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REVIEW

Dosage-Dependent Gene Regulation in Multicellular Eukaryotes: Implications for Dosage Compensation, Aneuploid Syndromes, and Quantitative Traits

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Evidence from a variety of data suggests that regulatory mechanisms in multicellular eukaryotes have evolved in such a manner that the stoichiometric relationship of the components of regulatory complexes affects target gene expression. This type of mechanism sets the level of gene expression and, as a consequence, the phenotypic characteristics. Because many types of regulatory processes exhibit dosage-dependent behavior, they would impact quantitative traits and contribute to their multigenic control in a semidominant fashion. Many dosage-dependent effects would also account for the extensive modulation of gene expression throughout the genome that occurs when chromosomes are added to or subtracted from the karyotype (aneuploidy). Moreover, because the majority of dosage-dependent regulators act negatively, this property can account for the up-regulation of genes in monosomics and hemizygous sex chromosomes to achieve dosage compensation. © 2001 Academic Press

Key Words: **gene regulation; dosage effects; aneuploidy; dosage compensation; quantitative traits; sex chromosomes; transcription factors.**

INTRODUCTION

Gene expression in bacteria and unicellular eukaryotes is highly responsive to environmental conditions, often turning on or off related genes under different circumstances. In multicellular eukaryotes, this is rarely so. Nevertheless, in this review, we argue that gene-expression mechanisms have evolved in multicellular eukaryotes to be externally responsive in a different manner. Selection on the phenotype forces the mechanisms of transcriptional regulation to be "rate limiting" on phenotypic characteristics in the diploid state via their effect on target genes. This sets the extent of growth, the size of organs, the amount of metabolites, etc., making their control dosage-dependent.

The evidence that a single process or target gene is affected by multiple dosage-dependent factors began to emerge over twenty years ago from different types of studies. Henikoff (1979) and Reuter and Wolf (1981) found a large number of dominant mutations in *Drosophila* that modify the phenomenon of position effect variegation—the mosaic expression of genes that often occurs with novel juxtapositions of euchromatin and heterochromatin. Henikoff predicted "it is reasonable to expect that the *Drosophila* genome contains hundreds of loci that can modify variegation." And indeed there are (Reuter and Wolf 1981; Sinclair *et al.*, 1983; Weiler and Wakimoto, 1995). The second line of evidence came from chromosomal dosage experiments in maize and *Drosophila*. Changing the dosage of chromosomal segments alters the expression levels of various genes encoded elsewhere in the genome (Birchler, 1979; Birchler and Newton, 1981; Sabl and Birchler, 1993). In some cases, the *trans*-acting effects were a positive, i.e., the target gene expression increased as did the copy number of the chromosomal segment. With a negative effect, gene expression decreases as the dosage of the effective chromosomal segment increases. A negative or "inverse effect" is the more common type in both maize and *Drosophila*. Two-thirds to three-quarters of the modifiers act "inversely." The reason for this is unknown. Eventually, single

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TABLE 1
Dosage-Dependent Modifiers of the *white* Eye Color Gene of Drosophila

Modifier	Effect on *white*	Type of regulator
abnormal, small, homeotic discs 2 (*ash2*)	Negative	Transcription factor
apterous (*ap*)	Negative	LIM family transcription factor
Beadex (*Bd*)	Negative	Transcription factor
brahma (*brm*)	Positive	Chromatin remodeling
cap-n-collar (*cnc*)	Negative	bZIP transcription factor
devenir (*dev*)	Negative	Trithorax-Group
Distalless (*Dll*)	Negative	Transcription factor
Enhancer of Polycomb (*E*(*Pc*))	Negative	Polycomb Group
Enhancer of white-spotted[*81d5*]	Positive	Unknown
Enhancer of zeste (*E*(*z*))	Negative	Polycomb Group
extra sex combs (*esc*)	Negative	Polycomb Group
hedgehog (*hh*)	Positive	Cell–cell signaling
Inverse regulator-a (*Inr-a*)	Negative	Unknown
kismet (*kis*)	Positive	Trithorax-Group
kohtalo (*kto*)	Negative	Trithorax-Group
l(*2*) 05208/Kruppel-*homolog1* (*Kr-h1*)	Positive	Transcription factor
l(*2*) *03405/Uba1*	Positive	Ubiquitin activating
l(*3*) *00305*	Negative	Unknown
l(*3*) *01969*	Negative	Unknown
l(*3*) *02104*	Negative	Unknown
l(*3*) *03670*	Negative	Unknown
l(*3*) *04063*	Negative	Unknown
l(*3*)*04026*	Negative	Unknown
l(*3*)*08232*	Negative	Unknown
l(*3*)*87Ca/Vha55*	Positive	Polycomb Group
Lightener of white (*Low*)	Positive	Unknown
mei-P19	Negative	Unknown
Modifier of white (*Mow*)	Negative	Unknown
modulo (*mod*)	Negative	DNA binding
osa (*osa*)	Positive	DNA binding
oxen (*ox*)	Negative	Diacyl glycerol kinase
Polycomblike (*Pcl*)	Negative	Polycomb Group
Posterior sex combs (*Psc*)	Negative	Polycomb Group
Regena (*Rga*)	Negative	Transcription factor
Ribonuclear protein at 97D	Negative	Nuclear RNA binding
Ribosomal protein PO/AP lyase (*PO*)	Negative	AP3 DNA endonuclease
scalloped (*sd*)	Negative	Transcription factor
Sex combs on midleg (*Scm*)	Negative	Transcription factor
skuld (*skd*)	Negative	Trithorax-Group
sugarless (*sgl*)	Negative	UDP-G6DH (signaling)
Suppressor of Polycomb (*Su*(*Pc*))	Negative	Polycomb Group
Trithoraxlike (*Trl*)	Positive	Transcription factor
Ultrafemale overexpression (*Ufo*)/*lola*	Negative	Transcription factor
urdur (*urd*)	Negative	Trithorax-Group
verthandi (*vtd*)	Negative	Trithorax-Group
Weakener of white (*Wow*)	Positive/negative	Unknown
wingless (*wg*)	Negative	Cell–cell signaling

Note. Modifiers of *white* were determined by the increase or decrease of pigment levels in flies carrying leaky point mutations of *white*. All the listed modifiers have an effect on *white* as heterozygotes with their respective normal allele, thus demonstrating the dosage-dependent nature of their action. Further descriptions and references for each gene can be found in Flybase (http://flybase.bio.indiana.edu:82/).

genes were identified that fulfilled the predictions as being responsible for the dosage series effects (e. g., Rabinow *et al.*, 1991). Functional copies of regulatory genes that are heterozygous with null mutations would be equivalent to a monosomic chromosomal segment and usually increase target gene expression. However, there is evidence that

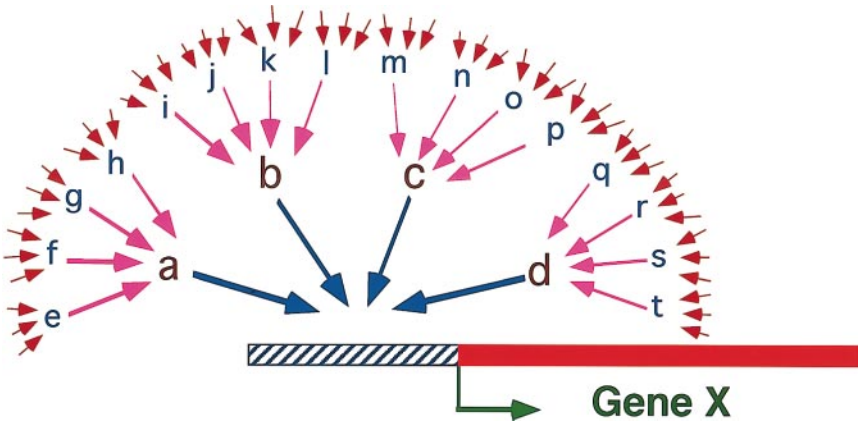

FIG. 1. Heuristic model of a dosage-regulatory hierarchy. Considering the expression of hypothetical gene X, if there are as few as four dosage-dependent regulatory genes that modify its expression directly, and for each of those there are four dosage-dependent modifiers and so on, it would take only three hierarchical levels to account for the scores of dosage-dependent modifiers of gene X. Because regulators a–d are dosage-dependent, any modulation of their concentration by e—t, etc. will be conveyed to gene X. In this manner, dosage effects would be transmitted through the hierarchy to the monitored gene or phenotype. It is recognized that reticulation among hierarchy branches, epistasis, and pleiotropy would complicate the interactions.

both positive and negative effects can be produced by the same gene under different circumstances (Birchler *et al.*, 1994; Bhadra *et al.*, 1997).

It is now clear that the modifiers of variegation and of gene expression overlap (Dorn *et al.*, 1993; Birchler *et al.*, 1994; Csink *et al.*, 1994; Bhadra and Birchler, 1996; Bhadra *et al.*, 1997; Frolov *et al.*, 1998; Frolov and Birchler 1998; Henikoff, 1996). These genes encode a heterogeneous collection of regulatory molecules ranging from transcription factors to chromatin proteins to members of signal transduction pathways that ultimately modulate transcription. The heterogeneous nature suggests that many aspects of gene regulation are subject to selection for rate-limiting action in the diploid state.

To define the number and nature of *trans*-acting dosage-dependent factors that influence a single target, leaky alleles of the X chromosome linked *white* eye color gene in *Drosophila* were used as a monitor (Table 1). These alleles provided an inexpensive and rapid screen for such modifiers. They allowed the detection of quite subtle phenotypic effects that even the most sensitive molecular technique might miss. It is important to document even the more subtle modifiers in such a model system in order to determine the types of genes that affect quantitative variation. In general, all such modifiers have the potential to influence artificial and natural selection for any characteristic under consideration. At least 47 dosage-dependent modifiers of *white* have been identified to date and it is likely that others exist. In fact, modifiers on the X chromosome are probably significantly underrepresented because *white* is also on the X, making screens there more difficult to conduct. Nevertheless, Muller (1950) provided evidence for dosage-dependent modifiers of *white* along the X with a predominance of negatively acting factors.

The majority of the *white* modifiers that have been defined at the molecular level are involved in various regulatory mechanisms. It has not been documented which ones act directly versus indirectly on the *white* gene, but it is highly probable that both are represented. It is also possible that steps subsequent to the transcription of *white* are modified to some degree, but there is little evidence for such effects. Because they all exhibit a dosage effect, it is likely that a hierarchy is operating in which an effect is transmitted through a cascade of concentration-dependent steps. Changing the concentration of a regulator at the top of the hierarchy changes the concentration of its targets, which in turn alters the amount of subsequent targets and so on until the ultimate housekeeping gene is modulated (Fig. 1). A hierarchy of dosage-dependent steps helps explain why there are so many factors that affect a single gene or process. With only a few hierarchical levels, numerous modifiers become effective on any monitored gene.

With so many modifiers of a single target gene, it is of interest to know the result of varying several of them simultaneously. The evidence suggests that, although interactions and epistasis are present, the net effect of multiple modifiers in most cases does not routinely surpass the effect of a single regulatory gene. In other words, a one-, two-, and three-dose series of a chromosome arm does not exceed the maximal effects of a dosage series of an individual factor. For example, in a direct correlation, the expression of a target gene expression might be reduced with one dose of a regulatory factor to a minimum of 50% of the normal two-dose diploid level and increased in three doses to a maximum of 150%. More often, an inverse correlation occurs in which having only one dose of a regulatory factor results in a target gene expression elevated to a maximum of 200% of the normal level and the

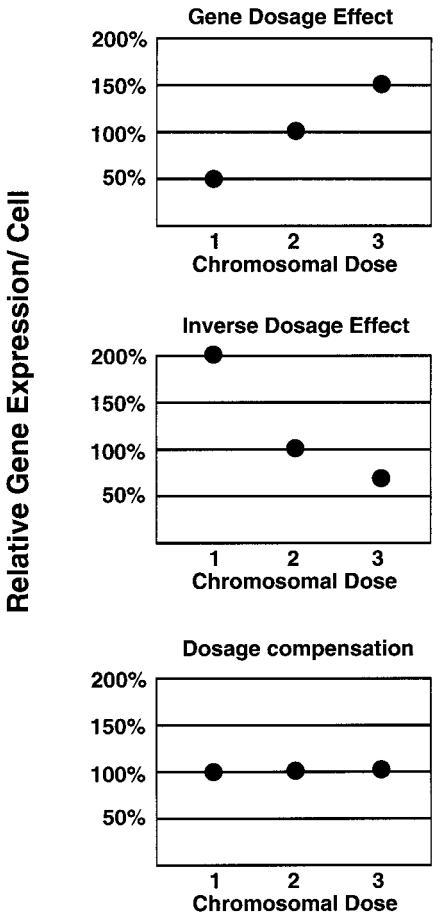

FIG. 2. Dosage effects and compensation. Relative gene expression per cell using 100% as the diploid level is used to illustrate the types of gene-expression effects observed in individuals with one, two, or three copies of a chromosome. When chromosomal segments carrying a specific gene are varied in a dosage series, the structural gene may exhibit a dosage effect as a reflection of the number of copies present (Top). The most common *trans*-acting dosage effect is an inverse correlation between the dosage of the varied chromosome arm and the expression of the gene per cell (Middle). When a structural gene is varied together with a segment of the genome that produces an inverse dosage effect upon its expression, the two effects of the structural gene dosage and the inverse effect cancel each other to result in dosage compensation (Bottom). Modified from Bhadra *et al.* (1999) by permission.

three-dose situation causes reductions in gene expression to a minimum of 67%. Despite the fact that changing the dosage of a single regulatory gene can achieve the direct or inverse correlative limits (Rabinow *et al.*, 1991), aneuploids for rather substantial fractions of the genome, that undoubtedly carry several modifiers of one gene, still produce dosage effects within the same range (Birchler, 1979; Birchler and Newton, 1981; Devlin *et al.*, 1988; Guo and Birchler, 1994; Auger *et al.*, 2001). It appears that the dosage effects result from an altered stoichiometry of regulatory factors encoded on the varied chromosome interacting with others whose concentrations are unchanged, as occurs, for example, with the *myc*/*max*/*mad* interactions (Grandori *et al.*, 2000).

Further insight into this issue comes from combining individual modifiers of *white*. Single, double, triple, and quadruple combinations of mutant modifiers, heterozygous with normal alleles, all produce an approximate doubling of *white* gene expression relative to normal rather than a progressive increase (Bhadra *et al.*, 1998). There is some degree of cumulative action among different genes, but generally the effects fall within the inverse correlation limits (Bhadra *et al.*, 1998). Thus, the most common aneuploid effect, regardless of the length of chromosome varied, is an inverse correlation between the dosage of the varied segment and the expression of the target gene.

Because the inverse effect is global in modifying target genes in the varied segment as well as in the remainder of the genome, the majority of genes experience dosage compensation in aneuploids involving substantial chromosomal length (Birchler, 1979, 1981; Birchler *et al.*, 1990; Birchler and Newton, 1981; Devlin *et al.*, 1982, 1988; Guo and Birchler, 1994). Therefore, rather than reflecting gene dosage, the expression levels of housekeeping genes present on varied chromosomal segments in a one-, two-, and three-dose series are often relatively constant. Obviously, at least some of the inversely acting regulatory genes in the varied segment must be dosage-dependent and those genes elsewhere in the genome with which they stoichiometrically interact must not be changed in expression in order to produce the *trans* effects and compensation of the target genes. Segments composed of only 5–10% of the genome exhibit compensation for many of the included genes in both maize (Birchler, 1979; Guo and Birchler, 1994) and *Drosophila* (Devlin *et al.*, 1982, 1988; Birchler *et al.*, 1990). Subdivision of these aneuploid regions showed that the basis of the dosage compensation was a cancellation of a gene-dosage effect by an inverse dosage effect produced by the same chromosome arm (Birchler, 1981, 1990). That is, when only a single copy of a chromosome arm is present, a housekeeping gene on that arm might be expected to be expressed at only 50% of the normal diploid amount. However, inversely acting regulators of that gene are also varied in the same region, causing the single copy of the housekeeping gene to be increased two-fold in expression. This situation results in a net expression level more or less equivalent to the diploid (see Fig. 2). When the same segment is present in three copies instead of the normal two, there are now three copies of the housekeeping gene. The concomitant inverse effect, however, reduces the expression of each copy to about two-thirds of the normal level. The effect of an increased number of structural genes is cancelled by the reduced expression of each copy. To summarize, as an aneuploid segment involves an increasing portion of the genome, the probability rises that genes on the varied segment will be dosage-compensated. Concomi-

tantly, the expression of genes elsewhere in the genome has a greater probability of being negatively affected.

DOSAGE COMPENSATION OF SEX CHROMOSOMES

Given that dosage compensation occurs for the majority of genes when virtually any chromosomal segment is varied in dosage, the issue arises of dosage compensation of X-linked genes (Muller, 1932). Throughout the plant and animal kingdoms, sex-determination mechanisms are often associated with changes in chromosomal dosage in which one member of a homologous pair of chromosomes has become degenerate or missing (Charlesworth, 1996). Modulation of gene expression must occur for the remaining active member of the pair because the situation is analogous to a monosomic condition, which, for any other chromosome of the complement, would be highly detrimental or lethal. The nature of this modulation has been examined in extensive detail in very few species with heteromorphic (or XO) sex chromosomes (only *Drosophila*, *Caenorhabditis elegans*, mammals). Two interpretations have been made. One is that there is an increase in expression of the single sex chromosome (*Drosophila*). The other is that there is a reduction to half the normal expression in the sex that possesses the two functional chromosomes (mammals; *C. elegans*) (reviewed by Cline and Meyer, 1996; Baker *et al.*, 1994). Because this latter scenario suggests that there is an evolution toward a condition similar to monosomy, which is otherwise lethal (Rosenbluth and Baillie, 1981; Rose *et al.*, 1984; Sigurdson *et al.*, 1984; see discussion by Graves *et al.*, 1998), the interpretation of the evolution of these systems should be reexamined. A scenario that should be considered is that a single X in males (or its equivalent in females with X inactivation) has doubled expression. If the mechanism of male increase also operates in females or there is a selection pressure to equalize autosomal expression following such changes, then the down-regulation of the Xs in females would follow, as suggested by Charlesworth (1978). The end product would be an equal per cell expression of male and female sex-linked genes, but a doubled per gene expression relative to the progenitor state before the formation of sex chromosomes.

Indeed, in mammals, one of the two X chromosomes in females is inactivated (reviewed by Lyon, 1999). However, recent evidence has suggested that a gene on the single X in male mice is up-regulated two-fold relative to its progenitor (Adler *et al.*, 1997; Graves *et al.*, 1998). While the change in expression is of the proper magnitude, it is unknown whether this is a response to an inverse dosage effect that typically occurs in experimental "monosomics" or whether it is brought about by some other mechanism. Further experiments will be necessary to understand more fully the evolution of gene expression from the sex chromosomes in mammals.

In *Drosophila*, it is clear that dosage compensation is achieved by an up-regulation of the single X chromosome in males (Arkhipova *et al.*, 1997). The basic mechanism has been suggested to rely on the inverse dosage effect (Birchler, 1977, 1979, 1996). The two-fold increase in expression of target X-linked genes in males is thought to be brought about by the net effect of altered stoichiometry of gene-specific transcription factor complex components encoded on the X versus those encoded on the autosomes. The involvement of the inverse effect in dosage compensation was demonstrated by the behavior of the X-derived *white* eye color gene transgenically inserted into an autosome in a background where the endogenous *white* was deleted from the X. In its new autosomal location, the expression of *white* was inversely modulated by the dosage of the X chromosome (Birchler, 1992). One copy of the X in males conditions the highest expression; two copies of the X in females are intermediate; and three copies in so-called metafemales are the lowest. When the *white* gene was examined on the X chromosome, an X dosage series consisting of males, females, and metafemales all had similar expression, indicating that dosage compensation extends to the three-copy X genotype (Birchler, 1992; see also, Margolis, 1934; Stern, 1960). Moreover, mutant alleles of *white* have been identified that fail to show dosage compensation in males, having lower expression than females. These alleles also fail to show compensation in metafemales, having greater expression than the normal females (Birchler, 1992). This finding indicates that the same mechanism of dosage compensation operates in both males and metafemales.

If the inverse dosage effect is responsible for X chromosomal dosage compensation, one might predict that a dosage series of the sex chromosome would produce an inverse response on the autosomes. Indeed, metafemales have the expression of many autosomal genes reduced to an apparent lower limit of two-thirds of the female level (Birchler *et al.*, 1989). However, in males, where the expression of genes has been subjected to evolutionary selection, the situation is not so simple. In this case, the expression of the autosomes is quite similar to females, although when differences do exist, the most common autosomal sexual dimorphism is a higher gene expression in males (Smith and Lucchesi, 1969; Birchler, 1984).

Clearly, the expression of the single X chromosome in males has been modified during evolution, because it is associated with a set of distinctive chromosomal proteins called the male-specific lethal (MSL) complex (see Fig. 3). This fact became known by analyzing a set of mutations that, when homozygous, were lethal to males, but for which females could survive (Belote and Lucchesi, 1980). Using antibodies against these proteins, it was found that, in males, they are specifically located on the X chromosome (Kuroda *et al.*, 1991). The complex is composed of at least the products of the *male-specific lethal 1*, *2*, and *3* loci, the *maleless* locus, the *males absent on the first* (*mof*) gene (Hilfiker *et al.*, 1997), the JIL1 kinase (Jin *et al.*, 1999, 2000),

and at least two noncoding RNAs (*roX1*, *roX2*) (Meller *et al.*, 1997). The *mof* gene product is a histone acetyltransferase specific for H4 Lys16 residues (Hilfiker *et al.*, 1997; Aktar and Becker, 2000). The JIL1 kinase phosphorylates histone H3 (Jin *et al.*, 1999, 2000). All of these genes are expressed in males and females, except *msl2*, whose encoded protein is present normally only in males. Its product is instrumental in targeting the complex to the X (Kelley *et al.*, 1995). This localization appears to initiate at several nucleation sites from which spreading occurs over a considerable distance (Bhadra *et al.*, 1999; Kelley *et al.*, 1999) to cover the chromosome. The presence of the complex on the X chromosome in males leads to an increase in the level of histone 4 lysine 16 acetylation (Turner *et al.*, 1992; Bone *et al.*, 1994). (It is assumed that histone phosphorylation catalyzed by JIL1 kinase is similarly affected, although this issue is still unresolved.) These chromosomal-labeling studies led to the hypothesis that the presence of the MSL complex on the X in males brought about the increased acetylation of H4 and that this modification caused a two-fold increase in X chromosomal gene expression to achieve dosage compensation. Again, however, the situation is not so simple.

When absolute levels of gene expression from the X and the autosomes were examined in larvae mutant at the *msl* loci, there was little impact on the X-linked genes sampled, but the most common effect on autosomal genes was an increase in expression in males (Hiebert and Birchler, 1994). Under these circumstances, the MSL complex is not bound to the X. Therefore, it is unlikely to be the primary determinant of dosage compensation, because, for the most part, dosage compensation of X-linked genes is still operative in its absence. Interestingly, the genome wide expression pattern is now as predicted by the prevalence of the inverse dosage effectors, namely, compensation of most target genes on the X and a near doubling of overall expression of the autosomes (Hiebert and Birchler, 1994; Birchler, 1996).

Further investigation of the binding properties of the MSL complex showed that a partial complex is uniformly distributed on all chromosomes in females (Bhadra *et al.*, 1999). The H4 acetylation is also uniform. A similar situation occurs in the dipteran Sciara, where a partial complex is present on all chromosomes in both males and females and shows no specific association with the sex chromosomes (Ruiz *et al.*, 2000). However, in *Drosophila* males, the acetylation is higher on the X and lower on the autosomes compared to females, because MOF is sequestered to the X in males (Fig. 4). In *msl* mutant males and females, there is no binding of the complex to any chromosome, but MOF binds uniformly at a low level on its own in the absence of the MSL complex (Fig. 4) and is catalytically active in modifying H4 (Bhadra *et al.*, 1999, 2000). Therefore, the acetylation level on the X in mutant *msl* males goes down, but goes up on the autosomes compared to normal males. When gene expression is monitored in situations in which the acetylation level increases or decreases on the autosomes, gene expression parallels the H4 acetylation level (Bhadra *et al.*, 1999, 2000). This finding is in accordance with the general rule that histone acetylation creates a more open chromatin configuration leading to increased gene expression (Brownell and Allis, 1996). The sequestration of histone modifiers to the X in males may have evolved to counteract the increased expression of the autosomes that might otherwise have occurred due to the inverse dosage effect produced by the single copy of the X chromosome.

In contrast, genes on the X do not respond to the high levels of H4 acetylation that are present in normal males (Bhadra *et al.*, 1999, 2000). Nor do genes on the X increase in expression when the acetylation level is raised by directing the MSL complex to the Xs in females (Bhadra *et al.*, 1999, 2000). These observations led to the concept that some component of the MSL complex inhibits genes from responding to the high level of acetylation. The results also indicate that the MSL complex itself is not the determinant of hyperactivation of the X. Indeed, when MOF alone is artificially targeted to a gene, the acetylation increases as well as the level of expression, but the latter is severalfold greater than needed to account for dosage compensation (Akhtar and Becker, 2000). The MSL complex on the X in males may inhibit this response so as to allow target genes to respond to the two-fold inverse dosage effect, which provides the proper level of modulation to achieve dosage compensation.

ANEUPLOID SYNDROMES

Having discussed the genome modifications of sex chromosomes needed to respond to the multitude of dosage-dependent regulatory genes, we can now turn our attention to how regulatory genes might cause aneuploid syndromes in situations where no selection pressure has occurred. In both plants and animals, increases or decreases in chromosomal dosage have a significant impact on the phenotype (Blakeslee *et al.*, 1920; Patterson *et al.*, 1937; Lindsley *et al.*, 1972; Epstein, 1986; Bond and Chandley, 1983). As the length of the varied region increases, the probability that lethality will result becomes greater. As a general rule, monosomy is more severe than trisomy, but both are less vigorous than the normal euploid.

It is likely that the various vigor relationships among aneuploids have their basis in the level of target gene expression in the respective genotypes (Birchler and Newton, 1981). With both positive and negative dosage effects operating in aneuploids, reductions of target gene expression will occur in both monosomics and trisomics as demonstrated in maize (Birchler and Newton, 1981; Guo and Birchler, 1994; Auger *et al.*, 2001). The positive effects result from noncompensated structural genes present on the varied chromosome and from positively acting *trans* effects on target genes elsewhere in the genome. In monosomics, the lowest reductions are typically 50% of the normal diploid amount. In trisomics, there are also reduc-

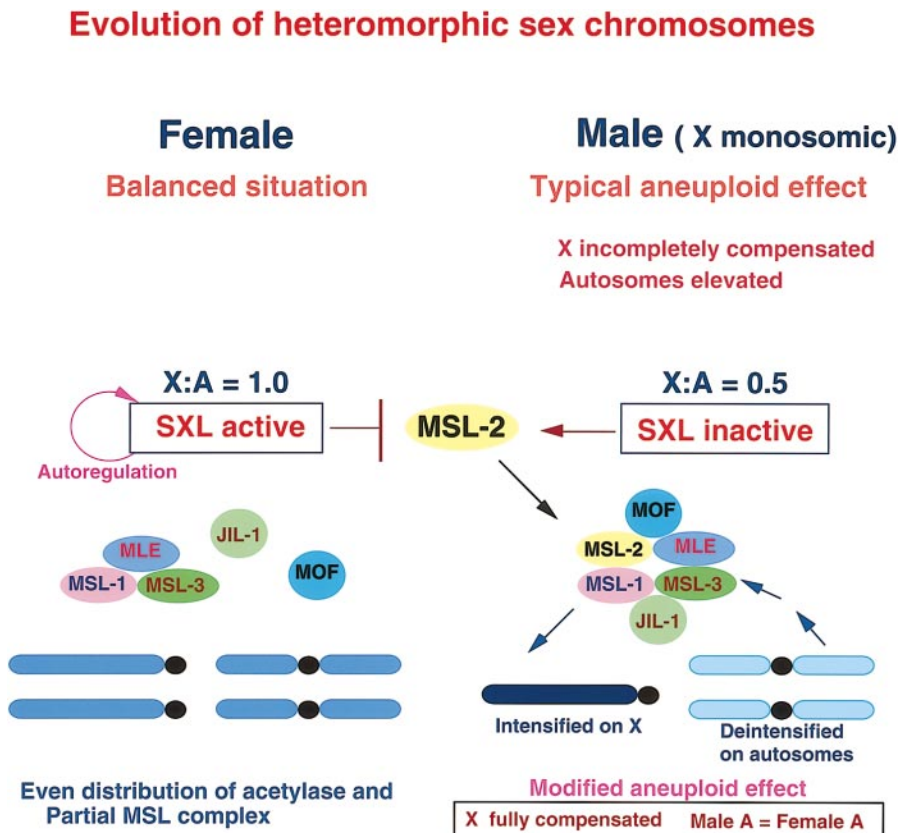

FIG. 3. Heteromorphic sex chromosomes and the modification of genomic expression in *Drosophila*. At the top of the figure, the situation is depicted that would result without evolutionary modifications of the sex chromosomes. Below is shown how the MSL complex is sequestered to the X in males at the expense of its presence on the autosomes. The *Sxl* gene is expressed only in females, where it blocks the expression of *msl2*, a critical component of the male-specific lethal complex for X sequestration. In females, there is a uniform distribution of histone acetyltransferase and JIL1 histone kinase as well as a partial MSL complex. In males, the presence of the MSL2 protein sequesters the complex together with the MOF histone acetylase and JIL1 kinase for association with the X chromosome. The consequence of this sequestration in males is that the autosomes are reduced for their level of histone acetylation and phosphorylation, while these chromatin modifications are increased on the X. Therefore, the inverse dosage effect of the X on the autosomes is muted by the reduced acetylation and phosphorylation. On the X, the chromatin modification alters the expression of a few genes, but for the majority, the MSL complex prevents a response to the high levels of acetylation and phosphorylation, allowing the two-fold inverse dosage effect to provide the proper level of X chromosomal dosage compensation.

tions in gene expression. In this case, they result from an inverse dosage effect on genes encoded elsewhere in the genome. The lowest reductions are at the 67% value relative to normal. Many effects only occur in either monosomics or trisomics rather than the whole dosage series, but the range still falls within these limits (Guo and Birchler, 1994; Auger *et al.*, 2001). If the expression of different sets of target genes are reduced in the monosomic and trisomic, then different gene products become rate limiting on vigor and the respective aneuploid syndromes would result (Birchler and Newton, 1981; Guo and Birchler, 1994). Aneuploidies of the various chromosomes of the karyotype will modify different groups of target genes and thus produce unique phenotypes. The classical thinking on this issue is that an imbalance of gene products from the varied region relative to the remainder of the genome is detrimental to the organism (Blakeslee *et al.*, 1920). The analysis of gene expression in aneuploids, however, demonstrates that dosage compensation occurs for the majority of varied genes but the remainder of the genome is modulated to varying degrees. The "imbalance" appears to be a reflection of the stoichiometric alterations of components of regulatory complexes rather than of the target gene expression, although it is difficult to separate the two. The situation is complex, but the facts that greater reductions of target gene expression occur in monosomics than in trisomics and that these changes more or less parallel the phenotypic consequences suggest that the aneuploid syndromes are caused by the dosage effects (Fig. 5).

In contrast to the extensive modulations of gene expres-

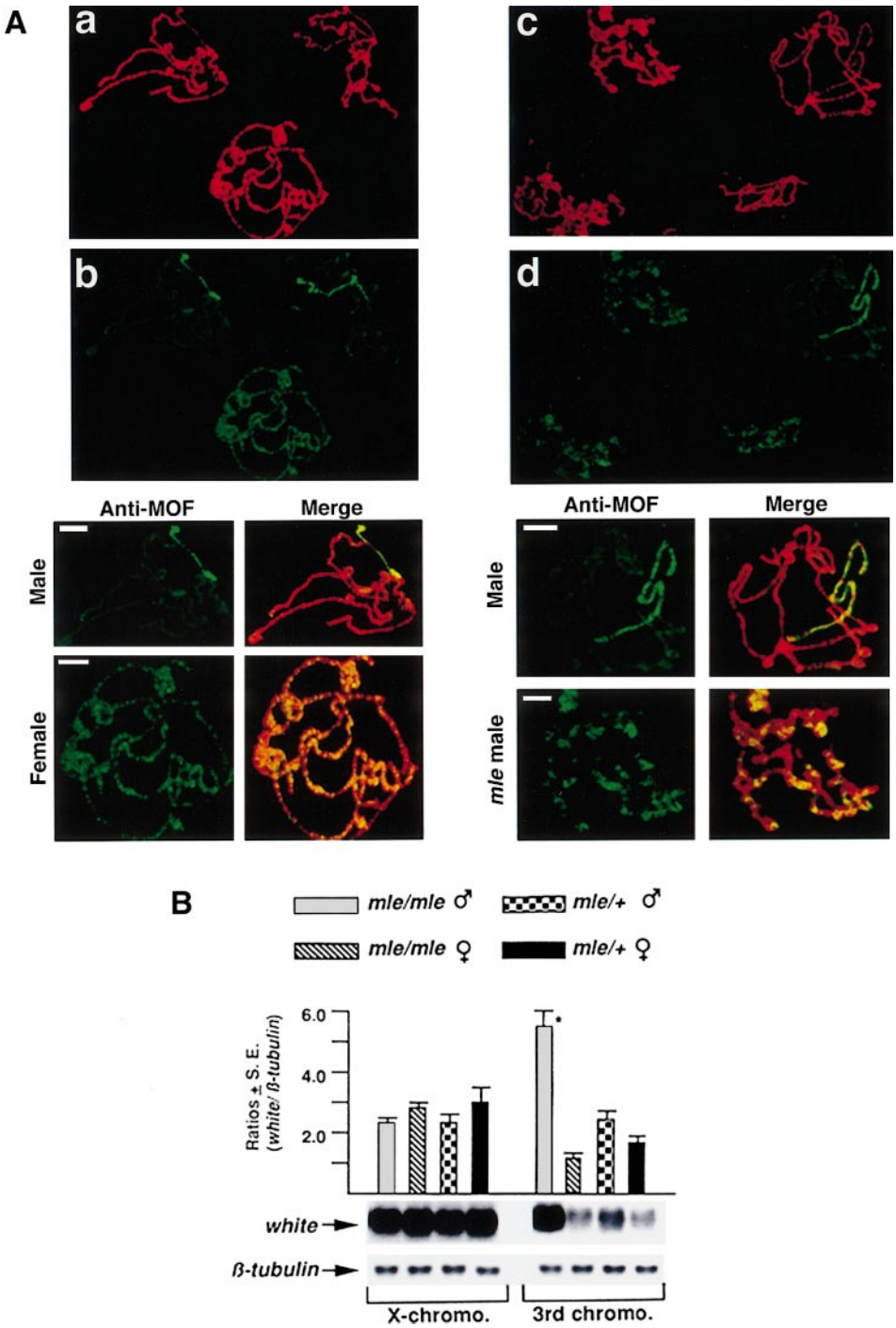

FIG. 4. Sequestration of MOF histone acetyltransferase to the X chromosome and the impact on gene expression. (A) Distribution of MOF in different genotypes. (a) Mixture of salivary gland polytene nuclei from normal males and females stained for DNA with propidium iodide (PI) (red). (b) The same nuclei probed with antibodies to MOF (green). A nucleus of each sex is enlarged below with the anti-MOF pattern and the image merged with PI. Note the enrichment of MOF on the X chromosome in males, but the uniform genomic distribution in females. (c) Mixture of salivary gland polytene nuclei from normal and *mle*/*mle* males. (d) The same nuclei probed with antibodies to MOF. Below are enlarged individual nuclei of each genotype. Note the sequestration of MOF to the X in normal males, but the return of MOF to all chromosomes in the *mle* mutant males, where no MSL complex is formed. Thus, normal males have greater amounts of MOF on the X and lesser amounts on the autosomes compared to females. In the *mle* mutant males, there is less MOF on the X and greater amounts on the autosomes compared to normal males. (B) Gene expression consequences of MOF sequestration. A full-length *white* transgene was

sion that occur with aneuploids, changes in the dosage of the whole genome in a ploidy series have much less impact (Lucchesi and Rawls, 1973; Birchler and Newton, 1981; Guo *et al.*, 1996; Birchler *et al.*, 1990; Rabinow *et al.*, 1991). In this case, there is a tendency for genes to exhibit a per cell expression that is directly correlated with ploidy. In most but not all cases, increased ploidy causes a correlated increase in cell size; thus, in general, the gene products are at a similar concentration per cell among different ploidies. Nevertheless, some genes have a greater or lesser expression with increasing ploidy (Guo *et al.*, 1996). These observations are consistent with the suggestion that the aneuploid effects result from the altered stoichiometry of transcriptional regulators.

RELATIONSHIP TO QUANTITATIVE TRAITS

The multiplicity of dosage effects on a single target gene (or a single phenotype) also suggests a basis for the polygenic nature of quantitative traits (Guo and Birchler, 1994; Bhadra *et al.*, 1998). Most mutations exhibit a strong dominant/recessive relationship with normal alleles (Stadler, 1928; Orr, 1991) and are the basis of discontinuous (qualitative) traits. However, a major tenet of quantitative genetics is that many traits exhibit an intermediate phenotype, at least to some degree, in hybrids between parents representing the extremes of the population. The genetic control of such phenotypes is continuously distributed in subsequent generations (for recent reviews, see Tanksley, 1993; Mackay, 1995). This behavior suggests a control by which there are many genes contributing to the trait and that, for many of them, the alleles from the different parents show a dosage effect to some degree in the hybrid. Thus, it is likely that much of quantitative variation has a basis in the behavior of dosage-dependent regulatory genes (Guo and Birchler, 1994; Byrne *et al.*, 1996; Doebley *et al.*, 1997; Lukens and Doebley, 1999; Frary *et al.*, 2000). Stated another way, with the knowledge that many dosage-dependent regulatory genes will affect any single gene or trait, one would predict the type of genetic control of the phenotype that quantitative characters generally exhibit. The dosage hierarchy described above unifies the polygenic nature of additive quantitative traits with the observation that any one characteristic is affected by multiple aneuploidies.

Two examples illustrate the nature, behavior, and hierarchy of quantitative trait loci. The *teosinte-branched* (*tb1*) locus of maize is a negatively acting transcription factor that is a repressor of organ growth (Doebley *et al.*, 1997). During the domestication of maize, the expression of *tb1* has been up-regulated approximately two-fold to suppress the highly branched morphology typical of the maize progenitor, teosinte. The up-regulation of *tb1* requires alleles at other loci, illustrating hierarchial interactions (Lukens and Doebley, 1999). Secondly, in tomato, fruit size is controlled by many loci. A major quantitative trait locus for this character, *fw2.2*, has been cloned and found to have homology to the human oncogene *ras*, which exerts a regulatory control of cell division (Frary *et al.*, 2000). The small-fruit allele of this gene is semidominant to the large-fruit allele. Transformation of the gene back into tomato showed that a transgenic copy of *fw2.2* reduced fruit size, indicating that this gene acts negatively on the quantitative trait. The common alleles of this locus appear to differ by changes that affect the expression rather than the structure of the protein itself, suggesting the potential for hierarchial modulations of *fw2.2* by other regulatory genes that would contribute to the overall quantitative trait.

RELATIONSHIP TO DEVELOPMENTAL PROCESSES

This type of concentration-dependent behavior is also exhibited by many regulators that control developmental decisions (Driever and Nusslein-Volhard, 1988; Struhl *et al.*, 1989; Warrior and Levine, 1990; Sauer and Jackle, 1991; Jiang and Levine, 1993; Weintraub, 1993; Cribb *et al.*, 1995; Kennison and Russell, 1987), including a dosage-dependent cascade from one regulator to another (e.g., Schulz and Tautz, 1994). Also, a screen for dosage-dependent modifiers of the ectopic expression of *Kruppel*, which is itself a dosage-dependent transcription factor, identified a second tier of regulatory genes that alter its expression (Abrell *et al.*, 2000). Both long-range and short-range gradients are involved in making morphological decisions and a change in concentration of these molecules will alter the phenotype (for review, see Christian, 2000). Indeed, some of the dosage-dependent regulators of *white* are also involved in developmental decisions (See Table 1). Moreover, the involvement of gradients in development is a reflection of the principle that many regulatory mechanisms will evolve to

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assayed on the X or on the third chromosome in normal and *mle*/*mle* males and females. Both transgene insertions are present in a background with a deletion of the normal *white* gene. Northern analysis of the four genotypes with the X insertion indicates that dosage compensation occurs even in the *mle*/*mle* genotype. For the transgene on the third chromosome, a slight sexual dimorphism in expression occurs between normal males and females with males being higher. In the *mle*/*mle* mutant males, the expression of the transgene is significantly (denoted by *) increased relative to the normal male. Histograms represent the mean ratios (± standard error) of triplicate measurements of the *white*/*tubulin* ratio in the four genotypes for each transgene insertion. The combined results from binding and gene-expression studies suggest that the *mle* mutants show no impact on X expression, but, on the autosomes, the increased MOF and hence H4 acetylation cause an increased expression. Modified from Bhadra *et al.* (1999) by permission.

be "rate limiting" on the phenotype in the diploid state and hence exhibit dosage-dependent behavior when assayed genetically.

The classical concept of "balance" has also been invoked as the basis of the developmental process of sex determination in *Drosophila* (Bridges, 1925). The immediate trigger is the splicing state of the *Sex-lethal* (*Sxl*) mRNA, which itself encodes an RNA splicing factor. In females, auto-splicing is established and self-perpetuating so that a functional protein is made (Bell *et al.*, 1988, 1991). Males do not express SXL. The presence or absence of SXL protein in turn affects a splicing cascade that ultimately determines the activity state of the transcription factor, *doublesex*, different forms of which control the majority of the differences between males and females (Burtis and Baker, 1989; Cline and Meyer, 1996). Although the action of *Sxl* is dichotomous, its expression is controlled by dosage-dependent transcription factors that differ in concentration between males and females early in development (Erickson and Cline, 1991, 1993). Nevertheless, sex determination can be influenced in a highly multigenic fashion. Dobzhansky and Schultz (1931) demonstrated this fact by examining X chromosome aneuploids of triploid intersexes, which rest on the threshold of the sex-determination switch. Triploid intersexes have two X chromosomes and three sets of autosomes and are composed of a mixture of male and female cells. By introducing deficiencies and duplications along the X in intersexes, many chromosomal segments were found that shifted the sexual differentiation. It is possible that, in the cells of intersexual flies, the dosage-dependent regulators of *Sxl* can be modulated easily from the threshold in either direction. In any one lineage during development, *Sxl* splicing will determine either the male or female state, but the number of lineages of either sex is likely to be influenced by the particular component of the dosage hierarchy that has been modulated in each individual aneuploid.

SUMMARY

It is becoming clear that the expression of most genes, metabolic pathways, or developmental processes is affected by multiple dosage-dependent transcriptional regulators. Loss-of-function mutations in regulatory genes will most often exhibit dosage-dependent, semidominant behavior, whereas those in target housekeeping genes will usually be recessive when assayed phenotypically. Because regulatory genes are themselves subject to dosage-dependent regulation, the expression of any structural gene is affected by numerous regulators acting through intermediates. As a result, for many different types of target structural genes, the controlling genes might overlap. The action of the regulatory genes has implications for developmental mechanisms, quantitative traits, aneuploid syndromes, and dosage compensation. Each of these processes has the potential to be modulated quite subtly by many modifiers. The meiotic assortment of regulatory gene variation will generate thousands if not millions of possible genotypes, resulting in a quantitatively distributed array of phenotypes upon which natural selection can act.

FUTURE ISSUES

Any mutation in a gene involved in regulatory or developmental processes that gives it an advantage for transmission to descendents over alternative alleles will be perpetuated. By impacting the phenotype via control over metabolic or developmental pathways, regulatory processes evolved to become "rate limiting" at the diploid level. An important future direction would be to investigate the interaction of the various modifiers on a single gene or phenotype. An interesting theoretical offshoot would be to examine the extent to which different branches of a dosage hierarchy compete against each other to affect the phenotype. The available data suggest that an equilibrium among at least several branches occurs such that each of these branches is capable of influencing the phenotype.

Despite the evidence that many regulatory processes are dosage-dependent, there are several means by which the mechanism can drift away from being rate limiting in the diploid state. First, new mutations might occur that alter the degree of effect of any regulatory pathway on the phenotype. Another consideration is the impact of pleiotropy. Many of the dosage-dependent factors are quite broad in their range of target loci, which could affect many different aspects of the total phenotype. If these different aspects are under different selective pressures, the consequences for the regulatory process could be conflicted. Also, neutral aspects of the phenotype will be shifted together with the aspects under selection. The use of genomic tools to study global patterns of gene expression will be important for addressing these issues from an evolutionary standpoint. It is important in such studies to determine the gene expression per cell or per DNA unit rather than relative to the expression of other genes, as is often applied in differential display or microarray analyses. This type of comparison is necessary because, in many aneuploids, a substantial fraction of all tested genes is modulated in the same direction, so relative measurements will mute the magnitude of the effect.

Although there is some evidence at the level of target gene expression for similar dosage effects in mammals (Klose and Putz, 1983; Reichert, 1986), few studies have been performed in vertebrates. However, given the similarities among the types of regulatory factors between *Drosophila* and vertebrates (e.g., Bel *et al.*, 1998), it is unlikely that significant differences exist with respect to these types of dosage effects, but similar studies would confirm or deny the relationship. Future studies concerning these issues in mammalian species will impact many subject areas, including the evolution of X inactivation, aneuploid syndromes/birth defects, and the basis of complex genetic traits.

Developmental processes in plants and animals are be-

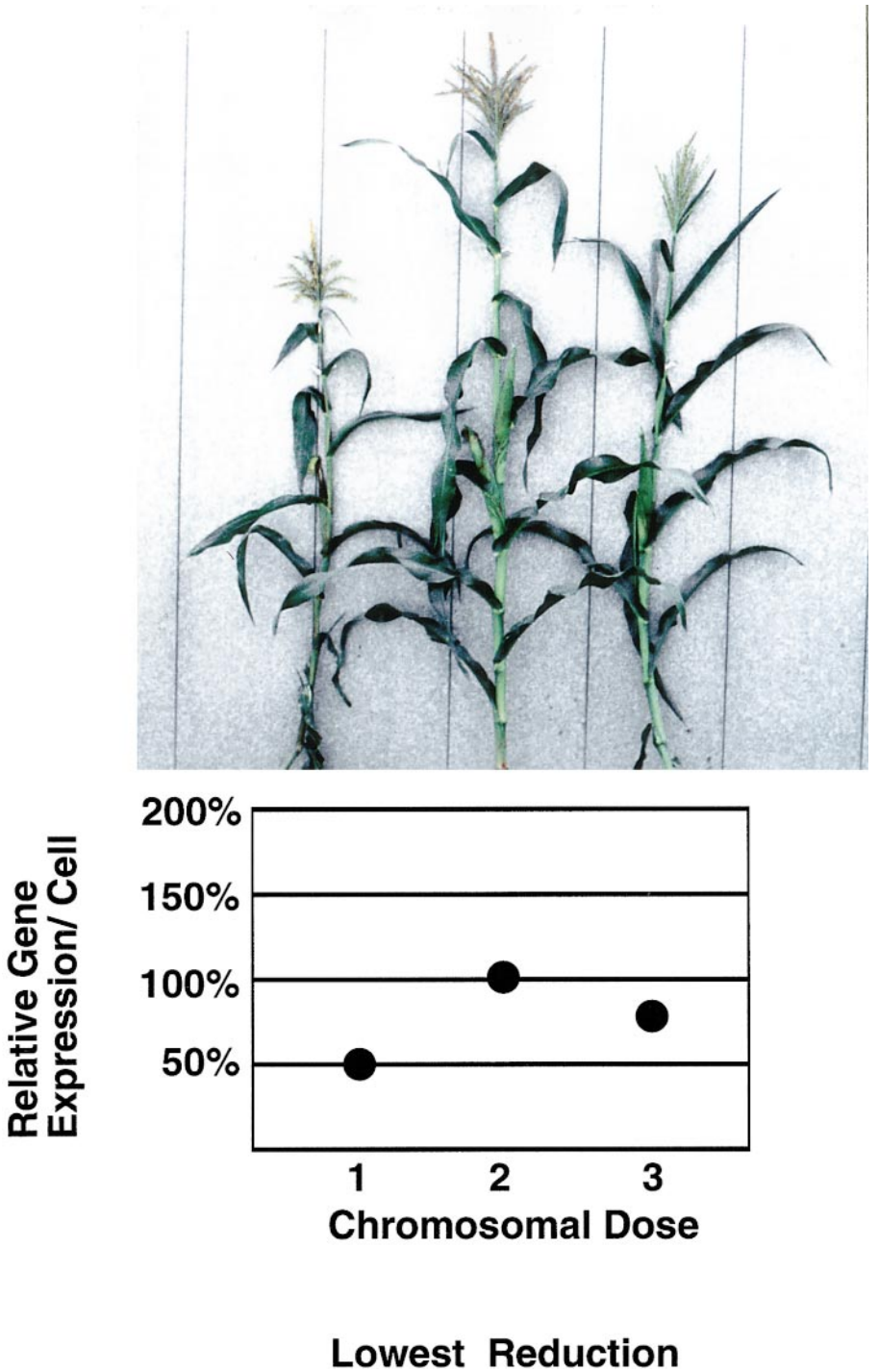

FIG. 5. Reductions in gene expression in aneuploids match the vigor relationship of a dosage series. Monosomic individuals have reductions due to structural gene dosage effects as well as from positively acting *trans*-acting effects. The lower limit is 50% of the normal diploid. Trisomics have reductions in gene expression due to the *trans*-acting inverse dosage effect. The most severe reductions are 67% of the normal diploid. If different sets of genes are reduced in expression in the monosomic and trisomic and are rate-limiting on growth under the respective situations, the typical vigor relationship of a dosage series would be realized. That is, monosomics are often less vigorous than euploid normals and trisomics are intermediate between the two. This relationship is depicted above with maize plants from a 1, 2, 3 dose series for the long arm of chromosome 7. The exact relationship within a dosage series differs from chromosome to chromosome, which is likely to be due to the different set of genes modulated in each case and the degree to which they impact the phenotype. (Photo by C. B. Carson)

lieved to have evolved independently, because the common ancestor for the two taxa was single-celled (Meyerowitz, 1999). Nevertheless, the types of dosage effects observed in the two kingdoms appear to be quite similar. There is a multitude of effects; there are both positive and negative modulations with the latter predominating; the range of modulation of target genes usually falls within a direct or inverse correlation with the dosage of the varied regulator. One explanation for this commonality is that the evolutionary precursor organism had regulatory mechanisms that behaved in this manner and continued to do so with an expansion of the number of regulators in the genome. Recent studies of chromatin remodeling factors in yeast indicate both negative and positive global modulations of gene expression (Sudarsanam *et al.*, 2000; Holstege *et al.*, 1998). However, aneuploid studies in yeast have suggested that the consequences in this species are not very detrimental and that the genes on the varied chromosome are not compensated (Hughes *et al.*, 2000). This interpretation assumes that substantial inverse effects were not operative. If they did indeed occur, gene expression would be reduced throughout the genome and unchanged from the varied chromosome, which would result in the same relative expression of the duplicated chromosome to the remainder of the genome. Nevertheless, the global positive and negative modulations suggest such action is evolutionarily primitive and provide a baseline from which the dosage effects might have evolved to their pervasive presence in multicellular eukaryotes.

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