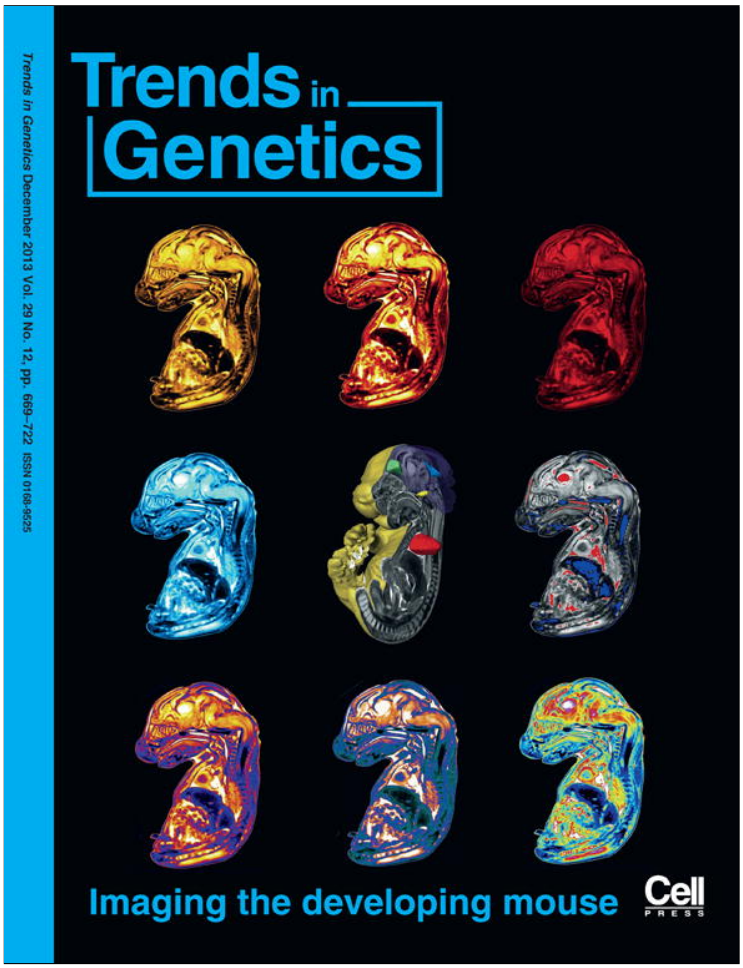

Review

Sex chromosome dosage compensation: definitely not for everyone

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Sex chromosomes often entail gene dose differences between the sexes, which if not compensated for, lead to differences between males and females in the expression of sex-linked genes. Recent work has shown that different organisms respond to sex chromosome dose in a variety of ways, ranging from complete sex chromosome dosage compensation in some species to active compensation of only a minority genes in other organisms. Although we still do not understand the implications of the diversity in sex chromosome dosage compensation, its realization has created exciting new opportunities to study the evolution, mechanism, and consequences of gene regulation. However, confusion remains as to what sorts of genes are likely to be dosage compensated, how dosage compensation evolves, and why complete dosage compensation appears to be limited to male heterogametic species. In this review, I survey the status of dosage compensation to answer these questions and identify current controversies in this fast-moving field.

Sex chromosome dose differs between males and females

In diploid species, sex determination is often linked to sex chromosomes, which follow one of two primary types. In male heterogamety (see Glossary), males have an XY genotype and are the heterogametic sex, and females are XX and are homogametic. Alternatively, many species are female heterogametic, with ZW females and homogametic males with a ZZ genotype. Regardless of which sex is heterogametic, sex chromosome pairs, meaning the X and Y or Z and W chromosomes, usually originate from a pair of autosomes, initially identical, that diverge from each other after recombination between them is suppressed. Once recombination is halted, the sex-limited Y or W chromosome deteriorates in both gene content and activity [1]. The degree of difference between sex chromosome pairs varies among species and, although many species with genetic sex determination show only small differences between the X and Y or Z and W chromosomes [2], some sex chromosome pairs show marked divergence from each other when the region of suppressed recombination is large.

The decay of genes and gene activity on the Y or W chromosome causes an imbalance between males and females in gene dose; whereas the homogametic sex retains two copies of all X- or Z-linked genes, genes lost from the sex-limited chromosome are present in only one copy in the heterogametic sex. In those species where recombination suppression spreads across the sex chromosomes and a larger share of the Y or W chromosome is gnawed away, dose differences between the sexes emerge for an increasing proportion of X- or Z-linked genes and the heterogametic sex becomes effectively monosomic for the X or Z chromosome. In some species, such as eutherian mammals, *Drosophila*, and many birds, only a few dozen genes remain on the Y or W chromosome [3–5], which leads to gene dose differences between the sexes for hundreds of genes on the X or Z chromosome.

Gene dose is often, although not always, correlated with gene transcription and translation levels [6,7]. This is

Glossary

Complete dosage compensation: a regulatory mechanism that affects the entire sex chromosome, leading to the hypertranscription of the single copy in the heterogametic sex.
Dose effect: the degree to which changing the number of copies of a gene alters expression levels.
Eutherian mammals: the clade within the mammals including the placentals, excluding the monotremes and marsupials.
Female heterogamety: sex chromosome inheritance system where the male has two Z chromosomes and the female one Z and one W. Examples of this type of sex chromosome system include birds, lepidopterans, and snakes, as well as some fish and amphibians.
Heteromorphy: the degree of divergence between the X and Y (or Z and W) paired sex chromosomes.
Hypertranscription: a regulatory process by which the rate of gene transcription is increased at a locus.
Incomplete dosage compensation: the situation, now observed in several species, whereby many genes on the sex chromosome are expressed less in the heterogametic sex due to reduced gene dose.
Male heterogamety: sex chromosome inheritance system where the female has two X chromosomes and the male one X and one Y. Examples of male heterogametic include mammals, *Drosophila*, most beetles, and *Caenorhabditis elegans*.
Monosomy: a form of aneuploidy where a normally diploid pair of chromosomes is reduced to one copy.

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because reducing the number of gene copies cuts the number of targets that the transcriptional machinery can work from to generate RNA, which can translate to differences in protein levels. Additionally, because genes do not work in isolation, sex chromosome dose differences between males and females can affect protein titers for not only X- and Z-linked loci, but also the many downstream autosomal genes that they regulate [8,9]. Because eukaryotic genomes have complex interconnected network structures, gene dose differences for a few hundred genes on the sex chromosomes could theoretically affect a large proportion of the genome.

Limited deletions of specific genes or restricted regions of autosomes can be tolerated in many cases; however, complete autosomal monosomies are generally lethal. Given the harmful effects of autosomal monosomy, it was assumed until recently (Box 1) that sex chromosome monosomy would need to be actively compensated for by hyperexpression of nearly all genes on the X (or Z) chromosome in the heterogametic sex. The assumption that complete sex chromosome dosage compensation is required to accompany sex chromosome divergence has changed somewhat over the past few years. Although some species, such as *Drosophila* [10] and *Caenorhabditis elegans* [11], do regulate the entire sex chromosome as a unit to achieve complete dosage compensation, where all (or nearly all) genes on the sex chromosome are restored to the diploid expression level in the heterogametic sex, it is now clear from the list of species that show incomplete dosage compensation (Table 1) that this sort of complex whole-chromosome regulation is not necessarily expected to accompany all heteromorphic sex chromosomes.

Studies in birds [12], *Schistosoma* [13], and other species illustrate that many organisms are resilient to dose effects. In these and other species with incomplete dosage compensation, the transcriptional differences between males and females resulting from reduced dose in the heterogametic sex persist for most genes on the sex

Box 1. Ohno's theory of sex chromosome dosage compensation

Although deletions of limited regions of any given chromosome can often be tolerable, monosomy of an entire autosome is frequently catastrophic for an organism. Given that sex chromosome divergence leads to male monosomy of the X or female monosomy of the Z chromosome, Susumu Ohno proposed over 40 years ago that the heterogametic sex would upregulate the single X or Z chromosome to compensate for sex chromosome monosomy [16]. This would return expression for genes on the X or Z chromosome in the heterogametic sex to the diploid level observed before gene activity decayed on the sex-limited Y or W chromosome. This theory of dosage compensation was supported by subsequent empirical work in the main model organisms, placental mammals [42,43], *Caenorhabditis elegans* [11,44], and *Drosophila*, all of which appeared to exhibit complete X chromosome dosage compensation as Ohno predicted. This in turn led to the widespread and long-standing assumption that complete sex chromosome dosage compensation was a requirement for any species with divergent sex chromosomes.

The first real crack in the theory of complete sex chromosome dosage compensation occurred in 2007 with the publication of two papers from separate groups showing that birds lacked complete Z chromosome dosage compensation [12,45] and, as a result, most Z-linked genes were expressed at lower levels in females because of reduced gene dose. At this point, it was not clear whether birds were simply the inevitable exception to every biological rule, or dosage compensation was less common than previously assumed. Since then, however, studies on a wide range of other organisms (Table 1, main text) have overturned the view that complete sex chromosome dosage compensation necessarily accompanies sex chromosome evolution. This has in turn led to a re-evaluation of the model organisms originally thought to exhibit dosage compensation, with some surprising results (see Box 3).

Table 1. Current status of dosage compensation[a]

Species or clade	X_{male}:AA_{male} or Z_{female}:AA_{female}	XX_{female}:AA_{female} or ZZ_{male}:AA_{male}	XX_{female}:X_{male} or Z_{female}:ZZ_{male}	Average sex chromosome dosage compensation	Refs
Male heterogametic species					
Caenorhabditis elegans	1	1	1	Complete	[11,44,46]
Drosophila melanogaster	1	1	1	Complete	[46,47]
Teleopsis dalmanni	1	1	1	Complete	[34]
Anopheles gambiae	1	1	1	Complete	[48,49]
Tribolium castaneum	1	>1	>1	Complete in males, females show overexpression	[27]
Gasterosteus aculeatus	<1	1	>1	Incomplete	[33]
Ornithorhynchus anatinus	<1	1	>1	Incomplete	[15]
Monodelphis domestica	1	1	1	Complete	[15]
Eutherian mammals[b]	<1	<1	1	Complete for dosage sensitive genes[e]	[15,21,22]
Silene latifolia	?	?	1	Complete	[17]
Female heterogametic species					
Schistosoma mansoni	<1	1	<1	Incomplete	[13]
Lepidoptera[c]	<1	1	<1	Incomplete	[14,31,50]
Aves[d]	<1	1	>1	Incomplete	[12,51–54]

[a]Recent studies have assessed the presence or absence of complete dosage compensation by comparing the average X or Z expression to the average autosomal expression in the heterogametic (X_{male}:AA_{male} or Z_{female}:AA_{female}) and homogametic (XX_{female}:AA_{female} or ZZ_{male}:AA_{male}) sex, and/or comparing average X and Z expression in females and males (XX_{female}:X_{male} or Z_{female}:ZZ_{male}).

[b]Assessed in human, chimpanzee, bonobo, gorilla, orangutan, Rhesus macaque, and mouse [15].

[c]Assessed in silk moth [31,50] and Indian meal moth [14].

[d]Assessed in chicken [12,45], Kentish plover [52], zebra finch [12], white throated warbler [53], and crow [54].

[e]See Box 3.

Box 2. How to assess dosage compensation

There are myriad ways to test for dosage compensation and measure dose effects of the sex chromosome. Most current studies are based on whole-transcriptome analysis, increasingly using next-generation RNA Sequencing (RNASeq) technologies. In many ways, the fairest test for dosage compensation compares expression of X- or Z-linked genes in both sexes to the ancestral expression level that existed before sex chromosome divergence [15,22]. Using this sort of phylogenetic context limits the comparison to those genes where the chromosomal location of orthologs can be identified across species, and works only for those genes that have not moved from their ancestral location, but does carry the benefit of measuring dose effects in response to sex chromosome divergence.

A more straightforward test for dosage compensation is to compare average expression for all genes expressed on the X or Z chromosome to the average autosomal expression in the heterogametic sex (X_{male}:AA_{male} or Z_{female}:AA_{female}). When the comparison does not differ statistically between them, complete sex chromosome dosage compensation is often concluded. In this sort of analysis, the comparison between average expression on the sex chromosome and the autosomes in the homogametic sex (XX_{female}:AA_{female}, or ZZ_{male}:AA_{male}) can be used to identify whether overtranscription occurs in the homogametic sex, as seen in *Tribolium* [27]. However, this test for dosage compensation assumes that the average expression for the ancestral X or Z chromosome was the same as that for the autosomes, and this may not always be the case [15].

Sex chromosomes often comprise regions of different ages, either due to fusions between existing sex chromosomes and autosomes [55] or because recombination ceased at different times for different regions of the sex chromosome [19,56]. This makes it possible to compare different regions of the same sex chromosome for variation in the efficacy of dosage compensation and dose effects [19,57,58] to understand dynamics in the rate of dosage compensation evolution (or lack thereof).

Comparing expression for the X or Z chromosome between males and females (XX_{female}:X_{male} or Z_{female}:ZZ_{male}) does not itself constitute a test of dosage compensation, because it does not indicate whether the heterogametic sex upregulates sex-linked genes. However, comparing female and male expression on the sex chromosomes can be used to assess the dose effect in species with incomplete sex chromosome dosage compensation.

In addition to RNA-based methods, it is also possible to use epigenetic markers to measure some types of sex chromosome dosage compensation [59–61]. This approach works only in those systems where dosage compensation is based on epigenetic reprogramming of transcription rates.

chromosome, with no obvious deleterious effects. Moreover, in a sharp turnaround from previous assumptions about the necessity of complete dosage compensation, a spate of recent studies on a wide range of organisms suggests that species with complete sex chromosome dosage compensation may be in the minority. Furthermore, some species that were previously thought to exhibit complete dosage compensation may, in fact, not (Box 3).

At the same time that these new results overturned the assumption that complete sex chromosome dosage compensation is required, they also led to several new questions. Why is sex chromosome dosage compensation complete in some organisms but not in others? For species with incomplete dosage compensation, is there a cost to the heterogametic sex for reduced expression of X- and Z-linked genes? These questions have recently been explored using different approaches, and the results have built a more nuanced picture of sex chromosome dosage compensation.

Not all genes with similar expression levels between males and females are dosage compensated

There is still some confusion as to what level of expression to expect as a result of halving gene dose in the heterogametic sex. Although it may seem reasonable to assume that halving gene dose should result in halved expression, copy number variation studies tell us that this is far from true in many cases.

For many genes, halving the gene dose does not produce any observable changes in expression, or dose effect. Dose effects are the difference in RNA or protein abundance in response to changes in gene dose (or copy number). In other words, dose effects are the difference in expression observed when copy number (or dose) of a gene is varied. It is increasingly clear that many loci, on or off the sex chromosomes, do not show dose effects, and expression is the same whether an individual has one or two copies. For example, genes with lower expression levels are less likely to show dose effects on the autosomes [7] as well as on sex chromosomes [14,15]. This may be because the rate of transcription is not saturated at lower expression levels. Additionally, dose effects are less likely for genes with higher levels of feedback regulation through genetic networks [7]. In these cases, regulatory interactions theoretically act to buffer out dose effects.

Studies of autosomal monosomy are, in many ways, good proxies for sex chromosome dose. However, autosomal monosomy generally occurs in a single generation, in contrast to changes in sex chromosome dose, which often occur more gradually as Y and W gene content decays gradually. Despite this key difference, studies of autosomal monosomy indicate that, for many of those genes that do show dose effects, halving the gene dose does not necessarily result in a 50% reduction in expression levels, but rather somewhere in between 50% and 100% of the expression expected from two copies [6,7]. The variation in dose effects means that even in species with no active sex chromosome dosage compensation mechanism, some sex-linked genes will not differ in expression between the sexes, and most genes will not show a 50% reduction in expression due to halved gene dose in the heterogametic sex.

Additionally, genes that lack dose effects, or lack the full 50% reduction in response to halving the gene dose, are not necessarily dosage compensated. Sex chromosome dosage compensation connotes an active transcriptional process, selected for in the heterogametic sex, that restores expression from the single X or Z chromosome to diploid levels [16]. Genes that lack dose effects cannot have been actively selected to increase expression because their expression did not fall with reduced gene dose. Rather, their expression levels are passively buffered by the transcriptional machinery or network interactions. This may seem a somewhat semantic argument; however, differentiating these two mechanisms, one direct and actively selected for and the other indirect and a consequence of other gene characteristics, is in fact important.

In systems with incomplete sex chromosome dosage compensation, variation in dose effect can lead to some confusion. Specifically, it can be difficult to determine whether similar expression in males and females for any

given sex-linked gene is due to direct dosage compensation [17], where selection has acted to increase expression in the heterogametic sex, or is simply an indirect consequence of a locus that does not experience a dose effect. Moreover, sex chromosomes show unique patterns of masculinization and feminization of gene expression [18], which can act against the evolution of dosage compensation [19] and further obscure the genetic and evolutionary forces shaping expression. Different analytical approaches to the study of dosage compensation are needed to differentiate these effects (Box 2).

Of those genes that do show dose effects, only a fraction are dosage sensitive, either because the X- or Z-linked gene itself is haplo-insufficient [20], or because it causes deleterious downstream regulatory effects in autosomal genes [9,15]. These dosage-sensitive genes, which are often components of large macromolecular complexes where stoichiometric balance is important, are the most likely to be actively dosage compensated through selection to upregulate transcription in the heterogametic sex [21,22]. In some organisms, selection for compensation at dosage-sensitive loci has theoretically led to the evolution of complex machinery that affects transcription rates for the entire X chromosome, as seen in the whole-chromosome regulatory apparati seen in *Drosophila* and *Caenorhabditis* [11,23,24], which compensate nearly all X-linked loci, including those that show no dosage sensitivity. In other cases, the decay of Y or W gene content has only caused selection for active dosage compensation of some genes [15,25], and compensation for these loci has not progressed to the evolution of a mechanism that regulates the entire sex chromosome.

Selection for dosage compensation is not usually sex specific: it affects both males and females

Selection for dosage compensation theoretically acts in the heterogametic sex [16] and although the mechanism of dosage compensation in *Drosophila* is male specific, and therefore has minimal effects on females [10,26], dosage compensation mechanisms in several other organisms affect gene expression in both sexes. This is because gene transcription rates are often strongly correlated in females and males and, therefore, selection in the heterogametic sex to increase transcription to compensate for reduced gene dose can cause overtranscription in the homogametic sex [21,27].

Overtranscription can be as deleterious for the homogametic sex as undertranscription in the heterogametic sex for dosage-sensitive loci [28], setting up the potential for conflict between females and males over optimal transcription rates [29]. Given the strong intersexual correlation in transcription, any increase in expression of the single X or Z chromosome in the heterogametic sex could also increase expression from the two X or Z chromosomes in the homogametic sex. In other words, doubling expression from the single X chromosome in males to compensate for reduced expression could cause as much as twofold higher expression in females, because each of her X chromosomes will themselves have doubled expression.

In some cases, conflict over optimal transcription rates for dosage compensation has been resolved. Overtranscription in the homogametic sex in some organisms been corrected through the evolution of a second mechanism, such as that in *C. elegans* hermaphrodites, which counteracts X chromosome hypertranscription [11]. Similarly, it is possible that female X inactivation in mammals has occurred for similar reasons [16,21] (although see Box 3). However, in many species, conflict over dosage compensation remains unresolved for a large proportion of loci. This can lead either to cases of overtranscription in the homogametic sex, as seen in *Tribolium* beetles [27], where females express X-linked genes more than the diploid autosomal average, or undertranscription in the heterogametic sex, as in the numerous cases of incomplete dosage compensation listed in Table 1. Theoretically, in these cases, the rate of transcription for any X- or Z-linked gene is the result of the balance between selection for hypertranscription in the heterogametic sex and selection against overtranscription in the homogametic sex. Additionally, we might expect the transcription rate to vary across genes on the sex chromosome relative to the costs of over- versus underexpression for any given locus.

Although the number of species for which sex chromosome dosage compensation has been assessed has grown rapidly over the past few years, the data set remains in many ways rather sparse. This makes it difficult to assess whether the species with unresolved conflict over optimal sex chromosome transcription rates are moving toward an ultimate resolution at every X- or Z-linked locus, which would lead to complete dosage compensation and equal expression from the sex chromosomes in both sexes. If this is true, then incomplete dosage compensation, at least for dosage-sensitive genes, is simply a transitory state, existing for a period following sex chromosome divergence and before complete dosage compensation is achieved. How and why compensation progresses in some species to cover dosage-insensitive genes via whole sex chromosome regulation is still difficult to envision.

Dosage compensation in not universal, but it is more common in XY than ZW species

In addition to being no longer considered universal, sex chromosome dosage compensation shows an unusual distribution pattern among male and female heterogametic species (Table 1). This pattern was first discernible in 2009, with the publication of results showing that Lepidoptera, in addition to birds, lacked complete Z chromosome dosage compensation [30,31], suggesting that complete sex chromosome dosage compensation was confined to male heterogametic species. Although some debate still remains as to the status of Z chromosome dosage compensation in lepidopterans [32], subsequent studies in additional ZW [13,14] and XY [15,27,33,34] species have added further data points to support this pattern. Taken together, these studies suggest that, although not all X chromosomes are accompanied by complete dosage compensation mechanisms [33], complete dosage compensation is thus far limited to male heterogametic species, and is incomplete in all female heterogametic species tested to date.

There are several potential reasons for this pattern, which are not necessarily mutually exclusive. First, the fact that mutations more often occur in males [35], a

Box 3. Mammals: do they or don't they?

The view of sex chromosome dosage compensation, in its most traditional sense [16], is that the X or Z chromosome will be upregulated in the heterogametic sex, and that this will result in the average expression of the X or Z chromosome being equal to average autosomal expression in both sexes. In reality, selection for sex chromosome dosage compensation can not only restore expression levels in the heterogametic sex, but also cause the homogametic sex to overexpress X-linked genes. The potential for overexpression in the homogametic sex led Ohno to propose that X chromosome inactivation in female placental mammals, which causes the Barr bodies observed in female somatic cells, evolved to resolve the conflict between males and females over optimal X chromosome transcription rate by equilibrating gene dose between them.

Ohno's postulate predicts that the average expression from the single active X chromosome in both sexes is equal to average autosomal expression (Figure IA). Empirical data supported this prediction [62,63] until a study in 2010 [64] indicated that the single active X chromosome in males and females was not in fact hypertranscribed, and average expression from the X chromosome in both sexes was less than the autosomal average (Figure IB). The data-filtering methods from this paper were loudly criticized [65,66], and additional data were used to show that average X expression equaled that from the autosomes according to Ohno's prediction [59].

The story took another twist a year later with several papers convergently showing evidence that, although dosage-sensitive genes do seem to be compensated [21], the single active X chromosome is not on average hyperexpressed in either sex (Figure I), and mean expression in females and males is less than that from autosomal loci [15,22]. Thus, the current evidence suggests that Ohno was only partly correct in his predictions, but there may very well be further amendments.

The disagreement over the status of mammalian X chromosome dosage compensation illustrates how decisions made during data analysis can radically affect results in whole-transcriptome analysis. Transcription is remarkably messy, and a large proportion of the genome is expressed at low levels that are arguably biologically irrelevant [67]. Decisions have to be made during the data analysis about what constitutes significant expression and, therefore, should be included; such decisions can alter perceived expression averages and, thus, conclusions as to the status of dosage compensation [41]. Including all genes in chromosome-wide averages, including those with no or low expression levels, compresses perceived dose effects between the sexes. Additionally, some sex chromosomes have a higher proportion of inactive genes than autosomes, and failure to filter these out reduces the average sex chromosome expression level compared to autosomes.

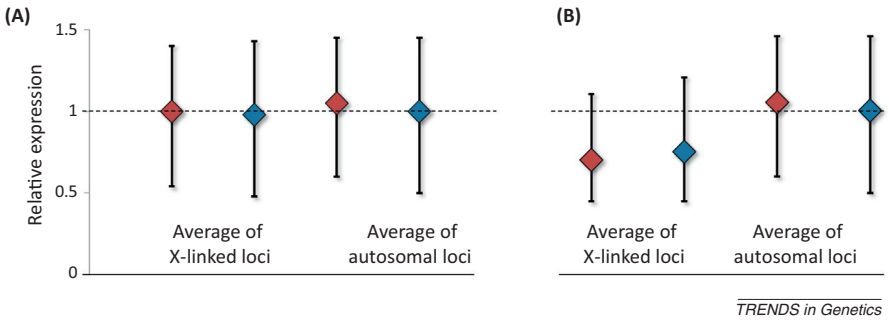

Figure I. Although average expression from the X chromosome is similar in males (blue) and females (red), considerable debate remains as to whether X-inactivation in female placental mammals is accompanied by hyper-expression of the single active X in both sexes **(A)** or not **(B)**. Current evidence suggests that the latter scenario is the case [15,21,22], but the debate seems to swing back and forth every few years, so stay tuned for further developments.

product of the fact that the number of germline cell divisions in many species is higher in spermatogenesis than in oogenesis, can affect the rate of evolution of different sex chromosomes in different ways. This male-driven mutation would cause Y chromosomes to accumulate deleterious mutations more quickly, and to degenerate more rapidly, than W chromosomes [36] because Y chromosomes are always present in males and W chromosomes never are. This would in turn lead to a more leisurely rate of W chromosome degeneration compared with the Y chromosome, although it is important to note that this difference in Y and W degeneration has never been documented from empirical data. However, if true, this difference between Y and W chromosomes in rates of gene decay could theoretically allow more time for the compensation of specific dosage-sensitive Z-linked loci and thereby reduce the overall selection on Z chromosomes for a chromosome-wide mechanism of dosage compensation compared to the X chromosome [37]. The resulting pattern of incomplete compensation, and overall male-bias in expression for the Z chromosome, would have few, if any deleterious effects in females, because dosage sensitive genes would theoretically be compensated.

Alternatively, Z chromosomes may be less able than X chromosomes to adapt to gene loss on the sex-limited chromosome due to a relative reduction in genetic diversity and population size. In many species, sexual selection acts primarily in males, leading to a female skew in the number of individuals reproducing and contributing to the next generation. This skew reduces the effective population size of the Z chromosome, and increases the effective population size of the X chromosome, relative to the autosomes, leading to both lower levels of genetic diversity and reduced rates of adaptive evolution on Z chromosomes compared with X chromosomes [38,39]. This potentially makes Z chromosomes less able, or just slower, than X chromosomes to adapt to changes in gene dose caused by degeneration of the sex-limited chromosome. If this is a true cause of the lack of complete Z chromosome dosage compensation, it suggests that only the most dosage-sensitive genes would experience sufficient selection to achieve compensation on Z chromosomes, and other, partially dosage-sensitive genes that have not achieved compensation would exert a deleterious effect on heterogametic females. Of course, this reduced adaptability of the Z chromosome may be counterbalanced by the reduced rate of W chromosome degeneration described above.

Another explanation for the association between complete sex chromosome dosage compensation and X chromosomes is based on the fact that selection for dosage compensation in the heterogametic sex can cause overexpression in the homogametic sex [29]. This sets up a conflict between males and females over optimal expression levels. This, combined with the fact that sexual selection often means that selection is stronger on males than on females, could explain the observed distribution of complete dosage compensation [30]. Under this scenario, selection in XY males would be stronger to upregulate their single X chromosome than selection in females against overexpression, leading to hyperexpression in the homogametic sex [27] until a second correcting mechanism evolves in females [21,40] (or, in the case of *C. elegans*, hermaphrodites [11]). Under female heterogamety, selection for upregulation of the Z chromosome in females would rarely over-ride selection against hyperexpression in males; therefore, the rate of evolution for dosage compensation in ZW systems would be retarded. If true, this places a significant burden on ZW females for incomplete sex chromosome dosage compensation. However, this conflict could be circumvented by compensating mechanisms with female-limited action, which would not cause deleterious overexpression in males.

It is important to point out that, although these alternatives all fit the available data (Table 1), characterization of dosage compensation in further species may well contradict the patterns observed and, therefore, the potential explanations. Additionally, these explanations remain verbal models at this point, and are difficult to differentiate from each other in terms of their predicted gene expression patterns.

Concluding remarks

Few key tenets of genetics are completely overturned in the space of a few years and, for that reason alone, recent progress that shows complete sex chromosome dosage compensation to be far from universal is remarkable. However, although these new data have advanced the study of sex chromosome dosage compensation on several fronts, they have also created several new questions.

It is astonishing, especially given the high level of scrutiny in this taxonomic group, that debate remains as to whether the X chromosome is upregulated in placental mammals to achieve dosage compensation as Ohno predicted [16]. The persistence of this question is not due to a lack of data, but rather likely stems from the fact that data processing, filtering, and analysis methods can strongly influence results and interpretation in studies of chromosome-wide gene expression averages [41]. Therefore, the question as to the status of eutherian X chromosome regulation may remain contentious for some time, until the field settles on best practices for processing and analysis of transcriptome data.

The new data showing that many species lack complete dosage compensation creates questions as to how and why selection to compensate dosage-sensitive genes has led to whole-chromosome mechanisms of sex chromosome regulation in some species. In this same vein, why has selection for dosage compensation not progressed to whole chromosome regulation in other species?

Finally, the current data suggest that complete sex chromosome dosage compensation is confined to XY species, although it is important to point out that data from additional species may alter this interpretation. If complete dosage compensation is indeed restricted to X chromosomes, what genetic and evolutionary properties explain this distribution? Also, what are the mechanisms by which the conflict over dosage-sensitive genes is resolved between the sexes in species lacking complete sex chromosome dosage compensation?

Answering these questions will require several things. First, the debate over the status of dosage compensation on the eutherian X chromosome illustrates the need to standardize data processing and analysis methods. This is important well beyond the question of sex chromosome dosage compensation in mammals, because it can also affect the perceived dose effects in species with incomplete sex chromosome dosage compensation, and more broadly in any transcriptome study. Second, scientists working on sex chromosome dosage compensation need to standardize their terminology. Often, the literature conflates gene expression that has been selected for hypertranscription in the heterogametic sex with those that lack dose effect into the single term 'dosage compensated'. This leads to the incorrect conclusion that all genes that lack dose effects between males and females have been selected for dosage compensation. Finally, the shift in the paradigm of complete sex chromosome dosage compensation illustrates how data from outside the traditional model organisms can turn a long-held theory on its head. Further studies in species with independently evolved sex chromosomes, such as frogs or fish, are needed to create a more cohesive picture of sex chromosome regulation, its distribution across sex chromosome types, and its evolutionary progression.

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