chromosome fusions that generate recombination 'cold spots' extended from the fusion point[83], as demonstrated experimentally in *Pristionchus* nematodes[104], could also predispose genomic regions to the origination of new sex chromosomes.

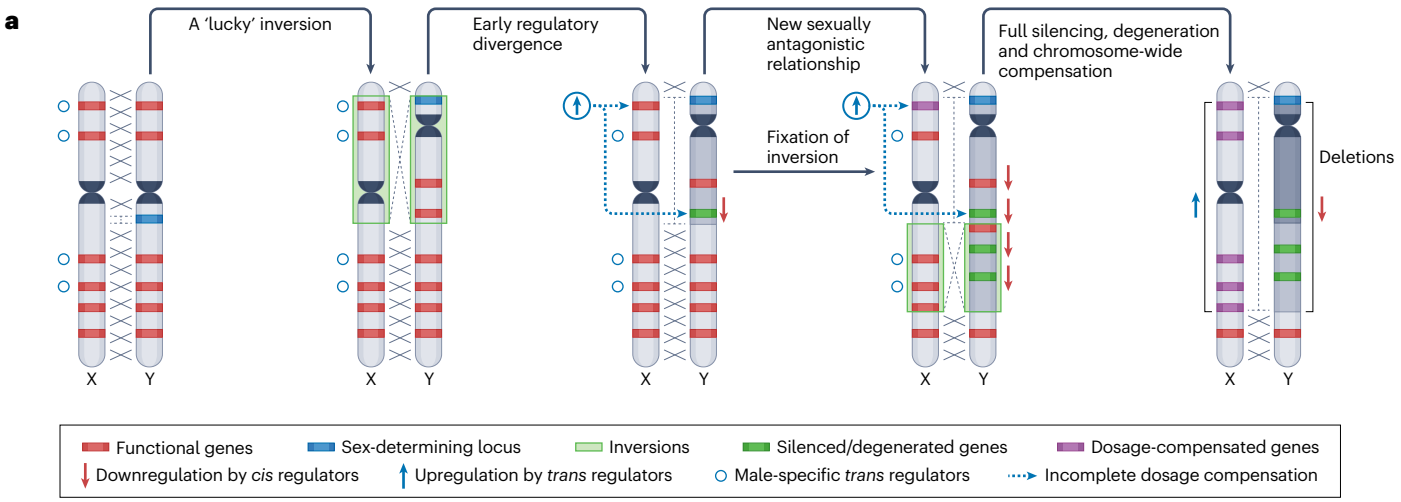
## Evolution of dosage compensation

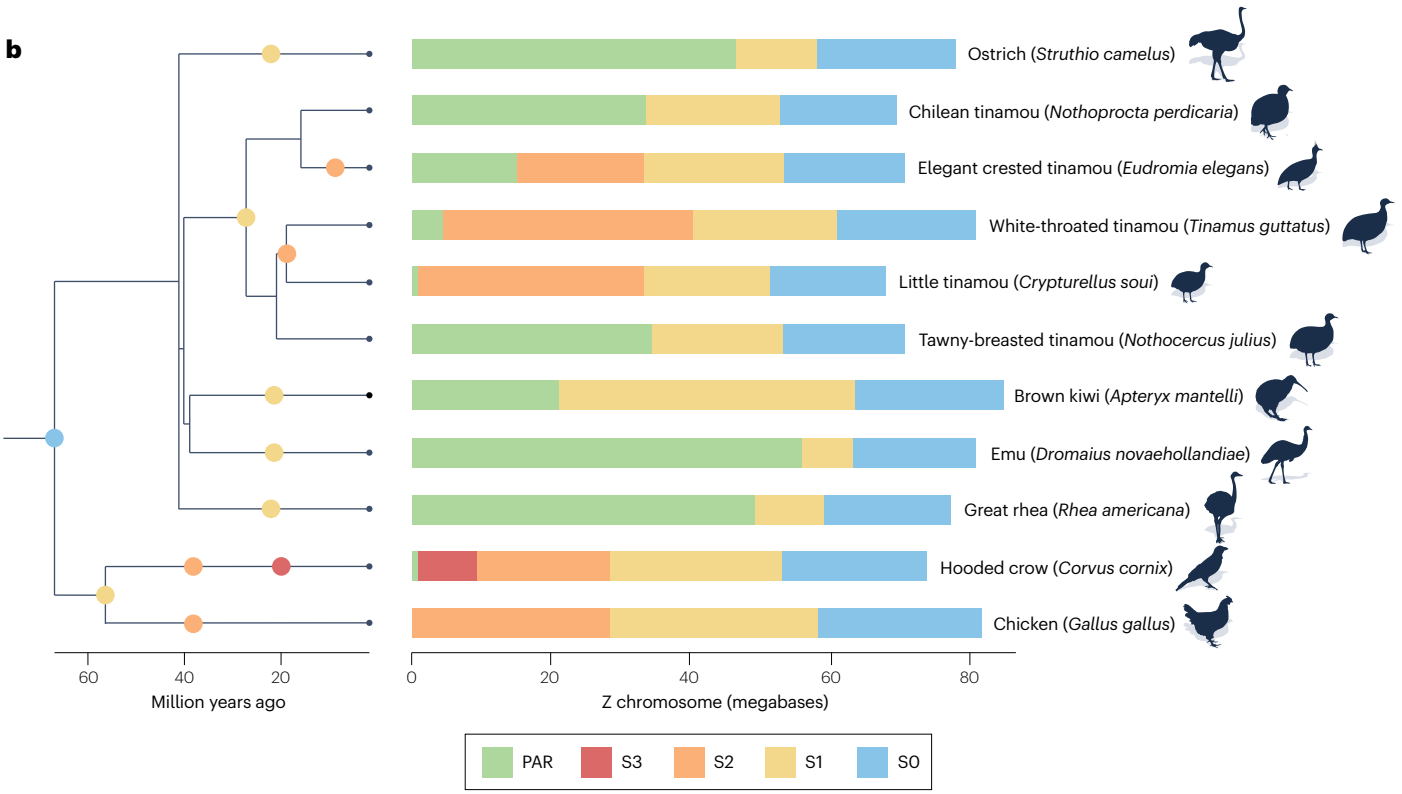
Following recombination suppression and Y-linked gene degradation, this reduced and imbalanced dosage (relative to that of autosomes) was predicted first by Susumo Ohno[12], a pioneer of evolutionary theory of sex chromosomes, to select for upregulation of X-linked genes in males by gaining a dosage-compensation complex (DCC). Ohno also predicted that some mechanism would have to evolve to avoid the DCC causing overexpression of X-linked genes in females. Interestingly, studies across a wide range of species with heteromorphic sex chromosomes have revealed a great diversity of extent and mechanism of dosage compensation[11,105]. For instance, prior work has shown that the chromosome-wide or global DCC can be restricted to males and upregulate gene expression (as in many insects) or restricted to females and either inactivate one X chromosome (randomly in eutherians, or the paternal X in marsupials[106]) or halve expression of both X chromosomes (as in the XX hermaphrodite nematodes and both Z chromosomes in male silkworms[107] and monarch butterflies[108]; Fig. 3b). Notably, global dosage compensation should be distinguished from transcriptional buffering or feedback mechanisms probably underlying the gene-by-gene or incomplete dosage compensation that also act on autosomes[109]. Also of note, the known XY systems may have evolved global dosage compensation more frequently than the ZW systems[110]. Here, we focus on the evolution of the well characterized global dosage compensation of the eutherians, *C. elegans* and *Drosophila*, whose mechanistic details have been comprehensively reviewed elsewhere[71,106,111,112].

### Origins of global dosage compensation

A critical step in the acquisition of global dosage compensation involves the evolution of a sex-specific transcription factor or long-non-coding RNA, as part of the DCC. For example, such long-non-coding RNAs include *roXs* for *Drosophila*, *Rsx* for marsupials and *Xist* for eutherians (Fig. 3b). This evolutionary process might also include the gain of the corresponding X-linked *cis*-regulatory binding sites that coordinate specific recognition by, and then the spreading of, the DCC along the X chromosome. Among the invertebrates characterized thus far, such transcription factors are intimately linked with the sex-determination cascade and often downstream of USDGs. For example, the *Drosophila* DCC gene, *msl-2*, is downstream of the sex-determination switch, *Sxl*[111], and the DCC genes in *C. elegans* (*sdc-2*)[71], silkworms (*Masc*)[113] and mosquitoes (*Guy1*, specifically in *Anopheles stephensi*)[114] all have important dual roles in both sex determination and global dosage-compensation processes (Figs. 2 and 3). Consistently, in both *D. melanogaster* and *C. elegans*, global dosage compensation occurs after sex determination and is initiated in early embryos, whereas X inactivation in eutherians occurs before sex determination and the two cascades are distinct (Fig. 5).

Most DCC component genes are ancient but originated with different functions — only acquiring the DCC-related function recently, probably after any lineage-specific USDG turnover. For example, the *Xist* gene acquired its DCC-related function in the eutherian ancestor (and therefore *Xist*-mediated dosage compensation is absent in marsupials and monotremes) and its sequence is derived from pseudogenization of part of the coding gene *Lnx3*, which is present in most vertebrates[115,116]. Another example is *msl-2*, which has become male-specifically expressed and encoded a key component of DCC in *Drosophila*. It is present in most metazoans,




and a recent study in mouse suggested that its ancestral function is critical for preserving biallelic expression[117], particularly that of dosage-sensitive genes. In fact, many other non-sex-specific components of DCC in *C. elegans* (FOX-1 and several condensin subunit proteins[71]) or those of *Xist*-interacting proteins in eutherians (for example, SPEN[118]) are deeply conserved between invertebrates and vertebrates (Fig. 3b). Therefore, current evidence suggests that DCC evolution involves either repurposing ancient regulatory genes or evolving new genes that interact with these ancient genes with a sex-specific expression.

## Spread of dosage compensation

Studies from model systems, such as *Drosophila* species and *C. elegans*, have demonstrated how proto-X chromosomes transition from DCC origination to global dosage compensation. Both *D. melanogaster* and *C. elegans* have evolved specific enrichment of sequence elements on the X chromosome for recruiting the DCCs (Fig. 5a). Insights have been gained into how the DCC and these *cis*-elements are propagated along the entire X chromosome by studying the neo-X chromosomes of different *Drosophila* species (for example, *D. miranda*)[119,120] as well as the genetically engineered neo-X chromosome in *C. elegans*[121].

**Fig. 4 | New model and study system of recombination suppression.** **a**, In this model that incorporates regulatory mutations, the original recombination suppression between sex chromosomes is assumed to evolve by chance instead of sexually antagonistic mutations. In such a 'lucky' inverted region (green rectangle) between the proto-X and proto-Y, mutations may occur in *cis*-regulatory elements on the Y chromosome that downregulate gene expression (red arrow) and promote the accumulation of deleterious mutations in the coding regions (green bar)[92]. This effect forms a feedback loop that further selects for downregulating gene expression of the Y-linked copy and incomplete dosage compensation (dotted arrow) that is mediated by mutations increasing expression (blue arrow) of the male-specific *trans*-factor (blue circle). Owing to stabilizing selection on the gene expression, recombination between the X- and Y-linked alleles are now selected against, which results in fixation of the lucky inversion[90]. **b**, Species such as some palaeognathous birds (ostrich, emu and tinamous) that carry long pseudoautosomal regions (PARs) and slowly differentiating sex-chromosome regions can be good models for testing the regulatory evolution and recombination suppression hypotheses between sex chromosomes. Blocks represent independently evolved evolutionary strata of sex chromosomes in birds, inferred by the divergence level between the Z and W chromosomes. Different colours refer to PARs and different ages of strata (S0 is the oldest and S3 is the youngest)[29,80]. Dots on the phylogeny indicate the origination time of respective evolutionary strata. Panel **b** is adapted with permission from ref. 29, Elsevier.

In *C. elegans*, the DCC was found to spread across the fusion breakpoint onto the proximal autosomal region, which is artificially fused to the X chromosome, indicating that DCC intrinsically spreads to loci near the target gene before evolving any *cis*-regulatory elements.

In *D. miranda*, at least 40% of the neo-X linked genes have become bound by the DCC within only 1.5 million years, suggesting that the DCC can quickly spread onto the young X chromosome. Furthermore, 60% of these genes bound by the DCC have a neo-Y homologue with intact open reading frames[122], indicating that many X-linked genes have been upregulated before they become hemizygous in males. Notably, such advanced dosage compensation is suggested by the regulatory model discussed above to select for downregulation and facilitate the protein degeneration of the Y-linked genes[92] (Fig. 4a). The acquisition and propagation of DCC recognition motifs can occur either through expansion of pre-existing simple repeats[120] or, as shown in *D. miranda*[119] and a teleost[123], recurrent insertions of transposable elements carrying the motifs. Interestingly, the neo-X chromosome of *D. busckii*, which evolved by fusion between the X chromosome and a small, ancestrally heterochromatic autosome (the dot chromosome), has not evolved global dosage compensation[124]. In *D. miranda*, the DCC preferentially spreads onto the ancestrally euchromatic regions of neo-X chromosome rather than the ancestrally heterochromatic regions, whose neo-Y homologues have suffered more severe downregulation or gene loss[122]. These results suggest a model in which the X chromosome evolves global dosage compensation in a manner that is constrained by the heterogeneity of chromatin landscape on the ancestral autosome, in addition to selection pressures to compensate for the loss of Y-linked genes (Fig. 5a).

### Incomplete dosage compensation

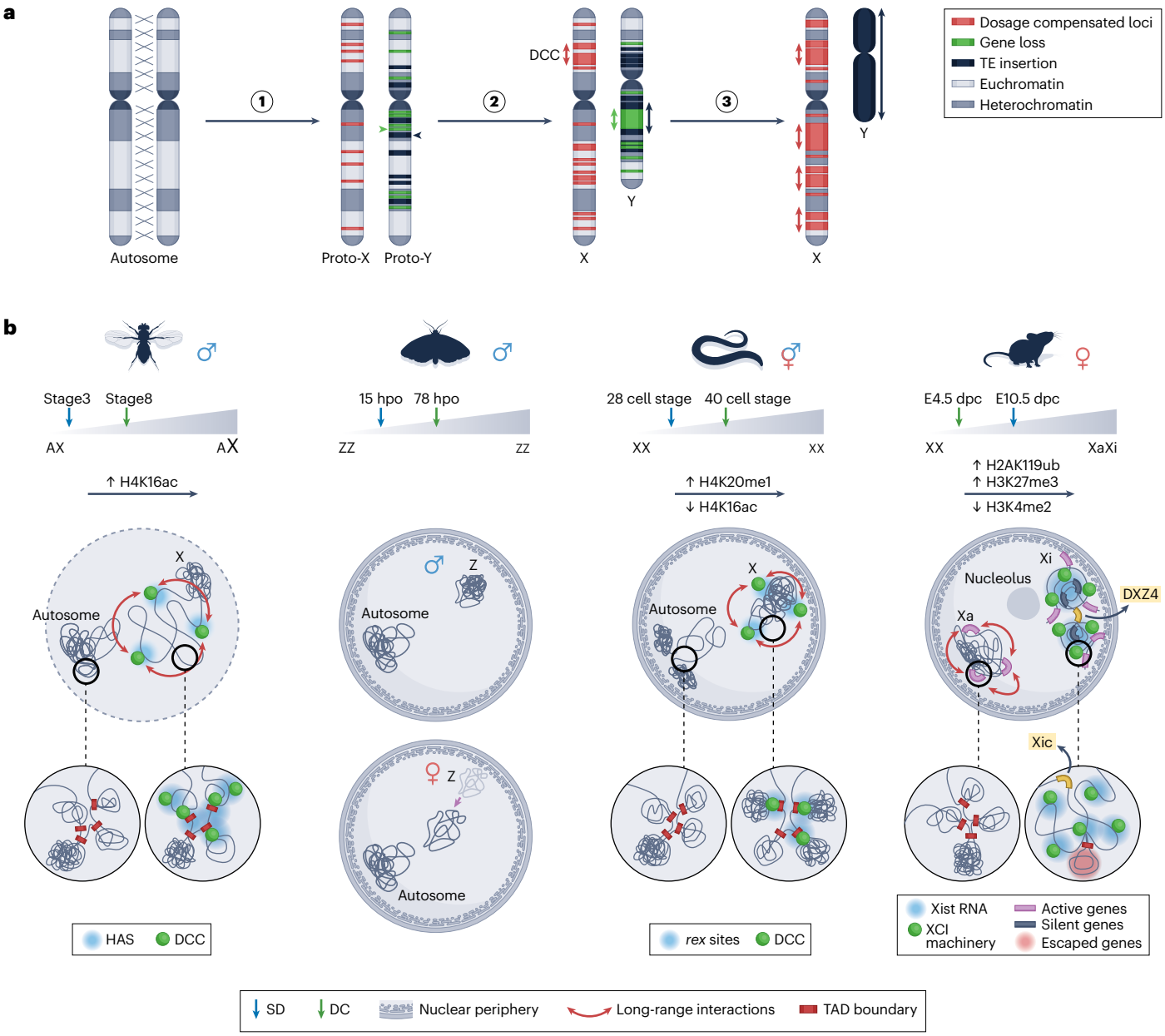
Some species, particularly many vertebrates with ZW sex chromosomes (for example, birds[125] and advanced snakes[126]), can cope with evolving only incomplete dosage compensation. For some of these species, their sex chromosomes may have diverged slowly (for example, palaeognaths in Fig. 4b) without a selective pressure strong enough for evolving global dosage compensation. In addition, sexual selection that usually targets males may favour male-biased expression of Z-linked genes (as they are more frequently inherited in males than W- and autosome-linked genes) and therefore counteract the evolution of global dosage compensation[110]. Interestingly, recent work suggests that the platypus and chicken have dosage compensation that is incomplete at the level of transcription but global at the level of translation[127]. Other work has further uncovered variation in dosage compensation between individual X/Z-linked genes across different cell types and developmental stages[128], within populations[129] and under different environmental conditions[130]. The underlying mechanisms of this variation in the extent (incomplete versus global) of the evolved dosage compensation are far from clear.

## Divergence of sex chromosomes in 3D space

In addition to the distinct sequence characteristics of X (reviewed[131]) and Y chromosomes[33] relative to autosomes, heteromorphic sex chromosomes have unique local epigenomic landscapes and higher-order nuclear architectures (that is, how the genome is folded and positioned in the somatic nucleus)[71,112]. Highly differentiated Y chromosomes have often evolved constitutive heterochromatin owing to transposable element accumulations and have become associated with the chromocentre (a condensed cytological chromatin compartment in the nucleus), as shown in both *Drosophila* and mouse[132,133]. By contrast, X chromosomes can evolve either regions of facultative heterochromatin or hyperactive euchromatin, dependent on the mechanism of global dosage compensation, if any. A key difference between global versus incomplete (or gene-by-gene) dosage compensation processes is the alteration of chromosome-wide topology, although the causal relationship between chromosome topology and nuclear positions with global dosage compensation, and more generally transcription regulation, is still debated (see below). Such topology can be detected by sequence- or image-based chromosome conformation capture methods (for example, Hi-C) and are reported as either long-range interactions or topologically associating domains (TADs) that exhibit stronger genomic interactions within than between each TAD unit[134].

### Genomic interactions

Despite the varied mechanisms of dosage compensation between *Drosophila* and *C. elegans*, both model systems exhibit long-range interactions between a subset of hierarchical *cis*-regulatory elements that differ in their capacity to recruit the DCC and are enriched on the X chromosome[135,136]. Some of these *cis*-regulatory elements have been demonstrated to have stronger or earlier DCC binding than others in *Drosophila* (PionX versus other HAS elements[137,138]) and *C. elegans* (high- versus low-affinity *rex* sites[136,139]). These long-range interactions may facilitate the spread of the DCC — either across the heterochromatic regions or onto the secondary weaker *cis*-regulatory elements — along the entire X chromosome in the 3D nuclear space. In hermaphrodites of *C. elegans*, TAD boundaries coincide with *rex* sites with high DCC occupancy. However, deletion of the DCC-dependent *rex* sites in *C. elegans* leads to disruption of TAD boundaries on X chromosomes, but does not affect global dosage compensation mechanisms to transcriptionally repress X-linked genes[140]. In female mice, through a sequence-independent mechanism, *Xist* RNA spreads onto a limited number of




distant loci that are close in 3D space and then diffuses to coat one of the X chromosomes to be inactivated in females[141]. This X chromosome then becomes segregated into two large megadomains mostly devoid of TADs and long-range interactions (except for regions that escaped X-inactivation), both of which are preserved on the other, activated X chromosome[112] (Fig. 5b).

### Interactions with nuclear bodies

Since the primary discovery of Barr bodies in the cytogenetic era, dosage compensation (or, more generally, transcriptional regulation) has been known to involve dynamic interactions between chromosomes (or specific genomic regions) with various nuclear bodies such as the nucleolus, nuclear periphery and speckles[142]. For instance, the inactivated (and compacted) X chromosome in female mice is located close to either the nucleolus or nuclear periphery, whereas the activated X chromosome is positioned at the periphery. Furthermore, deletion of the Lamin B receptor protein causes the inactivated X chromosome in mice to shift away from the nuclear periphery but notably has only a minor impact on the silenced genes[143]. During establishment of global dosage compensation in the silkworm, the female Z chromosome becomes more accessible in chromatin configuration and shifts towards the nuclear interior and the two male Z chromosomes become more compact, which corresponds to the upregulation of female Z-linked genes and downregulation of male Z-linked genes, respectively[107].

## Conclusions and perspectives

Sex chromosomes exhibit distinct genomic, epigenomic and 3D nuclear architecture patterns that reflect their different evolutionary histories and regulatory landscapes from autosomes. The role of recombination

**Fig. 5 | Evolution and chromatin features of dosage compensation.** **a**, Model of epigenomic evolution of sex chromosomes. Ancestral autosomes have a heterogenous distribution of euchromatin (white) and heterochromatin (dark grey) before they become sex chromosomes. The canonical model of sex-chromosome evolution assumes that dosage compensation evolves on X-linked genes in response to the loss of their Y-linked homologues, but this prediction is not supported by studies of neo-X and neo-Y chromosome evolution in *Drosophila miranda*[122]. Instead, the dosage-compensation complex (red) preferentially spreads in the ancestrally euchromatic regions of the neo-X chromosome, whose neo-Y homologues have not yet degenerated. Gene loss (green), TE insertions or heterochromatin spreading processes (black) preferentially occur in the ancestrally heterochromatin-dense regions of the neo-Y chromosome. **b**, Global dosage compensation in the model organisms *Drosophila*, silkworms, nematodes and mice. The top panels show the time of sex-determination initiation (blue arrow) and global dosage compensation (green arrow) during development for each species. Global dosage compensation occurs after sex determination is initiated in the three invertebrates, but before sex determination in mice. The different sizes of the X or Z chromosome indicates up- (larger) or downregulation (smaller) of X- or Z-linked genes, relative to autosomal (A in *Drosophila*) genes. The changing levels of histone modifications on the X (or Z) chromosome after establishment of dosage compensation are also shown. The bottom panels show the chromatin changes within the nucleus during dosage compensation. In *Drosophila*, X-linked chromatin becomes looser and more accessible[135,138], whereas the X (or Z) chromosomes of the other model species become more compact. The compensated X chromosomes of both *D. melanogaster* and *C. elegans* exhibit strong long-range interactions coordinated by the DCC complex and X-linked recruitment sites (HAS or *rex* sites) compared to autosomes[138,139]. In mice, the inactivated X chromosome in females loses most long-range interactions and topologically associated domain (TAD) structures and becomes segregated into two large mega-domains separated by the DXZ4 repeat[112]. Dosage compensation also causes the X chromosomes to shift nuclear positions in silkworms and mice[107,112,172]. DC, dosage compensation; DCC, dosage-compensation complex; dpc, days post-conception; HAS, high-affinity sites; hpo, hours post-oviposition; *rex*, recruitment elements on X; SD, sex determination; TE, transposable element; Xi, inactivated X chromosomes; Xa, activated X chromosome; Xic, X-chromosome inactivation centre.

suppression in sex-chromosome evolution – beginning with the first theoretical models by Nei[144] – has been researched now for more than 50 years. Such work has illuminated how X[131] and Y[145] chromosomes are expected to evolve, such as why they become heteromorphic[90,95] (Fig. 4a) or remain homomorphic (as in some teleosts, reptiles and amphibians) without proceeding to later phases of sex chromosome evolution (Fig. 1). These species (Fig. 2a) lend support to other models of sex-chromosome evolution (such as the 'fountain of youth' model[25]) that consider the effects of lineage-specific features of reproductive tissues (for example, natural sex reversals) that are usually absent in the classic genetic model organisms.

Future efforts are needed to integrate lineage-specific developmental biology (particularly species with different cell types that are dominant over other gonad cells during sex determination) with the evolution of sex-determination cascades and sex chromosomes. In mammals, sex determination is initiated in the gonad somatic supporting cells, which affects the decision of germ cells to subsequently differentiate into a sperm or egg. In zebrafish and chickens, depletion of germ cells can cause complete or partial sex reversals in the gonad or secondary sexual characters[146]. At the tissue level, the Müllerian duct gives rise to the oviduct in most vertebrates but is absent in teleosts. This tissue is inferred to have originated in the ancestor of jawed vertebrates and undergoes partial regression in males of cartilaginous fish and amphibians but regresses more completely in amniote males (Fig. 3b). Such lineage-specific differences in reproductive tissues are proposed to be associated with the gain of sex-determining function of certain genes (for example, *amh*) and may affect the potential for natural sex change[74]. These features might underlie the different 'usual suspects' of USDGs and their interactions with downstream 'parliamentary' genes between lineages (Fig. 3). Notably, single-cell transcriptome sequencing has offered some insights into the diversity of gonad development between species at the cellular level. For example, a recent study showed that chicken gonad supporting cells have a different progenitor compared to other vertebrates[147].

Research into sex-chromosome evolution will also probably continue to benefit from an increased understanding of epigenomic regulation and genome function in three dimensions. Emerging evidence suggests that both the spreading of dosage compensation and degeneration of Y chromosomes do not occur in a gene-by-gene manner but could involve global changes of chromatin architecture through phase separation in the 3D nuclear space (Fig. 5). Young or slowly diverging sex chromosomes (for example, those of *D. miranda*[122] and some birds) (Fig. 4b) are promising models not only for the regulatory evolution of sex chromosomes (Fig. 4a) but also the tempo and mode of epigenomic evolution. The rapid development of various genomic technologies would facilitate future interdisciplinary investigations into the mechanisms generating diversity of sex chromosomes.

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## Author contributions
Q.Z. contributed substantially to discussion of the content. All authors wrote the article and reviewed and/or edited the manuscript before submission.

## Competing interests
The authors declare no competing interests.

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