Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration

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Abstract | The human Y chromosome is intriguing not only because it harbours the master-switch gene that determines gender but also because of its unusual evolutionary history. The Y chromosome evolved from an autosome, and its evolution has been characterized by massive gene decay. Recent whole-genome and transcriptome analyses of Y chromosomes in humans and other primates, in *Drosophila* species and in plants have shed light on the current gene content of the Y chromosome, its origins and its long-term fate. Furthermore, comparative analysis of young and old Y chromosomes has given further insights into the evolutionary and molecular forces triggering Y-chromosome degeneration and into the evolutionary destiny of the Y chromosome.

Sex chromosomes
A pair of chromosome that determines the sex of an individual.

Genomic conflict
Conflict of different genes within an organism that arises when genes inside a genome are not transmitted by the same rules (such as mitochondria or nuclear genes) or when the transmission of a particular gene is increased to the detriment of other parts of the genome.

Y-chromosome degeneration
The process of gene loss from the Y chromosome.

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Sex chromosomes are derived from autosomes and have independently evolved many times in different lineages. For example, the human X and Y chromosomes originated ~200–300 million years ago in eutherian mammals[1,2] after the split of monotremes, and sex chromosomes evolved independently in birds and snakes, and multiple times in other reptiles, amphibians and fish; they also formed repeatedly in many invertebrate taxa and plants[3–5]. Sex chromosomes carry the master sex-determining genes, are subject to unique evolutionary forces[6,7] and play a prominent part in many evolutionary processes, such as speciation[8], adaptation[9,10] and genomic conflict[11]. Some Y chromosomes are known to undergo a process termed Y-chromosome degeneration, in which the Y chromosome loses most of its original genes over evolutionary time[3–7]; this article focuses on such degradation in humans, *Drosophila* spp. and some species of plants.

Two features set Y chromosomes apart from the rest of the genome: a lack of recombination on the Y chromosome over some or most of its length[6] and male-limited transmission of the non-recombining segment[10]. Investigating Y chromosomes is challenging; their lack of recombination prevents classical linkage-mapping studies, and their high content of repetitive and ampliconic sequences has excluded them from most genome sequencing projects[12]. Classical genetics studies have shown that Y chromosomes often harbour almost no genes[13], and indeed some species have completely lost their Y chromosome[14,15].

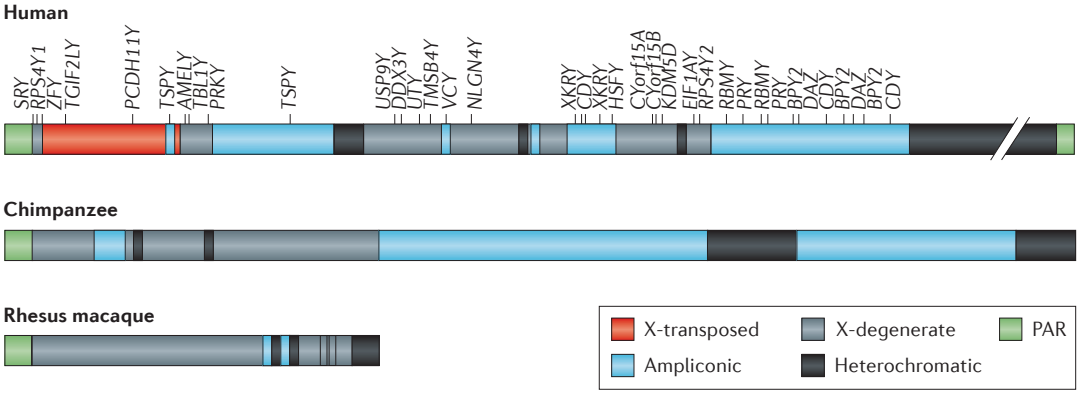
Recent genomic advances, however, have provided newly detailed views of Y-chromosome evolution and have revealed many novel and often surprising insights[12]. Analyses of Y-chromosome sequences have shed light on the DNA sequence composition and gene content of recently formed and ancient Y chromosomes in animals and plants. These studies have revealed the evolutionary forces operating on Y chromosomes and have provided novel insights into molecular and evolutionary mechanisms that initiate Y differentiation or that determine its long-term survival (in particular, the human Y chromosome); these recent insights are the focus of this article.

I first present a brief summary of whole-genome analyses of the genomic architecture of old Y chromosomes in primates and *Drosophila* spp., and follow this with a description of the evolutionary processes that drive Y-chromosome differentiation. I then present recent findings from genome-wide investigations of the molecular processes that initiate Y-chromosome degeneration in recently formed Y chromosomes of plants and *Drosophila* spp., and I conclude with a discussion of the long-term destiny of Y chromosomes and promising future directions of Y-chromosome research.

Genomic composition of old Y chromosomes

Old Y chromosomes, such as those of *Drosophila melanogaster* and humans, are often highly heterochromatic and have a large amount of repetitive and ampliconic DNA[15], making them notoriously difficult to sequence[12]. The genes carried by the sex chromosomes

Box 1 | **Primate Y chromosome as revealed from genomic studies**



Genomic and genetic analyses have revealed that the primate sex chromosomes are composed of a heterogeneous mix of sequences[16,20–22] with different evolutionary trajectories.

Pseudoautosomal regions (PARs). These sequences flank the male-specific region of the Y chromosome (MSY) and have homologues on the X chromosome. The PARs of the sex chromosomes recombine during meiosis[102]. The human Y chromosome has two PARs. The rhesus macaque and chimpanzee Y chromosomes have only one PAR that corresponds to the short-arm PAR in humans, and the precise boundary between that PAR and the MSY is identical in the three species.

Heterochromatin. The human Y chromosome contains a large heterochromatic block (~40 Mb long) that has not been sequenced. Both the chimpanzee and rhesus macaque Y chromosomes have much less heterochromatin.

X-transposed region. This is a 3.4 Mb region that is unique to humans, that transposed to the Y chromosome ~3–4 million years ago and that contains only two genes.

X-degenerate region. The X-degenerate region is a deteriorated version of the ancestral autosome that formed the Y chromosome. It contains 16 single-copy genes in humans with homologues on the X chromosome that mostly have housekeeping functions[16,18]. Genes located in the X-degenerate regions are well-conserved among primates. Specifically, all of the 16 X-degenerate genes are shared between human and rhesus macaques, whereas chimpanzees have lost four of them through inactivating mutations.

Ampliconic regions. The ampliconic region is a highly repetitive sequence. In humans, ampliconic regions contain around 60 genes belonging to nine different gene families that all have male-specific function and that are often located in palindromes[17,103] (BOX 3). Whereas humans and chimpanzees contain a substantial amount of ampliconic DNA, this sequence feature is almost absent from rhesus macaques, and the euchromatic segment of the MSY is notably smaller in rhesus macaques than in humans and chimpanzees. Only 0.5 Mb of the rhesus MSY euchromatin is ampliconic compared with 10.2 Mb and 14.7 Mb in humans and chimpanzees, respectively[19–21]. Also, fewer ampliconic regions are in palindromes in rhesus macaques; the human and chimpanzee MSYs have 8 and 19 palindromes that span 5.5 Mb and 7.5 Mb, respectively, whereas the rhesus MSY has only three palindromes, which collectively span 437 kb. Unlike in humans, most palindromes in chimpanzees exist in multiple copies so that each palindrome arm has multiple potential partners for both intra- and interpalindrome gene conversion. Despite the elaborate structure of the chimpanzee Y chromosome, there are no chimpanzee-specific ampliconic genes, yet three out of nine multi-copy, testis-expressed gene families present in humans are pseudogenized or are simply absent in chimpanzees. Thus, the gene repertoire of the chimpanzee MSY is considerably smaller and simpler than that of the human MSY. The chimpanzee MSY contains only two-thirds as many distinct genes or gene families as the human MSY and only half as many protein-coding transcription units.

of humans and other mammals overlap, suggesting that they all evolved from a common ancestral chromosome >200 million years ago, and all members of the *Drosophila* genus share a homologous ancestral sex chromosome pair that formed >60 million years ago; thus, the Y chromosomes of these model species are evolutionarily old, and the X and Y chromosomes are highly diverged from each other.

To date, only three primate species — humans, chimpanzees and rhesus macaques — have had most of their Y-chromosomal euchromatic DNA sequenced[16–21] (BOX 1). In addition, detailed molecular and bioinformatics analyses have allowed identification of most of the protein-coding genes located on the completely heterochromatic Y chromosome of *D. melanogaster*[22–30], but the vast majority of non-exonic DNA on the *D. melanogaster* Y chromosome has not been sequenced (BOX 2). Comparative analysis of primate Y chromosomes and *D. melanogaster* Y chromosomes has revealed common features that are shared between their Y chromosomes and that have independently evolved in these clades; the most striking characteristic is chromosome-wide decay in functional genes on the Y chromosome.

In humans, the euchromatic part of the Y chromosome is ~23 Mb in size and contains 78 protein-coding genes compared with 150 Mb of euchromatin and around 800 protein-coding genes on the X chromosome. Similarly, the *D. melanogaster* X chromosome contains

Recombination
The breaking and rejoining of DNA strands to form a new combination of genetic information.

Heterochromatic
Heterochromatin is a tightly packed form of DNA that is typically genetically inactive and contains repetitive sequences and few genes.

Box 2 | The *Drosophila melanogaster* Y chromosome

The ~40 Mb Y chromosome of *Drosophila melanogaster* is completely heterochromatic, and only parts of the coding sequence of Y-linked protein-coding genes have been sequenced[104]. Male *D. melanogaster* flies that lack a Y chromosome are viable but sterile, indicating that Y-linked genes have a role in spermatogenesis[22]. Investigations of Y-chromosome rearrangements identified six fertility factors on the *D. melanogaster* Y chromosome[22,23], and some of these fertility factors span several megabases of DNA and are over 100 times larger than the average *D. melanogaster* gene[23]. This dramatic increase in gene size is due to gigantic introns that largely consist of repetitive DNA and results in the formation of giant lampbrush-like loops during their transcription in spermatogenesis[105]. Bioinformatics analysis of the *D. melanogaster* genome identified 13 protein-coding genes (one of which is in multiple copies) and two RNA-coding genes, most of which have no homologues on the X chromosome; instead, their closest paralogues are autosomal, suggesting that these genes transposed onto the Y chromosome from an autosomal ancestral copy[24–26]. Comparison of the Y-chromosome gene content across the 12 sequenced *Drosophila* species revealed that only three of the *D. melanogaster* Y-linked genes are Y-linked in all species, and only five of the genes were inferred to be present in the most recent common ancestor of the 12 *Drosophila* species >60 million years ago[29], and the other genes were acquired more recently[27]. Thus, the gene content of the *D. melanogaster* Y chromosome shows a low level of conservation, and gene gains had a prominent role in the evolution of the *Drosophila* Y chromosome.

The only homologous region between the X and Y chromosomes in *D. melanogaster* is the ribosomal DNA locus, which is a tandemly repeated array consisting of hundreds of units encoding ribosomal RNA genes and physically accounts for ~10% of the entire Y chromosome[28]. The lack of homology between the X and the Y chromosomes has led to the suggestion that the Y chromosome in *D. melanogaster* is in fact derived from a supernumerary B chromosome after loss of the ancestral Y chromosome[30], but it can also simply mean that all original genes were lost from the Y chromosome over time.

Interestingly, although the *D. melanogaster* Y chromosome is degenerated, heterochromatic and contains very few genes, increasing evidence suggests that it has an important role in regulating the expression of numerous, possibly hundreds to thousands of autosomal and X-linked genes, which is presumably related to epigenetic modification of chromatin state by the Y chromosome[31–33]. That is, the Y chromosome of *D. melanogaster* — which accounts for 20% of the haploid genome of a male fly — is entirely heterochromatic and might function as a sink for proteins that are necessary for the establishment of heterochromatin. Indeed, the Y chromosome exerts an epigenetic effect in position effect variegation (PEV; the silencing of genes owing to their proximity to heterochromatin); an extra Y chromosome in the karyotype (XX-Y females and XY-Y males) suppresses PEV, whereas a missing Y chromosome (X0 males) enhances PEV[106]. The Y chromosome might interfere with gene expression either by recruiting proteins involved in chromatin remodelling (in PEV) or by disturbing some key transcription factors[107,108].

B chromosome
A supernumerary chromosome that is not essential for the life of a species and that is present in only some of the individuals of a species.

Position effect variegation
(PEV). The variable, heritable suppression of genes by their juxtaposition to heterochromatin or telomeres or by movement of a gene into a different nuclear domain or chromosomal context.

Palindromes
A DNA sequence composed of two inverted repeats (arms) separated by a short spacer.

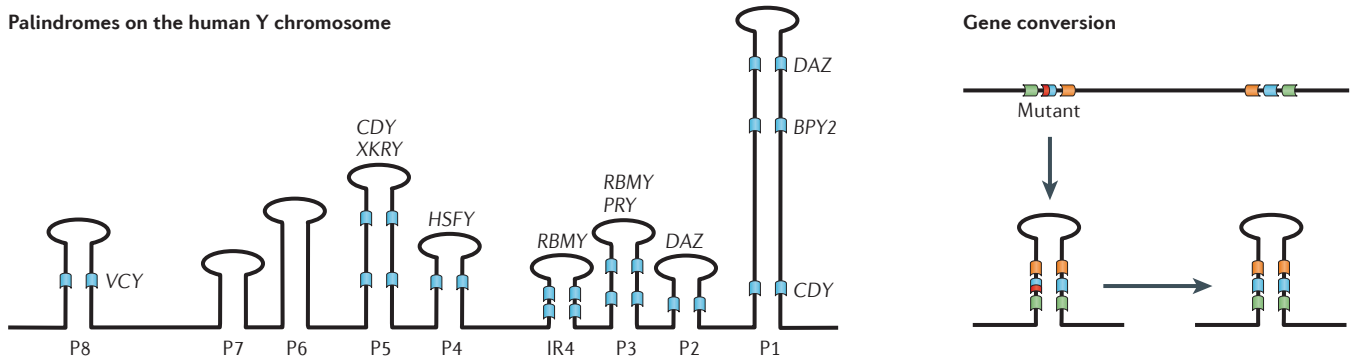
~22 Mb of euchromatic sequence and harbours around 2,200 genes, whereas the ~40 Mb Y chromosome is completely heterochromatic and contains only 13 known protein-coding genes. Y chromosomes instead often contain a large fraction of repetitive and non-functional DNA. Genome sequencing and transcriptome analysis have also confirmed that many genes located on the Y chromosome in both primates[18] and *Drosophila* spp.[22–25] have male-related function. Furthermore, sequencing of the human Y chromosome has revealed large ampliconic regions that contain genes belonging to different gene families[16]. These gene families commonly reside in palindromes, and gene conversion between members of a gene family may have aided their long-term survival on the Y chromosome[17] (BOX 3). Sequencing also revealed the presence of evolutionary strata (see below). It is also interesting that gene gain has had an important role in *Drosophila* spp. Y-chromosome evolution[27], and the *D. melanogaster* Y chromosome influences expression of possibly hundreds of autosomal and X-linked genes[31–33]. Whole-genome and transcriptome analysis has also revealed some other surprising twists and interesting characteristics of these chromosomes (for details, see BOXES 1,2), but they largely conform to the classical view that Y chromosomes represent highly degenerate versions of the X chromosome. Both the primate and the *D. melanogaster* Y chromosomes have lost most of their ancestral genes that were initially present on the autosome that formed the Y chromosome millions of years ago, and most of the remaining genes have a male-specific function.

Evolutionary forces on the Y chromosome

Sex chromosomes originate from autosomes[34,35]. They can arise in species with separate sexes in which sex is determined by environmental cues (such as in turtles, where temperature determines the sex of developing embryos) and can also arise in hermaphrodites (that is, individuals with both male and female sex organs). FIGURE 1 illustrates a potential path leading to heteromorphic sex chromosomes, but others are possible[34]. The first step in the evolution of Y chromosomes is likely to be the acquisition of a male-determining gene on one member of a pair of autosomes that will ultimately become the sex chromosomes (for example, a male-determining gene forming a proto-Y chromosome). Genetic sex determination with otherwise homomorphic sex chromosomes is observed in many taxa in amphibians, fish, reptiles and many invertebrates[15,36]. For heteromorphic sex chromosomes to originate after the acquisition of a male-determining gene, recombination needs to become suppressed between the homomorphic proto-sex chromosomes[5,34]. This allows the Y chromosome to evolve independently of its X homologue.

The potential role of sexually antagonistic mutations. Sexually antagonistic mutations[37] — which are beneficial to one sex but detrimental to the other[5,34] — are thought to provide the selective force to suppress recombination between nascent sex chromosomes. Sexually antagonistic mutations are more likely to become established in a population if they are more often transmitted through the sex that they benefit[10], and restriction of recombination between the nascent proto-X and proto-Y chromosomes may be a consequence of selection favouring genetic linkage between sexually antagonistic mutations and the sex-determining region. Evidence from mammals suggests that the elimination of recombination might be achieved through chromosomal inversions on the proto-sex chromosomes[1,2,38,39]. Inversions are known to suppress recombination locally in heterozygotes[38]. Thus, an inversion on one of the proto-sex chromosomes can repress recombination between the proto-X and proto-Y chromosomes in males and can allow them to accumulate mutations independently and to become genetically distinct. Suppression of recombination can evolve in multiple steps along the proto-sex chromosomes, as has happened in mammals[1], birds[40] and some plants[41,42]. In particular, comparison between genes in humans located

Box 3 | **Palindromes and gene conversion on the primate Y chromosome**



Genome sequence analysis of the human Y chromosome revealed that a substantial fraction of the chromosome is occupied by large repeat units (amplicons). Amplicons can be organized as tandem arrays or as inverted repeats (palindromes). In humans, there are eight palindromes that make up 54% (5.5 Mb) of the ampliconic sequence (palindrome 1 (P1)–P8 (see the figure)), and most contain several protein-coding genes that belong to different gene families (such as the deleted in azosperma (*DAZ*) gene cluster in P1 and P2 or the chromodomain protein, Y-linked (*CDY*) gene cluster in P1 and P5).

Palindromes allow for intrachromosomal gene conversion between members of a gene family through pairing of homologous genes that reside within palindromes on the Y chromosome. This may allow the restoration of a mutation-free gene copy at a locus with a deleterious mutation (depicted in red in the figure) through a gene conversion with another copy of the same gene family and has been shown to oppose Y-chromosome degeneration by analytical and computer simulation methods[109,110]. A consequence of gene conversion between the arms of a palindrome is a high amount of sequence identity between repeat units. In humans, 60% of ampliconic sequences (including all eight palindromes) show 99.9% or greater intrachromosomal sequence identity. A side-effect of the repetitive structure of amplicons is that they are evolutionarily rather unstable, have undergone dramatic restructuring across primates and have high mutation rates. Indeed, several cases of human male sterility are associated with deletions of Y-linked chromosomal segments that are the result of recombination events between repeat regions[111].

Heteromorphic sex chromosomes
A pair of chromosomes that is morphologically distinct.

Homomorphic sex chromosomes
A pair of chromosomes that is morphologically indistinguishable.

Proto-sex chromosomes
A new pair of chromosomes that (recently) acquired a sex-determining function but that otherwise contains identical genes.

Sexually antagonistic mutations
A mutation that is beneficial to one sex but detrimental to the other.

Genetic linkage
The tendency of genes that are proximal to each other on a chromosome to be inherited together.

Chromosomal inversions
A chromosome rearrangement in which a segment of a chromosome is reversed end to end.

in the X-degenerate region on the Y chromosome (BOX 1) and their homologues along the X chromosome has revealed that groups of X–Y gene pairs show heterogeneous levels of sequence divergence[1,2,43]. This implies that the Y chromosome did not stop recombining with the X chromosome at once over its entire length but instead did so in multiple successive steps. The regions along the sex chromosomes that stopped recombining at distinct time points are called evolutionary strata: the oldest stratum in humans (stratum 1) dates back over 240 million years, and the youngest stratum (stratum 5) originated only 30 million years ago. Sex-determining region Y (*SRY*), the master male-determining gene in mammals, is located in the oldest stratum[1], which is consistent with predictions that the emergence of a sex-determining gene can trigger the differentiation of homomorphic sex chromosomes.

The effects of a lack of recombination on the Y chromosome. Various evolutionary models have been proposed to explain the degeneration of the Y chromosome[44–49], and a common feature of these models is that the net efficacy of natural selection is strongly reduced on a non-recombining chromosome (BOX 4). Natural populations are subject to recurrent mutations, some of which increase survival or reproductive success (beneficial mutations), but most mutations have negative fitness consequences (deleterious mutations). Natural selection results in beneficial mutations becoming incorporated into the genome of a species, whereas deleterious mutations are selected against (allowing and maintaining adaptation[50]). On a recombining chromosome, selection can act independently on each mutation. In the absence of recombination, however, selection operates on entire chromosomes. An entire chromosome will be fixed in the population if a beneficial mutation arises on it, or an entire chromosome will be purged if it carries a deleterious mutation[51].

The consequence of chromosome-wide linkage is that mutations at different loci that segregate in the population can influence the fixation or loss of linked mutations (for more details, see BOX 4). Thus, Y chromosomes are expected to accumulate deleterious mutations and to incorporate fewer beneficial mutations. Consistent with these theoretical expectations, non-recombining Y chromosomes generally show lower levels of adaptation: that is, they show increased rates of accumulation of deleterious mutations and lower rates of adaptive evolution relative to recombining regions of the genome in various species[52–56]. However, although the general properties of these models are well understood theoretically, their relative contribution to the observed degeneration of Y chromosomes in natural populations is less clear[6]. In particular, the evolutionary dynamics of these models and the speed at which they cause gene decay on an evolving Y chromosome strongly depend both on selection coefficients and on mutation rates for beneficial and deleterious alleles[34,47,57]; these parameters are often poorly characterized.

The effect of male-limited transmission. Another unusual feature of Y chromosomes is that they are always transmitted from father to son and, unlike any other part of the genome, they are never passed through females.

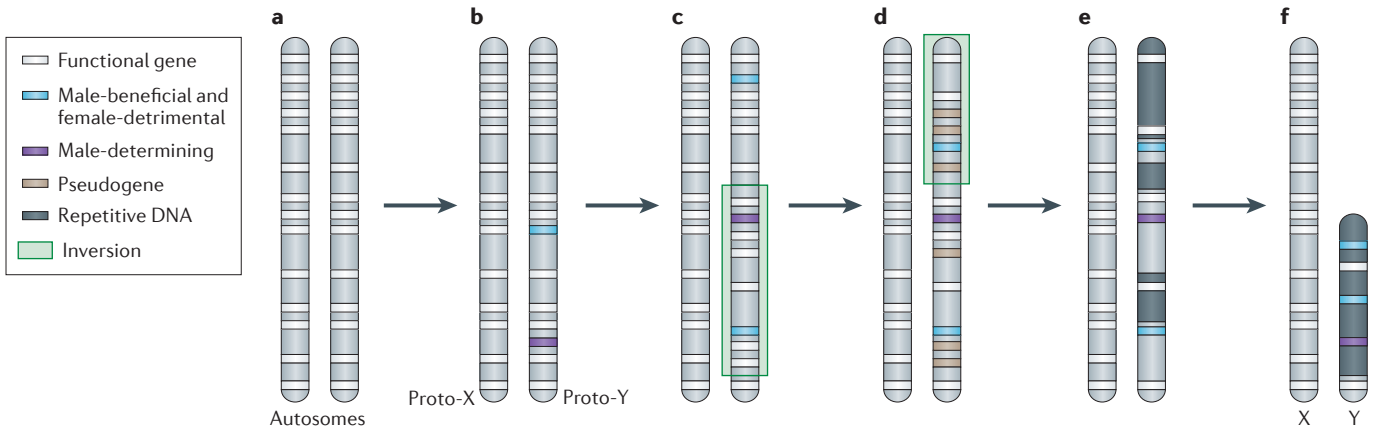


Figure 1 | **A model for the differentiation of sex chromosomes.** **a** | Sex chromosomes formed from ordinary autosomes, which contain identical sets of genes. **b** | A potential first step in the evolution of heteromorphic sex chromosomes is the acquisition of a sex-determining locus on a proto-sex chromosome, such as a male-determining gene. The emergence of separate sexes and sex chromosomes from a hermaphrodite ancestor requires a male and a female sterility mutation to occur on the proto-sex chromosomes[6]. **c** | Accumulation of sexually antagonistic mutations close to the sex-determining region select for suppression of recombination on the proto-sex chromosomes, which can be achieved by chromosomal inversion. **d** | The non-recombining region can increase if other mutations with sex-specific fitness effects accumulate on proto-sex chromosomes. Furthermore, a lack of recombination results in the accumulation of loss-of-function mutations at Y-linked genes (pseudogenization). **e** | Lack of recombination also results in an accumulation of repetitive DNA, which can lead to an increase of the size of the evolving Y chromosome. **f** | Large segments of non-functional DNA can be deleted in old Y chromosomes and can reduce their physical size. The evolutionary outcome of this process is heteromorphic sex chromosomes, in which the X chromosome largely resembles the autosome from which it derived, and the Y chromosome has lost most of its ancestral genes and may instead have accumulated repetitive DNA.

Male-limited transmission implies that the Y chromosome is an ideal part of the genome to carry genes that increase male fitness[5,34], as male-beneficial mutations on the Y chromosome are always transmitted through the sex in which they are advantageous and are sheltered from counter-selection in females if they are sexually antagonistic[10] (that is, if they are good for males but harmful to females). Thus, predictions for the gene content of Y chromosomes are clear; gene decay dominates, and surviving Y-linked genes are enriched for male-beneficial functions. Whole-genome sequence analyses in primates and *Drosophila* spp. have confirmed that the gene content of ancient Y chromosomes reflects these evolutionary forces (BOXES 1,2): that is, the vast majority of the ancestral genes have been lost from the Y chromosome, and many remaining genes, or genes that have been recruited secondarily to the Y chromosome, harbour male-specific functions[18,23].

Initial stages of Y-chromosome evolution

Little can be learned about the evolutionary forces and molecular mechanisms that drive gene decay from gene-poor ancient Y chromosomes. Recent genomic studies in young plant Y chromosomes and *Drosophila* neo-Y chromosomes, where degeneration is in progress, have revealed new insights into the origin of non-recombining Y chromosomes and how and why they degenerate.

Y-chromosome evolution in* Drosophila *neo-sex chromosomes. Neo-sex chromosomes of *Drosophila* spp. offer a powerful opportunity to study Y-chromosome degeneration in action in a comparative framework[58] (FIG. 2). *Drosophila* neo-sex chromosomes are formed by fusions of autosomes with the ancestral sex chromosomes that are shared among all members of the *Drosophila* genus (FIG. 2). Male *Drosophila* flies completely lack recombination; Y-fused chromosomes — called neo-Y chromosomes — are therefore male-limited, are sheltered from recombination and are thus subject to the same evolutionary forces as a true Y chromosome. The age of the fusion roughly correlates with the level of degeneration on the neo-Y chromosome with the caveat that the exact rate of degeneration depends on several species-specific factors, such as effective population size, number of genes present on the neo-Y chromosome or generation time. Several *Drosophila* species have recently formed 'neo-sex chromosomes', which, depending on how long they have been segregating as sex chromosomes, are at a different stage in their transition from an ordinary autosome to a heteromorphic pair of sex chromosomes. *Drosophila* neo-Y chromosomes thus allow the study of processes of gene decay on an evolving Y chromosome in a comparative chronological order. In particular, three *Drosophila* species with neo-sex chromosomes of different ages and different levels of degeneration have been characterized at the genomic level (namely, *Drosophila pseudoobscura*, *Drosophila miranda* and *Drosophila albomicans*; FIG. 2). Interestingly, the eutherian Y chromosome largely derives from a single autosomal region added to the ancestral sex chromosomes after the split from marsupials 80–130 million years ago[43]: that is, most of the human Y chromosome is also a 'neo-Y chromosome'.

Beneficial mutation
A mutation that increases the survivorship or fecundity (fitness) of its carrier.

Deleterious mutation
A mutation that decreases the survivorship or fecundity (fitness) of its carrier.

Dosage compensation
A process that balances expression of sex-linked and autosomal genes in the heterogametic sex.

***The neo-Y chromosome of* D. pseudoobscura.** *D. pseudoobscura* has a neo-sex chromosome pair that was formed ~15 million years ago. The neo-Y chromosome of *D. pseudoobscura* resembles the architecture of the ancestral Y chromosome of *Drosophila* and is entirely heterochromatic. It has not been fully assembled yet, but it appears to harbour only a handful of active genes[59]. Its former homologue, a neo-X chromosome, has maintained most of its roughly 3,000 ancestral protein-coding genes and has evolved all of the features that are typical of an X chromosome: that is, it is fully dosage-compensated, and it has evolved the gene content that is typical for an X chromosome (such as an excess of ovary genes and a deficiency of testis genes, as observed

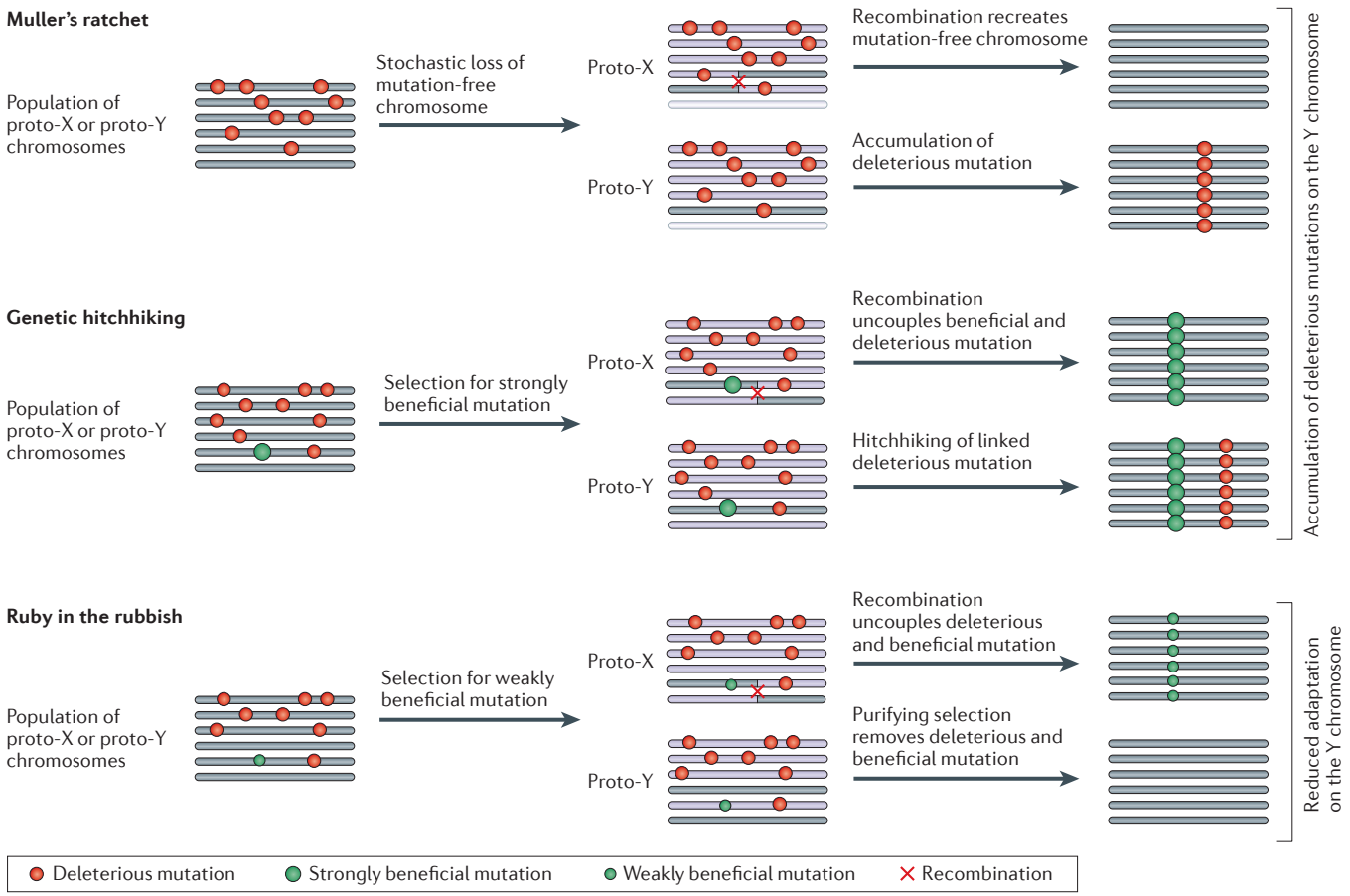
Box 4 | Population processes of Y-chromosome degeneration

Gene decay on a non-recombining Y chromosome is a consequence either of an accumulation of deleterious mutations at ancestral genes (Muller's ratchet[46] and genetic hitchhiking[44]) or of a lower rate of adaptation on Y-linked relative to homologous X-linked genes (ruby in the rubbish[45]). The figure shows a population of six recombining proto-X chromosomes or six non-recombining proto-Y chromosomes that are subject to beneficial and deleterious mutations. Chromosomes or chromosomal fragments that are lost in future generations are shown faded in the middle panel of the figure.

Muller's ratchet
This mechanism refers to the irreversible accumulation of deleterious mutations in a finite non-recombining population. In finite populations, chromosomes may accumulate mutations owing to stochastic effects. Recombination on the X chromosome allows the recreation of mutation-free chromosomes, whereas this loss is irreversible on a non-recombining Y chromosome and will result in the fixation of a particular deleterious mutation on the Y chromosome[112].

Genetic hitchhiking
Newly arising beneficial mutations can occur on a chromosome that also contains deleterious mutations. Owing to recombination, beneficial alleles can fix on the X chromosome without dragging along linked deleterious mutations. On a non-recombining Y chromosome, however, the fixation of the beneficial mutation will simultaneously fix the linked deleterious mutation. Genetic hitchhiking requires that the selective advantage of the beneficial mutation outweighs the effect of the linked deleterious allele so that the Y chromosome containing the beneficial mutation has a net selective advantage[47]. If this is not the case, the ruby in the rubbish process occurs (see below).

Ruby in the rubbish
Y chromosomes may undergo less adaptive evolution relative to the X chromosome owing to the linkage of beneficial alleles with deleterious mutations. Beneficial mutations of weak effect can become uncoupled from linked, strongly deleterious mutations by recombination on the X chromosome and become fixed in the population. By contrast, on non-recombining Y chromosomes, they will be eliminated by purifying selection. X-linked genes will continue to adapt and to incorporate beneficial mutations, whereas the Y chromosome will lag behind. Eventually, it can become advantageous for the male no longer to express its maladapted Y-linked genes and to silence or to inactivate them.



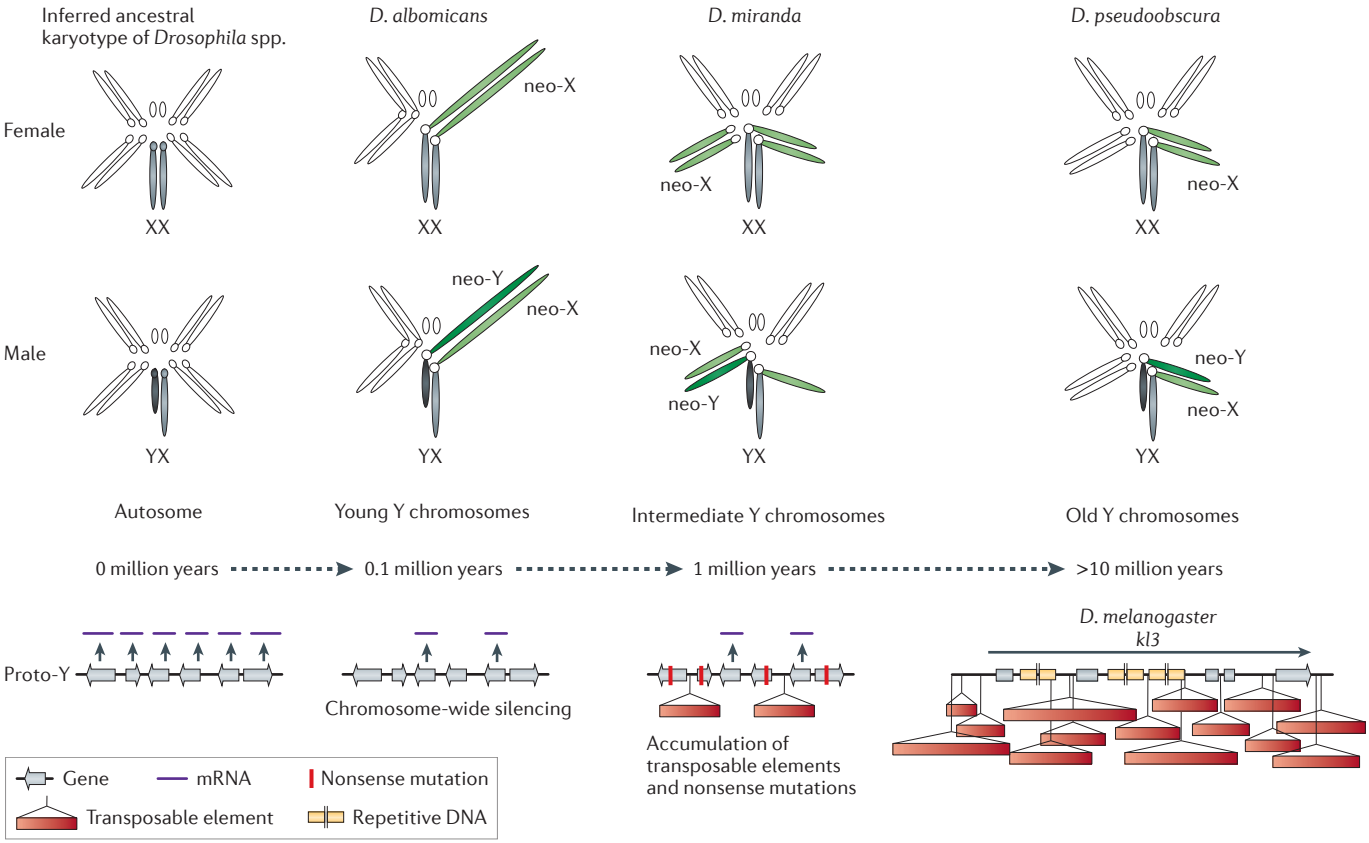


Figure 2 | **Neo-sex chromosomes in *Drosophila* spp.** Neo-sex chromosomes (in green) are formed by fusions of autosomes with the ancestral sex chromosomes (in grey), and the neo-X and neo-Y carry identical gene sets at the time of their origin (that is, 0 million years). To date, three *Drosophila* species with neo-Y chromosomes of different ages have been studied in detail and provide a dynamic picture of the molecular changes associated with Y differentiation; their karyotype is shown. Chromosome-wide downregulation of the expression of protein-coding genes before the accumulation of nonsense mutations at coding sequences is observed on the neo-Y chromosome in *Drosophila albomicans*, which originated ~0.1 million years ago. Thus, gene decay on the neo-Y chromosome appears to be initiated by chromosome-wide silencing possibly as a result of epigenetic modifications causing a change in chromatin structure. A general alteration of the genome architecture of the neo-Y chromosome has occurred within only 1 million years in *Drosophila miranda*. In particular, the neo-Y chromosome of this species is characterized by massive decay in gene function, including the fixation of stop codons and frame-shift mutations, large deletions and a general accumulation of repetitive transposable element DNA. The loss of gene function is accompanied by the beginning of heterochromatinization of the neo-Y chromosome. After 15 million years, almost no sequence similarity between the neo-Y chromosome and its former homologue can be detected, and the neo-Y has become entirely heterochromatic. This evolutionary stage has been reached in *Drosophila pseudoobscura* and resembles the general architecture of the ancestral Y chromosome in *Drosophila* spp. Genes on the ancestral Y chromosome of *Drosophila* spp., such as *kl3* in *D. melanogaster*, contain massive introns that are filled with repetitive DNA.

Muller's ratchet
The irreversible accumulation of deleterious mutations in a non-recombining population.

Genetic hitchhiking
The fixation of a deleterious mutation that is linked to a beneficial allele.

Ruby in the rubbish model
The selective elimination of a beneficial mutation that is linked to a deleterious allele.

for the ancestral X chromosome in *Drosophila* spp.[60–62]). Thus, an autosome can evolve into a fully differentiated sex chromosome pair within only 15 million years, and a non-recombining Y chromosome can lose thousands of genes within that time period.

***The neo-Y chromosome of* D. miranda.** The *D. miranda* neo-sex chromosome originated only ~1 million years ago[52], and its neo-Y chromosome shows features that are characteristic of both its autosomal origin and those of a degenerate Y chromosome. Cytological analysis shows that the neo-Y chromosome is partly heterochromatic[63]. Recent analysis of the genome and transcriptome of *D. miranda* has provided a comprehensive view of the evolutionary forces that operate on this young neo-Y chromosome[64–66]. Specifically, it is still largely homologous to the neo-X chromosome; more than 70% of neo-X sequences have homologues on the neo-Y chromosome, and more than 90% of the protein-coding genes are present on the neo-Y chromosome[66]. However, the neo-Y chromosome shows massive accumulation of repetitive DNA: almost half of the neo-Y sequence consists of transposable-element-derived DNA[53,65,67]. Further, of the roughly 3,000 genes that were initially present on the ancestral neo-Y chromosome, more than 40% have already become non-functional and have acquired

frame-shift mutations, stop codons or have been entirely deleted[66,68]. Thus, the whole protein-coding gene complement of the neo-Y chromosome has undergone dramatic, genome-wide degeneration in accordance with theoretical expectations of a reduced effectiveness of natural selection on a non-recombining chromosome[51]. In addition, whole-transcriptome analysis shows that gene decay at the DNA level is accompanied by chromosome-wide gene silencing. Specifically, a large fraction of neo-Y genes (~50%) is expressed at a lower level from the neo-Y chromosome relative to the neo-X chromosome[64–66]. Thus, Y-chromomsome degeneration can proceed rapidly on these neo-Y chromosomes in *Drosophila* spp.: more than a thousand genes have lost their function within only 1 million years. However, as a dosage compensation machinery exists in *Drosophila* spp.[60], Y-chromosome degeneration may proceed at a faster rate than it would if a new sex chromosome were formed *de novo* in the genome of a species without dosage compensation already in place.

***Initiation of neo-Y chromosome degeneration in* D. albomicans.** Many neo-Y genes are pseudogenized and downregulated in *D. miranda*, and the neo-Y chromosome is accumulating transposable element DNA and has partly become heterochromatic. However, the sequence of events is unclear. Does an accumulation of nonsense mutations on the neo-Y result in the production of many non-functional proteins from a degenerating neo-Y chromosome, which in turn selects for downregulation of these genes? Gene silencing could potentially be achieved through recruitment of transposable elements on the neo-Y chromosome, resulting in transcriptionally inactive heterochromatin or through the accumulation of mutations in regulatory regions. Alternatively, does gene silencing and/or heterochromatin formation occur first and do downregulated neo-Y genes then neutrally accumulate nonsense mutations?

Genome and transcriptome analysis of the younger neo-Y chromosome of *D. albomicans* (which is less than 0.1 million years old) partially answered these questions. In this species, the neo-Y chromosome (which contains almost 5,000 genes) shows almost no evidence of degeneration at the DNA sequence level: less than 2% of protein-coding genes are pseudogenes[69]. Intriguingly, however, a large fraction of these neo-Y genes (~30%) is expressed at a lower level than their neo-X homologues despite little decay of those genes at the protein level[70]. This suggests that transcriptional downregulation precedes degeneration of protein-coding genes. The formation of heterochromatin is a plausible explanation for this chromosome-wide downregulation, but it is also possible that the accumulation of mutations in promoter and intronic regulatory sequences can lead to lower expression. It will be of interest to identify the molecular changes that result in downregulation on the *D. albomicans* neo-Y chromosome.

The role of gene function in Y-chromosome degeneration. The study of neo-Y chromosomes has also allowed insight into the sequence of events leading to masculinization of Y genes. Intriguingly, the shrinking gene repertoire on the neo-Y chromosome of *D. miranda* shows the first signs of specialization of gene content for male-specific roles[66]. In particular, genes are not randomly lost from the neo-Y chromosome with regard to gene function[71]. Instead, genes with male-beneficial fitness effects are much more likely to be retained on the neo-Y chromosome when compared with random neo-Y genes[71], whereas genes with female-beneficial effects are preferentially lost[66,71]. Further, genes with inferred male-specific function (those with male-beneficial or with male-beneficial and female-detrimental effects) undergo increased rates of adaptive protein evolution on the neo-Y chromosome[66]. Also, neo-Y genes with reproductive function have evolved male-tissue-specific expression or have increased their expression on the neo-Y chromosome in male tissue[66]. Thus, in this model system at least, masculinization accompanies the early stages of Y-chromosome evolution. This supports the hitchhiking model of Y-chromosome degeneration (BOX 4): that is, the fixation of male-beneficial mutations can drag along segregating deleterious mutations. This model was initially criticized on the basis that it might be difficult for beneficial mutations to overcome the deleterious effects of linked detrimental mutations[34]. However, the observed masculinization of the *D. miranda* neo-Y chromosome demonstrates that beneficial mutations can fix on a gene-rich young Y chromosome[66] and may drag along linked deleterious mutations to fixation. Indeed, patterns of neutral variation in *D. miranda* neo-Y chromosomes in natural populations support adaptive evolution occurring on the neo-Y chromosome[72].

The initial stages of sex-chromosome evolution in plants. Unlike animals, most land plants are co-sexual, and male and female reproductive functions are found within a single individual. Only a small number of plant species (~6%) have evolved separate sexes, which is sometimes associated with the emergence of heteromorphic sex chromosomes[73,74]. Most plant sex chromosomes are evolutionarily young[73] and provide an interesting contrast to animal sex chromosomes.

Recent transcriptome analysis in *Silene latifolia*, the white campion, has provided some initial insights into the evolution of plant sex chromosomes[75,76]. The *S. latifolia* X and Y chromosomes were formed ~10 million years ago, may contain roughly 4,000 genes and are morphologically distinguishable. The Y chromosome is substantially larger than the X chromosome, which may be caused by an accumulation of repetitive DNA[77]. Also, YY plants are non-viable, suggesting that severely deleterious mutations have already accumulated on the *S. latifolia* Y chromosome[77]. Whereas the Y chromosome of this species has not been sequenced yet, recent transcriptome analysis has identified around 400 genes pairs from the X and Y chromosomes[75,76]. Rates of protein evolution are elevated for Y-linked genes in *S. latifolia* compared to their X-linked homologues[75,76]. In addition, levels of gene expression are significantly lower at Y-linked genes relative to their X homologues[75,76]. This indicates that the efficacy of purifying selection is reduced on the *S. latifolia*

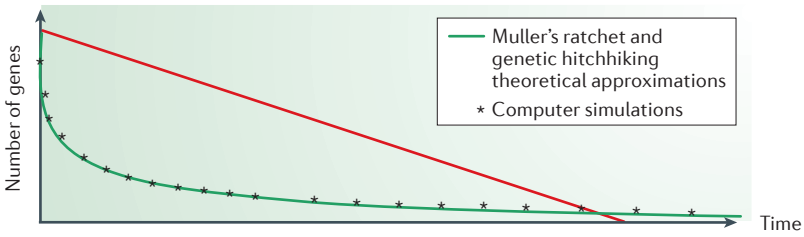


Figure 3 | **Evolutionary dynamics of Y-chromosome degeneration.** Population genetic processes to explain gene loss on a non-recombining chromosome do not predict continuous gene decay and eventual extinction of the Y chromosome[84,85]. A simple model of constant gene decay is shown by the red line. Instead, analytical approximations (green line) and computer simulations (asterisks) under the Muller's ratchet and genetic hitchhiking models suggest that gene-rich non-recombining Y chromosomes initially rapidly degenerate, but gene decay slows down over time and ultimately comes to an almost complete halt after a threshold number of Y-linked genes has been reached. In addition, empirical evidence from *Drosophila* neo-Y chromosome sequencing and primate Y chromosome gene content are consistent with this nonlinear degeneration.

Y chromosome, and deleterious mutations have started to accumulate, similarly to young animal Y or neo-Y chromosomes. However, the rate of gene loss may be lower in plants than in *Drosophila* spp. or humans, and more than 80% of Y-linked genes may still be functional[75,76]. This could indicate that Y chromosomes generally degenerate much more slowly in plants than in animals[75,76]. A potential explanation for these differences lies in the haploid expression from Y-chromosome-bearing gametes in plants but not animals. That is, in plants, pollen expresses many genes, whereas in animals, sperm show very limited gene expression[78]. Expression in the haploid stage may result in stronger selection pressure to maintain Y-linked genes in plants, whereas Y-linked genes are generally sheltered by their X homologues in animals.

Molecular characterization of young sex chromosomes in several plant species is ongoing[79], and recently the first Y-linked genomic sequence was published from papaya[42]. Sex determination in papaya is controlled by sex chromosomes: two different Y chromosomes control male and hermaphrodite development. A comparison between the hermaphrodite-specific Y sequence and the corresponding X-linked regions revealed two large-scale inversions forming two evolutionary strata, which probably caused recombination suppression between the X and Y chromosomes at 7 million and 2 million years ago. A dramatic increase in size of the Y chromosome was detected mostly owing to retrotransposon insertions on the Y chromosome[42]. Most of the genes present in the ancestral chromosome region that formed the sex chromosomes are still present on the current papaya Y chromosome, but gene decay proceeded more rapidly on the Y-linked relative to the X-linked region, indicating potential degradation of the Y chromosome[42]. In some plant species, such as *Rumex* spp., Y-chromosome degeneration has progressed even further, resembling the Y chromosome of many animals. Thus, although gene loss on plant Y chromosomes might be slower than in animals, degeneration of non-recombining chromosomes is a wider phenomenon that is shared between kingdoms.

Long-term survival of the Y chromosome

The process of Y-chromosome degeneration has prompted the suggestion that continuing gene loss will lead to the eventual disappearance of the human Y chromosome[80–83]. These predictions are based on a naive model of a constant rate of gene loss from the Y chromosome. However, recent theoretical and experimental studies have clearly demonstrated that Y-chromosome degeneration does not proceed in this simple linear fashion[21,84], and they refute these sensational claims of human Y-chromosome extinction (FIG. 3).

Predicting the future of the Y-chromosome using theoretical models. The evolutionary forces that drive gene loss on a non-recombining chromosome are well-understood (see above). Recent theoretical studies that model these evolutionary forces and population simulation studies have investigated the temporal dynamics of these models on an evolving Y chromosome and have demonstrated that the rate of gene decay is expected to decrease over evolutionary time and should come close to a complete halt on an old, gene-poor Y chromosome[84,85]. The reason for the predicted rapid decline in the rate of gene loss lies in the fact that old Y chromosomes have fewer active genes. The presence of fewer functional loci on the Y chromosome implies that the total number of beneficial and deleterious mutations — which are proportional to the number of functional genes on the Y chromosome — that can simultaneously segregate in the population is dramatically decreased. Interference among segregating mutations drives Y-chromosome degeneration (BOX 4), and the presence of fewer segregating mutations means less interference among them. Thus, eventually, the same processes that caused gene loss on the Y chromosome are expected to become exceedingly slow[84,85], and evolutionary theory does not predict that a Y chromosome would drive itself to extinction.

Neo-sex chromosomes and the future of Y chromosomes. Empirical observations of degenerating Y chromosomes of different ages are also inconsistent with a linear model of the rate of gene loss from the Y chromosome. If Y chromosomes degenerate at a constant rate, a Y chromosome twice as old should carry only half as many genes as the younger one. Probably the best empirical estimates of gene loss from Y chromosomes can be obtained in comparative data from neo-Y chromosomes in *Drosophila* spp. (FIG. 2). In *D. miranda*, the neo-Y chromosome formed ~1 million years ago. The neo-Y chromosome of this species has lost approximately 40% of its ancestral genes[66] — starting with ~3,000 and now reaching ~1,700 genes — and a constant rate of gene decay would imply that this neo-Y chromosome should go extinct after roughly 2–3 million years. However, if it is assumed that the same forces act on all neo-Y chromosomes, resulting in similar rates of decay, empirical data from the *D. pseudoobscura* neo-Y chromosome show the contrary. The neo-Y chromosome of this species was formed more than 15 million years ago and still has over a dozen genes left out of the original ~3,000; this number of genes is similar to that of the ancestral Y chromosome of *D. melanogaster*,

which is more than 60 million years old[27]. Thus, if these three species are extrapolated from, Y-chromosome degeneration in *Drosophila* spp. clearly does not follow a linear path; instead, gene decay is initially rapid, but it eventually equilibrates at a small number of stable genes. If anything, the ancestral Y chromosome of *Drosophila* spp. is actually gaining genes instead of losing them[27], and more than half of the genes that are currently present on the *D. melanogaster* Y chromosome were acquired less than 60 million years ago after the radiation of the *Drosophila* genus[27].

Primate Y chromosomes and the future of Y chromosomes. Comparative genomic studies in primates provide further empirical evidence against human Y-chromosome extinction[21]. Recent genomic data have shown that the rhesus macaque, which split from humans 30 million years ago, has an almost identical Y gene set as humans[21]. This implies that the gene content of the human Y chromosome has been stable in the past 30 million years and that the last common ancestor had already reached an equilibrium Y-chromosome gene number (FIG. 3). A linear rate of decay would have instead predicted that humans and rhesus macaques would show little gene overlap on their Y chromosomes as they should have lost genes independently since their split. These empirical data thus refute the hypothesis of the imminent loss of the human Y chromosome.

Interestingly, in some organisms, such as most nematodes, the Y chromosome has entirely disappeared (that is, they have XX/X0 sex determination[86]), which is the evolutionary end point of all erosion of genetic activity from the Y chromosome. However, for the complete elimination of the Y chromosome to occur, an alternative way of sex determination must evolve (such as determining sex from the ratio of X chromosomes to autosomes), and any genes required for male function must be translocated to other chromosomes. Thus, although the Y chromosome can ultimately be lost, it will do so only if alternative sex-determination mechanisms and male fertility functions on other chromosomes have evolved first, meaning that the Y chromosome can disappear without any negative fitness consequences. Otherwise, Y chromosomes can be a stable and important component of the genome in many species.

Conclusions and perspectives

Like many areas in biology, research on sex-chromosome evolution will greatly benefit from the genomic revolution. Many new models of sex-chromosome evolution, at all stages of differentiation (that is, nascent Y chromosomes as well as highly differentiated Y chromosomes), can now be analysed at the DNA sequence and chromatin levels, and sex-specific transcription profiles can be obtained. Although repetitive DNA and the ampliconic structure of many old Y chromosomes continue to pose serious challenges towards deciphering their genome sequence and currently require laborious and expensive approaches, new sequencing technologies that will provide much longer reads should allow us to make progress.

Furthermore, studying a diverse set of Y chromosomes will allow us to test whether certain features identified in model organisms are a general characteristic of Y chromosomes. For example, are amplicons unique to primates, or do they allow survival of Y-linked genes in other taxonomic groups as well? Do other taxa have similar megabase-sized introns just as *D. melanogaster* does? Are evolutionary strata, which have already been found in animals and plants, a general feature of sex-chromosome evolution? In addition, such data will allow us to investigate further whether the processes that are currently hypothesized to drive Y-chromosome evolution do, in fact, do so. For example, do sexually antagonistic mutations indeed accumulate along the recombining portion of the sex chromosomes, and do they drive the suppression of recombination along the proto-sex chromosomes? It will also be of great interest to study more Y chromosomes from plants, particularly young ones, to establish whether the slow pace of Y-chromosome degeneration is a general property in plants. Haploid Y-bearing cells in animals (sperm) show little gene expression but are expressed in plants (pollen), and haploid purifying selection may slow down Y-chromosome degeneration in plants.

Another challenge in our understanding is why not all homomorphic sex chromosomes stop recombining with each other and thus become heteromorphic over long evolutionary times. For example, birds have homologous sex chromosomes that formed ~120 million years ago[87] and are similar in age to the mammalian sex chromosomes[88]. Yet, whereas all mammals and most bird lineages have highly differentiated sex chromosomes, in some groups of birds, such as ratites, the sex chromosomes remain homomorphic[89]. Similar differences in the progression from homomorphic to heteromorphic sex chromosomes are seen among snake lineages. Comparisons of the genomic sequences and transcriptomes between taxa with homomorphic and heteromorphic sex chromosomes should provide clues to this puzzle.

Birds and snakes have female heterogametic sex chromosomes: that is, females have a non-recombining W chromosome. Contrasting patterns of evolution on Y versus W chromosomes should reveal whether W chromosomes show similar patterns of degeneration but also whether they show specialization of their gene content to reflect the female-limited transmission of a W chromosome. Also, in fish, reptiles and amphibians, there are several closely related groups that differ in their female or male heterogamety[90], and examination of these species groups will allow us to contrast evolutionary forces occurring on Y versus W chromosomes. Another interesting area of study here will be in the high occurrence of nascent sex chromosomes in fish, reptiles and amphibians[91–98]. Tackling their patterns of sequence evolution at the molecular level will greatly increase our understanding of the molecular basis and evolutionary processes of the beginnings of Y-chromosome degeneration.

Finally, invertebrates, which constitute the vast majority of animal species, have a multitude of sex-determining mechanisms, and Y and W chromosomes

Female heterogametic
Female heterogamety describes a species in which males have two Z chromosomes and females have a Z and a W chromosome.

Male heterogamety
A species in which females have two X chromosomes and males have an X and a Y chromosome.

have independently evolved many times in many different taxa[15]. Recent efforts have started to characterize transitions of sex chromosomes in insects[99–101], but many other systems with interesting biology and karyotypes provide a treasure trove to study sex-chromosome evolution[15]. To conclude, a comparative analysis of Y chromosomes at different stages of differentiation across the tree of life will give further insights into the characteristics and the evolutionary forces that act on sex chromosomes.

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