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REVIEW ARTICLE

Dosage compensation and its roles in evolution of sex chromosomes and phenotypic dimorphism: lessons from *Drosophila, C.elegans* and mammals

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Abstract In many sexually reproducing species, sex is determined by cytologically distinguishable 'sex chromosomes'. The popular view is that the consequence of heteromorphic sex chromosomes is detrimental, and evolutionary emergence of dosage compensation mechanism is expected for two fold upregulation of X linked genes in order to restore the balance for the haplo-X in the sex against the diplo X of the other. Since, male and female share nearly identical genome in most animals, and since antagonistic selection operate for the expression divergence of the sex biased genes between sexes for mating type distinction, dosage compensation system is evolved in many species to link global transcription profile of the genome through histone variants and epigenetic modification of the genes for driving sex determination function. Whole genome transcriptome analyses and the investigations on the profiling of accessible chromatin components in male and female at different phase of development of *Drosophila, C. elegans* and mammal revealed that 50–60% X and autosomal genes of the genomes are expressed under sex specific selection through allelic bias (except some required dosage sensitive genes) expression, ranging from absent to complete compensation. The review focuses the recent development of dosage compensation research and illustrates its roles in sex chromosome evolution and sexual dimorphism in *Drosophila, C. elegans* and mammals.

Keywords Sexual dimorphism · Sex chromosomes · X chromosome · Dosage compensation

Introduction

Sex determination often involves cytogenetically distinguishable sex chromosomes, that are evolved independently many times in both animals and plants. When heteromorphic sex chromosomes appear in males, it is referred to as XY/XO system, and homomorphic sex chromosomes are referred to as XX females. Conversely, when heteromorphic sex chromosome appears in females, it is referred to as ZW system and homomorphic sexes are ZZ males. In many animals, primary sexual phenotype is determined by the number of X chromosome relative to number of set of autosomes (X: A ratio), including *C. elegans* and *Drosophila* [17, 41, 67]. In *C. elegans*, Y chromosome is absent, unlike *Drosophila* where it contains genes essential for spermatogenesis although it has no role in sex determination process. In the group of animals, when X: A ratio is 0.75–1.0, female phenotype (including fertile hermaphrodite *C. elegans*) appears, however, range of X:A ratios for males varies from 0.5 to 0.67 [17, 41]. While in *C. elegans* with intermediate X:A ratio (0.67–0.75), show variable intersexual phenotypes [67], *Drosophila* display intersex phenotypes with the range of X:A ratio 0.67–0.85. From these observations, it appears that although X:A balance mechanism is functional in different groups of animals for determination of sex, its mode of action is varied from species to species. In mammals, however, maleness is normally determined by dominant Y-borne *Sry* gene, although a series of papers have claimed that remnant of a dosage dependent sex determining (dosage sensitive sex reversal gene—DSS) mechanism is still present in mammals [13, 141]. Thus, although an XXY genotype is

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phenotypically male in mice and humans, sex chromosome dosage can causes phenotypic variations in human. Together, it appears that the relative role of the sex chromosome(s) in fostering sex determination process varied considerably from species to species and sometime confusing. On the other hand, a number of positive association between sex chromosomes and sexually dimorphic phenotypes indicate that there is indeed some connection in sex determination system.

Earlier, Ohno [107] and Charlsewroth [22, 24] hypothesized that the X and Y chromosomes are evolved from an autosome pair due to the presence of a dominant sex determining gene. According to their model, heteromorphic sex chromosomes—such as XY or ZW have arisen independently when male/or female determining genes appear on an autosomal pair. When one member of the pair acquired a testis determining factor, another allele of opposite sex should be accumulated on the other homologue, and there will be selection for suppression of recombination to keep the sex specific cassette of gene together [25]. Since the Y chromosome passes from fathers to sons, it is subjected to selection only in males. This reduction in recombination may have the outcome of selection against crossing over between alleles linked to the sex determination locus that had different effects in males and females. Once recombination will reduce, Muller ratchet, genetic hitchhiking and comparable processes may lead to the accumulation of deleterious recessive alleles on the Y and this functional impairment eventually result in the Y chromosome's physical degeneration [7, 8, 22, 31]. Thus, in many taxa, a striking common feature of the Y chromosome is small in size and maintains a few genes required for males. It accumulates an unusual abundance of repetitive DNA sequences and fragments of transposable elements [6]. The limited genetic content and functional degeneration of Y chromosome results in 'monosomy' condition of X in males—a phenomenon that is typically fatal when it happens to autosomes. During the process of evolution of the heteromorphic sex chromosomes, natural selection played an important role to evolve a mechanism for compensation of the genes in the inactivated or lost segment of the Y chromosome. Thus, according to the model, dosage compensation of X linked genes is an evolutionary strategy required to equalize gene expression between individuals possessing different numbers of X chromosome(s) for sex determination and dosage compensation evolved independently in various taxa, many times over [21, 23, 83].

On the other hand, several lines of evidence indicated that in mammals, the *Sry* system of sex determination has introduced recently to replace the X:A system and the system is not universal in mammals [141]. Furthermore, in many species Y is not physically present for male sex determination (e.g. in many nematodes, Orthoptera). In *Drosophila*, XXY:AA genotype is functionally and phenotypically female, while XO:AA genotype is sterile males [17, 41] and in *C. elegans* XO:AA is male and XX:AA is female. These results are inconsistent with the prediction of the model that the diversity of sex chromosome systems in different animals is caused by hijacking of a chromosome pair with a new sex determination locus. In reality, there is no strong evidence in support of the process of evolution of the sex chromosomes in the route [11, 31, 142].

In past decade, the roles of dosage compensation in sex determination process have been intensively investigated by various authors [41, 52, 53, 141, 150] using whole genome transcriptome analyses and the mapping of the landscape of accessible chromatin in male and female of different animals. As data accumulates, noval features of dosage compensation mechanisms and their putative roles in the sex chromosomes evolution in different animals are emerging. For investigating the molecular solution of the dosage compensation mechanisms, most investigators have so far been restricted their studies to the three animal groups—the nematodes, *Drosophila* and mammals. In this review attempt has therefore been made first to highlight the major milestones and the latest achievements in dosage compensation research on the three animals, followed by discussion on the roles of dosage compensation mechanisms in evolving sex chromosome morphology and sexual dimorphism in the three animals.

Dosage compensation: historical and present concept

Muller [104] first described the phenomenon of dosage compensation. Using the X linked eye colour mutant, white apricot (w^a), he showed that although females carry two copies X linked w^a mutant, while males have only one copy w^a locus, two sexes are alike in eye colour. By adding extra copies of w^a gene as duplications, Muller [103] had shown that, although females with three copies of this locus, or male with two copies, both appeared darker in eye colour than flies with usual two or one copies in their respective sexes, males eye colour appeared much darker than females. This difference in effect of w^a duplication is a manifestation of dosage compensation. He claimed that dosage compensation is a complex form of adaptation (negative control) in which compensator gene works. Technically, w^a was a mutant allele of X-linked genes which he compared. Later, Smith and Lucchesi [122] repeated by spectrophotometric measurement of extracted eye pigments of normal alleles and noted that dosage compensation applies to normal alleles as well.

Evidence linking dosage compensation in mammals was first reported by Barr and Bertram [14] who discovered

cytologically inactivated X chromosome from female cat neuron. Ohno et al. [108] first suggested that the Barr body could be an inactivated X chromosome in female. Later, Lyon [89] explained correctly that the Barr body is an inactivated X chromosome in female for dosage compensation.

In contrast to the mammalian dosage compensation, Mukherjee and Beermann [101], by using ^{3}H-uridine autoradiography, first showed that dosage equivalence for X linked genes between males and females of *Drosophila* is achieved by hyper-transcriptive activity of the male X chromosome. Thereafter, dosage compensation research has been initiated in different laboratories using different model organisms. The latest development of dosage compensation research in three model organisms are discussed below.

Dosage compensation mechanism in *C. elegans*

As mentioned, in *C. elegans*, X:A ratio dictates both sex determination and X chromosome expression pattern. In XX females, the genes of both X chromosomes express nearly half the rate as XO males to account for dosage compensation. This requires the action of dosage compensation complex formed by at least nine proteins, several of which are involved in sex determination [67, 99, 106]. These components are inherited from oocyte. The gene products of the Dosage Compensation Complex (DCC) are supplied maternally to both XX and XO embryos, with one exception: SDC2 is expressed exclusively in XX embryos, where it mediates both the development of a hermaphrodite phenotype and the recruitment of the different proteins to the DCC [41, 43, 45]. The DCC proteins then form a condensin complex, which play a role in chromatin modifications, resulting decreased level of expression of the X chromosome [4, 127].

Dosage compensation mechanism in *Drosophila*

Initially dosage compensation research in *Drosophila,* was focused on its occurrence in different species of the genus *Drosophila* and their mode of operation. Using ^{3}H-uridine autoradiography technique, Mukherjee and his coworker [26, 44, 78] published a series of papers in which they had shown that dosage compensation in different species of *Drosophila* operates through piece meal mechanism. They also showed that there is a link between faster rate of replication of male X chromosome and hyper transcriptive activity of male X [33, 77]. The 'rule of cellular autonomous regulation' of dosage compensation in *Drosophila* was also recorded by his group [33, 76]. On the other hand, Lucchesi and others [84–87, 126] demonstrated that post-transcriptional mechanism is functioning for X chromosomal dosage compensation in *Drosophila.* Lucchesi and his co-workers [87, 94, 95] then measured the X chromosomal transcriptive activity pattern of some aneuploids and hyperploids, using ^{3}H-uridine autoradiography, and claimed that the X chromosomal activity of male can be regulated at five different levels by rate limiting transacting autosomal factors.

Based on these studies, Lucchesi and others [12, 15, 85, 110] then searched for transacting regulatory genes from autosomes by choosing male lethal mutants, that may control the male X chromosomal activity. They identified altogether five genes from autosomes [12, 58, 75]. They are *maleless* (*mle), male specific lethals (msl-1, msl-2, msl-3),* and *male absent on the first* (*mof).* For convenience, all four male lethal genes and *mof* collectively referred as the *msl* genes. By analyzing binding affinity of the proteins of the genes to the polytene X chromosome(s) of male and female third instar larvae, they claimed that the genetic regulatory components for establishing the higher order organization of male X polytene chromosome is the DCC [75, 88, 131]. It consists of five proteins called MSLs (MSL1, MSL2, MSL3, MLE and MOF) and two non-coding RNAs roX1 and roX2 [58, 75, 97]. The expression of MSL2 in males mediated the assembly of DCC on the male X chromosome. The MSL2 expression is regulated by the synthesis of *Sex lethal* (SXL) protein in sex specific manner. They also proposed a working model for MSL complex mediated regulation of male X chromosome [3, 12, 42, 58, 65, 88, 131]. According to the model, DCC bind to male X chromosome to enrich acetylation of histone H4 at lysine (H4K16ac) on the male X chromosome, due to its MOF activity. The acetylation of histones is linked to transcriptional hyperactivation of male X chromosome. In females, *Sxl* gene product translation pattern turn off the DCC formation by repressing the MSL-2 translation.

A critical evaluation of the above model of dosage compensation regulation should consider six important aspects of the data upon which the generalizations are derived. The first is that the time of action of the DCC for proposed two fold expression of the X chromosome in male is quite late; i.e. around the onset of gastrulation [30]. In contrast, available data indicated that two fold activity of the X chromosomes occur at early phase of development in female *Drosophila* [60, 82]. The second concern is that the functional activity of *msl* mutations is sex specific, i.e. the loss of function mutations of the genes kill only males which may imply that female level of X chromosome organization is by default, unlike *C. elegans* and mammals. Third aspect is that the nature of interactions between *Sxl* and *msl's* are incomplete and produce intersex phenotypes

[121] which may indicate that the *msl's* are responsible for sex differentiation rather than dosage compensation. The fourth aspect is that the loss of either non-coding RNA *roX1* or *roX2* is tolerated, but in double mutants there is no dosage compensation, suggesting that they are functionally redundant [97]. The fifth aspect is that it is still unknown how MSL complex recognizes the X chromosome for dosage compensation [46, 73, 114, 134]. The final consideration is that MSL dependent regulatory mechanism fails to explain the binding affinity of MSL complex in male aneuploids and intersex X chromosome(s). However, a secondary level of regulation by DCC for hyperactivation of the male X chromosome has not yet been ruled out [34].

On the other hand, by analyzing template activity pattern of the polytene X chromosome(s) of male and female from fixed cytological preparations, using different exogenous RNA polymerases and necessary conditions favourable for RNA synthesis, Chatterjee and Mukherjee [34], have shown that template capacity of the X chromosome for "dosage compensation in *Drosophila* is primarily a property of inherent organization of the X chromosome". When chromatin template activity of the polytene chromosomes of *Drosophila* has been measured after high molar salt extraction, an increase in template activity on both X and autosomes was noted. However, the increase is significantly less in the male X chromosome than that of the autosomes of some nuclei. The results have been interpreted to have suggested that "X chromosome hyperactivity of the male, might be guided by an inherent modulation of structure of the X chromatin" [37]. This view is strengthened from the observation that single X chromosome of male bind higher amount of non-histone chromosomal proteins for modulating male level organization [36] and these proteins preferentially bind to the male X chromosome throughout the development [29]. Using male aneuploids with different size of X chromosome fragments, Chatterjee [27] further showed that the X chromosome contains some discrete elements that can alter the conformation of X chromatin in response to their dose and thereby make the X linked genes to express in sex biased transcriptional apparatus. He also identified one male aneuploidy where X chromosomal activity was displayed with varying degree of condensation of euchromatic regions of X chromosome, starting from 'male' level over a wide range of intermediate level to a normal 'female' level (mosaic) (Fig. 1a–c) [28]. Based on these observations, he pointed out that "the X regulatory sequences involved in dosage compensation in *Drosophila* are located on the X chromosome and are polygenic". He also claimed that to achieve dosage compensation there may be other level of control that can finely modulate the organization of the X chromosome in response to changing X:A ratio. Genetic data showed that some aneuploids indeed transform sexual

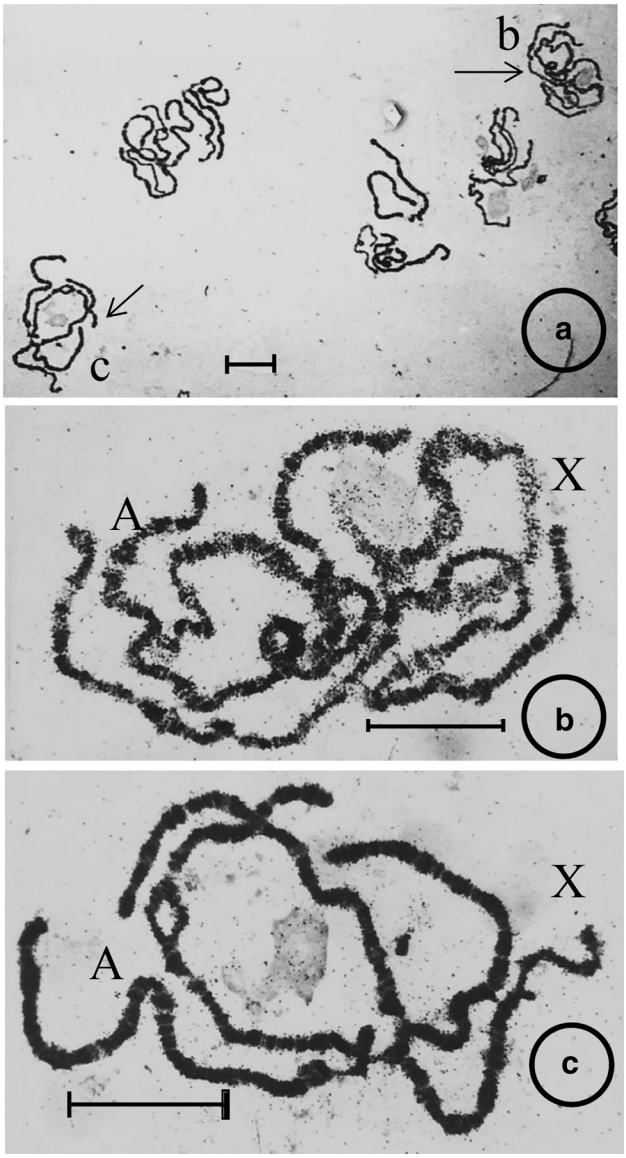

Fig. 1 Autoradiographs showing the polytene chromosome morphology and ^{3}H-UMP incorporation pattern over X and autosomes of an aneuploid larva with Dp8C-20F of *D. melanogaster,* **a** at low magnification, showing two different types of nuclei in the field (arrows), **b** a nucleus from higher magnification from **a** showing X chromosomes with 'male' level of X chromosome organization, and **c** a nucleus from higher magnification from **a** with 'female' level organization of X chromosome. *X* X chromosome, *A* autosome. The scale is given in 10 μm. Figure 1b adapted from Chatterjee 1990 [28], with permission

phenotype of individuals [125]. One interesting feature has emerged from segmental aneuploidy experiments that in every nuclei, the entire X and the duplicated segment in trans condition are expressed at same level. Latter, Mukherjee and Chatterjee [102] showed that functional organization of both X and autosomes was severely affected in triploid intersexes with X:A ratio 0.67 compared to triploids (Fig. 2a, b). Taken together, it appears

that in *Drosophila*: (a) chromosome wide regulatory mechanism exists on discrete location of the X chromosome for its sex biased expression in dose dependent manner (see below); (b) the organization of X chromosome is set primarily between 'male' to 'female' level. Chatterjee [30] also formulated a model to explain the relationship of X:A ratio to sex determination and dosage compensation (Fig. 3).

Later, Chatterjee and Chatterjee [32] proposed a model in which they claimed that lineage segregation of the X chromosomes (i.e. Xp or Xm) is the key regulator of somatic dosage compensation. They argued that, since male generally receive X chromosome from females, and since epigenetic state of female germ line X chromosome is normally hyperactive due to specific depletion of HP1a and H3K9me2 association (germ line depletion of Piwi leads to a loss of silencing of this group of TEs) [63], hyperactivity is an 'inherent organization' of the male X chromosome. Furthermore, necessary components of dosage compensation are inherited from oocytes. On the other hand, since females receive X chromosomes from both the parents, and since epigenetic state of male X chromosome follow germ line gene silencing pathways, it is expected that one X chromosome in female is upregulated and other X chromosome is silenced to neutralize the differences in X linked gene dose between male and female (e.g. mammalian female Xs). They further argued that since male somatic X chromosome is normally hyperactive in all taxa due to its lineage specific transmission, it is not necessary to modulate X chromosome further for hyperactivity in somatic cells of *Drosophila*. They, therefore argued that like *C. elegans*, down regulation of either both Xs or one X in females may play a role in regulation of dosage compensation in *Drosophila*. In brief, the key predictions of the model were that: (a) hyperactivity of male X chromosome is the 'inherent property' of male cells in the three species; (b) inactive condition of the 'paternal X chromosome' is biologically reversible in female ovum; (c) since female receive two X chromosomes from both the parents, the accessible state of the two X chromosomes can be 're-programmed' either by lineage segregation of X chromosomal activity, or by epigenetic remodelling of X chromosome materials at early embryo for dosage compensation. The predictions of the model were extensively verified in mammalian embryos [133, 143] and *Drosophila* embryos [60, 82] (see below) from different laboratories and noted that accessible chromatin remodeling of female X chromosomes is a dynamic process in very early stage of female embryos by transposable elements based silencing mechanism (see below).

For experimental verification of the model, Chatterjee et al. [38] analyzed functional morphology of the X

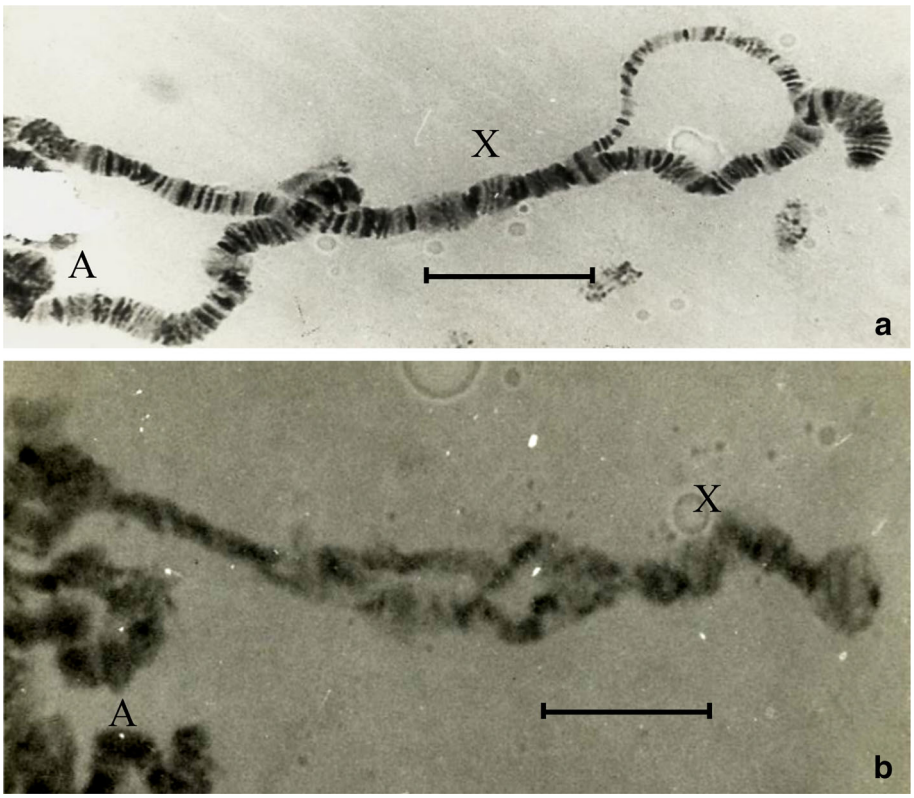

Fig. 2 Photomicrographs showing the polytene chromosome morphology of **a** triploid female (3X;3A), and **b** an triploid intersex (XXY;3A). Note that in intersex nucleus, functional morphology of both X chromosomes and autosomes are affected. *X* X chromosome, *A* autosome. Bar 10 μm. Adapted from Mukherjee and Chatterjee 1992 [102], with permission

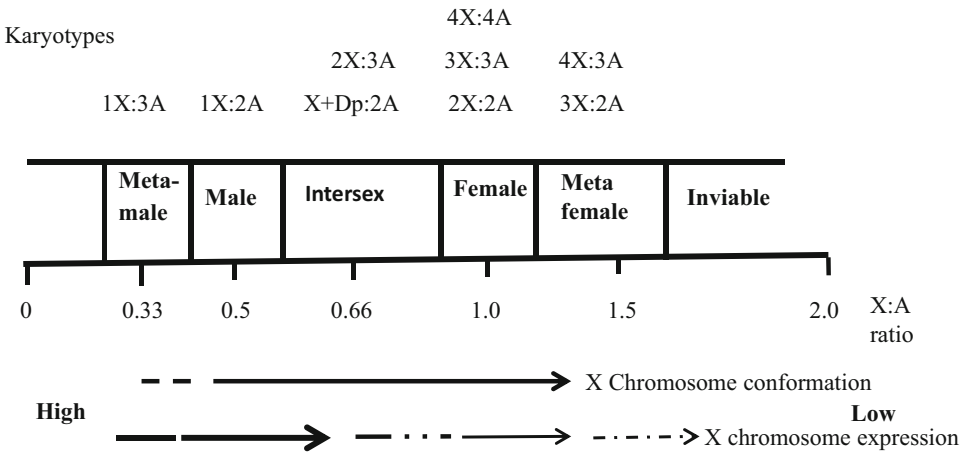

Fig. 3 The chart showing the sexual phenotypes and X chromosome expression pattern in relation to the the X:A ratio of *Drosophila*. Below the sexual phenotypes, the X chromosomal conformation and X chromosomal activity patterns of the individuals are shown. Adapted from Chatterjee 1992 [30], with permission

chromosome using *In(1)BM2* rearrangement strain of *D. melanogaster* that normally induce position effect variegation (PEV) phenotype in male X chromosome. Using aneuploids interaction assay, they showed that aneuploids carrying one variegated X plus a piece of X chromosome of different fragments not only suppress PEV phenotype of the male X chromosome, but also cause repression of male level organization in both X chromosome elements. They argued that since variegated phenotype of X chromosome is caused by long distance heterochromatin spreading through transposable elements (TEs), and since the heterochromatin spreading on the male X chromosome can be modified by additional amount of X material, the redistribution of the major heterochromatin markers from PEV induced X chromosome to the duplicated segment of X cause reduced or negligible heterochromatin marks on the PEV induced male X chromosome. In brief, they claimed that normally pericentric heterochromatin region of X chromosome harbor a class of 'TEs' for regulation of dosage compensation or other activities. This is mainly because of the unique properties of TEs for silencing activities of some genes and their transposition abilities. To establish female specific function of X chromosomes, the intercalary heterochromatin binding proteins are induced through transposons and replacement of canonical histones variants of zygotes [38, 113, 130] routed through male lineage for sexual dimorphism (discussed below). Since X chromosome has allocyclic behavior, cell cycle function is implicated in the dosage compensation process. In this context, it may be noted here that alteration in dose of chromatin regulator proteins by hypomorphic mutations of under mentioned genes demonstrated the importance of stoichiometric balance among the protein regulators for maintaining the male X chromosome structure and function. For example, by alteration (either by hypomorphic or loss of function mutations) of global concentration of heterochromatin proteins [e.g. HP1a or SU(VAR) 205; and SU(VAR)3-7] [123], or SUUR [116, 148], or euchromatic proteins (viz. Jil-1, a histone kinase H3S10) [146], (ISWI) [49, 120], it is possible to induce or suppress the PEV in male X chromosome. It is, therefore, reasonable to think that the functional morphology of male X can be modulated by changing stoichiometric balance of chromatin regulator proteins through transposon triggering and the phenomenon is mutually interdependent.

Dosage compensation mechanism in mammals

As discussed above, mammals inactivate one of their two X chromosomes in females for dosage compensation. The inactivated X chromosome is cytogenetically known as Barr body [100]. One of the consequences of X inactivation is that in most tissues of the female is functionally hemizygous for X linked genes [48]. On the other hand, the male is hemizygous. It implies that loss of one allele of a gene on one X chromosome on homogametic sex has a minimal fitness consequences. Indeed, the monosomic condition (45 XO turner female) in human is viable. Thus, it reasonable to think that in females, there is a selection for combinations of X-linked genes which are favourable for females, similarly, in males, there is a selection of combinations of genes favourable for males. The random X inactivation means that selection will ensure complete set of favorable allele of the X chromosome in female. The lack of post-transcriptional X-chromosome dosage compensation in human has also been established by a number of authors [40].

Several lines of data also confirmed that mammals have adopted a system in which one of the two X chromosomes in a female becomes transcriptionally silent (monoallelic) from the embryoninc stage in a mosaic fashion throughout life [18, 70]. Since necessary components of dosage compensation are inherited from oocyte, mammalian dosage

compensation neither requires a sex specific DCC nor chromosome-specific targeting, except all X chromosomes carry the X inactivation centre (XIC) locus. Within the XIC there are several genes that code for long non-coding RNAs [18, 52]. The XIST (X-inactive specific transcript) code for a large noncoding RNA. In mouse, XIST is transcribed exclusively from the future inactive X (Xi), and spread in cis from the X inactivation centre to coat the whole X, triggering silencing [52, 112]. In humans XIST upregulation at the blastomere stage precedes XIC, implying that the choice of which X to be inactivated establish downstream of XIST upregulation [52, 112]. In addition, stabilization of the inactive state maintains with epigenetic modifications including recruitment of the Xi with macroH2A and DNA methylation at CpG islands [18, 52]. The modifications involve a multilayered silencing complex that establishes transcriptional repression of the Xi [18, 52], and the repressive mark enrichment stabilizes somatic heritability of the inactive state. The chromatin structure of the active X in mammals and base line transcription rates of X linked genes then appear the same between sexes. Notably, the epigenetic signature established in inactive X chromosome (Xi facultative heterochromatin) is different to that of constitutive heterochromatin [18, 144].

Lyon [90] hypothesized that LINE elements may play a role in the spreading and stabilization of the Xist RNA on the X chromosome (although higher frequency of LINE elements on the human X is detected than on the autosomes, the accumulation of LINE elements is not detected in the mouse XIC region). Recently, it has been shown that many XIC genes evolved by accumulating TE repeats in their coding sequences [18].

In brief, it appears that in mammals, there is effectively a single active X chromosome in both sexes. Earlier Chatterjee [31. p. 203] proposed that "the random inactivation of one of the X chromosomes of the females in eutherian mammals could be advantageous from the point of view of selective advantages. Normally, it would be detrimental to a cell to have both the mutant and wild type alleles functioning within same cells…, the females that have mosaic heterozygous expression, do not show full effects of the deleterious recessive genes. It is possible that normal allele of the cell populations often provides enough product of an essential gene to correct the defect of the cells that caused by the mutant allele".

While X chromosome dosage compensation is apparent in placental mammals, its status in other mammals is less clear. In marsupials, there is no evidence for an X inactivation center (Xic), and the Xist gene. The inactive marsupial X lost epigenetic modifications associated with transcription during interphase. It was observed that, the nuclear territory that harbor Xi was devoid of RNA pol II [39].These observations indicate that like eutherian mammals, the marsupial Xi is situated in a transcriptionally inert nuclear compartment. Interestingly, marsupial Xi also bears an epigenetic signature similar to that of pericentric heterochromatin [1].

In the prototherians (montremes), the X and the Y are almost similar in the X length, the only difference being the length of the short arm. None of the platypus X chromosomes share homology with the therian X chromosome [135] including sex specific genes on the X and Y chromosome, and the ratio of X and autosomes. It is possible that depending on the type and strength of selection, evolution occurs for multiple sex chromosomes in animals. This involves genome wide gene regulation for sexual differentiation and dosage compensation. The sex chromosomes and functional status (cytological appearance) of X and Y chromosome in males and females of different mammal groups have been extensively reviewed elsewhere [18, 52, 62, 81, 105, 141].

Dosage compensation and functional genomics

Progress in evolutionary genomics and transcriptomes has made possible to compare expression contribution of X and autosomes for sexual dimorphism. Using microarray analysis, it has been shown that the expression of X-linked genes in all sampled tissue is, on average, approximately equal to that from autosomal genes both in females and males [64, 80, 105].

Notably, global transcription profiling in different animals has indicated that thousands of genes distributed throughout the genome contribute to sexual dimorphisms for both the gonad and the soma [69, 72, 109]. The expression comparison between X linked genes and autosomal genes indicated that transcription profiling of sex biased gene expression are not limited to X chromosome but autosomes also harbor the major share of sex biased genes in most species including *Drosophila*, and mice.

With the use of next generation DNA sequencing, that can provide digital measurement of gene expression (RNA seq) profile of the genome, it has also been documented that in *Drosophila* about 30–60% transcriptome is expressed differently in males and females (sex biased). The analyses of X linked genes revealed that a tiny fraction of X linked genes evolved for upregulation. However, genes on the X chromosome of hemizygous male show little or no upregulation [136]. Based on these data, Zhang and Oliver [145] argued that the evolution of distinct chromatin structure responsible for dosage compensation in male has affected the female Xs in *Drosophila.*

As pointed above, in mammals, transcription from loci on the Xi is partial, never 100% monoallelic (inactivated)

or 100% biallelic (escapee). There is a locus specific probability that a gene on the Xi is transcribed [1]. The small fraction of genes, that are dosage sensitive, may have different dosage compensation mechanisms. Two fold expression of X linked genes in males are largely absent.

Unfortunately, the available data do not allow us to correlate the role of X specific gene expression on the sex biased autosomal gene expression pattern and their link. However, the synchronous sex specific transcription profiles between X and autosomal genes provide indirect evidence that a co-ordination of sex specific gene expression pattern between X and autosomes exists.

By extrapolating from microarray-based gene expression data across the entire genome of male and female *D. melanogaster,* several authors, [93, 109, 111, 117] have suggested significant deficit of genes with male biased expression on the X chromosome. In adults, about 5% of X linked genes exhibited male biased expression compared to 8–11% on autosome arms. In general, there is a significant excess of gene duplications in which the new autosomal gene have arisen from an X linked parental gene through retrotransposon (see below) [54, 128, 147]. In female mice and humans, most escape genes from X chromosome are expressed for developmental stage related differences between sexes.

Comparisons of the chromatin landscape of males and females and dosage compensation

Microarray based gene expression data suggested that in early embryos, dosage compensation in mammals is achieved by first doubling global expression levels of the Xs in females, followed by an inactivation of one X in females [105]. Similarly, in *Drosophila*, dosage compensation operates in female embryonic cells by expressing two fold levels of X linked genes compared to the male X chromosome, followed by reduction of transcription of the two X chromosomes in female [60, 82]. The differences in expression of male and female X chromosome(s) are progressively lost at some critical stage of development. The reason was not clear at that time.

Investigations on the chromatin landscape in males and females, at different stages of development provide the mapping of accessible chromatin between sex. In this technique, ChIP-seq profiles for six different histone modifications are primarily used [150]. When histone modifications are characterized from sex specific chromatin state of *Drosophila,* it was noted that six different forms of histone modifications are associated in the genome of two sexes. They are: H3K4me1 (associated with enhancers and introns); H3K4me3 (associated with active promoters and transcription start sites); H3K9me2 (associated with constitutive heterochromatin); H3K27me3 (associated with polycomb repressed regions); H3K36me3 (associated with transcription elongation); and H4K16ac (associated with transcribed region). Brawn and Bachtrog [19] characterized the histone modification profiles of *Drosophila* genome of both sexes, and noted the following: (a) heterochromatic regions in both males and females contain high density of transposable elements compared to the genomic regions that are not associated with H3K9me2 in either sex (transposable element densities in the two sexes are 21 vs 6%); (b) the genome wide heterochromatin/euchromatin balance differ between the sexes [2, 19]. According to the data of Brawn and Bachtrog [19], approximately 10.2% of the genome was associated with the H3K9me2 state in females, and only 5.8% in males, and 15.5% was associated with the H3K27me3 state in females, and 10.7% in males. It implies that females have approximately 1.8 times higher heterochromatin than males, and approximately 1.4 times higher polycomb repressed regions in the euchromatic portion of the genome than males. A similar excess of H3K9me2 was seen in the assembled portion of the genome of *D. melanogaster* females compared to that in males. Based on these data, Brawn and Bachtrog [19] proposed that transposable elements trigger heterochromatin formation and that the euchromatin/heterochromatin balance differs between sexes in *Drosophila.* They also indicated that most differences in the chromatin landscape between sexes are direct or indirect consequences of sex chromosomes. Sex biased expression is associated with sex specific chromatin modifications. Brawn and Bachtrog [19] also documented that male X chromatin is enriched of active chromatin state (H4K16ac associated state). Interestingly, they noted that a marked depletion of H4K16ac marks on the male autosomes, relative to the female autosomes. These data clearly indicated that dosage compensation in males causes a genome wide effect by redistributing active chromatin marks. They further noted that less repressive chromatin was assembled mostly euchromatic portion of the genome in males, and a higher density of transposable elements in heterochromatic regions found only in females. Thus, the differences in chromatin structure between males and females are not limited to the sex chromosomes but extend genome wise. In fact, sex specific chromatin landscape is induced largely as a consequence of stoichiometric redistribution of active and repressive chromatin marks in the genome. The sex-specific chromatin landscape of *D. miranda* further support the fact that expression profile of a chromosome is reflected by functional state of the chromosome caused by modulation of histone on the chromosome [150].

Similar results were recorded by Wu et al. [143] in mammalian pre-implantation embryos. They observed that

at the four cell stage of embryo, the expression pattern of the both X and autosomal genes are biallelic. Chromatin regulator genes are generally upragulated at early stage (2-8 cell stage). Progressively, the accessible chromatin landscape of the female X's and autosomes is shaped by transposable elements. After some cell divisions, the accessible chromatin landscape of the X chromosomes of female was towards mostly monoallelic [133]. Similar situation was also noted in *C. elegans* [4].

Together it appears that the expression pattern of the genes in two X chromosomes and autosomes is largely readjusted by paramutation (epigenetic silencing) at early embryos in females for dosage compensation in the three species. The different methylation patterns and other chromatin modifications that are imposed on the DNA sequences for sex biased expression can be inherited in this way. Transposable elements play profound role in epigenetic modifications for sex biased expression of the genome by inducing heterochromatin formation. In fact, transposable elements are structural components of *D. melanogaster* heterochromatin [51, 115].

What is emerging from the three different mechanisms of dosage compensation?

Based on the above observations, it appears the following: (a) The epigenetic modifications for dosage compensation are mainly inherited from oocyte and sperm. Male X chromosome is normally hyperactive for its lineage specific transmission. On the other hand, since female receive two X chromosomes from both the parents: the epigenetic state of parent-of-origin dependent Xp chromosome, follow germline silencing pathways, and Xm is upregulated. However, at early embryonic stage of female, maternal and paternal X chromosomes showed largely comparable open chromatin landscapes transcriptomes despite the presence of wide spread allele specific DNA methylation due to reprogramming of the epigenome. The functional activities of the two X chromosomes are thereafter readjusted during early embryonic stages of the females, either by inactivation of one of the X chromosome or by inducing intercalary heterochromatin in the selected alleles in one of the two X chromosomes using transposable elements or by replacement of canonical histones with other variants [113, 130] routed through male lineage, resulting in similar level of expression pattern of the X-linked genes in both sexes. While the *C. elegans* and *D. melanogaster* dosage compensation mechanisms are comparable [38], mammals inactivate one of their two X chromosomes in females for dosage compensation. (b) Transcription inactive of X chromosome in mammals be never total 'monoallelic' nor total 'biallelic' (escapee). Two fold expression of X linked genes are largely absent in all taxa. (c) Epigenetic landscape of the X chromosome(s)/whole genome is different in the male and female. Obviously, sex biased genes are expressed in the X chromosome and autosomes through selection in dose dependent manner. Epigenetic marks change the transcriptome across the genome in response to sex biased signal. Thus, the epigenome orchestrate dynamic regulation of the genome in response to changing sexual environment of the animal. Together, it appears that, in the three animals, dosage compensation mechanism operates in females by allele specific down regulation of the expression of two X chromosomes. Thus, a single principle is operating for establishment of dosage compensation in the three animals, for sex determination (see below) (Fig. 4).

Sexual antagonistic genes drove chromosomal sex determination and dosage compensation

The sexual dimorphism exists in some lineages where there are no sex chromosomes. Yet, genes encoding for sexual dimorphic traits can reside in the chromosomes and expressed sex specifically. Best examples are the environmental sex determination system bearing animals where thousands of sex determining genes in a genome, can be regulated in response to changing 'environmental factors' for producing mating type of distinction. Thus, sex determination can be operated in animals, even if organism lacks sex specific chromosomes. This finding indicates that it is not always possible for all organism to adopt stable genetic tool of sex determination, although necessary sex determination and differentiation genes are accumulated in the genome for their sexual dimorphic phenotypes for sexual reproduction. Consistent with the idea it has been observed that the mechanism of sex determination varies from species to species according to their life history parameter, adaptive divergence and environment.

However, many organisms have pronounced sex chromosomes. The process, the time scale and the result of sex chromosome evolution has been highly debated. In this section of review, I shall describe the current understanding on the process of evolution of sex chromosomes and dosage compensation.

The sex biased genes are the product of either male or female specific evolutionary pressure for establishing mate recognition phenotypes that mediate species reproduction [53, 54, 93, 118, 119, 142]. These genes may be distributed non-randomly in a distinct genomic location. Experimental evidences from *Drosophila*, mammals and birds support the contention that due to regulatory idiosyncrasies of chromosome, sex biased genes are distributed non-randomly. In consequence, a chromosome can be enriched

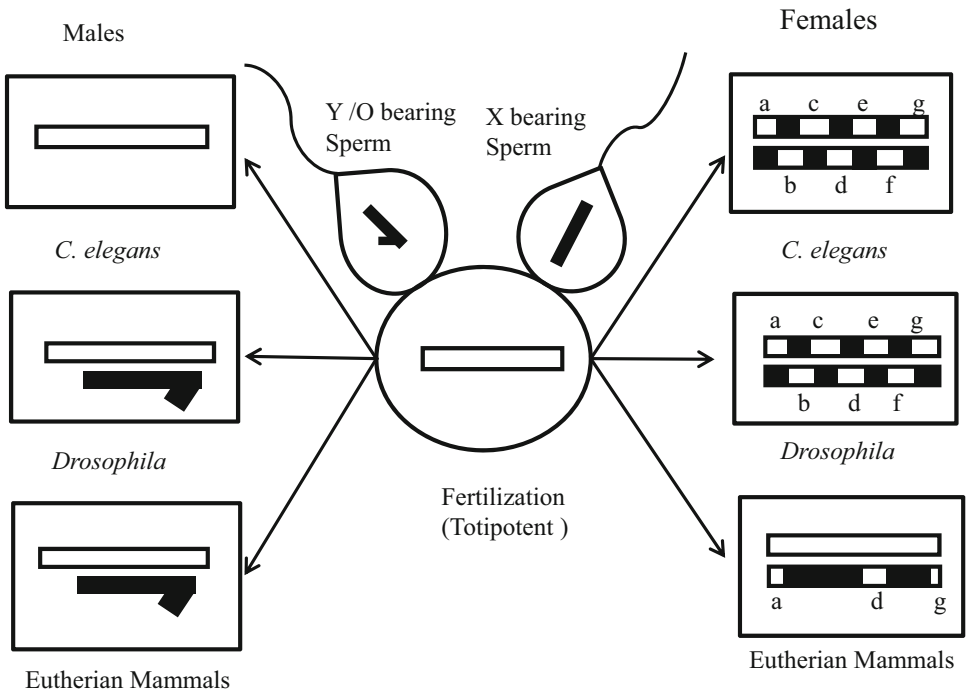

Fig. 4 The carton model showing expression pattern of the X chromosomes of males and females during early embryogenesis for resetting somatic dosage compensation. In males, MSCI occur during spermatogenesis, and epigenetic marks on histones and histone variants of sex chromosomes are inherited to the egg during fertilization. On the other hand, during oogenesis genomic imprint is erased and active X chromosome is reset. Thus hyperactive X chromosome is the 'inherent property' of the male somatic cells. On the other hand, in female, the mature lineages of accessible state of the two X chromosomes are reprogrammed either by lineage segregation of the X chromosome activity (i.e. monoallelic e.g. eutherian mammals) or by inducing allele specific intercalary heterochromatin (monoallelic) through transposon (e.g. *Drosophila* and *C. elegans*). See text. Filled bar paternal, hollow bar maternal X chromosome and hatched bars showing the accessible state of the chromatin in female Xs after epigenetic reprogramming during early embryogenesis

with genes associated with sex and reproduction. When the chromosome carry the genes for both male and female bias expression, sexual antagonistic reaction is inhabitable—that are beneficial for one sex and detrimental for the other. When sexual antagonistic loci are disproportionately accumulated on proto-sex chromosome(s) and autosomes, and when one chromosome of a pair can operate either in opposite direction for sex determination, the evolutionary forces exploited the evolving sex chromosome of the animal to differentiate as sex chromosome by readjusting the other sexually antagonistic genes in autosomes. In fact, sex specific evolutionary pressure shape the asymmetry of distribution of sex biased genes between the chromosomes for establishing the biological coherent pattern of X:A signal in dose dependent manner (i.e. genic balance) for determination of sexual phenotypes- male and female. The sex dependent selection pressure screen the antagonistic fitness effects of the X chromosome for both the sexes through sexual selection [59]. There is fulcrum balance between female and male specific selection pressures but that is generally optimal for neither sex alone until some regulatory mechanisms evolve to separate the male and female specific expression of the X chromosome. The balance between sex specific regulators determine sex specific chromosome organization (particularly by chromosome wide enrichment of certain satellite related repeats, some mono and dinucleotide repeats and transposon etc.) to coordinate the sex biased expression of the X chromosome [50, 68, 140]. For instance, in *Drosophila*, X specific regulatory sequences (i.e. 1.688 g^3/cm repeats or 359 bp sequence) are distributed along the X chromosome in the different discrete locations for regulating cis-acting genes on the X chromosomes, and their long range interaction [38, 71]. According to the sex specific pattern optima of a species, the level of organization of the X and autosome is also established in response to the change in X:A ratio. Indeed, the balance between X and autosomal regulators determine the sex specific chromosome organization pattern and effectively resolves the conflicts between male and female antagonistic genes possibly through a sex regulatory gene [e.g. *Sex lethal(Sxl)* in *Drosophila* and *Xol -1* (XO lethal -1) in *C. elegans*] activation. The experimental data with *Drosophila* model suggest that changes in X:A ratio not only affect the template capacity of X chromatin materials but also alter the sex differentiation pattern of the animal (Fig. 5a, b).

Indeed, X chromosomal activity can be maintained in mosaic fashion in a male aneuploid with large duplicated segment of X chromosome (Fig. 1). These features of the X chromosome are consistent with the theory that X regulatory elements have profound role in epigenetic information establishment on the X chromosome(s) for sex biased transcription, rather than exclusively through autosomal factor(s), although a secondary level of autosomal regulation has not yet been ruled out. Furthermore, breakdown of sexual antagonistic relationship in 'metamale' or 'metafemale' not only limited to abnormal sexual phenotype of the individuals, but also cause defective epigenetic information establishment on X and autosomal genes, resulting epigenetic abnormalities in germline. Similarly, it has been observed that in triploid intersex *Drosophila*, with X:A ratio 0.67, defective epigenetic information establishment between X and autosome [102] results not only limited sexual phenotypes but also cause epigenetic abnormalities in germ line differentiation (Fig. 5c, d). These data together indicate that sexual antagonistic selection contribute not only to establish the epigenetic information establishment on the X and autosomal genes through dosage compensation for the benefit of both sexes but also to protect and regulate what epigenetic information is proper and heritable through subsequent generation. However, in conventional comparisons of gene expression profile of the X chromosomes between sexes may be overlooked, owing to compensatory changes that may obscure the existing inter-sex differences.

In all taxa, however, the quantity of sexual antagonistic genes on the X chromosome may not have the same relation to the average level of autosomal gene products in two sexes. Precisely, chromosome wise distribution of sexually antagonistic genes in the genome is not similar in all species. This view is strengthened from the observations that, while in *D. melanogaster,* there is a deficit of male biased genes on the X chromosome, the *C. elegans* X chromosome carry more male biased genes compared to *Drosophila*. How in mammals, primary sex determination mechanism became separated from X:A counting mechanism by co-opting *Sry* gene is poorly understood. However, the fact is that mammalian X carry a disproportionately high number of testis differentiation genes [138]. Together, it appears that differences in sex biased gene content on X chromosome between taxa may cause fundamental differences in the mechanism of dosage compensation.

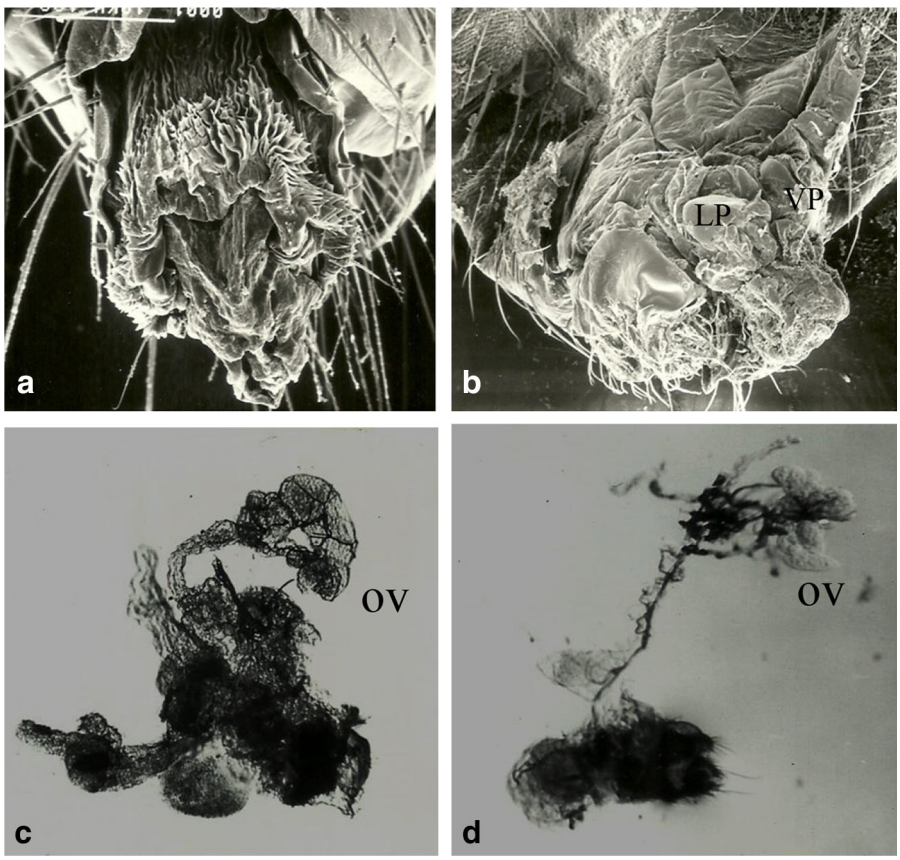

Fig. 5 Examples of the SEM views of terminalia and internal reproductive organs of triploid intersexes of *Drosophila.* **a** an individual where all female genital derivatives have been poorly differentiated; **b** an individual where both male and female genital derivatives (e.g. LP, lateral plates, VP, vaginal plates) are present; **c** an internal reproductive organ with poorly developed ovary like gonad; **d** an internal reproductive organ with poorly developed duct system and ovary like gonad

Dosage compensation maintains sexual antagonistic fitness of the X through gene trafficking

Many studies have documented strong signatures of positive selection in rapid evolution of reproductive genes [72, 93, 128, 147]. One possible route of continuous accumulation of sexually antagonistic genes is during gametogenesis. Male biased genes in the gonads are generally associated with spermatogenesis. Therefore, the higher rate of evolution of the class of sex biased genes is expected. It may be noted here that one unusual feature of male gametogenesis is that sex chromosomes have to undergo precisely regulated conformational changes- meiotic sex chromosome inactivation (MSCI). It is common among species with XY sex chromosomes [66, 79, 132]. The significance of MSCI process is still unknown. The characteristic features of MSCI suggest that: (a) the inactivation of sex chromosomes during meiosis may occur for silencing the meiotic drives (or other selfish DNA elements) to avoid potential damage from recombination between sex chromosomes and autosomes during male meiosis; (b) the inactivation of X linked genes during male meiosis help to incorporate recessive male mutations, and/or demasculinization of the gene content efficiently from X chromosome through transposable elements (TEs) activity; (c) some TEs may implicate in non-allelic gene conversion mechanism to accelerate the evolutionary fine-tuning of sexual fitness of the individual [55]. Although there is no strong evidence in favour of these assumptions, the analyses of X chromosome activities generally is consistent with some of these predictions. Thus, understanding the selective pressures underlying the evolution of MSCI is challenging.

In contrast, during oogenesis, the imprinted paternal X (Xp) is reactivated and euchromatinized for enabling meiotic pairing and recombination between two X chromosomes. However, the selective pressure for asymmetrical oogenic meiosis and choice of chromosome complements for egg from four cells are unknown. Therefore, an approach may be useful in understanding the early steps in genome selection in eggs with known evolutionary parameter. In several instances, it has been documented that the recombination bearing X and autosomes with beneficial alleles are selected for ovum for fertilization. In general, limited information are available on the rates of adaptive evolution on the process of oogenesis and the driving force of fixation of female biased genes on the X chromosome.

Nevertheless, sexual antagonistic genes are accumulated on the X chromosome during gametogenesis over time [10, 54, 128]. The consequences of rapid rate of accumulation of antagonistic genes on the X chromosome may lead to qualitative and quantitative unknown modification at the level of gene expression relative to ancestral genomic state, resulting in a shift or unbalance in regulatory system in sex determination [109, 147]. Therefore, it is necessary to adopt a mechanism to avoid intersexual correlation of transcription on X and autosomes (see above). Thus, the genes on X chromosome undergo continual adjustment compared to autosomal genes to maintain the genetic systems of sex determination, and sex specific fitness optima. As a result, the genes on the X chromosomes are faced with strong selection process and respond adaptively to both their sex biased transmission, interaction with the dosage compensation mechanism. Thus, while in females, there is a selection of combination of X linked genes which are favourable for females, in males there is a selection of combination of genes favourable for males.

To keep balance of sexual antagonistic genes on X and autosomes (genome), a system has been adopted by many animals to redistribute these genes in the genome through gene trafficking using retrotransposon [10, 57, 136, 137, 147]. However, not all genes are redistributed unconditionally. Obviously selection acts on the genes that are to be distributed. In fact, a deficit of X linked male biased genes is not a rule for X chromosome. The specificity of redistribution of sexual antagonistic genes may be assessed through several criteria: Firstly, phenotypic importance and fitness potential of the gene in a population may be considered under convergent selection for both male and female. One direct evidence is that most of the newly transposed autosomal genes in *Drosophila* are expressed in testis [10, 69]. The X linked genes required in the late spermatogenesis would be selected against in favour of autosomal copies. Secondly, when a female-advantageous sexually antagonistic alleles on the X chromosome is accumulated, there is a need to counter select a male advantageous sexually antagonistic X linked allele. Dominant female advantageous alleles are favourable for females. Similarly, if the accumulated female antagonistic genes are partly dominant, then the female-beneficial mutations allow to accumulate on the X chromosome, while male beneficial mutations are to be removed from the X. In particular, it appears that when an X linked recessive male biased mutation is accumulated, the selection process act on the gene for testing its fitness in terms co-adaptability with female biased genes (i.e. whether it can masked in diploid female and can keep the mutation hidden from selection in females). Female beneficial mutations are fixed more easily on the X because the X chromosome spends two-thirds of its time in females and thus, it is more often under selection in the background of the sex. Therefore, the fixation probability of an X linked sexually antagonistic mutation is depending on its nature of interaction with the

other genes, fitness drive of the sex etc. Together, it appears that a selective force is operating on the movement of the X linked genes on the genome for having sexual fitness of both sexes.

Since, dosage compensation accommodates the level of sexual antagonism, for establishing sex biased gene expression pattern on X, and since sex specific X chromosome expression directly interfere with subsequent transcriptional modification of the genome, it is expected that sexually antagonistic genes on the X chromosome are faced with continuous selection for maintaining sex specific fitness of the animal. Dosage compensation also limits subsequent transcription factor binding or chromatin remodeling, of the newly accumulated genes in the X chromosome. Thus, it has been noted that newly acquired X linked male-biased genes that are not adapted for dosage compensation mechanism, allow trafficking the gene off from the X chromosome of *D. melanogaster* [10]. Bachtrog and her coworkers [150] have shown that in *D. pseudoobscura,* where newly evolved XR [homologus to *D. melanogaster* autosomal arm 3L—Muller D element] acquire dosage compensation mechanism by redistribution of ancestral sex biased autosomal genes through gene trafficking mechanism from D element to other autosomes for establishing stable X:A signaling system over a reasonably short period of time frame (e.g. 13 MY). Parallel process occurs in mammals where preferential movement of X chromosome genes to autosomes via retrotransposon was recorded. Since, the mammalian X chromosome is enriched with testis specific genes, no difference between male and female biased gene expression for X linked genes in mammals was noted. In *C. elegans* also, the X seems to be depleted in genes with male biased expression [109].

Together, it appears that sexually antagonistic genes on the X chromosome are faced with continuous selection for maintaining sex specific fitness of the animal in context of time, environment and population. Continuous adaptive evolution of the X chromosome (the faster X effect) is the 'rule' of male heterogametic species where dosage compensation actively helps in limiting or interfering the evolution of sex biased genes on the X chromosome in response to sexual fitness of the genome. A systematic evaluation of gene function by RNAi further showed that genes on the X chromosome are less likely than autosomal genes to have essential functions, suggesting the X and autosomes have evolved a segregation of genes by class, through gene trafficking, perhaps to escape cell lethal consequences of male X inactivation in the male germline at least for the male heterogametic species.

How dosage compensation help in selection of sexual dimorphism?

Sexual reproduction in itself does not require dimorphic phenotypes. However, establishment of sexual dimorphism by sex determination process clearly indicate that sex specific regulators of X chromosomes play an important role in differentiation process of dimorphic phenotypes for both sexes. The male and female specific evolutionary pressures shape the sexual dimorphism in many animals to show mating type distinction and sex specific fitness of the individual. The choice of sexual dimorphism has profound impact on the evolutionary trajectory of gene underlying. However, the constrain on the evolution of dimorphism is probably conflicting selection pressures between the sexes [92, 145]. Lineage specific adaptive evolution play pivotal role in sexual dimorphism in animals. The sex-neural tissues ultimately mediate the sexual dimorphism of a species.

The question is therefore, how dosage compensation accommodates sex specific phenotypes? As described above, sex chromosomes harbor the primary signal for gene expression networks of both sexes. As a consequence of the bifunctional switch of sex determination cascade is activated through epigenetic information establishment on the X chromosome in dose dependent manner, it is widely accepted that dosage compensation yield male and female phenotypes. Reasoning from *Drosophila* model is that defective epigenetic information establishment on X chromosome not only cause abnormal autosomal gene expression pattern in triploid intersexes, (Fig. 2b) but also change germ line differentiation. It has also been noted that defective epigenetic information establishment on X chromosome results abnormalities in parent-to-offspring inheritance of epigenetic information for sexual development [92]. The genome sequencing and transcriptome-profiling data also provide evidence that the majority of dimorphism is the result of expression differences of genes that are present in both sexes, are regulated through sex determination hierarchy [5, 92, 150]. However, it is not yet apparent how sexual dimorphism is regulated in hemizygous vs homozygous expression pattern of the X chromosomal genes in different animals.

It therefore, appears that dosage compensation coordinate the sex specific selective forces of the genome of a species and allow similar level expression of sex biased genes on X chromosome in males and females that in turn regulate the expression pattern of other hierarchies of the genes in the genome to orchestrate sex specific aspects of development, morphogenesis, differentiation and adult function. Sex determination mechanisms accommodate the species specific divergence of the distribution pattern of

sexual antagonistic genes on X and autosomes and evolved different mechanisms of dosage compensation to co-ordinate the sex specific gene function on the X chromosome and autosomes. Sex determination mechanism was therefore, evolved as a sum of the evolutionary decisions that led to set a genetic regulatory mechanism to implement bifunctional switch from the nearly identical genome for mating type distinction not by involving any changes in DNA base sequences, but to respond the conflicting selection between the sexes for their functionality in dose sensative manner. Earlier, Chatterjee [31, p. 203] proposed that "evolution of dosage compensation … allow dosage differences between sexes and these differences could be useful to the organism by emphasizing and reinforcing mating type distinction".

Dosage compensation drove dimorphic sex chromosomes

Sex chromosomes originate from autosomes [11, 22, 31, 141]. However, there are some discordance about the processes of heteromorphic sex chromosomes evolution in different animals [7, 11, 31]. As described above, molecular necessity of epigenetic information establishment on X chromosome thorough dosage compensation is the primary step for implementation of sex specific cascade in *Drosophila*. The evolutionary pathway is also facilitated a balanced correlation between sex chromosome and autosomal gene expression pattern for implementation of epigenetic information for sex biased transcription. When a chromosome of a pair(s) of proto-sex chromosome build up a system of sexually antagonistic genes (see above), the evolutionary forces allow to express the entire X chromosome in sex specific manner, rather than a single dominant masculinizing gene expression [11, 16]. In the evolutionary sequence, when an evolving X chromosome acquires dosage compensation mechanism that can operate through sex biased modifications to its chromatin structure, it results changes in the entire evolutionary dynamics of epigenetic information establishment in the sex chromosome. The heterochromatin formation on the proto-Y occurs progressively to restore proper gene dose balance, since it would have deleterious consequences, if silencing does not occur at potentially functional genes on proto Y. The antagonizing effects of active transcription and associated differences in chromatin structure on proto-Y could be established progressively through selection by the evolution of epigenetic modification on diverging sex chromosomes. Thus, proto-Y linked genes would be down regulated individually by transposable element insertion resulting in heterochromatinization. Consequently, the Y chromosome progressively accumulates deleterious mutations at higher frequency through selective degenerative force and forced to select male lineage group by accumulating advantageous male limited genes slowly to avoid recombination. In brief, evolution of Y chromosome involves two processes: (a) the partial or complete suppression of crossing over between the X and Y chromosomes and (b) an enhancement of the accumulation of repetitive DNA sequences through transposable elements leading to degeneration of Y chromosome [7, 8, 124]. This transition of Y chromosome is facilitated by emergence of dosage compensation mechanism on the proto- X chromosome to increase fitness of the sex determination process of the animal. Thus, sex difference selection help to recruit the type of genes carried on X and Y chromosome. Earlier, Chatterjee [31, p. 203] proposed that "the evolution of heteromorphic sex chromosomes were the consequence of the evolution of dosage compensation and not *vise versa"*.

Direct evidence of sex specific driving force shape the sex chromosomes come from the *D. miranda,* [9, 150] where the neo X and neo Y chromosomes are evolved from building up a positive selective pressure to abolish recombination between close linkage between sexually antagonistic mutations.

The asymmetric transmission of Y chromosome can also contribute to its degeneration via antagonistic zygotic drive. For example, complete elimination of the Y chromosome has been recorded in many taxa including *C. elegans.* Some mammals, however, retain a small, gene poor Y chromosome for facilitating sex determination process. The components of human Y chromosome include one large heterochromatin block and an euchromatic segment where most genes reside. Comparisons of the gene content on the Y chromosome in different mammalian species indicated that different subsets of the same gene set are located on the X in different species [141]. Curiously, the epigenome of the Y chromosome in *Drosophila* is very different from mammals. Although, Y chromosome harbors 15 protein coding genes in *D. melanogaster*, it carries large amount of DNA [21]. The gene content of the Y is also younger than the other chromosomes. The Y chromosome of the *Drosophila* does not share any single copy genes with the X chromosome [20, 21]. It contains variation in repeat number of the multicopy rDNA locus, which can cause differential PEV especially on male X chromosome. Experimental evidences showed that the Y chromosome of *Drosophila* serves as a heterochromatin sink [98]. Some authors therefore, claimed that the *Drosophila* Y chromosome is not a degenerated X, rather it has originated from B chromosome (supernumerary dispensable chromosomes) that evolved the ability to pair with the X [21, 149]. They believed that, to regulate global heterochromatin balance, and retention of different class of

transposable elements especially for regulation of dosage compensation in males (see above) [47, 74, 98], the present Y chromosome has been reintroduced from B chromosome [21]. For male limited transmission, the Y chromosome recruited some male biased genes via transposon recently. Although the pathway might seem odd, it had independently happened in other species [16, 149] in which the Y chromosomes originated from B chromosome present in many species.

Together, it appears that for having a stable genetic tool of sex determination process, different species have evolved dimorphic sex chromosomes by which modulation of sex biased epigenome can be expedited through the expression pattern of sex chromosomes for establishing sexual dimorphic phenotypes. A complex selection pressures acts either directly or indirectly, through dosage compensation mechanism for many changes within cells by which bipotential differentiation mechanism can be established easily for sexual reproduction.

Conclusion

The emerging evidences from past several years indicated that heteromorphic sex chromosomes of male heterogametic species, are evolved through selection pressure for shaping the distribution pattern of sexual antagonistic genes on the X and autosomes in such a manner that sex specific regulatory mechanisms can be operated either by the dose sensitive mechanism of X chromosome(s) or by lineage specific inheritance of Y chromosome for sex determination. Dosage compensation coordinates the transcription apparatus of sexual antagonistic genes of the genome through epigenetic information establishment on X chromosomal genes for triggering sex regulatory gene hierarchies. Once the sex determination hierarchy initiates the function, it orchestrates the regulation of the genome either by hormonal signal (e.g. *Drosophila* and mammals) [56, 91] or by cell lineage pathway (e.g. in *C.elegans*) [129]. Precisely, dosage compensation process acts as 'bifunctional switch' of sex regulatory cascade at early stage of development in male heterogametic species. Defective epigenetic establishment on X chromosome (i.e. intersexual syndrome) results not only abnormalities in sex determination (i.e. the bipotential primordia of the imaginal discs can not initiate sex specific differentiation), but also cause epigenetic abnormalities in germline. Once sex determination cascade starts functioning, dosage compensation has no major role in epigenetic modifications in the genome. A great deal of evidences indicated that the primary processes for sex differentiation require regulation of sex specific epigenetic marks across all regions of the chromatin including enhancer, promoter and intergenic regions of the genome, as well as in exon and introns. These factors modulate the folding of nucleosome fibres to reconfigure the chromatin for fine tuning of transcription of the X chromosome(s) through epigenetic changes for sex specific differentiation. Sex specific genetic systems establish sex specific transcription apparatus utilizing post translational modifications of histone, DNA methylation and chromatin associated complexes for changing sex specific transcription apparatus of the genome. Transposon activities are recruited to mediate the silencing and/or activating the sex specific gene expression pattern of the individual. Si RNA that are derived from TEs, are involved in genome defense and transgenerational inheritance of heterochromatin identity ensuring genome plasticity. Taken together, it appears that male and female specific epigenetic program are reset to remodel the X chromatin for dosage compensation for initiating mating type distinction of sex. The value of dosage compensation and sex determination research is discussed by Chatterjee [35] that "although sex differences caused basic changes in the organization of cells, it has been observed that results of animal studies are frequently not reported by sex, nor are they included in some text book studies, such as those on cell growth and aging etc. Developmental and molecular events leading to the establishment of sexual dimorphism are not only fascinating problems for developmental biologist but also essential for the survival of the species. As such, knowledge on sex differences may be taken into account in planning and interpreting research".

Little is known how female heterogametic species coordinate the sex biased expression of their genome. Available data indicate that dosage compensation system comparable to male heterogametic species may not be required in avian species [61, 96, 139]. Thus the convergent evolution of the X and Z is puzzling. There may be two reasons for such exception: (a) lineage specific 'ancestral' adaptive mechanism is still functioning in these species, since birds are evolved from reptiles where sex determination systems are mostly regulated by environmental cues; (b) the genes shape the morphological, behavioral, reproductive potential of each sex, initial coordination does not require dosage compensation mechanism in these species, due to large amount of nutrients present in the egg. That the nutrients modulate the genetic program of sexual development has been documented for many taxa including the bees, and Hymenoptera.

In brief, it could be emphasized that in most male heterogamous species, dosage compensation co-ordinate sex specific gene hierarchies through epigenetic information establishment on the X chromosome. Sex biased epigenetic information is established on the X chromosome using transposable elements through a genetic system. However, the cellular regulatory process that target TEs in accurate location of the genome is still not clear. Also,

present data do not allow us to determine how adaptive divergence of a species not only change its sexual reproduction mechanism in response to their environmental cues but also can alter the mechanism of regulation of sex biased genes for their sexual phenotypes. To make further progress, it will be critical to determine the biochemical mechanisms that facilitate sex determination process in other animals lacking dosage compensation process.

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