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Dosage Compensation: How to Be Compensated…Or Not?

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Diverse dosage compensation mechanisms have evolved across species to equalize gene expression between sexes and between the sex chromosomes and autosomes. New results show that two opposite modes of dosage compensation can occur within one species, the monarch butterfly.

In diploid organisms, maintenance of the correct gene dosage is essential for normal cellular function and development [1]. For example, aneuploidies are responsible for approximately 46% of spontaneous abortions in humans [2]. Very few babies survive with aneuploidies on one of the gene-poor chromosomes such as 21, 18 or 13, or on the large gene-rich X chromosome [3]. However, X chromosome aneuploidies cause fewer phenotypic effects than autosomal aneuploidies [4]. What makes X chromosome aneuploidies more tolerated than those on autosomes, even with its high gene content?

To answer this question, we have to investigate the diverse mechanisms that evolved to equalize the sex-chromosome dosage imbalance across species. Although sex determination and dosage compensation strategies can vary widely within a clade, no previous study has reported that multiple modes could coexist within one species. A paper by Gu *et al.* [5] in this issue of *Current Biology* now shows that a dual mode of dosage compensation occurs in a female heterogametic species: the monarch butterfly (*Danaus plexippus*).

Sex determination can be very diverse across species, with mechanism as divergent as environmental and chromosomal/genetic determination within the same clade. Chromosomal/genetic sex determination is mainly regulated by a pair of heteromorphic sex chromosomes of two main classes: male heterogamety (female XX, male XY) or female heterogamety (female ZW, male ZZ) [6]. Sex chromosomes originate from a pair of homologous autosomes called the proto-X and proto-Y, if we are using male heterogamety as an example, but the same mechanism can also apply to female heterogamety [7]. The Y chromosome starts evolving when a male determining gene appears on an autosome forming a sex-specific region of the proto-Y. Therefore, proto-X and proto-Y can no longer recombine due to the lack of homologous sequences, resulting in degeneration of the Y chromosome (Figure 1). However, a critical issue is what happens when each X-linked gene only has one functional allele in males, as opposed to when two alleles existed before Y-chromosome degeneration. Moreover, the single copy of the X in males leads to an imbalance in gene expression relative to diploid autosomal genes. To avoid the 'peril' of one X, Ohno hypothesized that upregulation of X-linked genes in the heterogametic sex would be necessary to restore the balance [8]. Diverse mechanisms have evolved independently across species to equalize gene dosage between males and females and the X-chromosome and autosomes.

In mode I (Figure 1), *Drosophila* attain dosage compensation via a male specific lethal (MSL) complex, which specifically targets the male X-chromosome through a zinc finger linker protein CLAMP and doubles the transcription of the single X chromosome [9]. This one-step strategy compensates X-linked gene expression relative to autosomes (A) and restores the balance between males and females simultaneously. Therefore, no further compensation steps are required.

The second mode of dosage compensation (Figure 1) is based on the idea that down-regulation of one of the two X-chromosomes in females would decrease gene dosage of X-linked genes compared with autosomal genes. Therefore, the X-linked genes in both XY males and XX females are proposed to double in both sexes as a first step which results in over-transcription in females (tetrasomy) and corrects the gene dosage imbalance between the male X-chromosome and autosomes [10]. Next, silencing of one of the two upregulated female X-chromosomes corrects for the tetrasomy which caused an imbalance between the female X-chromosomes and autosomes.

Diverse mechanisms evolved to avoid X-chromosome tetrasomy such as, for example, random X-chromosome inactivation in mammalian females via the heterochromatic Barr body [11] or, alternatively, halving the expression from both X-chromosomes in *Caenorhabditis elegans* XX hermaphrodites. Regulation of X-inactivation in mammals involves a long non-coding RNA, *Xist*, which recruits the Polycomb repressive complexes PRC2, which spreads along the X-chromosome to silence gene expression [12]. In contrast to the complete silencing of one X-chromosome in mammals, an interesting variation occurs in XX *C. elegans* hermaphrodites where a specialized condensin complex limits local RNA polymerase II (Pol II) recruitment to X-chromosomes, which halves the expression from both Xs [13].

The study by Gu *et al.* [5] identified for the first time a dual-mode (mode III in Figure 1) dosage compensation strategy in monarch butterfly (*Danaus plexippus*), a female heterogametic species (female ZW, male ZZ). The Z chromosome in monarch butterfly is composed of a newly derived Z (Neo-Z) from an autosome and an ancestral Z (Anc-Z). This genetic

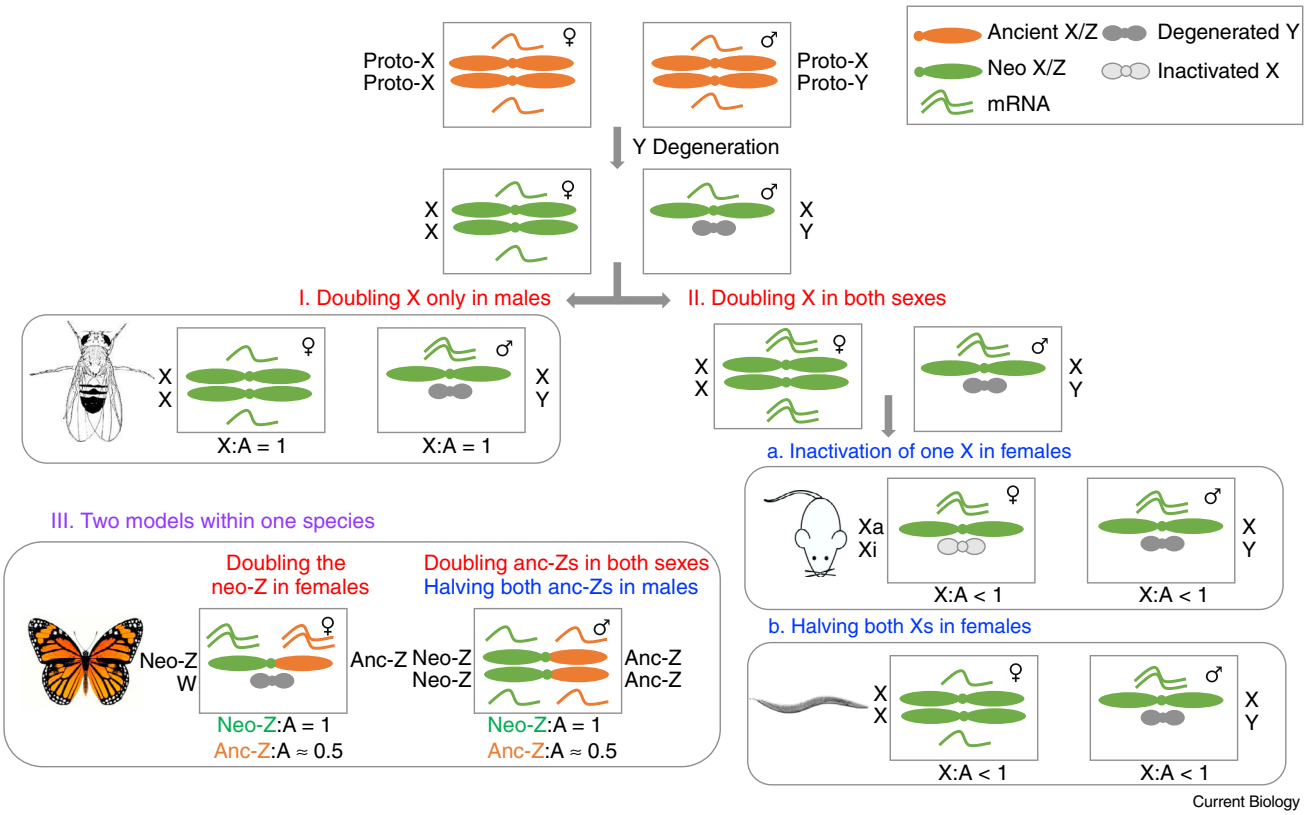

Figure 1. Evolutionary strategies of dosage compensation across species.
Sex chromosomes originated from autosome pairs: proto-X and proto-Y. During sex chromosome evolution, formation of sex-specific regions stopped recombination between proto-X and proto-Y, which drove Y degeneration. Ohno hypothesized that the X-linked expression level must be restored relative to autosomes in males to avoid the 'peril' of having only one X-chromosome. Diverse mechanisms evolved to perform dosage compensation across species. In mode I, *Drosophila* doubles the X-linked genes in males only, and no further step is necessary. The X:A ratio in *Drosophila* is 1 for complete dosage compensation. In mode II, X-linked gene expression is doubled in both sexes and results in hyper-transcription in females. Therefore, the secondary steps evolved to inactivate one of two X-chromosomes in mammalian females or halve expression from both X-chromosomes in *C. elegans* females. The X:A ratio in mammals and *C. elegans* is less than 1, causing incomplete dosage compensation. The new study by Gu *et al.* shows that monarch butterfly, a female heterogametic species (female ZW, male ZZ), uses mode I on its neo-Z and mode II for anc-Z. This is the first-time that two modes of dosage compensation have been found to occur on one single sex chromosome. The neo-Z:A ratio in monarch butterfly is 1 for complete dosage compensation, while the anc-Z:A ratio is close to 0.5, meaning that there is a lack of dosage compensation relative to autosomes.

system provides a unique opportunity to study two groups of sex-linked genes with distinct evolutionary paths on a single sex chromosome. Gu *et al.* found that the ancestral Z exhibits a mode II-like pattern because both Anc-Z chromosomes halve their expression in males (ZZ), similar to *C. elegans* hermaphrodites (XX). In contrast, the newly derived Z behaves more like *Drosophila* (mode I). The Neo-Z shows a female (ZW)-specific two-fold transcriptional upregulation, which results in complete dosage compensation of this newly derived segment. Moreover, the H4K16ac chromatin mark, which promotes transcription activation in *Drosophila* dosage compensation, differentially occupies the Anc-Z compared with the Neo-Z. Monarch butterfly dosage compensation is the first example where two modes of dosage compensation occur on a single sex-chromosome in one species. The detailed molecular mechanism behind this dual regulation system requires further investigation but it is possible that it requires the H4K16ac chromatin mark.

Although the molecular mechanisms that drive X-chromosome repression in mammals and *C. elegans* are well-established, the proposed first-step of two-fold X-upregulation remains speculative. To date, *Drosophila* is the only species that has a well-defined X-upregulation mechanism and this normally occurs only in XY males, while females have two active Xs which are balanced with autosomes already. A complete dosage compensation (i.e. X:A ratio = 1) has been reported in both sexes of *Drosophila*. However, the mechanisms of X upregulation in mammals and *C. elegans* still remain under investigation and the compensation level is largely incomplete (i.e. X:A ratio < 1). Strikingly, lack of dosage compensation (i.e. X:A ratio = 0.5) has been universally observed among female-heterogametic (ZW system) species, including birds and *Lepidoptera* (moths and butterflies).

Unlike X chromosome inactivation, which has been actively studied for over a half century, experiments that investigate X-chromosome upregulation at the genome-wide level have only been

possible in recent decades when transcriptomics became available. Multiple studies have performed microarray or RNA sequencing methods in a number of male-heterogametic (XY system) species [14–19] to test Ohno's hypothesis that there is dosage compensation between sex chromosomes and autosomes. Support for Ohno's hypothesis came from studies which tested subsets of genes, such as actively expressed genes [15], large protein-coding genes [17], or ubiquitously expressed house-keeping genes [18]. Upregulation of these genes reached an X:A expression ratio close to or higher than 1 in diverse somatic cells. Therefore, these genes were considered as 'dosage-sensitive' because their expression was upregulated. However, there are also studies which refute Ohno's theory [16,19]. Lin *et al.* [19] argued that due to the unavailability of the ancestral proto-X (X) and proto autosomes (A), most current tests are indirect as they make comparisons between the present-day X (X) and present-day autosome (A) and are thus inconclusive. They claimed that by directly comparing the human X with X orthologs identified from chicken, the expression of X-linked genes is roughly half of that on autosomes. However, the number of orthologous genes which were studied was very limited. Additionally, proteomic studies also provided conflicting testing results: The proteomics data from mouse and *C. elegans* yielded an X:A ratio of 1 [15], whereas analysis of human data suggested an X:A ratio of 0.5 [19].

The debate over Ohno's hypothesis continues as more and more new species are analyzed using genomic approaches. In their new paper on the monarch butterfly, Gu *et al.* also tested for Z-chromosome upregulation compared with autosomes. Interestingly, Neo-Z, which has mode I *Drosophila*-like dosage compensation, shows complete dosage compensation (Neo-Z:A = 1) as in *Drosophila*. In contrast, genes on Anc-Z, which manifest mode II *C. elegan*-like dosage compensation, exhibit on average nearly half the autosome levels (Anc-Z:A = 0.5) in both sexes. Therefore, there is no transcriptional upregulation from the Anc-Z, consistent with previous investigations of the Z-chromosome in other female-heterogametic species (birds and *Lepidoptera*) [20]. Surprisingly, Gu *et al.* revealed that the monarch butterfly has two distinct regulatory mechanisms for dosage compensation on a single chromosome, suggesting that dosage compensation mechanisms are even more diverse than previously expected.

The new dual mode of dosage compensation identified in monarch butterfly provides a very strong new model system to study the evolution of dosage compensation. Unexpectedly, the neo-sex chromosome that recently derived from an autosome is independent from pre-existing dosage compensation mechanisms that regulate the ancestral sex-chromosome. It will be very exciting to understand how two opposing dosage compensation systems are precisely regulated on the molecular level on one chromosome and how they influence each other during the course of evolution. One key question that could be answered in the future will be: does dosage compensation evolve on the 'gene-by-gene' level or by a 'global' mechanism? The emergence of two divergent strategies within the same species will provide many exciting opportunities to understand dosage compensation at the mechanistic level.

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