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# Gene Dosage Compensation and the Evolution of Sex Chromosomes

## In diploid organisms compensation evolved gradually and led to heteromorphic sex chromosomes.

John C. Lucchesi

*Summary.* Dosage compensation is a mechanism by means of which the activity of X-linked or Z-linked genes is made equal in the two sexes of organisms with an XX compared to XY or ZZ compared to ZW basis of sex determination. In mammals, compensation is achieved by the inactivation of one X chromosome in somatic cells of females. In *Drosophila*, compensation does not involve inactivation. The two X chromosomes in females as well as the single X in males are regulated, and individual genes are thought to respond independently to the regulatory mechanism. It is proposed that in both groups of organisms the evolution of heteromorphic sex chromosomes was gradual and occurred as the direct result of the evolution of dosage compensation rather than the reverse.

In diploid, sexually reproducing organisms, the genetic information responsible for initiating the various manifestations of sexual differentiation is often restricted to one pair of homologous chromosomes, that is, the sex chromosomes. In many instances, evolution has led to a visible structural modification of one of these homologs while the other remained unchanged. The sex that carries the heteromorphic pair of chromosomes (XY or ZW) is termed heterogametic; the opposite sex, with two unchanged homomorphic chromosomes (XX or ZZ) is said to be homogametic.

Inherent to such a system for sex determination is an inequality in the dosage of genes present on the X or Z chromosomes, in males and females. Yet, many of these genes mediate basic functions, not related to sex differentiation. The phenotypic product of such genes is usually found to be equivalent in the two sexes, indicating that a compensatory mechanism is operative, presumably for the purpose of preventing differential selection between the sexes. This manifestation, termed "dosage compensation" by H. J. Muller *et al.* (*1*), has been studied in the fruit fly *Drosophila* and in mammals. In the mammals, compensation is achieved by the inactivation of one X chromosome in somatic cells of females. The molecular basis for this phenomenon is not known, nor are the circumstances of its appearance in the class Mammalia understood, although the evolution of compensation in the group has been the subject of speculation (*2*). In *Drosophila*, compensation does not involve chromosome inactivation. Both X chromosomes in females as well as the single X in males appear to be regulated and individual genes are thought to respond independently to the regulatory mechanism. Different species within this genus can be arranged in a series that may represent a recapitulation of actual evolutionary steps in the history of the regulatory phenomenon of dosage compensation.

The main purpose of this article is to develop the thesis that the evolution of heteromorphic sex chromosomes is the direct consequence of the evolution of dosage compensation, and not the reverse. In a primitive diploid organism with a simple, two-allele mating-type system of sex determination, dosage compensation was probable; its occurrence led to sex chromosome heteromorphism. The evolution of these two phenomena was gradual, beginning at

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some point adjacent to the locus responsible for mating type determination and progressing, in piecemeal fashion, along the chromosome. The secondary objective is to focus attention on the *Drosophila* story and to suggest that it should serve as a conceptual guide for a renewed effort to elucidate the mammalian system.

In this article I propose to summarize as succinctly as possible our factual understanding of dosage compensation and to discuss the development of a model to explain its mode of operation in *Drosophila*. Attention is focused on the evolution of sex chromosomes, concentrating in particular on the karyotypic differences generated by centric, whole-arm fusions in this genus. Finally, dosage compensation in mammals is described and discussed in the perspective of the foregoing considerations.

## Evolution of Sex Chromosomes

The simplest possible mechanism for sex determination, based on a single allelic difference at one locus, is illustrated in Fig. 1. In organisms with predominantly haploid life cycles, suitable mating type differentiation is achieved by gene expression during the haplophase alone. In a diploid organism, gene expression would occur in the diplophase; the surface characteristics of the gonial cells and of the resulting gametes could reflect the presence or absence of one of the alleles in the parental genotype and thereby establish one of two possible mating types. The chromosomes that bear the alleles *A* and *a* need not be differentiated. Such a situation may prevail among those lower metazoan groups where many species are hermaphroditic (*3*), in certain higher invertebrates such as mosquitos and midges, and in lower vertebrates such as fishes, most amphibians, and most reptiles (*4*).

Differentiation between the sex-determining chromosomes will occur as a consequence of "genetic isolation" of the homolog which bears the allele restricted to one mating type or sex (*A* allele in Fig. 1b). Mutations, which most frequently lead to a reduction or absence of gene product, would occur at random along these chromosomes but would accumulate in the neighborhood of the locus of *A* since the closer a mutant is to *A*, the lesser the probability that it would cross over to the *a*-bearing homolog with subsequent opportunity for homozygosis and elimination. This effect might be enhanced if *A*/*a* individuals were to exhibit a reduction in recombination resulting from some indirect physiological effect of the *A* allele or from the presence of an inversion in the *A*-bearing chromosome. Dysfunctional genes adjacent to the locus of *A* could become constitutively heterochromatic or be eliminated by the occurrence of random deficiencies. In any event, the process would spread along the chromosome.

Such a series of intermediate evolutionary steps is suggested by the karyotypic differences presented by various families of snakes (*4, 5*). Among Boidae, the Z and W homologous chromosomes are homomorphic. Many members of the family Colubridae exhibit a heteromorphic pair of chromosomes in the female karyotype; here, the Z and W chromosomes differ by a pericentric inversion. In the more highly evolved Crotalidae and Viperidae, the heteromorphism of the two sex elements is maximal, with the W chromosome greatly reduced in size.

The type of mutations described above would lead to gene dosage differences between the sexes, and these differences could be useful to the organism by emphasizing and reinforcing mating type distinctions. Conversely, they could represent a selective disadvantage sufficient for the evolution of a compensating mechanism equating gene activity in the heterogametic and homogametic genotypes, that is, dosage compensation.

## Dosage Compensation in *Drosophila*

The similarity in expression of X-linked genes, in females having two X chromosomes and in males having only one X, was first observed when phenotypes such as eye-pigment production were being compared (*1*). Later, dosage compensation was observed during chromosomal RNA synthesis, by autoradiographic measurement of the rate of incorporation of labeled precursors into RNA along the giant polytenic chromosomes in the salivary gland cells of *Drosophila* larvae (*6*). It was observed to influence the level of enzymic activity (*7*). Recently, a correlation between the compensated synthesis of RNA at an X-linked polytene chromosome puff and the amount of a specific protein in the larval salivary gland was demonstrated (*8*). In general, the congruity of the observations made at the levels of phenotypic products, enzyme activity, and chromosomal RNA synthesis provides reasonable support for the contention that compensation operates during transcription.

Two aspects of the regulatory phenomenon should be mentioned. The first distinguishes its manifestation in *Drosophila* from that in mammals. In *Drosophila*, both X chromosomes are active in each cell of the female soma, although their total output is equivalent to that of the single male X. This was suggested by the absence of phenotypic mosaicism in females heterozygous for

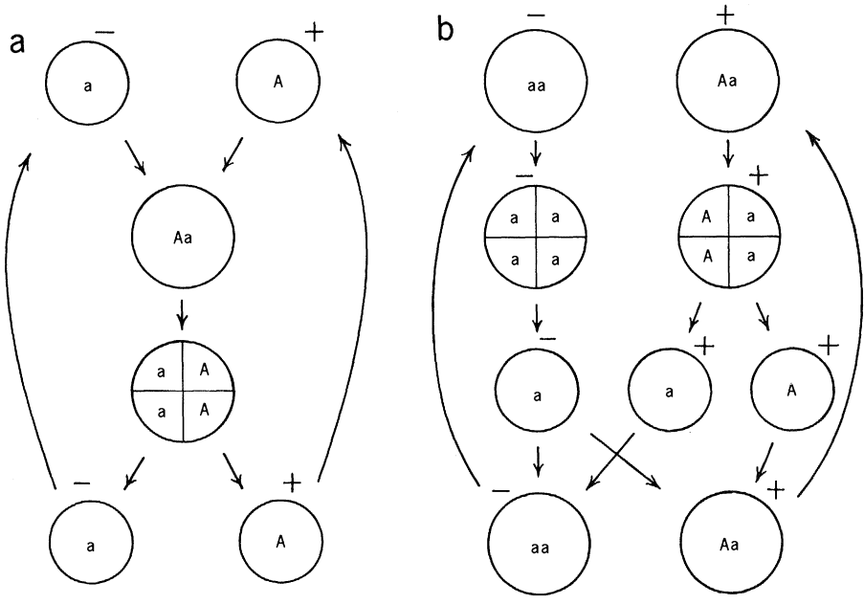


Fig. 1. (a) Diagram of the life cycle of an organism passing most of its life in the haplophase. *A* and *a* are alleles of a gene determining the mating type. (b) Reproductive cycle of an organism where the diplophase is the dominant part of the cycle. *A* and *a* are alleles of a sex-determining gene.

X-linked mutant alleles affecting hypodermal characteristics: +/*y* (where *y* stands for yellow bristles and hairs), +/*f* (*f* represents forked bristles and hairs), +/*w* (*w* represents pigmentless ommatidia) are all uniformly wild type. Proof that both X chromosomes are active simultaneously in cells of females was provided by the study of allozymes (*9*). Females heterozygous for two electrophoretic variant alleles of the enzyme 6-phosphogluconate dehydrogenase (6-PGD) exhibit the two parental enzymes and a hybrid enzyme of intermediate electrophoretic mobility; that the latter was not an artifact of extraction was indicated by the failure to reproduce it in mixtures of parental extracts.

A second characteristic of dosage compensation in *Drosophila* is that an X-linked gene or a group of genes need not be part of the whole X chromosome in order to be regulated; a segment of X chromosome translocated elsewhere in the genome remains dosage compensated (*7*, *10*). The complementary observation is that the genetic activity of an autosomal segment is unaltered by its translocation to the X chromosome; it is not modulated by the regulatory mechanism responsible for dosage compensation (*11*). The autonomous behavior of small fragments in X-autosome translocations suggests that X-linked structural genes possess individual means of responding to the compensatory mechanism. Of course, the size of the translocated fragments in these types of studies is too large to allow one to distinguish between the regulation of individual genes compared to that of small clusters of genes.

The great ease with which genotypic manipulations can be performed in *Drosophila* has made it possible to study chromosomal variations other than those represented by normal diploid males and females. X-linked gene activity has been measured in triploid females 3X;3A and intersexes 2X;3A (*12*-*15*) as well as in metafemales 3X;2A (*14*-*16*). Most recently, X-linked gene activity was established in XY;3A metamales (*17*).

The conclusions that can be drawn from the work with heteroploids are represented in Fig. 2. The total product per cell for an X-linked gene is the same in males, females, and metafemales. Since all three forms have the same autosomal complement, it would appear that the activity of X-linked genes is regulated in such a fashion that it is in harmony with that of autosomal genes. In triploids, whether they are female, intersexes, or metamales, the total gene product per cell is 50 percent greater than that in diploids. Here again, X-linked gene activity is concordant with that of the three sets of autosomes. There are, therefore, at least five possible degrees of function for an X chromosome with the highest being exhibited by metamales and the lowest by metafemales. This renders the X a model system where gene activity can be preset by selecting the appropriate combination of X chromosomes and sets of autosomes, following the simple formula: activity per gene dose = ratio of sets of autosomes to number of X chromosomes (see Fig. 2).

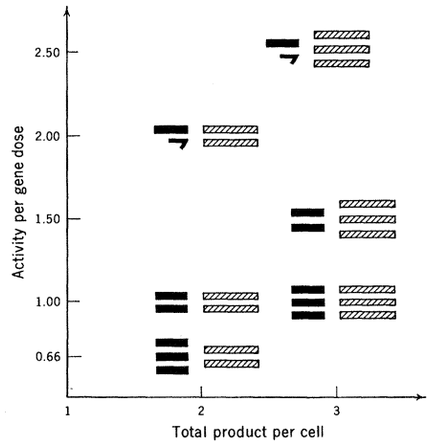


Fig. 2. Measurements of X-linked gene activity in various *D. melanogaster* karyotypes. Thick solid lines are the X chromosomes; cross-hatched symbols represent sets of autosomes; J-shaped symbols are Y chromosomes (*45*).

Two general models can be formulated to account for the type of regulation just described. The first, originally conceived by Muller (*18*) would maintain that dosage compensation can be attained by negative regulation, whereby X-linked gene activity is decreased such that the amount of cellular product resulting from two gene doses in a female is reduced to that resulting from a single dose in a male. The assumption is made that there are on the X chromosome certain genes (compensators), whose activity is not dosage compensated and whose products have an overall repressive effect on X-linked genes. The second model (*13*) suggests that equalization of X-linked gene products in males and females is achieved by positive regulation, that is, by a mechanism that enhances gene activity. The assumption is made that there is an autosomal gene (or genes) whose activity is dosage dependent and whose product is necessary for the transcription of X-linked genes. The concentration of regulatory molecules is relatively low and the number of X-linked gene sites competing for them is relatively high. The probability of transcription of an X-linked gene would be directly proportional to the concentration of regulatory molecules and to the number of doses of the gene and inversely proportional to the number of X chromosomes in the genome. All X-linked genes may be affected by the same factor produced by a single regulator. It is, of course, possible that functionally related genes on the X are grouped and that each group has its own regulatory factor and gene. In either case, the factor (or factors) is specific for X-linked genes and does not alter autosomal gene transcription.

A series of experiments were performed (*19*) in an attempt to test the two principal models of dosage compensation. The results failed to provide evidence for discrete X-linked regulatory genes or segments significantly altering the activity of other genes on the X chromosome. Furthermore, the results are not inconsistent with the positive regulation model of dosage compensation described above.

Some insight into the evolution of such a regulatory mechanism may be achieved by studying certain selected species of *Drosophila*, whose karyotypes have undergone whole chromosome arm translocation—so-called Robertsonian fusions—during the course of their emergence.

## Sex Chromosomes of Some *Drosophila* Species

The basic karyotypic configuration of the genus is six chromosome arms. Differences among species consist of numerous inversions, and of rearrangements of the chromosomal arms via translocations and fusions. The genetic content of the chromosomal arms has been largely maintained so that homology tables for different species can be constructed (*20*). It is generally believed that the ancestral chromosomal arrangement of the genus consisted of five acrocentric rod chromosomes (A through E) and one very small chromosome (F). During the course of evolution, according to the tabulation of Patterson and Stone (*20*), 54 different fusions have established themselves, 32 among autosomes and 12 between the X and an autosome. An example of the latter case is *D. americana*. In this species, an X and B fusion has occurred (Fig. 3b). Females are homozygous for this element while males retain the B arm as a free chromosome; both sexes, therefore, have two doses of

B. The karyotype of *D. pseudoobscura* (Fig. 3c) exhibits an X-autosome fusion, involving the D arm. Females are homozygous and males hemizygous for the X·D element which is, thereby, justifiably renamed XL·XR (the raised dot represents the centromere). The gene dosage difference with respect to both arms of this X, in males and females, appears to be compensated (*21*). It may be reasonable to assume that *D. americana* represents an early stage and *D. pseudoobscura* the end result of the evolution of an autosomal arm into a sex chromosome element.

## Evolution of Dosage Compensation in *Drosophila*

Among species of the obscura group in the subgenus *Sophophora* to which *D. pseudoobscura* belongs, *D. subobscura* is considered more primitive on the basis of morphological characteristics and geographical distribution, and on the basis of enzyme differences (*22*). This species has a rod X chromosome. The ancestral karyotype of *D. pseudoobscura* may have included an X and D fusion in a fashion analogous to the current *D. americana* karyotype: the females of the species were homozygous for X·D while the males bore X·D and a free D element. The free D element was limited to males and, because of the low level or absence of recombination in this sex, was genetically isolated. As deleterious mutations (to no or reduced activity) occurred on the free D element, a selective pressure was created favoring compensatory mutations on the translocated homolog. As further mutations accumulated on the free element, its genetic content became increasingly degenerate and perhaps heterochromatic. Concomitantly, an increasing number of genes on the D arm of X·D were represented by compensated alleles. The culmination of this process was the complete transformation of X·D into a dosage compensated sex chromosome, allowing the loss of the inactive free D element (*23*).

A second mechanism for the evolution of new sex chromosomes may involve the translocation of an autosomal element to the Y chromosome. An example of this occurrence may be found in *D. athabasca*. Several populations of this species whose males exhibit a Y and B fusion (Fig. 3d) have been described (*24*). In this case, the translocated element is male-limited and, after the accumulation of deleterious mutations, would become dysfunctional; the free homolog, of necessity, would become dosage compensated. An intermediate step in the evolutionary process just postulated may be witnessed in the last species to be discussed, *D. miranda* (Fig. 3e). It would appear that *D. miranda* is in the process of evolving a second X chromosome. One C element has become translocated to the Y and is degenerating while the homologous autosomal element is presumably becoming dosage compensated. Evidence for this is provided by the presence of distinctly euchromatic sequences on the Y chromosome; in other *Drosophila* species the Y is wholly heterochromatic. The free C element (renamed $X_2$) pairs with the Y and the X chromosome ($X_1$). The Y and the two X chromosomes regularly go to opposite poles during anaphase I of spermatogenesis, ensuring a stable pattern of sex chromosome segregation that is consistent with the balance theory of sex determination. Further evidence for residual homology of the Y and $X_2$ is provided by the presence of one active gene (of three genes examined) on the Y homologous to genes in the $X_2$ and C element of *D. pseudoobscura*. This was determined in interspecific hybrids produced by crossing *D. miranda* and *D. pseudoobscura* (*25*).

An autoradiographic study of chromosomal RNA synthesis in *D. miranda* has been performed (*26*). The rate of RNA synthesis along the single $X_1$ chromosome in males equals that of both $X_1$ chromosomes in females. The $X_2$, on the other hand, exhibits regional differences in transcriptional activity in the two sexes: the distal 10 percent of this chromosome is not dosage compensated while the majority of an interior segment representing 30 percent of its length is compensated.

The brief survey of selected species just discussed provides the logical basis of a scheme for the evolution of new sex elements. A chromosomal arm is translocated to either the X or the Y. In the case of a Y-autosome fusion, the autosomal element is male-limited by the very fact of its attachment to the Y chromosome. In the case of an X-autosome fusion it is the homologous, free element which is male-limited. In both instances, an unbalanced system would result from the accumulation of dysfunctional genes in the male-limited autosomal arm; the linkage group in question would be represented in the genome by two effective doses of some genes and a single effective dose of others. Pressures would be generated for new regulatory genes to function as activators or enhancers of the new sex-linked element; new regulatory sites associated with the structural genes on this element and capable of responding to autosomal regulatory factors would also be needed.

It is not unreasonable to assume that such a process yielded the original sex chromosomes of the genome. Here, physical proximity (and, therefore, tight genetic linkage) to the gene or gene-cluster responsible for sex determination would mediate the sex-limitation of genetic material.

## Dosage Compensation in Mammals

Current understanding of the phenomenon can be traced to three specific events, namely, the discovery of sex chromatin, the cytological demonstration that it consists of a single X chromosome, and the formalization of the inactive X hypothesis. First it was reported that a heterochromatic nuclear structure was often visible in preparations of female cat neurons, but never in those of male cats (*27*). This sex chromatin body or Barr body was observed in somatic cells of females of diverse mammalian species, including man. Ten years later, the nature of sex-limited heterochromatin was elucidated by following chromosome condensation in early and midprophase nuclei of regenerating rat liver cells (*28*). As the remainder of the chromosome complement condensed into discernible elements, it was evident that a single chromosome constituted the Barr body of diploid females. Since this structure is absent in males, the logical candidate was one of the two X chromosomes. In 1961, Lyon formulated her famous hypothesis (*29*). She suggested that the heterochromatic X of female somatic cells was genetically inactive, that heterochromatization occurred early during embryonic development, and that it was random with respect to the X chromosome affected in a given cell, while all descendants of the cell would have the same X chromosome inactivated. Supporting evidence for this hypothesis was provided by the demonstration that single cell clones of skin fibroblasts derived from females heterozygous for an electrophoretic variant of an X-linked enzyme (glucose-6-phosphate dehydrogenase) exhibit either one or the other of the two enzymic forms but never a mixture of both forms (*30*).

The type of mosaicism just described is conspicuously absent in certain marsupial species. In kangaroos, for example, unions between a male and a female bearing different electrophoretic variants

of the enzyme phosphoglycerate kinase produce daughters that invariably exhibit the maternal form of the enzyme; this observation fosters the conclusion that dosage compensation in these animals is mediated by paternal X inactivation (*31*). While total inactivation is almost always seen, there are a few instances where both paternal and maternal gene products appear to be formed in the same cell (*32*).

Various models have been formulated to explain the basis of X chromosome inactivation in marsupials and in eutherian mammals (*33*). Most recently it was proposed that the regulatory mechanism in eutherian mammals was derived from the marsupial mechanism (*34*). Although it has been challenged (*35*), this model will be briefly described for the purpose of illustrating the general trend of current speculation on the subject of Barr body formation. In marsupials this scheme calls for a sensitive site (ss) on the maternally inherited X chromosome ($X^M$); this site produces a few regulatory molecules that associate with a receptor immediately adjacent to the sensitive site. An essential assumption is that the regulatory product of the sensitive site associates only with the receptor located on the same chromosome and does not transfer to the homolog. When the receptor site is bound, the chromosome remains euchromatic and active. Passage through the male results in the inactivation of the sensitive site; no regulatory molecules are produced and none are associated with the receptor of this chromosome ($X^P$), which therefore becomes heterochromatic and inactive. In eutherian mammals, the sensitive and receptor sites are separated and the sensitive site is relocated onto an autosome. Passage of the sensitive site through a male still inactivates it so that only the maternally derived site produces a regulatory molecule (*36, 37*), which associates with the receptor site of the $X^P$ or $X^M$ at random, leading to the random inactivation of the maternal or paternal X chromosomes.

The molecular basis of X chromosome inactivation has been the subject of considerable speculation (*37, 38*). Regardless of the precise mechanism involved, it is clear that inactivation is triggered at a particular locus (or a few loci), subsequently spreading to the whole chromosome. This conclusion is based on the presence of specific genes on the murine X, such as $O^{hv}$ (*39*) or *Xce* (*40*), which affect the expression of other X-linked genes. Different *Xce* alleles appear to alter the probability that the X chromosome which carries them will become inactivated, thereby affecting the expression of various X-linked recessives in heterozygous females; $O^{hv}$ seems to have a comparable effect.

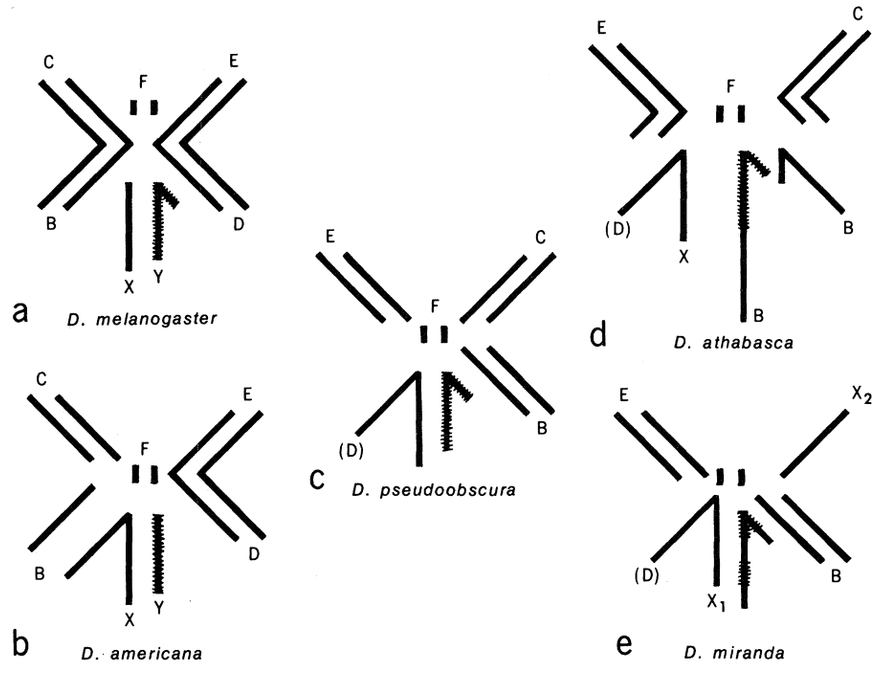


Fig. 3. Metaphase configurations of males from different *Drosophila* species.

## Evolutionary Considerations in Mammals

Some of the features of the sequence of events to be described have been proposed by Lyon (*41*). Major innovations are suggestions for (i) gradual, gene-by-gene evolution of compensation and (ii) the concomitant gradual evolution of the heteromorphism of the sex chromosomes.

Primordial mammals are assumed to have had a pair of homomorphic sex chromosomes and to have relied on two different alleles (*A* and *a*) of a single gene for sex determination. The *A* allele may have been responsible for the induction of testis differentiation; within the testis, the *a* allele would be inactivated, at least during gametogenesis. Inactivation in primary spermatocytes of the X chromosome (analogous to the *a*-bearing chromosome) is known to occur in a wide range of animal groups, and therefore is considered by Lyon to have preceded the evolution of dosage compensation in mammals. Mutations leading to a reduction or absence of gene product would occur at random along the *A*- or *a*-bearing chromosome but would accumulate in the neighborhood of the sex-limited, male-determining *A* allele since the closer any mutant is to *A*, the less the probability that it would cross over to the *a*-bearing homolog, become homozygous, and be eliminated. Such mutations create a selective pressure for the retention of compensatory events in the homologous chromosome. The events in question may have consisted of regulatory mutations such as those postulated for *Drosophila* as was discussed above. They may have consisted of tandem or near-tandem duplications (*42*). Since the precise nature of the compensatory events is not clear, the following discussion is based on the assumption that they consisted, in fact, of gene duplications. This assumption is made for the sole purpose of emphasizing that a piecemeal and gradual evolution of dosage compensation can still be effectively invoked.

For example, let us consider a structural gene *g* closely linked to the *A* locus. A male bearing an inactive mutant allele of *g* could have the constitution $Ag^-/ag^+$; a male with a compensatory duplication would be $Ag^-/ag^+g^+$. In females, such a duplication would have an adverse effect unless it were, somehow, inactivated. This could be realized if the duplicated segment were associated with a special type of mutation of the *a* allele; the new allele, *a'*, having been suppressed in the testis during gametogenesis, would remain inactive in the soma of the developing female embryo. The duplication, functionally linked to *a'*, would also remain inactive; females of the constitution $a'[g^+g^+]/ag^+$ (where

the brackets indicate inactivation) would exhibit reduced gene product and provide selection in favor of the duplication (that is, in favor of an $a'[g^+g^+]/ag^+g^+$ genotype). When transmitted by a female, $a'[g^+g^+]$ would remain active in somatic tissues, and result in an excess of gene product in both males and females. In males, association of $a'g^+g^+$ with an inactive allele of $g$ on the $A$-bearing chromosome would be favored ($Ag^-/a'g^+g^+$); in females selection would favor homozygosis for the $a'$-bearing chromosome in question, that is $a'[g^+g^+]/a'g^+g^+$ (*43*).

The process just outlined would repeat itself for loci adjacent to $g$, resulting in the progressive evolution of a sex chromosome whose genetic content is present in duplicate and whose transcriptional activity is under the control of a specific major sex-determining "gene." This gene (represented by the allele $a'$) provides that the sex chromosome is inactive during spermatogenesis and remains inactive in the somatic cells of female embryos. The homologous sex chromosome bears the $A$ allele of the sex-determining gene and very little else, a situation not unlike that encountered in modern-day marsupials.

The similarities between the proposed sequence of evolutionary steps in fruit flies and in mammals are evident. These are (i) gradual decay of the genetic information linked to the sex-limited form of a sex-determining factor; and (ii) simultaneous, piecemeal evolution of a compensatory mechanism. A salient difference in the two evolutionary paths is the proposed occurrence of *cis*-dominant regulatory sites associated with X-linked structural genes of *Drosophila* while the mammalian model relies on the occurrence of tandem duplications. It is conceivable that basic differences in the physical organization of the genome (*44*) may be responsible for the divergence of solutions to the problem caused by heterogamety in these diploid organisms. For example, the particular distribution of repetitive DNA sequences characteristic of primordial mammals may have facilitated the occurrence of unequal crossing over to a far greater extent than was possible in ancestral fruit flies. The former group of organisms would have relied primarily on the spontaneous generation of tandem duplications while the latter would have made use of random regulatory site mutations in order to compensate for the dysgenic events accumulating on the sex-limited segment of the genome.

## Concluding Remarks

Much of our belief in biological evolution is based on the facts uncovered by paleontology and genetics. Paleontology has provided the reality that evolution is responsible for the existing biotas; genetics has allowed the simulation of what we conceive to be the basic mechanisms involved. The discovery of new species of fossils continues to occur with remarkable frequency, and new methods for the analysis of new and old finds are constantly being devised by geologists and physicists. The various forces and phenomena thought to affect the genetic makeup of natural populations are being investigated with new and powerful tools provided by biochemists and mathematicians. Yet, for an individual to profess an insight into the biological changes that have occurred through time remains an act of faith of considerable magnitude.

Within the limitations just set forth, the purpose of this article has been to spin a relatively reasonable evolutionary tale in the hope of expanding the current perception of a highly significant example of genetic regulation in eukaryotes.

### References and Notes


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23. Various considerations may be taken into account in attempting to envision the likelihood of such an occurrence. Foremost among them is the possibility, formulated by Dr. Brian Charlesworth (*Proc. Natl. Acad. Sci. U.S.A.*, in press), that slightly deleterious mutations may accumulate on a genetically isolated chromosome and spread throughout a population by way of "Muller's ratchet" [J. Felsenstein, *Genetics* **78**, 737 (1974)], a mechanism conceived by H. J. Muller in a different context. Each genetically isolated chromosome would carry a different set of mutant alleles whose number would continue to increase. Their cumulative effects, although yielding little differential selection among the males of the population, would provide the necessary background for compensatory mutations tending to partially restore fitness relative to females. The bias of my article is that compensation would occur on an individual gene (or on an individual transcriptional unit) basis.
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42. The importance of gene duplication as an evolutionary process has been thoroughly discussed by S. Ohno [*Evolution by Gene Duplication* (Springer-Verlag, New York, 1970)]. That such duplications do, in fact, exist on the human X chromosome has been argued by M. F. Lyon (*41*).
43. Once again, various considerations may be taken into account in envisioning the likelihood of this type of event. It is possible that individual gene duplications have little effect on the fitness of individuals which bear them and, therefore, occur with some significant frequency in a population. Thus, a compensatory event may precede the more deleterious dysgenic mutations. Furthermore, while the vast majority of mutations in a given gene lead to reduction or loss of gene product, all such mutations could be compensated by the same gene duplication.
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