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Dosage compensation evolution in plants: theories, controversies and mechanisms

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Abstract

In a minority of flowering plants, separate sexes are genetically determined by sex chromosomes. The Y chromosome has a non-recombining region that degenerates, causing a reduced expression of Y genes. In some species, the lower Y expression is accompanied by dosage compensation, a mechanism that re-equalizes male and female expression and/or brings XY male expression back to its ancestral level. Here, we review work on dosage compensation in plants, which started as early as the late 60s with cytological approaches. The use of transcriptomics fired a controversy as to whether dosage compensation existed in plants. Further work revealed that various plants exhibit partial dosage compensation, including a few species with young and homomorphic sex chromosomes. We are starting to understand the mechanisms responsible for DC in some plants, but in most species we lack the data to differentiate between global and gene-by-gene dosage compensation. Also, it is unknown why some species evolve many dosage compensated genes while others do not. Finally, the forces that drive DC evolution remain mysterious, both in plants and animals. We review the multiple evolutionary theories that have been proposed to explain DC patterns in eukaryotes with XY or ZW sex chromosomes.

Key words: sex chromosomes, Y degeneration, dosage balance, dosage-sensitive genes, cis-regulatory sequence divergence, imprinting.

Introduction

Sex chromosomes originate from autosomes, after acquiring sex-determining genes [1]. In male heterogametic systems males are XY and females XX, while in female heterogametic systems females are ZW and males ZZ. The X chromosomes recombine in females, however, recombination is suppressed between the X and the Y around the sex-determining region, which drives degeneration of the Y chromosome [2]. The same applies to ZW systems with W degeneration, but for simplicity we hereafter use the XY nomenclature. The genes located in the X-Y non-recombining region are called sex-linked genes and the region that still recombines between the X and the Y is called the pseudo-autosomal region (PAR). Traditionally, Y degeneration has been considered a consequence of recombination suppression [3]. Y degeneration leads to lower expression of Y genes and eventually complete gene silencing and loss, reducing gene dosage in males compared to females (Figure 1.a). This dosage imbalance is likely deleterious for dosage-sensitive genes (i.e. genes that require a precise expression level relative to other genes in order to achieve their functions, [4]). The traditional view of sex chromosome evolution offers that the dosage imbalance in XY males caused by Y degeneration leads to the evolution of a compensatory mechanism called dosage compensation (hereafter DC). However, a recent theory proposes an alternative order of events for the evolution of sex chromosomes and DC, where early DC is the ultimate cause (not the consequence) of X-Y recombination suppression maintenance [5]. More details on this new theory are included later in this review.

DC has been characterized in some species and is mediated by a change in expression of the X (or Z) chromosome [6]. Three canonical mechanisms have been discovered and a considerable amount of work has been done to characterize their molecular details (Figure 1.b) [6]. In *Drosophila melanogaster* and the green anole lizard, the extensive Y degeneration

is compensated by a straight doubling of the X chromosome expression in males [7,8](Figure 1.b). In *D. melanogaster* this is achieved by the MSL complex [6].

In placental mammals, dosage compensation is less straightforward: X chromosome expression is doubled in both sexes and one of the two X chromosomes is inactivated in females, so that the ancestral expression level is maintained (Figure 1.b) [9]. This original view of mammalian DC evolution has been refined by the findings that, while X-chromosome inactivation is a chromosome-wide phenomenon orchestrated by the non-coding RNA *Xist* [10], doubling of X expression is achieved on a gene-by-gene basis and does not affect all X genes [11,12]. Indeed, X expression is upregulated only in a few genes that are broadly expressed and dosage-sensitive [13–15]. For another subset of X genes, dosage compensation is achieved by downregulating interacting autosomal genes [16]. Interestingly, some genes located on the X chromosome escape inactivation, mainly genes located on the short arm of the X chromosome, a segment that recently stopped recombining [17]. The reason why upregulation of the X is local (gene-by-gene regulation), whereas X inactivation is global (chromosome-wide epigenetic modification) is not yet understood. Ohno [9] hypothesized that X upregulation first evolved to compensate for Y degeneration and that X inactivation evolved afterwards to re-establish ancestral expression levels in females. However, a demonstration of the order of events is still lacking [18] and an alternative scenario is discussed later in this review [19]. In adult tissues of placental mammals, X-inactivation is random, affecting the paternal or maternal X with equal probability and the inactive X varies from cell to cell. On the other hand, in marsupials X-inactivation is non-random and the paternal X is systematically inactivated (Figure 1.b) [20]. In marsupials, DC is thus associated with genomic imprinting (the differential expression of maternal and paternal alleles).

In *Caenorhabidtis elegans*, an androecious species (with XX hermaphrodites and X0 males), DC is achieved in somatic tissue through X upregulation in both sexes followed by a

hermaphrodite-specific two-fold lower transcription from of the two X chromosomes [21–23] (Figure 1.b). However, only a few dosage-sensitive X genes are upregulated in *C. elegans* to maintain ancestral expression levels [24]. X upregulation and X downregulation are achieved by independent mechanisms. X down-regulation is accomplished by the dosage compensation complex, which is composed of proteins homologous to the members of the condensing complex. In *C. elegans* germline (meiotic and proliferating cells), transcription of the single X in males and transcription of the two Xs in hermaphrodites is repressed by chromatin remodeling MES proteins (maternal-effect sterile), whose loss leads to sterility. The single X chromosome in males acquires additional repressive histone modifications, due to its unpaired status during meiosis. This example illustrates that different tissues may have different sex chromosome transcription levels.

The literature distinguishes the evolution of dosage compensation that relies on a specific molecular mechanism (such as the *Drosophila* MSL-driven X upregulation) from immediate compensatory upregulation of the X copy in males following the decreased Y expression, a mechanism called buffering. Indeed, when one copy of a gene is less expressed, the cell machinery might be more available to express the other gene copy at higher levels, or adjustments in gene expression networks might automatically happen through feedback loops. Buffering effects have been observed in *Drosophila* when one copy of an autosomal gene was deleted, the remaining hemizygous gene expression was approximately two-thirds that of normal diploid expression, rather than the expected 50% [25]. Similar results were obtained in yeast [26]. The distinction between specific dosage compensation and buffering mechanisms relies on the discovery of the underlying dosage compensation mechanism, which has so far been elucidated in few species.

Over the past decade, thanks to the advance of next-generation sequencing and in particular RNA-seq, DC has been studied and documented in various other animal species

[27,28]. These studies have revealed that the patterns of DC observed in animals are incredibly diverse. Gu and Walters [27] proposed a nomenclature with four DC types depending on two criteria. The first criteria is whether expression is equal between males and females and is called "dosage balance" [27], but we hereafter refer to it as sex-equality. Sex equality is indicated by an expression ratio XY_{male}/XX_{female} centered around one. The second criteria to classify DC types is whether ancestral expression levels are recovered in XY males. This phenomenon is called dosage compensation *sensu stricto* by Gu and Walters [27], but hereafter referred to as ancestral expression recovery. An expression ratio XY_{male}/AA centered around 1 indicates ancestral expression recovery (where AA stands for autosomes or the orthologous autosomes in a closely related outgroup without sex chromosomes). In this review, we refer to DC in its broad assertion as either sex equality or ancestral expression recovery or both. In some species DC mechanisms are global and affect the entire X chromosome (as in *Drosophila* X upregulation, mammals X inactivation, *C. elegans* X inhibition), whereas in other species DC is incomplete and local or gene-by-gene [28,29]. For example, DC evolved only for a minority of genes in female heterogametic snakes [30] and chicken [31]. Surprisingly, in birds DC seems to be mediated by chromosome-wide changes in epigenetic marks in spite of impacting the expression of only a few genes [32]. It can be challenging to classify a species as having complete/global versus incomplete/local DC. For example, placental mammals and *C. elegans* are traditional examples of complete and global DC and yet, as explained previously, many of their genes lack X upregulation or escape X inactivation, suggesting that their DC is incomplete. These semantic difficulties have driven many controversies in the field, as illustrated below. For the majority of species, the precise mechanisms of DC have not yet been elucidated. Moreover, how DC evolves and why there is such a diversity of patterns is currently not understood.

The early steps of DC evolution have long been erased in old systems and only young sex chromosomes hold the potential to reveal how DC originated. *Drosophila miranda* carries a young (~1 My old) neo-X/neo-Y system following the fusion of an autosome with the sex chromosomes. Study of *D. miranda* has shown that the MSL complex was recruited on the neo-X to achieve DC [33,34]. However, this *D. miranda* neo-X DC relies on the co-option of a pre-existing mechanism. *De novo* evolution of DC has been difficult to study in the canonical animal systems because these sex chromosomes are over a hundred million years old [35]. Plant sex chromosomes are generally much younger than in animals because separate sexes (dioecy) is a derived trait in plants, unlike animals where separate sexes (gonochorism) are ancestral [36]. Great advances have recently been made on our understanding of plant sex chromosome evolution [37,38] thanks to next-generation sequencing and the development of methods to study sex chromosomes in non-model organisms [39–42]. For a long time, it was thought that DC did not exist in plants [43], in spite of early cytological evidence for DC happening in the plant *Silene latifolia* [44]. Here we review the early controversies that led to the final consensus that partial DC exists in the plant *S. latifolia*. We then detail recent research investigating the mechanism of DC in *S. latifolia*. In a third part, we summarize the state of knowledge on DC in other plant species. Finally, we review theoretical work on the evolutionary forces that might drive DC evolution in any eukaryote with XY or ZW sex chromosomes. We end by a discussion on open questions in the field of DC evolution.

Early cytological evidence suggests X chromosome inactivation in *Silene latifolia* XY system

S. latifolia is a dioecious plant with XY sex chromosomes [45]. The X, the Y and the autosomes are strongly dimorphic in size, which led *S. latifolia* to become a model for the study of sex chromosomes using cytogenetics since the 1940s [46–49]. Sex chromosomes evolved ~11 million years ago in that species [50], making it an ideal model to study potential early steps of DC evolution. Moreover, it is possible to compare the evolution of *S. latifolia* sex chromosomes to their homologous autosomal pair in closely related non-dioecious species [45,51], making it easier to infer ancestral autosomal states of sex chromosomes.

Supplementary Table S1 recapitulates DC studies carried in *S. latifolia*, from as early as the 60s up to now. Early cytological work in *S. latifolia* (previously reviewed in [44]), has shown that one X in females is late-replicating and enriched in silencing epigenetic marks, including DNA methylation. The second X in females and the single X in males are early-replicating, hypomethylated and enriched in active epigenetic marks (Figure 2.a) [52–58]. These studies suggest that DC operates in *S. latifolia* and that one X may be hypermethylated and inactive in females, similarly to mammals [59].

If indeed X chromosome inactivation occurs in *S. latifolia*, it can be erased without deleterious consequences as shown by the apparent lack of vegetative or reproductive disturbance in *S. latifolia* females treated by a DNA hypomethylating drug, 5-azacytidine (5-azaC) [60], however the study did not report any fitness measure. On the other hand, in males 5-azaC treatment led to a sex change to hermaphroditism which is transmissible through the Y chromosome [60,61], suggesting that DNA methylation of specific Y genes controls the sex in

S. latifolia. A limitation of these early cytological studies of *S. latifolia* DC is that gene expression was not measured.

Transcriptomic evidence of incomplete dosage compensation in *S. latfolia*

With the advent of RNA-seq, *S. latifolia* was the first plant in which Y degeneration was studied and DC tested transcriptome-wide (Supplementary Table S1) [62–64]. Chibalina and Filatov [63] used a cross sequenced by RNA-seq to identify sex-linked transcripts. By studying the segregation of alleles from parents to progeny, they were able to classify transcripts as autosomal, X/Y or X-hemizygous (when the Y allele is absent from the transcriptome). The authors then compared male and female expression levels for 124 X-hemizygous genes and found that male expression was significantly lower than female expression (right side of Figure 2.b). They concluded that there was no DC in *S. latifolia*.

Muyle *et al.* [62] used RNA-seq data on brothers and sisters from an inbred line to identify X/Y genes in *S. latidolia*. Genes that were significantly differentially expressed between males and females (sex-biased genes) were discarded to test for DC. Indeed, sex-biased genes typically have over two-fold differences in expression between sexes, whereas X/Y genes with a degenerated Y copy are expected to have two-fold or lower differences in expression between sexes. Therefore, sex-biased gene expression cannot be explained by Y degeneration alone and might reflect evolution under sex-specific selection. For this reason, sex-biased genes are expected to be involved in sex-specific functions and DC is likely not necessary for these genes. In the 1,350 non-sex-biased X/Y genes that remained, X expression increased when Y expression decreased (Figure 2.b). The authors concluded that there was a global DC mechanism acting in *S. latifolia*. Sex chromosomes evolved 11 My ago in *S.*

latifolia [50], which means that dosage compensation can evolve *de novo* relatively quickly, which was unexpected.

Results of Muyle *et al.* [62] were at first criticized due to the absence of DC observed in X-hemizygous genes [63]. Indeed, a study of the X-hemizygous gene *SlWus1* by qRT-PCR revealed that expression was halved in males compared to females, suggesting there was no DC for this gene [65]. Moreover, both Xs were expressed in females for *SlWus1* [65], contradicting early cytological evidence for X inactivation in *S. latifolia*. Bergero *et al.* [66] used RNA-seq data on a cross to identify X-hemizygous genes and were able to validate 99 of them by PCR. Using this gene set, they confirmed results by Chibalina and Filatov [63]: X-hemizygous gene expression was halved in males compared to females (right side of Figure 2.b). The authors concluded that DC was absent from *S. latifolia* [66]. X-hemizygous genes lack any Y expression, for this reason it was thought that these genes would be under stronger selection to evolve DC [66]. However, an alternative hypothesis might be that X-hemizygous genes lost their Y expression precisely because they were not sensitive to dosage. For this reason X-hemizygous genes may be under low selective pressure to maintain Y expression levels and to evolve DC [67]. X-hemizygous genes tend to be lowly expressed in *S. latifolia* males and, importantly, also in females [68]. As far as the level of expression correlates with dosage sensitivity, this observation may corroborate the idea that many X hemizygous genes are not strongly dosage sensitive, although some of them could very well be. X-hemizygous genes also tend to be under low selective pressure on coding sequence and already lowly expressed before becoming X-hemizygous in *Rumex* [69]. The level of constraint on non-synonymous sites may correlate with the degree of dosage sensitivity (for instance the fitness effect of a stop codon necessarily scales with the effect of halving expression). As far as this correlation holds, these *Rumex* observations also corroborate the idea that many X-

hemizygous genes are not strongly dosage sensitive, explaining why many of them are not dosage compensated in the first place.

The first draft of the *S. latifolia* genome finally clarified discrepancies among transcriptomic DC studies in *S. latifolia* [70]. Even though only part of the genome was assembled, 233 X-hemizygous genes were identified, exceeding previous RNA-seq approaches. The ratio of X expression in males over XX expression in females (X_{male}/XX_{female}) was found to be bimodal in *S. latifolia* X-hemizygous genes (Figure 2.c) [70]. The first peak, with X_{male}/XX_{female} close to 0.5 reveals genes without DC, while the second peak around 1 indicates full dosage compensation. Papadopulos *et al.* [70] concluded that approximately half of X-hemizygous genes are dosage compensated in *S. latifolia*. It is unclear why these dosage compensated X-hemizygous genes were missed from previous studies [63,66]. Papadopulos *et al.* [70] also studied X_{male}/XX_{female} expression ratio in 627 X/Y genes with varying degrees of Y silencing, which revealed that many of them may be fully dosage compensated [70]. However, a precise estimate of the percentage of dosage compensated genes in *S. latifolia* is still lacking. There is currently no consensus in the DC literature on how to categorize individual genes as dosage compensated or not. Arbitrary thresholds on expression ratio between males and females could be used. Alternatively, male and female expression in multiple individuals could be statistically compared, but the conclusions would depend on the species sample size. Finally, the epigenetic state of the genes could be combined with expression data in order to determine if a gene is dosage compensated.

Using an RNA-seq approach to identify sex-linked genes is very efficient and powerful in species without a reference genome [39,40]. However, it has some limits that could explain discrepancies between the RNA-seq based study of DC (Figure 2.b, right side) and the genome-based approach (Figure 2.c). First, in a cross sequenced by RNA-seq, X-hemizygous genes can only be identified through X polymorphisms, which are less common

than X/Y polymorphisms that allow the identification of X/Y genes. X-hemizygous genes are therefore more likely to be missed by an RNA-seq approach. Second, during *de novo* transcriptome reference assembly, highly divergent X-Y alleles are likely to be assembled into different contigs, leading to the erroneous labeling of X/Y genes as X-hemizygous. Finally, X-hemizygous genes tend to be lowly expressed in both males and females [68], making their identification through RNA-seq more difficult. These three biases make the RNA-seq approach less suitable than genome sequencing to study DC in X-hemizygous genes. Papadopulos *et al.* [70] study on DC in X-hemizygous genes allowed the *Silene* community to agree that dosage compensation is partial (i.e. incomplete) in *S. latifolia*. Some sex-linked genes are not dosage compensated, but many are. In addition to the conflicting findings of the different studies, that are not entirely resolved, part of the controversy around the existence of DC in *S. latifolia* also came from the use of different definitions. Indeed, some researchers required all genes to be dosage compensated to qualify the species as having evolved DC. However, this may be too restrictive: studies in placental mammals and *C. elegans* have revealed how DC may vary across cell types, developmental stages and different classes of genes (genes with high expression levels and genes that interact with multiple other genes are more likely to evolve DC [28,29]). In that regard, *S. latifolia* incomplete DC may not be that different from that of placentals and *C. elegans*.

Dosage compensation in *S. latifolia* is mediated by imprinting and epigenetic differences between the the two Xs in females

The discovery of dosage compensation in the plant *S. latifolia* was important as it showed that dosage compensation is not an animal-specific phenomenon. It added to other examples of convergent evolution between animal and plant sex chromosomes [71]. Yet,

many questions remained on *S. latifolia* DC, mostly related to its mechanism and a link was missing between early cytological findings suggesting X inactivation and more recent transcriptomic studies in *S. latifolia*. Moreover, a major limitation of previous transcriptomic studies was that patterns of DC could be explained by simple buffering mechanisms. Indeed, buffering mechanisms could explain higher X expression observed in *S. latifolia* males without requiring the evolution of a specific DC mechanism with its own molecular machinery (such as the Drosophila MSL complex). Another limitation of previous transcriptomic studies in *S. latifolia* is that only sex-equality was tested and it remained unknown whether ancestral expression levels were recovered in males.

To address these limitations, a study used two non-dioecious outgroups without sex chromosomes to infer ancestral expression levels in *S. latifolia* [62]. They inferred that X expression increased in males compared to outgroups. Interestingly, the maternal X chromosome was also upregulated in females, while the paternal X maintained expression levels similar to the outgroups (Figure 2.d). In XY systems, the X in males is maternally inherited. Hence, this pattern suggests a DC mechanism based on parental epigenetic marks that would upregulate the maternal X in both males and females. Importantly, buffering mechanisms could not explain maternal X upregulation happening also in females, suggesting that a specific mechanism of DC has evolved in *S. latifolia*, mediated by genomic imprinting [72]. Maternal X upregulation in *S. latifolia* compensates for Y degeneration in males and reestablishes ancestral expression levels. However, it results in over-expression of sex-linked genes in females (Figure 2.d), which may be detrimental. This finding has striking resemblance to the first step of the two-stage process proposed by Ohno for the evolution of DC in mammals (i.e. first X upregulation later followed by X inactivation [9]).

Intriguingly, Krasovec *et al.* [73] did not find any difference in expression between the maternal and the paternal X of *S. latifolia* females for 163 X/Y genes, contradicting Muyle *et*

al. [72] results obtained on 640 X/Y genes. *Ide*ally, data from Krasovec *et al.* [73] should be reanalyzed using Muyle *et al.* [72] bioinformatic pipeline and conversely. The differences observed between the two studies could come from the different gene sets, a methodological issue, or the fact that Muyle *et al.* [72] used an outgroup to infer the direction of expression changes. If the differences persist independently from the bioinformatic pipeline used, it could mean that different *S. latifolia* populations have different DC mechanisms or imprints, or that the different tissues sampled and their developmental stages impact DC (Supplementary Table S1).

Krasovec *et al.* [73] also studied the effect of artificial Y deletions on DC patterns in *S. latifolia*, with the idea of testing whether deleting Y genes leads to their immediate DC by simple buffering mechanisms. For most of the Y deletions studied by the authors, the single X expression in mutant males was comparable to X expression in control males without Y deletions (i.e. absence of buffering effects in deletion mutants). This observation suggests that buffering mechanisms are not widespread on the *S. latifolia* X and therefore buffering cannot account for the observed DC patterns in that species (Figure 2.b). Intriguingly, when Y deletions occurred in a specific part of the p arm (in between the two sex-determining factors), they caused a global doubling of X expression in mutant males compared to control males without Y deletions. This X upregulation compensated for the loss of deleted Y genes. However, X upregulation also affected X genes which Y homolog had not been deleted, causing a global excess of sex-chromosome expression in mutants compared to control males without deletions, which might be deleterious. The authors called this phenomenon immediate DC. This work suggests that a trans-factor exists on the *S. latifolia* Y that normally downregulates X expression. When this unknown Y factor is artificially deleted, the X becomes upregulated in males. This trans-factor did not contribute to DC evolution in nature,

maybe because it is deleterious to globally overexpress the X chromosome for genes that still retain Y expression.

The maternal X chromosome upregulation found in *S. latifolia* males and females [72] brings a new light to interpret early cytological results. The hypermethylated X in females could be the paternal X (expressed at ancestral levels), while the hypomethylated X could be the upregulated maternal X in males and females. Hypermethylation could be the ancestral epigenetic state of the X chromosome, as suggested by the fact that the paternal X is expressed at ancestral levels. Epigenetic studies in other *Silene* species without sex chromosomes could help clarifying this point.

A recent study corroborated this hypothesis using various antibodies against active and repressive histone marks and DNA methylation. Immunostaining of root meristematic tissue in *S. latifolia* females showed that the active histone marks H3K4me2 and H3K9ac were enriched on one of the two Xs (named X1, Supplementary Figure S1), while DNA methylation was enriched on the other X (named X2) [74]. Interestingly, X1 has a two- to threefold stronger active histone mark signal than X2 or the autosomes, suggesting it is upregulated. On the other hand, X2 shows weaker active histone mark signals than either the autosomes or X1, suggesting it is downregulated (Supplementary Figure S1.c-d). Unfortunately, it remains unknown whether X1 and X2 are maternal or paternal, an important point that requires future work. Furthermore, it will be interesting to test whether the late-replicating X chromosome corresponds to X2 (the hypermethylated X with lower levels of active histone marks). Some cytological studies detected different DNA methylation intensities between the shorter and longer arm of the X (Figure 2.a) [55], a pattern that was not confirmed by Bačovský *et al.* [74] nor Vyskot *et al.* [54]. However, when using less condensed chromosomes, a clear difference in epigenetic marks was observed between the longer and shorter arm of X1 in females and X in males (Bačovský, unpublished results). It is

possible that the tissue type and cell cycle stage at which epigenetic marks are studied matters, which could explain discrepancies among studies (Figure 2.a). In any case, cytological work in *S. latifolia* suggests that DC is a chromosome-wide phenomenon, rather than a gene-by-gene process. If indeed hypermethylation is the ancestral epigenetic state of the X chromosome, it would make sense for the shorter arm to be hypermethylated because this arm contains the PAR plus a region that recently stopped recombining that likely has little Y degeneration and dosage compensation. On the other hand, the longer X arm contains regions that stopped recombining a longer time ago that are likely more degenerated and dosage compensated. A better genome reference for *S. latifolia* and methylation data in outgroups will definitely help testing these hypotheses.

Because Bacovsky *et al.* [74] analysed the chromatin chromosome-wide, it remained unknown whether gene expression was affected by these epigenetic patterns. To try and address this limitation, Rodríguez-Lorenzo *et al.* [75] quantified histone marks using chromatin immunoprecipitation followed by RT-PCR on six X/Y genes. They found that the promoters of the six X alleles in males were associated with activation of transcription marks (H4Ac, H3K4me3 and H3K27me3), suggesting that the male X is upregulated. On the other hand, the promoters of the six X alleles in females were associated with both active and repressive marks, which could be due to one X being upregulated and the other downregulated [75]. Unfortunately, the two Xs in females (and their parental origin) could not be distinguished in that study.

If indeed one X is upregulated and/or one X downregulated in *S. latifolia* females, the next question will be how this happens and how such a mechanism evolved. In mammals, X chromosome inactivation is initiated by the long non-coding RNA X-inactive specific transcript (*Xist*), which coats the inactive X from which it is transcribed [10]. The spread of inactivation along the entire X chromosome is facilitated by long interspersed elements 1

(LINE-1), which are retrotransposons predominantly enriched on the X compared to autosomes [76–78]. A common idea is that LINE-1 sequence is synonymous to *Xist* RNA-binding sites and that LINE-1 domains influence the higher-order topological folding of the X chromosome, favoring efficient in *cis* spreading of *Xist* RNA coating within gene-rich territories. Indeed, LINE-1 transcription is maintained mainly on the X chromosome at the time that X inactivation is first established [79]. Interestingly, some transposable elements (TEs), such as *Ogre* retrotransposons, are enriched on the X of *S. latifolia* compared to the autosomes and are almost absent from the Y, similarly to LINE-1 on the mammalian X [80–83]. A hypothesis that would be of interest to test is whether the specific accumulation of some TEs on *S. latifolia* X chromosome plays a role in chromosome-wide epigenetic marks in a similar way as it does in mammals.

Patterns of incomplete dosage compensation are common in other plants

Following the discovery of partial DC in *S. latifolia*, numerous studies have looked for DC patterns in other plant species (summarized in Supplementary Table S2). An important question is whether DC is a general feature of plant sex chromosomes.

Cannabis sativa and ***Humulus lupulus*** (Cannabaceae) have homologous XY sex chromosomes [84] that evolved at least 20-30 My ago [84,85]. In *C. sativa*, the X and the Y are of similar size (homomorphism), while in *H. lupulus* the X is larger than the Y (heteromorphism) [86–88]. The sex chromosomes are well differentiated in both species and Y degeneration is advanced. Indeed, 70% of sex-linked genes have lost Y expression in *C. sativa* and in both species the Y is significantly less expressed than the X in males (Supplementary Table S2). Partial DC was observed in *C. sativa* and *H. lupulus* with a clear increase in X expression in males as Y expression decreased, although X-hemizygous genes

were poorly dosage compensated in *C. sativa*, similarly to observations based on RNA-seq data in *S. latifolia* [84,85].

Similar patterns to *S. latifolia* DC were again observed in ***Coccinia grandis*** (Cucurbitaceae). This species has strongly heteromorphic XY sex chromosomes with a larger Y that evolved ~3 My ago [89]. The decrease in Y expression was compensated by an increase in male X expression for X/Y genes [90]. However, a detailed analysis of X-hemizygous genes revealed that only half of them is dosage compensated (Supplementary Table S2).

In ***Rumex hastatulus*** and ***Rumex rothschildianus*** (Polygonaceae), sex chromosomes have independently evolved 9-16 and 8–11 My ago respectively [69,91]. The ancestral sex chromosomes are XY in *R. hastatulus* but a neo-sex chromosome recently evolved through a fusion between the X and an autosome, leading to polymorphic populations with XY or XY1Y2 sex chromosomes. Y degeneration led to the loss of Y expression in ~28% of sex-linked genes and a decreased Y expression in X/Y genes (Supplementary Table S2) [91]. For old X/Y genes, male over female expression ratio was centered on one in *R. hastatulus* in spite of Y degeneration, indicating DC. However, DC was absent for most X-hemizygous genes (Supplementary Table S2) [91], which led the authors to conclude that DC was absent from *R. hastatulus*. These results are once again reminiscent of *S. latifolia* partial DC patterns. In *R. rothschildianus*, Y degeneration was more extensive, with about 92% of Y genes that were not expressed [69]. X/Y genes exhibited a male over female expression ratio centered on one in *R. rothschildianus*, suggesting again DC. However, only a quarter of selectively constrained X-hemizygous genes evolved DC. Interestingly, X-hemizygous genes were dosage compensated through downregulation of female expression compared to orthologous autosomal expression in outgroups. These results demonstrate that XX female downregulation can contribute to DC in plants [69]. Down-regulation of expression in females cannot be

explained by buffering mechanisms following the loss of the Y copy and implies that a specific DC mechanism evolved for *R. rothschildianus* X-hemizygous genes. Sequencing of the *R. rothschildianus* genome would help determine in the future whether this female down-regulation mechanism is a gene-by-gene process or affects a region of the X chromosome.

Patterns of partial DC similar to *S. latifolia* have therefore been generalized to five additional species with heteromorphic sex chromosomes and intermediate to strong Y degeneration. As these plants belong to four different plant families, these results suggest that partial DC could be a widespread feature of plants with heteromorphic sex chromosomes, rather than a particular characteristic of *S. latifolia*. DC patterns were less pronounced in homomorphic plant sex chromosomes. For instance, ***Carica papaya*** (Caricaceae) is a dioecious tree with XY homomorphic sex chromosomes that evolved ~7 My ago [92]. Evidence for dosage compensation was only found for a few non-recombining X/Y genes (7 out of 50) [93], and partial DC was found for some X-hemizygous genes (Supplementary Table S2) [94].

Silene otites (Caryophyllaceae) carries young homomorphic ZW sex chromosomes that evolved ~0.6 My ago [95]. In this system too, only a few genes were found to be compensated following W degeneration (Supplementary Table S2) [96]. It is nonetheless striking to find DC in such a recent system. This observation strengthens the idea that Y (or W) degeneration and DC co-evolve from the start of sex chromosome evolution. Interestingly, *S. otites* is the first plant with ZW sex chromosomes in which dosage compensation patterns have been reported. In animals, dosage compensation may be more common in XY than in ZW systems, although this has been disputed [29,43]. It will be interesting to see in future studies whether plants exhibit a similar trend.

Silene pseudotites is a close relative of *S. otites* but bears independently evolved XY sex chromosomes. *S. pseudotites* Y degeneration is very weak, as expected given its young age (~0.3 My old) [95]. No evidence of dosage compensation was found in *S. pseudotites* homomorphic XY sex chromosomes either [96]. Despite signs of early X/Y regulatory changes, no patterns of DC were found either in the dioecious herb ***Mercurialis annua*** (Euphorbiaceae)[97]. However, there might be few dosage compensated genes in *M. annua* that may have been missed by the linear model approach used by the authors. This species has a recently evolved homomorphic XY system (~1.5 My old) with very weak Y degeneration (Supplementary Table S2).

Importantly, in all these studies, no specific DC mechanism has been identified (except for *S. latifolia* and *R. rothschildianus*). It will be important to investigate whether such mechanisms have evolved in other species or if simple buffering in males explains the observed patterns. Furthermore, only sex equality was considered (except for *S. latifolia*, *S. otites* and *R. rothschildianus*) and the use of closely related outgroups without sex chromosomes might help determine whether ancestral expression levels have been restored in males.

Theories on dosage compensation evolution

Since its discovery by Muller [98], the phenomenon of DC has been considered an adaptive response to the loss of Y gene expression. As such, it was implicitly accepted that DC would occur late in the evolution of sex chromosomes, only after Y degeneration had reached a significant level such as multiple Y gene losses [3,99,100]. DC is usually ignored in models of Y degeneration [2,101], as the two processes are thought to occur in turn. Nonetheless, degeneration of some Y genes in a chromosomal region could trigger DC of the

entire gene block, which could then accelerate degeneration of the remaining functional Y genes [102]. This effect was investigated through simulations introducing a fitness cost for deleterious Y overexpression for genes that are not degenerated but dosage compensated [102]. This model interestingly showed that DC could either accelerate or slow down the rate of Y degeneration. However, the prominent view in the literature has been that there is a linear causality between degeneration and DC [99], which implies that DC occurs relatively late compared to degeneration. A linear causality is more convenient to model and conceptually easier.

This conception was recently challenged by the development of models incorporating both cis- and trans-regulatory variation [5,19]. Cis-regulators (such as enhancers) are sequences that control the expression of the allele located on the same chromatid, while trans-regulators (such as transcription factors) can be distant in the genome and influence the expression of both alleles of a gene (Figure 3.a). These models suggested that Y silencing (and DC of dosage-sensitive genes) might evolve early, slightly ahead of Y protein degeneration [19]. After recombination suppression, the X and the Y cis-regulators diverge and both can undergo silencing mutations. However, silencing mutations cannot fix in X cis-regulators because they would become homozygous in females and cause a strong fitness reduction (due to an expression deficit). On the other hand, Y cis-regulators remain heterozygous in males and silencing mutations can fix on the Y without suppressing expression in males (Figure 3.b). Hence, regulatory divergence preferentially leads to a lower expression of Y genes, which makes deleterious mutations on the Y more recessive and less purged by selection. This is expected to accelerate mutation accumulation in Y genes (Figure 3.c), which in turn selects for further Y silencing, and so on. Ultimately, this positive feedback loop causes full Y protein degeneration and silencing (Figure 3.d). This process can occur in absence of selective interference, but selective interference can contribute to the

process and accelerate degeneration. If a given X/Y gene is dosage sensitive and under strong stabilizing selection to maintain sex equality, Y degeneration is only possible if X expression becomes concomitantly upregulated in males. Expression can be upregulated in males through the evolution of a stronger male-specific trans-regulator (Figure 3.e), or by increasing the strength of the X cis-regulator and weakening the female-specific trans-regulator (Figure 3.f), or a mix of both options. The outcome depends on the relative mutation rates on cis and trans-regulators and the availability of sex-specific trans-regulators that target specific sex-linked genes (or sex chromosome regions). This concomitant evolution of Y degeneration and DC maintains equal male and female expression throughout the process (Figure 3.g). DC can therefore evolve smoothly without intermediate steps where fitness is dramatically low in males (after Y degeneration and before X upregulation) or females (after X upregulation and before X inactivation), as in Ohno's scenario [9].

A major difference between the cis-regulatory divergence model [19] and the more classical view of sex chromosome evolution is that Y silencing, Y coding sequence degeneration and DC evolve hand-in-hand in the cis-regulatory divergence model, with regulatory changes occurring slightly ahead of the accumulation of deleterious mutations in coding sequences. Several studies have recently pointed out that regulatory changes happened early on sex chromosomes [19], which is consistent with this theory. For instance, it was at the time a surprise that *S. latifolia* had evolved DC *de novo* over the course of 11 My [59]. It is now commonly accepted in the field that DC can evolve relatively early following Y degeneration, because DC was described in recently evolved sex chromosomes of animals [103–105] and plants with sex chromosomes as recent as 0.6 My (*S. otites*, Supplementary Table S2). However, more detailed data on the relative occurrence of regulatory evolution versus Y coding sequence degeneration would be needed to better assess this theory and

compare its predictions with the more classical view where regulatory changes occur after Y degeneration.

Interestingly, the cis-regulatory divergence theory has the potential to explain recombination suppression along sex chromosomes [5]. Traditionally, recombination suppression on sex chromosomes was thought to be selected by sexually antagonistic alleles (alleles that are beneficial in one sex but deleterious in the other) [106]. However, empirical support for this theory remains scarce. Alternatively, recombination suppression could occur by chance, through the fixation of "lucky" Y inversions carrying very few deleterious mutations (compared to other portions of this chromosome in the population). Early regulatory divergence, and DC of dosage sensitive genes would then automatically create sex-antagonistic effects. Indeed, after DC emergence, X cis-regulators recombined on a Y cause overexpression in males, while Y cis-regulators recombined on the X cause lower expression in females. These sex-antagonistic effects are unrelated to sexual dimorphism (the differences between female and male phenotype), and only occur because one sex is heterogametic. They are sufficient to maintain recombination suppression between the X and the Y. Otherwise, it would be favorable for the X and the Y to recombine, to remove deleterious mutations accumulated on the Y. This theory places DC at the start of sex chromosome evolution and reverses the steps of the traditional view of sex chromosome evolution.

Finally, another theory proposes that DC in the form of X chromosome imprinting (such as paternal X inactivation in marsupials) may be a consequence of an intragenomic parental conflict (*i.e.* divergent selective pressures between the maternal and the paternal reproductive function) [107,108]. In flowering plants, marsupials and placental mammals, embryos are attached to their mother after fertilization and nourished through specific tissues such as the endosperm or the placenta. Theory suggests that mothers are under selection to limit transferred amounts of resources to provide equal amounts to all offspring and to benefit

their own survival and future progeny (although mothers may also benefit from differential allocation of resources to offspring of differing quality [109]). On the other hand, fathers are under selection to sire offspring that extract the most possible resources from the mother to benefit the offspring survival, at the expense of half-sibs from different fathers [110,111]. Theory predicts that this parental conflict leads embryonic growth enhancers to be paternally expressed and maternally silenced, while embryonic growth inhibitors are maternally expressed and paternally silenced [112]. Because the X spends two thirds of its time in females, it is expected to favor the interest of mothers. Hence, Haig [107,108] made the hypothesis that the X should be enriched in embryonic growth inhibitors. Fathers that transmit a silenced X to their daughters would then presumably have daughters that are able to extract more resources from their mother, possibly explaining why the paternal X is systematically silenced in marsupials [107,108].

Haig's theory offers an interesting perspective on the possible role of kin and parental conflicts on sex-chromosome evolution. However, it suffers from several issues. First, it is not clear why the X should initially be enriched in growth inhibitors, rather than contain both growth inhibitors and growth enhancers, as any autosome. The model does not work if growth enhancers are also present on the X. Second, for the model to apply, selection pressure to imprint growth inhibitors on the X needs to be stronger than selection to adjust expression levels of all other X genes. Indeed, globally silencing the X is likely to be detrimental for all dosage sensitive genes, which is likely to be quantitatively much stronger than the selection favoring imprinting of a few growth inhibitors. Local imprint of growth inhibitors would definitely be less detrimental than halving female expression of all X genes. Another difficulty of the theory comes from the fact that most tissues in placental mammals have random X inactivation (except for some extraembryonic tissues [113]). Haig [107,108] proposes that random X inactivation could have evolved from ancestral paternal X

inactivation to restore the advantage of diploidy and the masking of recessive deleterious mutations in females [107,108]. However, this very selection against X hemizygosity should have prevented the evolution of paternal X silencing in the first place. Finally, if parental conflict indeed led to imprinted X inactivation, it is unclear why this imprint would be maintained in adult tissues when resources are not acquired from the mother anymore and embryonic growth regulators are not expressed. Given these limitations, it seems unlikely that parental conflicts could drive the evolution of global X imprints in species with sex chromosomes (such as mammals and *S. latifolia*). However, it does not mean that imprinting is not important for DC evolution.

The association between DC and sex-of-origin imprints of sex chromosomes may have a more direct cause. Imprinting marks could easily be used to evolve DC, thanks to a reliable mechanism of maternal/paternal chromosome identification to either silence the paternal X in females (as in marsupials) or upregulate the maternal X in males and females (as in *S. latifolia*). Silencing the paternal X is simple because it is a rule that works in both sexes and leads to sex equality when the Y is silenced. It can probably evolve easily because it does not interfere with maternal X expression, which is necessary in males. It also does not require the very complex machinery that is necessary to count Xs and randomly inactivate one (as in placental mammals). Hence, parental imprinting may be a reliable and simple way to evolve global DC, rather than an elaborate mechanism involved in parental conflicts.

Open questions on dosage compensation evolution

Given the recent theoretical developments regarding the potential role of cis-regulatory divergence in DC evolution [19], a first obvious question is what drives DC evolution. It is possible that one model is dominant over the other – either cis-regulatory

divergence or the more traditional view of recombination suppression followed by Y degeneration due to selective interference eventually leading to DC evolution. Another possibility is that both models apply jointly and it might be difficult to disentangle the contribution of each phenomenon.

Effective population size (N_e) has been proposed as a potential factor influencing the evolution of DC and possibly explaining why some species lack DC [114]. However, Chen *et al.* [12] studied 25 animal species and found no correlation between N_e and the level of dosage compensation (measured by X/A and Z/A expression ratios). However, the effect of N_e on DC evolution is likely more complex than previously envisioned. If recombination suppression evolves first, followed by Y degeneration and then DC (as in the traditional view), then the effect of N_e may depend on the limiting factor (i.e. the step with the slowest evolutionary rate). If Y degeneration is the limiting step, DC evolution is likely to be faster in species with small N_e, where interference and Y degeneration is strongest. If DC is the limiting step, the overall process may be faster in species with larger N_e, where selection is more efficient. In the cis-regulatory divergence model, the effect of N_e is probably complex too. If recombination suppression is the key limiting factor, then the overall evolution of sex chromosomes may be faster in species with large N_e [5]. However, if degeneration and DC are limiting, then the reverse trend may be expected [19]. In any case, predictions are difficult to make as the different components of sex chromosome evolution (recombination arrest, degeneration and DC) are all dependent on N_e in different ways.

Another factor potentially influencing the evolution of DC is the size of the non-recombining region. However, its effect is again likely to be complex. In the traditional view, a large non-recombining region with many genes leads to stronger selective interference and faster Y degeneration [101]. This would lead to a fast decrease in male fitness, potentially leading to a stronger selection to evolve DC. On the other hand, in the case of the cis-

regulatory divergence model, small non-recombining regions (or strata) can evolve strong Y degeneration and DC. This is not possible to achieve with the traditional theory of sex chromosome evolution because selective interference is too weak on a small non-recombining region. The effect of the non-recombining region size on DC evolution is therefore dependent on which model best applies to sex chromosome evolution (traditional view versus cis-regulatory divergence).

A question that is often asked in DC reviews is why some species have DC and others do not. Studies summarized in Supplementary Table S2 show that dosage compensation is not ubiquitous in plants, especially for plants bearing homomorphic sex chromosomes with little degeneration. However, species lacking DC might just not have sufficient Y degeneration for selection to drive DC evolution. Another possibility is that only a few dosage sensitive genes are dosage compensated and these genes might be missed when looking for global DC patterns, such as for example in *M. annua* [97]. If a gene is dosage sensitive, a reduction in Y expression would be highly detrimental to male fitness and is expected to select for DC. Few aneuploidies are viable and fertile, illustrating that most chromosomes carry dosage sensitive genes whose expression cannot be decreased. Sex chromosomes are unlikely to be an exception to this rule and we recommend searching for gene-by-gene DC patterns in species that have ongoing Y degeneration and apparently lack DC.

Another question that remains open is why some species exhibit global DC, while in others DC is regulated locally, gene-by-gene. A possible explanation is that some non-recombining regions might contain more dosage-sensitive genes than others, just by chance [12,69]. If a high number of genes are dosage sensitive, global DC patterns are expected. This effect could have been determinant in the lack of global X upregulation in mammals [12]. A better assessment of the level of gene dosage sensitivity on sex chromosomes would be

useful, especially if this information can be obtained independently from the degree of DC and Y silencing.

Very few models compare the rate of evolution of global versus local DC. In the cis-regulatory divergence model, global and local DC can evolve at about the same rate [19]. However, this apparent resemblance masks different dynamics. Local DC evolves smoothly and gradually, gene-by-gene, depending on the timing of regulatory divergence. Global DC evolution is delayed at first, but when a tipping point is reached (once several Y genes are partially silenced and have accumulated deleterious mutations), global DC evolves and causes the acceleration of Y degeneration, leading to full silencing of all Y genes [19]. Presumably, the same antagonistic effect of global DC would occur in models based on selective interference, where pleiotropy of a global regulator would first hinder DC evolution, but then precipitate it once the process is initiated [102]. However, this type of model comparison presupposes that both local and global regulators are available to achieve DC evolution.It is unlikely that a global trans-regulator would specifically target genes on a particular X region homologous to a degenerated Y non-recombining region. Such global trans-regulators are probably imperfect, having pleiotropic effects on many autosomal or sex-linked genes outside of the Y degenerated region (similar to the aforementioned *S. latifolia* trans-factor detected by Y deletions [73]). In comparison, trans-regulators that affect a single gene with limited pleiotropic effects may be available in many cases (for instance through specific local mechanisms such as RNA interference or specific transcription factors). Hence, we hypothesize that it is likely that DC evolves first on a gene-by-gene basis. Local DC may later be replaced by a global mechanism, but these transitions need to be investigated. More empirical work is needed to elucidate how DC evolves initially. Research on mechanisms have been almost exclusively limited to cases of global DC and little is known about how

local DC is achieved. More theoretical work is also required to elucidate the different pathways and conditions that may lead to the evolution of global versus local DC.

Conclusions and perspectives

Dosage compensation in plants is an ancient field of research, which started in the late 60s with cytological approaches. Even though DC was convincingly suggested in *S. latifolia* by early cytological studies, this body of work was overlooked for many decades during which DC in plants was highly controversial [40]. Progress in our understanding of DC in animals [27] helped the plant community to agree that partial dosage compensation exists in many plant species (Supplementary Table S2). Indeed, initial disagreements stemmed from the use of different definitions of DC and divergent opinions on whether all genes should be dosage compensated [28]. It is now understood that genes that are insensitive to dosage are unlikely to evolve sex equality or ancestral expression recovery, even in canonical systems such as *C. elegans* and placental mammals.

The mechanisms responsible for DC in plants are only starting to be described and many open questions remain in this field, offering a rich possibility of future scientific investigation, both empirical and theoretical. Due to the general lack of well assembled dioecious plant genomes, it is currently difficult to assess whether DC is a chromosome-wide or a gene-by-gene process in many plants. Chromosome-wide analyses using cytogenetic tools is an obvious way to tackle this issue in the case of complex genomes. Another important question is whether it is possible for a species with Y degeneration and dosage sensitive genes to lack any DC. A better characterization of dosage sensitive genes may help us understand why in some species only a few genes are dosage compensated, while in others global DC patterns are observed. Finally, the nature of the forces that drive DC evolution

remains debated as many alternative theories have been proposed to explain DC evolution (selection on dosage following Y degeneration, X-Y cis-regulatory divergence and parental conflicts). These theories remain to be compared more explicitly using empirical data. For example, young sex chromosome systems both in animals and plants will be insightful to test whether Y regulatory degeneration precedes Y protein degeneration.

Figure legends

Figure 1: Dosage compensation in *Drosophila*, mammals and *Caenorhabditis elegans*. a) Y degeneration results in dosage imbalance between males and females (hermaphrodites in *C. elegans*) and between sex chromosomes and autosomes in XY males. **b)** The single X is upregulated in *Drosophila* males. Either the maternal X (X_m) or the paternal X (X_p) is inactivated in placental mammals. In marsupials, X_p is inactivated. In *C. elegans*, both Xs are down-regulated in hermaphrodites. In mammals and *C. elegans*, only a few dosage-sensitive genes have their X expression upregulated, hence the dashed green X chromosomes in the sketch.

Figure 2: Dosage compensation (DC) patterns in *Silene* *latifolia*. The figures are schemes (not real data) combined from different studies. **a)** Chromatin marks on male and female sex chromosomes [54,55,57,74]. Based on the cytological evidence using replication labeling and methylcytosine DNA immunostaining, females have one X chromosome with higher level of 5-methylcytosine (5mC) residues (hypermethylation) and at the same time late-replication (red and minus marks). The second X chromosome in females shows an opposite pattern, hypomethylation and early-replication (green and plus marks). It remains to be clarified if both arms of the hypomethylated X chromosome differ in the level of DNA methylation and replication timing, as results differ among studies. In males, the single X is hypomethylated and early-replicated and the Y chromosome shows similar level of DNA methylation compared to the autosomes. Transcriptionally active chromatin histone marks (H3K9ac and H3K4me) were found enriched on the hypomethylated X in males and females but depleted on the hypermethylated X in females. **b)** Expression levels in males and females for different degrees of Y silencing (schemed from [62,66,70]). The ratio of Y over X expression in males is used as a proxy for Y silencing, from weak Y silencing to the left, to absence of Y expression to the right. For X/Y genes, X expression increases in males as Y expression is reduced. For X-hemizygous genes (without Y expression), males exhibit dosage imbalance. **c)** Bimodal distribution of X expression in males over XX expression in females in X-hemizygous genes detected through genome sequencing (schemed from [70]). A ratio close to 0.5 indicates no DC, while a ratio close to 1 reveals full DC. **d)** Maternal X (X_m), paternal X (X_p) and Y expression in *S. latifolia* sex-linked genes compared to orthologous autosomal genes in hermaphrodite outgroups without sex chromosomes (schemed from [72]). X_m is upregulated in both males and females, which compensates for lower Y expression in males, but X_p maintained ancestral expression, leading to X overexpression in *S. latifolia* females compared to ancestral expression levels, at least for some genes.

Figure 3: The cis-regulatory divergence theory for dosage compensation (DC) evolution [19]. a) Cis-regulators regulate the expression level of the allele located on the same chromatid. Trans-regulators on the other hand can be distantly located in the genome (here on an autosome A) and affect the expression level of both alleles of a gene. Males are represented on the left with their X0 and Y0 alleles, females to the right with two X0 alleles. mT0 stands for male-specific trans-regulator allele 0, an autosomal trans-regulator expressed only in males. Similarly, fT0 stands for female-specific trans-regulator allele 0. **b)** After recombination suppression, X and Y cis-regulator will diverge. Y cis-regulators are always heterozygous in males and can fix silencing mutations without suppressing expression in males (as represented by the smaller size of the Y1 cis-regulator compared to Y0). **c)** A weaker Y expression makes the Y allele less visible to selection, leading to an accumulation of Y mutations (represented by stars in Y2). **d)** Further silencing of the mutated Y allele is selectively favored to minimize the fitness effect of accumulated deleterious mutations, and so on, leading to complete silencing of the Y3 allele. **e)** Y degeneration of a dosage-sensitive gene is only possible if concomitant DC evolves. Male expression is upregulated by a stronger male-specific trans-regulator mT1 (represented by a larger and darker blue square compared to mT0). **f)** Alternatively, a stronger cis-regulator evolves in X1 (represented by a larger square compared to X0). This increases expression in both males and females. Expression in females can be decreased through the evolution of a weaker female-specific trans regulator fT1 (represented by a smaller and lighter square compared to fT0). **g)** For dosage sensitive genes, expression is maintained equal between males and females throughout the process of Y degeneration and DC evolution.

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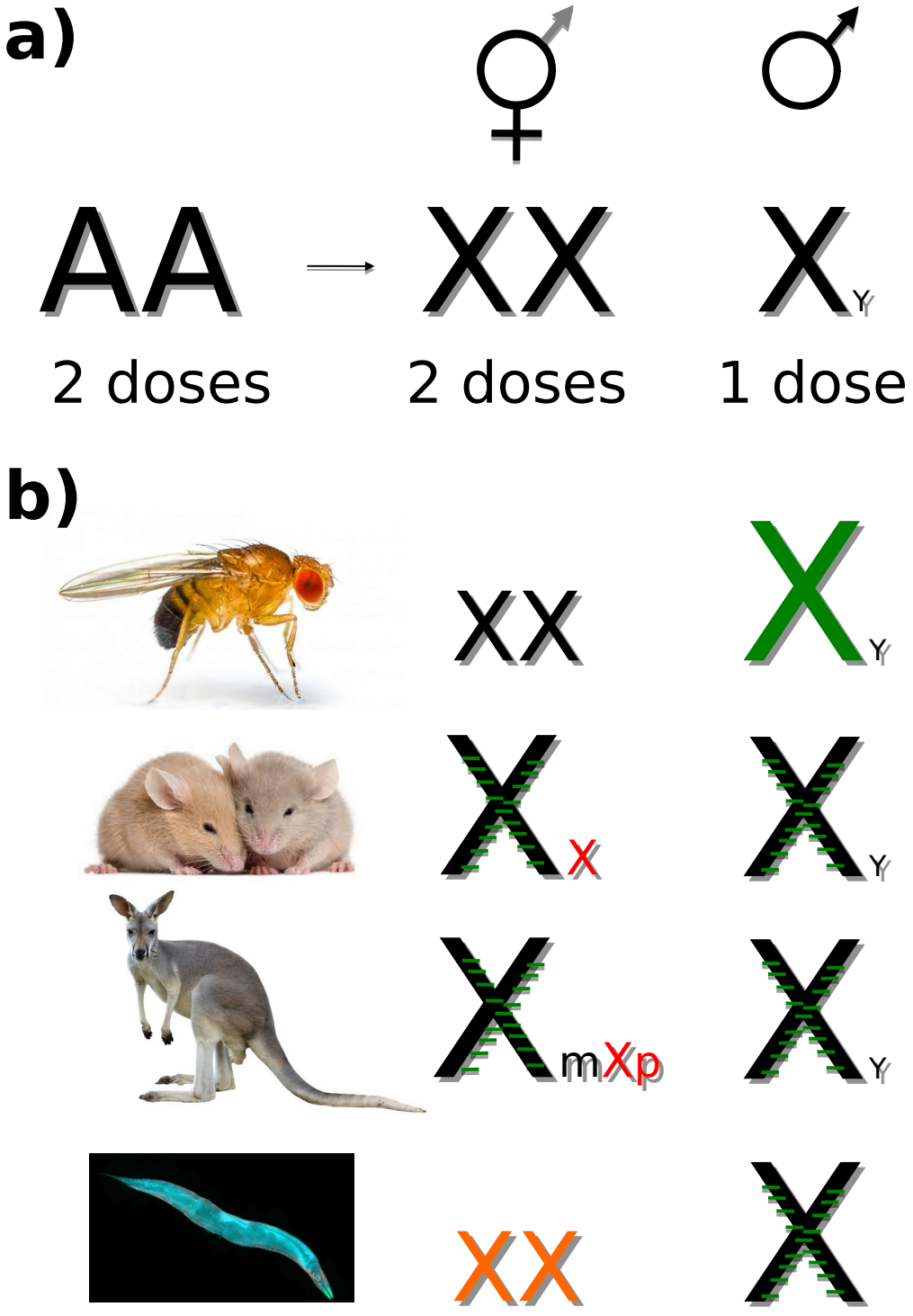
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765 Fig 1

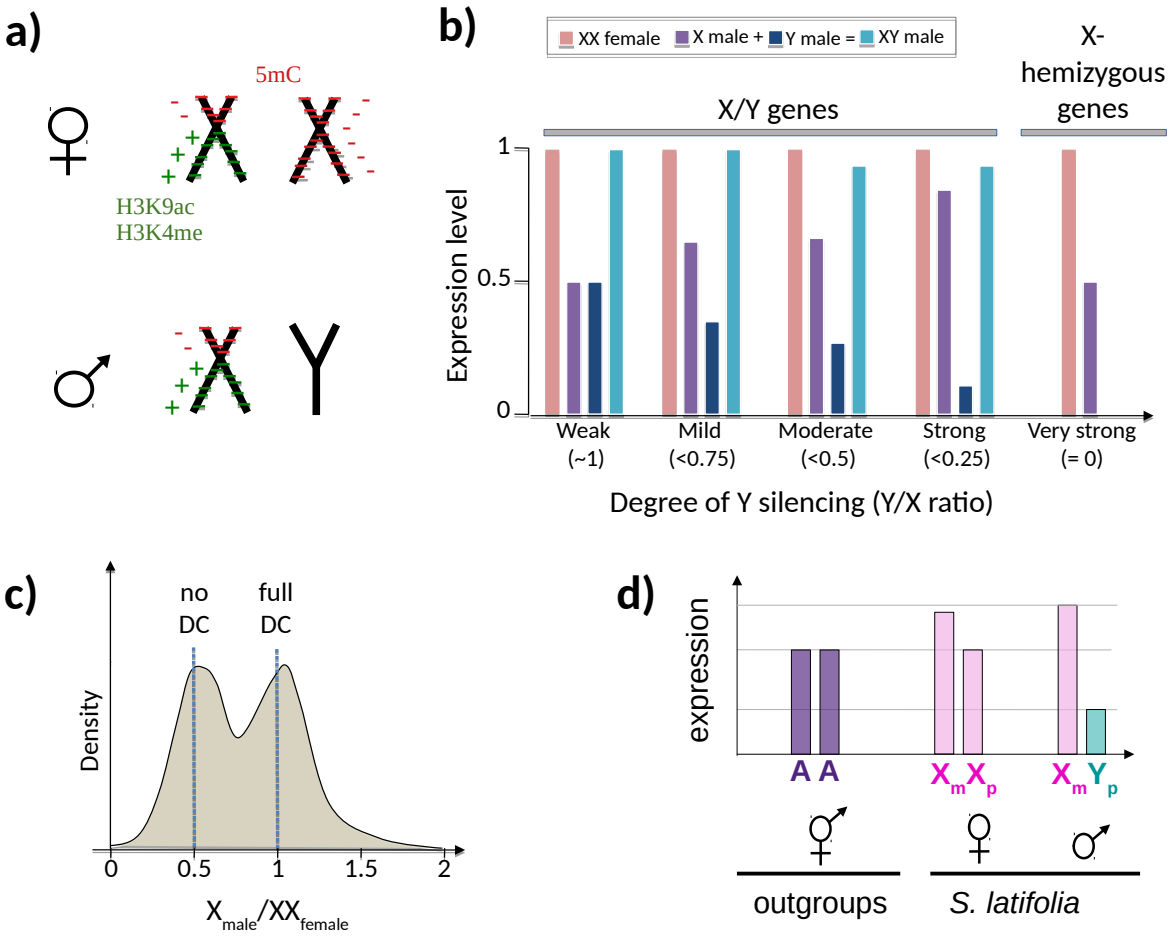
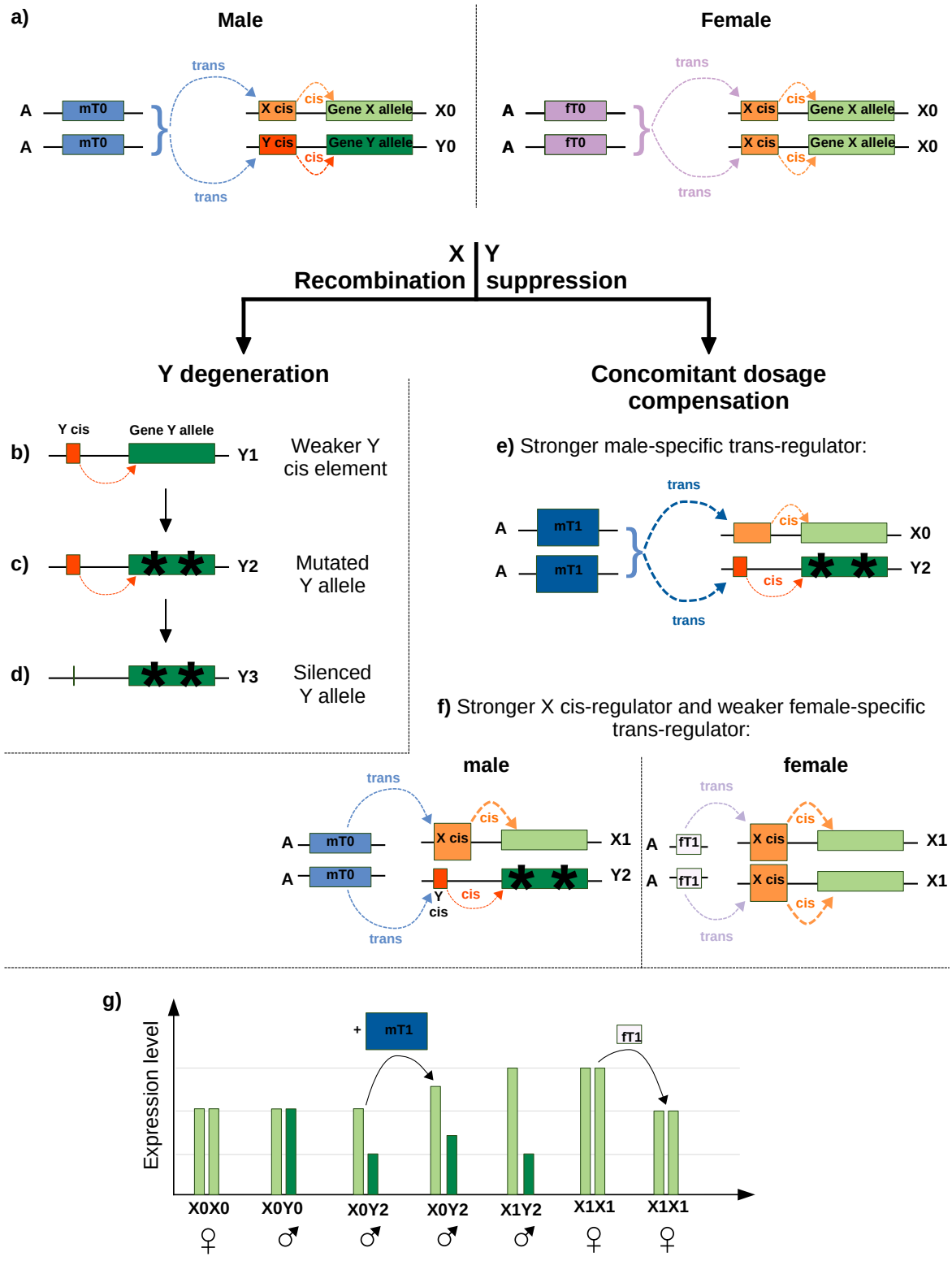

a)
5mC
H3K9ac
H3K4me
b)
XX female
X male +
Y male =
XY male
X-hemizygous genes
X/Y genes
Expression level
1
0.5
0
Weak (~1)
Mild (<0.75)
Moderate (<0.5)
Strong (<0.25)
Very strong (= 0)
Degree of Y silencing (Y/X ratio)
c)
no DC
full DC
Density
0
0.5
1
1.5
2
Xmale/XXfemale
d)
expression
A A
XmXp
XmYp
outgroups
S. latifolia

Fig 2


a)
Male
Female
trans
cis
A
mT0
X cis
Gene X allele
X0
Y cis
Gene Y allele
Y0
fT0
X
Recombination
Y
suppression
Y degeneration
Concomitant dosage compensation
b)
Y cis
Gene Y allele
Y1
Weaker Y cis element
c)
Y2
Mutated Y allele
d)
Y3
Silenced Y allele
e) Stronger male-specific trans-regulator:
mT1
f) Stronger X cis-regulator and weaker female-specific trans-regulator:
male
female
X1
fT1
g)
Expression level
+
X0X0
X0Y0
X0Y2
X0Y2
X1Y2
X1X1
X1X1

770 Fig 3