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The Influence of Whole-Arm Trisomy on Gene Expression in Drosophila

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ABSTRACT

The biochemical consequences of extensive aneuploidy in Drosophila have been examined by measuring the levels of specific proteins in larvae trisomic for entire chromosome arms. By far the most common effect is a reduction in gene product levels (per gene template) by one-third from the diploid quantity, consistent with the model that concentration-dependent repressors of these loci reside on the duplicated chromosome arms. Most loci appear sensitive to such repression in one or more of the trisomies examined, suggesting that such regulatory loci might be quite common. Repression of gene-product levels in trisomies may significantly contribute to their inviability. Few loci are activated in trisomies implying that most factors necessary for gene expression are in excess. While autosomal trisomies can repress the expression of both *X*-linked and autosomal loci, *X*-chromosomal trisomies have little effect on most autosomal genes. A family of genes coding for larval serum proteins do not respond similarly in trisomies, suggesting that regulation operates on a process which is not common to their coordinate regulation. Finally, *Adh* genes transposed to new chromosomal positions maintain their ability to be repressed in *3L* trisomies suggesting that this response to regulation involves a closely linked *cis*-acting regulatory element.

MANY examples of gene regulation in eukaryotes involve large changes in transcriptional activity (for example, see ASHBURNER and BONNER 1979). In contrast, the regulatory effects associated with dosage compensation are much more subtle. For example, in Drosophila, *X*-chromosome dosage compensation invokes, at most, a fourfold alteration in gene expression (LUCCHESI 1977). This response is controlled at the level of transcription and requires *trans*-acting gene function (BELOTE and LUCCHESI 1980). We previously observed that many autosomal genes duplicated in whole-arm trisomies of Drosophila can also produce compensated (diploid) levels of gene product (DEVLIN, HOLM and GRIGLIATTI 1982; DEVLIN, GRIGLIATTI and HOLM 1985). The reduction in gene expression in response to autosomal trisomy is on the order of one-third and, for most genes, is controlled at the transcriptional level (DEVLIN, GRIGLIATTI and HOLM 1984; GHOSH 1985).

The magnitude of transcriptional modulation associated with autosomal dosage compensation is quite small and, as a consequence, its functional significance seems curious in view of the observations that among strains, and among individuals within a strain, considerable variability in gene activity is tolerated (LAURIE-AHLBERG *et al.* 1980). Therefore, it seems unlikely that autosomal compensation reflects a regulatory attempt by trisomies to compensate for their genetic imbalance, but rather is a consequence of control mechanisms that normally operate in diploids.

In those cases where the quantity of a regulatory molecule affects the kinetics of a biosynthetic step, potential exists for end product regulation (KACSER and BURNS 1981). Because many of these steps require the action of enzymes or cofactors encoded elsewhere in the genome, *trans*-acting genetic modifiers which affect the final levels of active gene product are quite common (PAIGEN 1979, 1980). Some *trans*-acting regulatory genes change the gene product by direct modification, or by altering the *in vivo* stability of the RNA or protein (GANSCHOW and SCHIMKE 1969; TWARDZIK, GRELL and JACOBSON 1971; BEWLEY and LUCCHESI 1977; JOHNSON, FINNERTY and HARTL 1981; KING and MCDONALD 1983). Others do not appear to modify the final gene product (RAWLS and LUCCHESI 1974) and may therefore exert their effect at the level of transcription or RNA processing. The mechanism by which such genes act is likely to be quite varied, and systems utilizing "positive" or "negative" control can be envisaged (GUARENTE 1984). Conceivably, varying the dose of these *trans*-acting regulatory genes in trisomies could alter the expression of the structural genes that they control (RAWLS and LUCCHESI 1974).

The purpose of this investigation is to explore the extent to which gene modulation occurs in Drosophila trisomies. In previous studies we examined the effect of trisomy on the expression of genes within the duplicated arm (DEVLIN, HOLM and GRIGLIATTI 1982; DEVLIN, GRIGLIATTI and HOLM 1985). In this paper we examine the effect of autosomal trisomy on the

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expression of genes located elsewhere in the genome. To determine whether the response of genes to trisomy is mediated by *cis*-acting control elements and/or chromosomal environment we examined the expression of two families of genes, one of natural evolutionary origin and the other artificially created by *P*-element mediated transformation. We found that the majority of loci tested could be repressed by a *trans*-acting mechanism in at least one of the autosomal trisomies examined, whereas few activations of expression were observed. Modulation appears to operate independently of other regulatory mechanisms (such as *X*-chromosome dosage compensation) and does not obligatorily affect related genes in similar ways.

MATERIALS AND METHODS

Genetic stocks and crosses: Trisomies were produced by crossing two euploid strains, one with standard chromosomes and one bearing a single compound chromosome (FITZ-EARLE and HOLM 1978). The stocks used in each experiment are listed in the tables (except for the measurements in Table 1 for *Pgi* in *2L* trisomies which were made from experiments using a cross between the *F(2L) b pr/F(2L) b pr;F(2R) bw/F(2R) bw* and the *C(2L)lt;F(2R) bw/F(2R) bw* strains). For the experiments in which we examined the effect of autosomal trisomy on *X*-linked enzyme activity, an isogenic-*X* chromosome (*iso-X*) from the Oregon-R stock was introduced into the compound-free stocks by nondisjunction to yield the following strains: *iso-X/iso-X/C(3L)VG1, ru st;F(3R)VD1,e^s/F(3R)VD1,e^s*. This latter stock was also used in the experiments involving larval serum proteins in *3L* trisomies. To measure the level of expression of individual *Adh* genes in *2L* trisomies, a compound-free stock bearing null mutations for *Adh* was constructed. *b Adh^{N2} osp pr cn* females, treated with 2500 rad of γ-irradiation, were mated to compound-second bearing males. A newly induced *C(2L)b Adh^{N2} osp pr* chromosome was introduced into a compound-free stock by nondisjunction to produce the following strain: *C(2L)b Adh^{N2} osp pr;F(2R)bw Pin/F(2R)bw Pin*. Females of this stock were crossed to males bearing an *Adh* gene transposed to a new chromosome position (GOLDBERG, POSAKONY and MANIATIS 1983; J. POSAKONY, personal communication), and having null mutations for the native *Adh* genes. From these crosses, female trisomic-*2L* larvae were selected for analysis since all ADH enzyme activity in these individuals was encoded by the transposed gene. To produce trisomies for *3L* with *Adh* genes in various chromosomal positions, males from the transformed lines were crossed to *b Adh^{N2} pr cn;C(3L)VG1 ru st;F(3R)VD1 e^s/F(3R)VD1 e^s* females and female larvae were selected for analysis.

Enzyme assays and analysis of larval serum proteins: Gene product levels were determined from homogenates (25 mg live weight/ml of 10 mM PO_4, 1 mM phenylthiourea, pH 8.0) of wandering third instar larvae that had been collected from the side of half pint culture bottles. This stage was chosen for analysis since the detrimental effects of aneuploidy (*i.e.*, reduced size and viability) were less pronounced than at later stages of development. We have attempted to minimize these affects by raising diploid and trisomic individuals in uncrowded cultures and by assaying product levels using an unbiased sampling procedure between the different genotypes. The following gene-enzyme systems were examined (in the format: gene, enzyme, enzyme abbreviation, enzyme commission number): *Pgk*, phosphoglycerate kinase, PGK, EC.2.7.2.3; *Gpdh*, α-glycerol-3-phosphate dehydrogenase, GPDH, EC 1.1.1.8; *Adh*, alcohol dehydrogenase, ADH, EC 1.1.1.1; *Idh*, isocitrate dehydrogenase, IDH, EC 1.1.1.42; *Pgd*, 6-phosphogluconate dehydrogenase, 6PGD, EC 1.1.1.44; *Ak*, arginine kinase, AK, EC 2.7.3.3; *Pgm*, phosphoglucomutase, EC 2.7.5.1; *Cat*, catalase, CAT, EC 1.11.1.6; *Men*, malic enzyme, ME, EC 1.1.1.40; *Pgi*, phosphoglucoisomerase, PGI, EC 5.3.1.9; *Fum*, fumarase, Fum, EC 4.2.1.2; *Gpt*, glutamate-pyruvate transaminase, GPT, EC 2.6.2.1; *Zw*, glucose-6-phosphate dehydrogenase, G6PD, EC 1.1.1.49. The assays for these enzymes have been described (DEVLIN, HOLM and GRIGLIATTI 1982, 1985; DEVLIN, GRIGLIATTI and HOLM 1985). The assay conditions for Aldolase (*Ald*, aldolase, ALD, EC 4.1.2.13) were: 0.1 M Tris (pH 7.5), 0.2 mM NADH, 1 mM fructose-1,6-diphosphate, 1 unit glucose-6-phosphate dehydrogenase/ml, 10 units triosephosphate isomerase/ml and for aldehyde oxidase (*Aldox*, aldehyde oxidase, AO, EC 1.2.3.1) the conditions were 0.16 M PO_4, pH 7.5, 0.8 mM EDTA, 0.08% bovine serum albumin, 120 μg/ml phenylmethosulfate, 0.002% dichloroindophenol, 60 mM acetaldehyde. In the experiment involving *Adh* transformants, ADH enzyme activity was assayed in 0.05 M Na_2CO_3, 2 mM NAD, 0.275 M butan-2-ol, pH 9.0 (SOFER and URSPRUNG 1968). Enzyme activities are expressed relative to the total level of protein in the extract or, in the case of measurements in Table 10, on a live-weight basis. These standards were used to normalize for experimental variation, although clearly these determinations are only an estimate of the amount of product synthesized per gene copy. Measurements were not standardized to DNA content since third instar larvae are comprised of a great deal of polytene tissue and significant differences in the level of polyteny or the amounts of these tissues may exist between diploids and trisomies. We have found that measurements standardized either to total protein or to live weight are suitable for detecting differences in gene-product levels caused by altered gene dose both within and between diploids and trisomies (DEVLIN, HOLM and GRIGLIATTI 1982).

Larval serum proteins were analysed in two electrophoretic systems. For analysis by SDS-gel electrophoresis, samples of hemolymph from wandering third-instar larvae (using only females in experiments with autosomal trisomies) were collected in 10 mM PO_4, 1 mM phenylthiourea (pH 7.5) to which an equal volume of LAEMMLI's (1970) SDS complexing buffer was added. After boiling for 10 min, the samples were run on 9% SDS gels (BROCK and ROBERTS 1980). After staining with Coomassie Blue R250 and destaining, the gels were scanned with a Beckman DU-8 scanning spectrophotometer. The amounts of individual LSPs were expressed as the percentage of total LSP in the sample. This analysis was successful only for measurements of LSP-2 and LSP-1γ. LSP-1α and LSP-1β were not separated by this method.

To resolve the α and β subunits of LSP-1, hemolymph was collected in 10 mM $Na_2B_4O_7$, 1 mM phenylthiourea, 0.01% bromophenol blue (pH 10.0) and separated by electrophoresis on Cellogel (Kalex Scientific) cellulose-acetate strips in 50 mM Na barbital, pH 9.4 (ROBERTS and EVANS-ROBERTS 1979b). The strips were stained with Coomassie blue, destained and the bands of interest were excised and dissolved in glacial acetic acid for twelve hours (ROBERTS and EVANS-ROBERTS 1979a). The optical density of the samples was measured at 595 nm.

Enzyme activities or serum protein levels are expressed as the mean (with associated standard errors) of separate

TABLE 1

Activity of second and third chromosome enzyme loci in *2L* trisomies

	Enzyme locus					
	2R[a]	*3L*			*3R*	
Genotype	*Pgi*	*Ak*	*Pgm*	*Cat*	*Aldox*	*Men*
y/y (diploid)	7.59 ± 0.42 (6)	10.5 ± 0.76 (5)	1.41 ± 0.10 (5)	7.70 ± 0.64 (5)	9.49 ± 0.45 (5)	0.470 ± 0.052 (5)
y/y; C(2L)lt; F(2R)bw/ F(2R)bw (diploid)	7.25 ± 0.21 (6)	11.4 ± 0.91 (5)	1.36 ± 0.08 (5)	7.06 ± 0.54 (5)	10.9 ± 0.86 (5)	0.395 ± 0.063 (5)
y/y; C(2L)lt/+/F(2R)bw (trisomic-*2L*)	5.35 ± 0.17 (6)	10.6 ± 0.62 (5)	1.27 ± 0.11 (5)	8.06 ± 0.35 (5)	13.2 ± 0.93 (5)	0.432 ± 0.050 (5)
Estimated mean	7.42 ± 0.12	10.9 ± 0.49	1.39 ± 0.05	7.38 ± 0.31	10.21 ± 0.44	0.433 ± 0.034
Repressed estimate	4.95 ± 0.08	7.27 ± 0.33	0.927 ± 0.03	4.92 ± 0.21	6.81 ± 0.29	0.289 ± 0.023

[a] See MATERIALS AND METHODS for genotype. Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parentheses are the sample sizes. Repressed estimate is two-thirds the estimated mean.

extracts. Dose-dependent estimates and estimated means are the values expected in trisomies if no regulation is occurring. They were calculated, for the former, as the sum of the activity found in the compound-free strain plus one-half the activity in the standard strain, and for the latter, simply as the mean of the activities found in these two strains. Such estimates reflect the parental contribution to trisomies of the gene being considered. Repressed estimates are calculated as two-thirds of the dose-dependent or mean estimates.

RESULTS

Enzyme loci

Previous investigations have shown that genes within the duplicated regions of whole-arm autosomal trisomies can produce compensated levels of gene products. The present study constitutes a survey of the expression of genes located outside the trisomic arms. We have examined the expression of 15 enzyme loci in larvae trisomic for one autosomal arm (*2L*, *2R* or *3L*) or for the *X* chromosome. The results of these analyses have revealed several effects of aneuploidy on gene expression in Drosophila. For the purposes of this paper the term "unlinked" refers to chromosome arms other than the arm duplicated by the trisomic condition.

Genes outside the duplicated region can be repressed: The activities of six enzymes in trisomic-*2L* larvae and their diploid parental strains are presented in Table 1. PGI is encoded by a gene located on *2R*. The structural genes for AK, PGM and CAT are located on *3L*, and for ALDOX and ME on *3R*. These loci remain diploid in all three strains examined. Also shown are estimates of enzyme activities expected if: (1) no alteration of activity occurred and (2) if gene activity was repressed by one-third. The reader is also directed to Figure 1 where the expression of each gene in trisomies, relative to its expression in diploids, is graphically represented. In *2L* trisomies (Table 1), four of the six enzymes (AK, PGM, CAT and ME) were present at quantities close to that observed in the diploid controls, that is their activity was not affected by trisomy of the unlinked chromosome arm. However, two of the enzyme loci displayed altered levels of activity. *Aldox*, located on *3R*, produced approximately 30% more enzyme in *2L* trisomies than in diploids. This is significant since it represents one of only two cases that we have observed of activation of gene expression by an autosomal trisomy (ADH activity in *2L* trisomies was also higher than expected). The other unlinked locus affected by the trisomic-*2L* condition was *Pgi*, located on *2R*. The activity of this gene-enzyme was reduced by close to one-third in the trisomic-*2L* strains. Therefore, repression of gene expression in autosomal trisomies is not limited to genes within the duplicated arm.

A similar analysis was performed with trisomies for *3L*. Seven enzyme loci, located on autosomal arms other than *3L*, were examined. In contrast to our findings for *2L*, six of the seven gene-enzyme systems showed reduced levels of activity in *3L* trisomies relative to the parental diploid strains. Four of the loci (*Pgk*, *Gpdh*, *Adh* and *Pgi*) in these trisomies exhibited enzyme activities at levels approximately two-thirds of those found in diploids (Table 2); two of the loci (*Men* and *Ald*, both on *3R*) displayed levels of activity intermediate between the two estimated values. The other autosomal gene, *Aldox*, was unaffected by the duplication of *3L*. Thus, trisomy for *3L* appears to repress the expression of more loci than does trisomy for *2L*, although not all loci are equally affected.

The last autosomal trisomy examined was for the right arm of chromosome two. Because these aneuploids are poorly viable, we could measure the expression of only one gene located outside of *2R*. This gene, *Gpdh* on *2L*, was partially repressed in this trisomic-*2R* strain (Table 3).

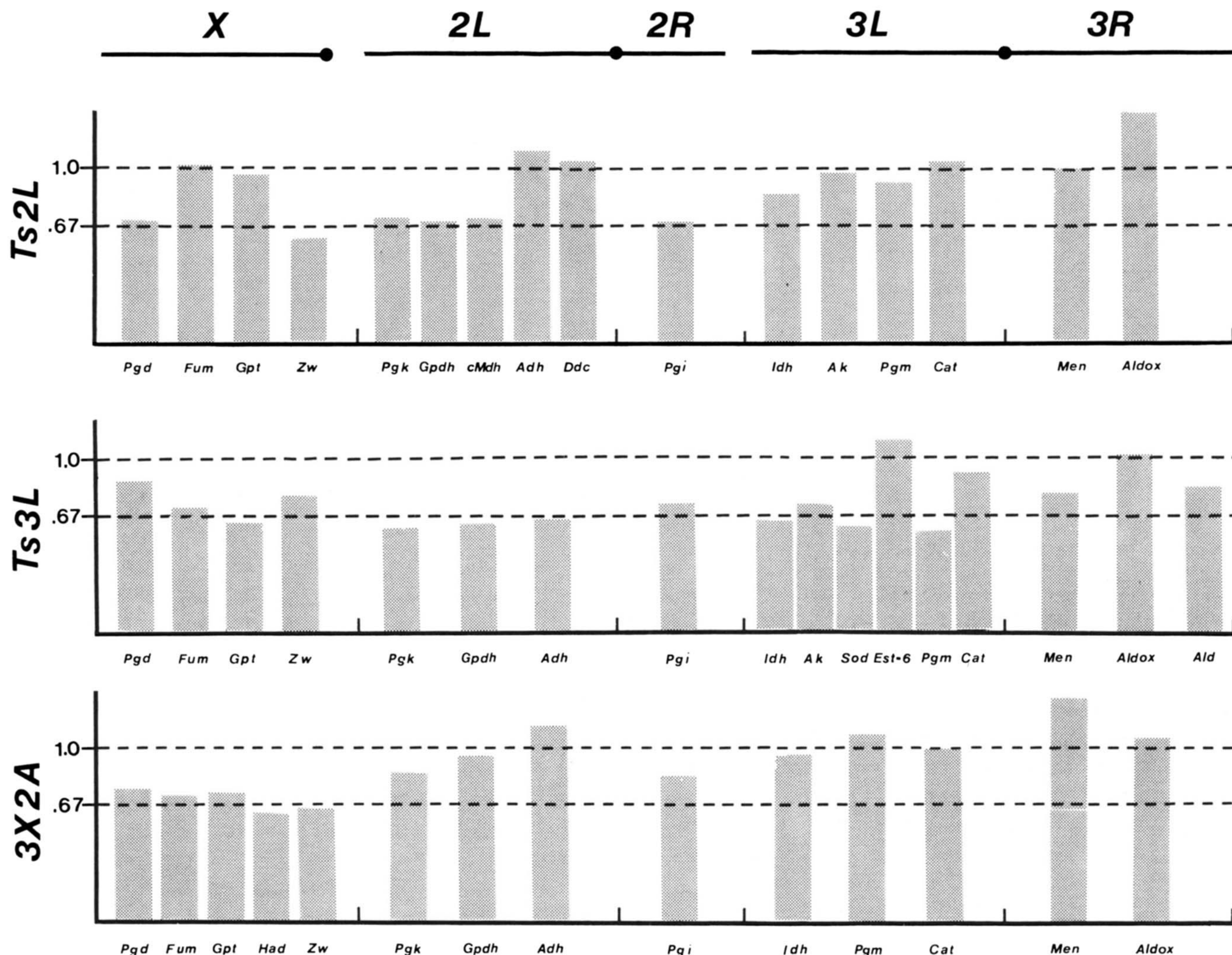

FIGURE 1.—Summary of the expression level of enzyme loci in *X*-chromosomal and autosomal trisomies. On each *x* axis, a value of 1.0 represents the diploid level of expression per gene. Some of this data is summarized from previous publications (DEVLIN, HOLM and GRIGLIATTI 1982, 1985; DEVLIN, GRIGLIATTI and HOLM 1985).

TABLE 2

Activity of second and third chromosome enzyme loci in *3L* trisomies

	Enzyme locus						
	2L			*2R*	*3R*		
Genotype	*Pgk*	*Gpdh*	*Adh*	*Pgi*	*Men*	*Aldox*	*Ald*
Oregon R (diploid)	5.17 ± 0.35 (5)	1.64 ± 0.03 (6)	0.388 ± 0.021 (6)	8.05 ± 0.52 (6)	0.452 ± 0.048 (7)	5.14 ± 0.48 (6)	1.14 ± 0.06 (4)
C(3L)VG1, ru st; F(3R)VD1, e^s/ *F(3R)VD1,* e^s (diploid)	4.39 ± 0.16 (4)	0.967 ± 0.021 (6)	0.344 ± 0.015 (6)	6.28 ± 0.35 (6)	0.364 ± 0.022 (6)	5.93 ± 0.46 (6)	1.25 ± 0.04 (4)
C(3L)VG1, ru st/+/ *F(3R)VD1,* e^s (trisomic-*3L*)	2.87 ± 0.10 (5)	0.811 ± 0.026 (6)	0.235 ± 0.015 (6)	5.31 ± 0.27 (6)	0.330 ± 0.011 (7)	5.61 ± 0.19 (6)	0.983 ± 0.057 (5)
Estimated mean	4.78 ± 0.10	1.31 ± 0.01	0.366 ± 0.006	7.17 ± 0.16	0.408 ± 0.014	5.54 ± 0.17	1.19 ± 0.02
Repressed estimate	3.19 ± 0.07	0.876 ± 0.006	0.244 ± 0.004	4.78 ± 0.10	0.272 ± 0.009	3.69 ± 0.11	0.797 ± 0.011

Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parentheses are the sample sizes. Repressed estimate is two-thirds the estimated mean.

TABLE 3

Activity of a *2L*-linked enzyme locus in *2R* trisomies

Genotype	Enzyme locus *Gpdh*
Oregon R (diploid)	0.998 ± 0.028 (4)
F(2L)pr/F(2L)pr; C(2R)px (diploid)	0.646 ± 0.059 (4)
F(2L)pr/+/C(2R)px (trisomic-*2R*)	0.639 ± 0.049 (6)
Estimated mean	0.822 ± 0.016
Repressed estimate	0.548 ± 0.010

Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parentheses are the sample sizes. Repressed estimate is two-thirds the estimated mean.

Clearly, trisomy for the major autosomal arms in *Drosophila melanogaster* can have striking effects on gene expression, not only on genes located within the duplicated arm but also on genes located on other arms or linkage groups. The most common effect is to reduce gene activity per template by approximately one-third of that found in euploids. This type of repression would be expected if trans-acting repressors of these structural genes were duplicated in the trisomic condition.

Most autosomal genes are unaffected by *X*-chromosomal trisomy: If *X*-chromosomal and autosomal trisomy are comparable conditions, then trisomy for the *X* chromosome might be expected to repress the expression of some autosomal genes. The activity of eight gene-enzyme systems, with loci representing each of the four major autosomal arms, were examined in males, females and metafemales (Table 4). All of these loci produced similar levels of product in males and females, despite their nonisogenic condition. In metafemales, six of these autosomal loci were unaffected (*Gpdh, Adh, Idh, Pgm, Cat* and *Aldox*). The other two loci were moderately repressed; *Pgk* and *Pgi* produced about 15% less enzyme in metafemales than in euploid males or females. Hence it appears that trisomy *X* has little effect on the expression of most autosomal genes. It should be noted that not all loci escape suppression in metafemales. Many *X*-linked loci synthesize diploid levels of product in these hyperploids (LUCCHESI 1983). This includes the *X*-linked gene encoding the 1-α variant of larval serum protein (DEVLIN, HOLM and GRIGLIATTI 1985). A related autosomal gene, located on *3L*, also displays a reduction in expression in metafemales (see below). However, apart from this exception, few autosomal loci appear to show the specific repression (one-third) in metafemales that is observed in autosomal trisomies. Therefore, it appears that the *X* and the autosomes do not exercise equivalent regulatory effects on autosomal gene expression.

Decreasing the *X/A* ratio in trisomies does not result in increased levels of *X*-linked gene expression: A fundamental aspect of the control system operating in male-female dosage compensation is that increases in *X*-linked gene expression are directly correlated with changes in the number of autosome sets. For example, the level of transcription of an *X*-linked gene in a triploid intersex (2*X*3*A*) is 50% greater than in diploid females (2*X*2*A*). It is not known if complete autosome sets are required to elicit this effect. Conceivably, the increased autosomal content of the genome that results in trisomies could activate the expression of *X*-linked loci. To test this possibility, the activities of four *X*-linked enzymes were monitored both in trisomy-*2L* and in trisomy-*3L* strains isogenic for the same *X* chromosome.

Marked variations in expression were observed for genes on this iso-*X* chromosome in the different eu-

TABLE 4

Activity of autosomal enzyme loci in metafemales

	Enzyme locus							
	2L			*2R*	*3L*			*3R*
Genotype	*Pgk*	*Gpdh*	*Adh*	*Pgi*	*Idh*	*Pgm*	*Cat*	*Aldox*
Oregon R +/*O* (male)	3.48 ± 0.23 (6)	0.714 ± 0.043 (5)	0.125 ± 0.006 (5)	3.89 ± 0.22 (6)	0.369 ± 0.013 (6)	0.633 ± 0.049 (4)	4.13 ± 0.27 (6)	6.19 ± 0.25 (5)
C(1)RM, y pn v/Y (female)	3.24 ± 0.15 (6)	0.682 ± 0.053 (6)	0.154 ± 0.012 (6)	3.57 ± 0.04 (6)	0.330 ± 0.014 (6)	0.784 ± 0.115 (5)	3.91 ± 0.32 (6)	5.42 ± 0.25 (5)
C(1)RM, y pn v/+ (metafemale)	2.91 ± 0.08 (6)	0.668 ± 0.039 (6)	0.162 ± 0.018 (6)	3.14 ± 0.40 (6)	0.339 ± 0.026 (6)	0.772 ± 0.083 (6)	3.95 ± 0.38 (6)	6.17 ± 0.33 (5)
Estimated mean	3.36 ± 0.07	0.698 ± 0.017	0.139 ± 0.004	3.73 ± 0.06	0.349 ± 0.005	0.709 ± 0.034	4.02 ± 0.10	5.81 ± 0.09
Repressed estimate	2.24 ± 0.05	0.465 ± 0.011	0.093 ± 0.003	2.49 ± 0.04	0.233 ± 0.003	0.472 ± 0.023	2.68 ± 0.06	3.87 ± 0.06

Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parentheses are the sample sizes. Repressed estimate is two-thirds the estimated mean.

TABLE 5

X-linked enzyme levels in diploid and trisomic larvae

Genotype	Enzyme locus			
	Pgd	*Fum*	*Gpt*	*Zw*
DIPLOIDS				
iso-*X* Oregon R (diploid)	0.158 ± 0.003 (14)	0.258 ± 0.014 (14)	1.19 ± 0.02 (14)	0.131 ± 0.003 (26)
iso-*X*; *C(2L)lt*; *F(2R)bw*/*F(2R)bw* (diploid)	0.184 ± 0.003 (16)	0.167 ± 0.003 (16)	1.12 ± 0.01 (16)	0.167 ± 0.003 (16)
iso-*X*; *C(3L)ru st*; *F(3R)e^s*/*F(3R)e^s* (diploid)	0.192 ± 0.002 (14)	0.241 ± 0.004 (14)	1.46 ± 0.014 (14)	0.225 ± 0.004 (14)
TRISOMIC *2L*				
iso-*X*; *C(2L)lt*/+/*F(2R)bw* (trisomic-*2L*)	0.124 ± 0.001 (16)	0.229 ± 0.003 (16)	1.12 ± 0.01 (16)	0.087 ± 0.001 (16)
Estimated mean	0.171 ± 0.001	0.213 ± 0.003	1.16 ± 0.01	0.149 ± 0.001
Repressed estimate	0.114 ± 0.001	0.142 ± 0.002	0.770 ± 0.003	0.099 ± 0.001
TRISOMIC *3L*				
iso-*X*; *C(3L)ru st*/+/*F(3R)e^s* (trisomic-*3L*)	0.161 ± 0.002 (18)	0.185 ± 0.005 (18)	0.856 ± 0.024 (18)	0.145 ± 0.003 (18)
Estimated mean	0.175 ± 0.001	0.249 ± 0.004	1.33 ± 0.01	0.178 ± 0.001
Repressed estimate	0.122 ± 0.001	0.166 ± 0.003	0.883 ± 0.004	0.119 ± 0.001

For each enzyme in each genotype, activities were determined separately for males and females and then pooled for analysis. Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parentheses are the sample sizes. Repressed estimate is two-thirds the compensated estimate.

ploid parental stocks (for examples, see *Fum* between the Oregon R and the second chromosome compound-free strains or *Zw* between the two compound-free strains in Table 5). However, despite these differences, the activities for these enzymes were not elevated in the trisomic strains. In trisomic-*2L* larvae, these loci remained dosage compensated (see male/female ratios in Table 6) between males and females, whereas a slight reduction in male/female product ratios may exist for trisomy *3L*.

X-linked genes can be repressed by autosomal trisomy: Male and female data were pooled for the analysis of expression between diploids and trisomies. When a comparison is made between autosomal genotypes (Table 5), two observations are evident. First, both autosomal trisomies examined failed to activate any of the four *X*-linked loci examined. Second, each locus was repressed by one-third in at least one of the trisomies. It would appear that *X*-linked loci can respond to autosomal aneuploidy in a manner similar to that of autosomal loci.

TABLE 6

Male/Female ratios of X-linked enzyme levels in diploid and trisomic larvae

Genotye	Enzyme locus			
	Pgd	*Fum*	*Gpt*	*Zw*
iso-*X* Oregon R (diploid)	1.12	0.962	0.935	0.826
iso-*X*; *C(2L)lt*; *F(2R)bw*/*F(2R)bw* (diploid)	1.02	1.13	1.21	1.06
iso-*X*; *C(3L)ru st*; *F(3R)e^s*/*F(3R)e^s* (diploid)	0.934	1.07	1.10	1.00
iso-*X*; *C(2L)lt*/+/*F(2R)bw* (trisomic-*2L*)	0.929	1.03	1.14	1.11
iso-*X*; *C(3L)ru st*/+/*F(3R)e^s* (trisomic-*3L*)	0.776	0.897	0.967	0.876

The male/female ratios shown were determined from the enzyme activity means obtained from equal numbers of extracts for each sex. The pooled activities are shown in Table 5.

Expression of gene families in trisomies

Larval serum protein genes: The results of the previous section suggest that each gene responds to the trisomic condition independently. Some property associated with each gene or its expression must render it susceptible to regulation in trisomies. We have examined the expression of a group of genes of recent evolutionary divergence (BROCK and ROBERTS 1983) to determine if they respond similarly to autosomal trisomy. The larval serum proteins are coded by two major groups of genes: LSP-2 is encoded by a single gene located on *3L* (ROBERTS and EVANS-ROBERTS 1979b), whereas LSP-1 is produced by three genes. While these related genes are similar in sequence

TABLE 7

Quantity of LSPs in diploid and trisomic-*3L* hemolymph from female larvae

Genotype	LSP ratio			Total LSP-2 protein
	2/1α	2/1β	1γ/2	
iso-*X* Oregon R (diploid)	1.88 ± 0.11 (8)	1.35 ± 0.09 (8)	1.03 ± 0.09 (11)	0.739 ± 0.052 (8)
iso-*X*; *C(3L)ru st*; *F(3R)e^s/F(3R)e^s* (diploid)	1.59 ± 0.08 (8)	1.54 ± 0.09 (8)	0.905 ± 0.09 (11)	1.01 ± 0.07 (8)
iso-*X*; *C(3L)ru st/+/F(3R)e^s* (trisomic-*3L*)	2.65 ± 0.08 (8)	2.65 ± 0.16 (8)	1.07 ± 0.09 (16)	1.29 ± 0.03 (8)
Dose-dependent estimate	2.53 ± 0.05	2.22 ± 0.05		1.38 ± 0.04
Repressed estimate	1.69 ± 0.03	1.48 ± 0.03		0.92 ± 0.03

Measurements in right-most column are in units of absorbance by Coomassie blue at 595 nm.

composition (SMITH *et al.* 1981), each is located on a different chromosome arm (*LSP-1α* on the *X*, *LSP-1β* on *2L*, and *LSP-1γ* on *3L*). LSP-1 product levels have been shown to be dosage dependent over a large range of gene numbers when small duplications and deficiencies are employed (ROBERTS, BLACKWELL and LOUGHLIN 1984).

Measurements of LSPs in diploids and trisomies were made from samples of hemolymph collected from wandering third-instar larvae. The amount of each LSP-1 was measured relative to LSP-2 to provide an internal standard. Total amounts of LSP-2, determined from hemolymph samples taken from trisomic-*3L* larvae and their diploid parental strains, are shown in column 5 of Table 7. Elevated, dose-dependent levels of this protein were observed in *3L* trisomies (about 40% over the expected compensated level) suggesting that *LSP-2* gene expression is unaffected by the duplication, that is the amount of product synthesized is proportional to the number of templates. The level of this protein relative to the LSP-1 proteins is also in agreement; the ratios LSP-2/LSP-1α and LSP-2/LSP-1β suggest that LSP-2 is dose dependent in *3L* trisomies and that the expression of the unlinked *LSP-1α* and *LSP-1β* genes are unaffected. Relative to LSP-1γ, equivalent amounts of LSP-2 are observed in the two diploid and in the trisomic strains. Since *LSP-1γ* and *LSP-2* are both located on *3L*, we can deduce that LSP-1γ protein levels are also elevated in trisomic-*3L* larvae. Thus, the expression of each of the four LSP genes in *3L* trisomies was similar to that observed in diploids; the total amount of gene product that accumulates is proportional to the number of templates for each LSP gene.

The quantities of the three LSP-1 subunits produced in trisomic-*2L* larvae are shown in Table 8. The amount of LSP-1γ, relative to LSP-2, does not differ between these trisomies and their diploid parents. Since the structural genes for both of these proteins are located on *3L*, and both are present in two doses in all three genotypes, it would appear that neither gene is affected by a duplication of *2L*. However, the *LSP-1β* gene, which is duplicated in the trisomic condition, produces a level of protein equivalent to that observed in diploids. Thus, this gene is compensating for its increased dosage in *2L* trisomies by synthesizing one-third less product per template. The other LSP-1 gene examined (*LSP-1α* on the *X*) also produced one-third less product in *2L* trisomies than in euploids. Thus, even very closely related genes may respond differently to the regulatory effects exerted by aneuploidy; two of the *LSP-1* genes appear to downregulate while the other is unaffected. The two genes that responded similarly, *LSP-1α* and *LSP-1β*, are more closely related to each other than they are to the gene that failed to respond (SMITH *et al.* 1981). While this relationship seemed to provide a possible explanation for their correlated expression, the activities of these three genes in trisomies for the *X* chromosome suggested otherwise. The expression of *LSP-1β* was not affected in trisomic-*X* larvae (Table 9). In contrast, LSP-1γ protein levels were reduced dramatically in metafemales. This is the only autosomal gene that we have examined whose product is markedly reduced in quantity in metafemales from the level found in diploids. We previously have shown that the *X*-linked *LSP-1α* gene was compensated in metafemales despite the inability of this gene to completely dosage compensate between males and females (DEVLIN, HOLM and GRIGLIATTI 1985). The quantities of LSP-2, relative to the LSP-1 subunits, are similar in metafemales, females and males (Table 9). Thus, duplication of the *X* chromosome does not appear to alter the expression of all LSPs similarly. Indeed, the two genes that do respond similarly (*LSP-1α* and *LSP-1γ*) are the least related in the family. These observations suggest that genes which are evolutionarily closely related do not necessarily respond similarly to the regulatory effects of trisomy (summarized in Figure 2).

***Adh* transformants in trisomies:** The preceding section revealed that very similar genes can respond

TABLE 8

Quantity of LSPs in diploid and trisomic-*2L* hemolymph from female larvae

Genotype	LSP ratio 1β/2	1α/2	1γ/2
Oregon R (diploid)	0.937 ± 0.029 (8)	0.646 ± 0.046 (8)	1.03 ± 0.03 (16)
C(2L)lt; F(2R)bw/F(2R)bw (diploid)	0.833 ± 0.053 (9)	0.664 ± 0.023 (9)	0.827 ± 0.067 (6)
C(2L)lt/+/F(2R)bw (trisomic-*2L*)	0.813 ± 0.024 (9)	0.424 ± 0.022 (9)	1.02 ± 0.17 (6)
Dose-dependent estimate	1.30 ± 0.03	0.655 ± 0.012	0.929 ± 0.032
Repressed estimate	0.868 ± 0.020	0.436 ± 0.008	0.619 ± 0.021

TABLE 9

Quantity of LSPs in males, females and metafemales

Genotype	LSP ratio 1β/2	1γ/2
Oregon R +/*O* (male)	1.06 ± 0.07 (8)	1.04 ± 0.15 (9)
C(1)RM, y pn v/Y (female)	0.756 ± 0.046 (8)	1.04 ± 0.08 (9)
C(1)RM, y pn v/+ (metafemale)	0.913 ± 0.088 (7)	0.521 ± 0.080 (9)
Estimated mean	0.908 ± 0.021	1.04 ± 0.04
Repressed estimate	0.606 ± 0.014	0.693 ± 0.030

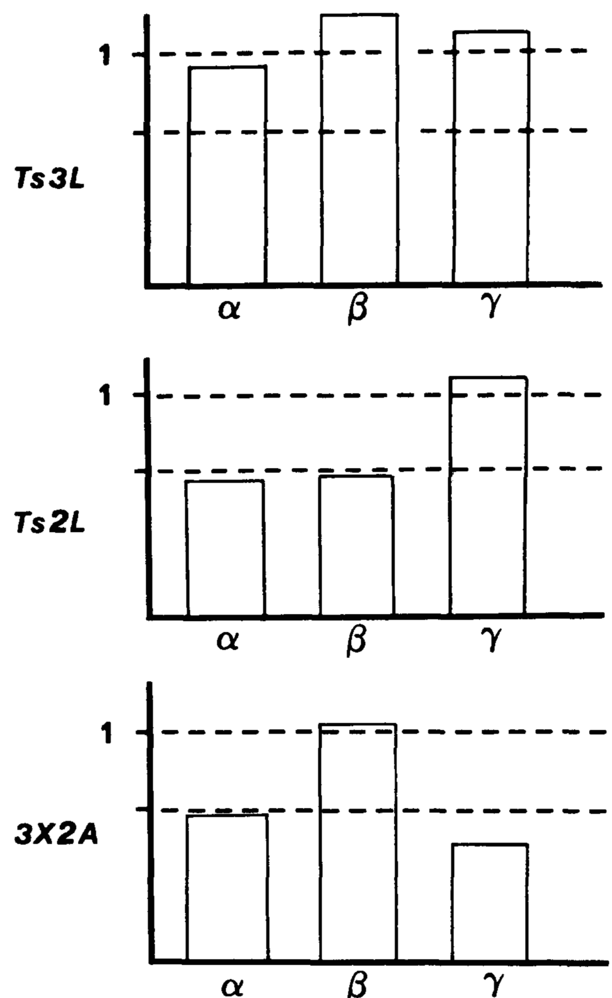

FIGURE 2.—Summary of the expression of *LSP-1* genes in *X*-chromosomal and autosomal trisomies. A value of 1.0 on each *x* axis represents the diploid level of expression per gene. The expression of *LSP-1α* and *LSP-1β* in metafemales is taken from DEVLIN, HOLM and GRIGLIATTI (1985).

differently to the same whole-arm trisomic condition. Two explanations for this result seem plausible. Members of the LSP gene family, while similar at the structural gene level, could have very different regulatory sequences. Alternatively, the response of the *LSP-1* genes to trisomy may be controlled by local differences in chromosome structure. An altered mode of gene expression could arise in trisomies if the duplicated arm carried a regulatory gene capable of modifying the chromatin domain containing the gene being examined.

The objectives of the following experiments were to determine, first, if the expression of a gene not normally affected by a particular trisomy could be rendered sensitive to modulation by transposition to a new chromosomal location and, second, whether a gene (and its associated flanking sequences) which is repressed by a trisomic condition maintains this ability in non-native chromosomal environments. To answer these questions, the expression of single *Adh* structural genes was examined in wild-type and in five transformed strains (the diploid strains possessing transposed copies of *Adh* were synthesized and provided by J. POSAKONY and D. GOLDBERG). The expression of *Adh* genes from each of these six strains was examined in diploids, *2L* trisomies and *3L* trisomies. The same *Adh* gene was examined in all cases (the Fast allele from the strain originally used to clone *Adh*) (GOLDBERG 1980), although the transformed

TABLE 10

Expression of *Adh* from various chromosomal positions in diploid and trisomic-*2L* female larvae

Strain	Chromosomal position	Karyotypic condition		Ratio
		Diploid	Trisomic-*2L*	
w; *Adh*^{F}	35B	2.09 ± 0.16 (4)	2.36 ± 0.22 (6)	1.13
tAP-1	19E	1.75 ± 0.10 (4)	2.02 ± 0.17 (6)	1.15
tAP-3	36A	0.893 ± 0.08 (4)	1.19 ± 0.02 (6)	1.33
tAP-5	17C	2.29 ± 0.11 (4)	2.29 ± 0.18 (6)	1.00
tAP-14	89A	0.339 ± 0.043 (4)	0.493 ± 0.034 (6)	1.45
tAP-17	12A	0.333 ± 0.016 (4)	0.463 ± 0.029 (6)	1.39

Enzyme activity is expressed as the change in absorbance per min per ml of extract per gene copy. Numbers in parentheses are the sample sizes.

strains differed in the amount of flanking *Adh* DNA that they possessed. Strains tAP-1, -3 and -5 have 11.8-kb inserts, including the *Adh* gene, whereas tAP-14 and -17 have only 4.8 kb of *Adh* region DNA. The data in column 3 of Table 10 show that the expression of a single *Adh* gene in diploids can vary considerably when the gene occupies different chromosomal positions. The two strains which display the lowest activity (about 15% of the wild-type level) are both transpositions of only 4.8 kb of *Adh* region DNA. The activities of transposed *Adh* genes in the trisomic-2L condition are also shown. The activities of these genes in trisomies resembles the pattern observed in diploids: *tAP-14* and *tAP-17* had the lowest amount of enzyme activity, *tAP-3* was intermediate, and *tAP-1* and *tAP-5* had nearly wild-type levels. In no case did the transposed *Adh* genes acquire the ability to be repressed in *2L* trisomies.

The expression of *Adh* in its normal position is slightly activated by a duplication of *2L* in these experiments (trisomy: diploid ratio = 1.13), which agrees with our previous findings (see Figure 1). The *Adh* transformants were also activated in trisomies, but to varying degrees. Figure 3 depicts the relationship between the amount of enzyme present in diploids *vs.* the level of activation of this quantity in *2L* trisomies. It is clear that the ability of the *Adh* gene to be activated in *2L* trisomies is negatively correlated with its diploid level of expression. It is noteworthy that the regression line intercepts the *x* axis at 1.49; this would predict that an *Adh* gene expressing at just above the zero level would be activated 1.5-fold by a duplication of *2L*.

The expression of these transposed *Adh* genes was also examined in *3L* trisomies. The expression of this gene (Fast allele) in its native position is repressed by one-third in these trisomies (Table 2). Figure 4 shows that *Adh* genes in non-native chromosome positions can also be repressed by a duplication of *3L*. Strains tAP-5, tAP-3 and tAP-1, which in diploids have levels of activity similar to the wild-type strain (F), are clearly reduced in ADH activity in *3L* trisomies. However, an effect with the low activity strains (tAP-14 and tAP-17) was not obvious.

DISCUSSION

An examination has been made of the numbers and types of genes that are affected by large degrees of aneuploidy in Drosophila (summarized in Figure 1). Not all genes in a given trisomic condition are affected in the same way. If general physiological disturbances were responsible for altered modes of gene expression in trisomies (*e.g.*, reduced RNA polymerase, ribosomes, etc.) then the expression of most genes might be expected to be affected similarly. However, a particular gene can be affected differently by the three trisomies. For example, *Gpdh* is repressed by one-third in *2L* and *3L* trisomies, but not in metafemales. Distinct effects observed for individual loci between different trisomic strains, and the individuality of the enzyme profiles for each trisomy, argue that discrete regulatory mechanisms are operating. While our observations imply the existence of discrete regulatory mechanisms, we have not formally identified any regulatory genes. However, the following discussion assumes that such genes exist and should be identifiable.

Negative regulation in trisomies: The most common regulatory effect observed in trisomies is a one-third repression of gene expression relative to diploids. Table 11 shows that, of the gene-trisomy combinations that differ from the diploid level, 70% are reduced by one-third. This is the type of response that is predicted for loci controlled by concentration-dependent repressors whose structural genes reside on the duplicated arm and are expressed in a dose-dependent manner. Many loci show this type of regulation; of the thirteen loci that were measured in all three trisomies, only three (23%) were not repressed by one-third in at least one of the trisomies. Since approximately 40% of the genome was not duplicated by any of the three trisomies examined, it seems likely that these loci are not exceptional and that negative regulators of their expression may be located either on *2R* or on *3R*. Reductions in the amounts of gene products in trisomies have also been observed for a few loci in yeast (GULLOV and FRIIS 1980), plants (MCDANIEL and RAMAGE 1970; CARLSON 1972; SMITH and CONKLIN 1975; BIRCHLER 1979, 1981; BIRCHLER and NEWTON 1981) and mammals (KLOSE and PUTZ 1983). Alterations in gene product levels in aneuploids presumably result in the wide spectrum of

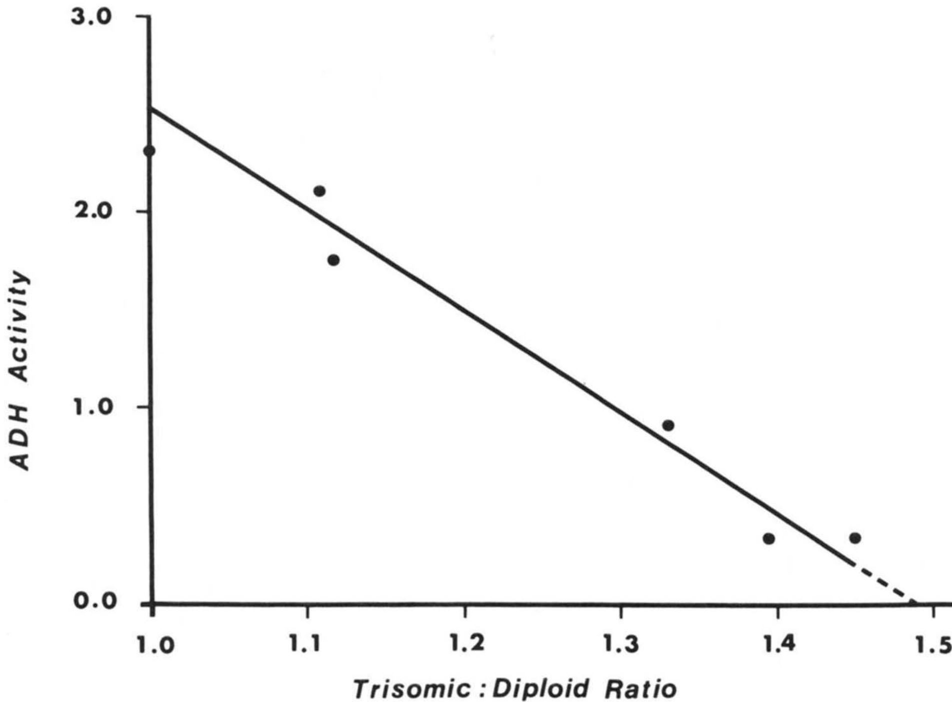

FIGURE 3.—Relationship between ADH enzyme activity in diploids and *2L* trisomies bearing transformed *Adh* genes. The ADH enzyme activity (expressed as the change in absorbance at 340 nm per min per ml of extract) in diploids is plotted on the *y* axis. The ratio of the activity observed in *2L* trisomies to the activity observed in diploids is plotted on the *x* axis. Regression parameters: $r = -0.979$, $b = -0.195$, x intercept = 1.49.

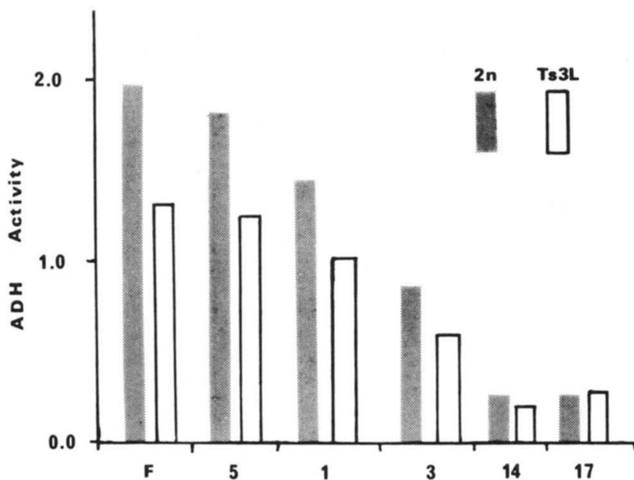

FIGURE 4.—Expression of *Adh* genes in diploids (*hatched bars*) and *3L* trisomies (*open bars*). ADH activity units are the same as in Figure 3.

TABLE 11

Summary of regulatory effects in trisomies

Regulatory effect	Trisomic chromosome			Total
	2L	*3L*	*X*	
I. Loci on Duplicated Arm				
Unaffected	1	3	0	4
Activated	1	0	0	1
Partially repressed	0	0	1	1
Fully compensated	4	4	5	13
II. Unlinked Loci				
Unaffected	6	3	7	16
Activated	1	0	1	2
Partially repressed	1	3	2	6
Repressed by 1/3	5	7	1	13
Total examined	19	20	17	56

Summarized from Figures 1 and 2.

phenotypic and physiological disruptions observed (BLAKESLEE 1922; BRIDGES 1922; LINDSLEY *et al.* 1972) and the actions of regulatory gene products might be in part responsible for these affects (YIELDING 1967; SMITH and CONKLIN 1975; BIRCHLER and NEWTON 1981). The recessive nature of most null mutations suggests that many gene products can be individually tolerated at less than diploid levels with little effect on fitness. However, small reductions in product levels from many genes in trisomies may cumulatively have a negative effect. Hence, the many disruptions observed for trisomies might arise as much from reduced product levels for genes throughout the genome as from overproduction from those genes located within the duplicated region.

Our results imply that most of the enzyme-loci measured in these studies are under the control of at least one negative regulatory gene. Assuming that these regulatory loci also operate in diploids, gene expression would appear to be controlled, in part, by the quantities of repressors that are produced. A comparison of the functions of the genes that were studied reveals that many of their respective enzymes operate in all cells and are physiologically vital ("housekeeping genes"). However, several of the genes examined in this study are inducible, or developmentally regulated, and also appear to be negatively regulated. Even the expression of complex loci such as *scute* or *bithorax* apear to be repressible by concen-

tration-dependent modifiers (LEWIS 1978; BOTAS, DEL PRADO and GARCIA-BELLIDO 1982; KENNISON and RUSSELL 1987). Thus, modulation of gene expression (not induction or inactivation) by negative repressors may operate on most types of genes, independently of developmental cues and in concert with other regulatory mechanisms. Indeed, systems of positive and negative control could be operating simultaneously.

While the functions of such *trans*-acting modulatory loci are unknown, at least some appear to affect RNA synthesis (DEVLIN, GRIGLIATTI and HOLM 1984; GHOSH 1985). Consequently, these regulatory gene products may be fundamental components of the transcription apparatus, and could affect either initiation or elongation. However, because different genes respond independently and differentially to the regulatory effects, the control mechanism may operate on a variety of elements involved in gene expression (including post-transcriptional processes). These regulatory genes may play a specific role in development and/or they may be involved in maintaining homeostasis in euploids as well as trisomies.

The actual number of negative regulatory loci operating in trisomies is not known. A one-regulator to one-gene relationship clearly does not exist, since several loci are modulated in more than one trisomy. Because the number of loci within a chromosome arm is large, it is likely that several regulatory loci are duplicated in each trisomy. A mean of 1.38 trisomies affected each locus for the three aneuploid conditions tested. By an extension of this figure, if the whole genome were surveyed, we would expect an average of 2.3 trisomies to affect each locus. Since there must be at least one regulatory gene on each trisomic arm for an affected locus then, on average, a minimum of between two and three such regulatory loci exist for each gene. Unless the average structural gene can also act as a regulatory gene, or there are more regulatory genes than structural genes, each regulatory gene probably affects the expression of more than one genetic locus. At this point, however, we cannot distinguish between (1) many regulators each operating on a few genes, or (2) a few regulatory loci each operating on many genes.

The modulations which occur in autosomal trisomies have not evolved to cope with variations in gene dose, since natural aneuploidy is not associated with any of these chromosome arms. In the absence of regular alterations in chromosome number, such as those that occur for the sex chromosomes, there would be little selective pressure for regulatory genes and the loci they control to be maintained on the same chromosome arm. Indeed, over a long evolutionary history, rearrangements such as pericentric inversions, translocations, and transpositions would disperse such regulatory loci. This is consistent with the observation that less than half of the loci that show repression in autosomal trisomies are located on the aneuploid arm (Table 11). In contrast, all of the enzyme-loci that were repressed in *X*-chromosomal trisomies were located on the aneuploid arm.

It seems paradoxical that autosomal trisomies can repress the expression of *X*-linked as well as autosomal loci whereas *X*-chromosomal trisomies predominantly affect *X*-linked genes (an exception should be recalled; one autosomal gene, *LSP-1γ*, was repressed in metafemales). Two simple explanations seem plausible. In evolutionary terms, it may not be advantageous to have regulatory genes (operating on autosomal genes whose product levels have adaptive significance) on a chromosome that normally varies in dose. While this should not present a problem if these genes are dosage compensated between males and females, evidence has been presented which suggests that *X*-chromosome dosage compensation may be incomplete (MARONI, KAPLAN and PLAUT 1974). Alternatively, modifiers which regulate autosomal genes may be as abundant on the *X* chromosome as on the autosomes. In metafemale larvae, unlike their autosomal counterparts, elevated quantities of regulatory gene product may not be synthesized because the majority of these genes are themselves dosage compensated at this stage of development. Compensation of such regulatory genes could occur by an extension of regular male-female dosage compensation or by hyperploid compensation of the type observed on the autosomes (DEVLIN, HOLM and GRIGLIATTI 1985).

We have little evidence to suggest how repression might be regulated at the molecular level. For one locus (*Gpdh*), we know that all alleles are active in each cell and that regulation is not mediated by feedback inhibition or by competition for a limited quantity of a factor required for gene expression (DEVLIN, HOLM and GRIGLIATTI 1982). Previous results have shown that the majority of dosage-mediated regulation occurs *in vivo* at the transcriptional level (DEVLIN, GRIGLIATTI and HOLM 1984; GHOSH 1985), although this has not been demonstrated for any single locus. BHADRA and CHATTERJEE (1986) have shown that this regulation is not observed for *in vitro* transcription studies, suggesting that the responsible regulatory molecules (perhaps diffusible) or chromosome structures are lost during preparation of the polytene chromosome templates. If modulation of *Adh* expression by *3L* trisomies is transcriptionally controlled, then the responsible cis-acting regulatory elements are located close to the structural gene, since an *Adh* gene transposed to new chromosomal positions can still be repressed. Conceivably, regulatory molecules could interact with the promoter or other flanking control sequences. In this regard, while the three closely related LSP-1 genes are coordinately regulated at the

RNA level (POWELL *et al.* 1984), we have observed that they respond differently to the various trisomic conditions. Regulation apparently operates on a component of gene expression which is not common (DELANEY *et al.* 1986) to all three genes (*i.e.*, chromosome position, etc.).

Positive regulation in trisomies: Few loci appear to be activated in the trisomic condition. Table 11 shows that of the 56 gene-trisomy combinations examined, only three exhibit levels of product (per template) that are significantly above diploid amounts (ADH and ALDOX in *2L* trisomies, and ME in metafemales). In Drosophila, there are relatively few additional cases of *trans*-activation of gene expression by gene duplication reported (BENTLEY and WILLIAMSON 1985), although increased levels of unlinked enzymes in human trisomy 21 have been observed (MELLMAN *et al.* 1964; MATTEI *et al.* 1982). The paucity of gene activations in trisomies could arise because loci capable of positive regulation of other genes are themselves subjected to autosomal dosage compensation. Alternatively, loci that are capable of regulating other genes in a positive and directly correlated fashion might be extremely rare. This would imply that most of the components required for biosynthesis are present in excess quantities. Indeed, this possibility, which has been suggested as the basis of dominance at the phenotypic level (MULLER 1950; KACSER and BURNS 1981), is further strengthened by the fact that most genes synthesize levels of product that are directly dependent on their dose. Thus, components involved in gene expression appear not to be rate limiting. The lack of gene activation associated with trisomies offers indirect evidence that, for the most part, negative regulatory genes do not operate on other negative regulators.

The expression of *Adh* in various chromosome positions has provided indirect evidence that positive effectors of gene modulation do exist, but that their level of synthesis can be sufficient in wild-type cells so that increased quantities are functionally irrelevant. In diploid strains, different chromosome positions reduce the expression of *Adh* to varying degrees. In trisomies for *2L*, the expression of *Adh* is activated by an amount that is dependent on the diploid expression level of the gene. A gene that is expressed at the wild-type level in euploids is only slightly activated by hyperploidy for *2L*. However, *Adh* genes which have their expression reduced by chromosomal position effects are subject to activity increases as high as 45% in *2L* trisomies. These results indicate that a locus may exist on *2L* which positively regulates *Adh*. An extra copy of this gene in trisomies would result in 1.5 times the diploid level of regulatory product. When *Adh* gene expression is low, the activator may operate in a concentration-dependent way to increase the expression of this locus. When the *Adh* gene is fully expressed (*i.e.*, in its native chromosomal position), the activator may be incapable of increasing expression further because an upper limit for *Adh* gene activity may be specified by other factors. A similar situation has been observed when the expression of a gene is simultaneously under the influence of two transcription enhancers (PELLHAM and BIENZ 1982). It should be noted that a specific transcription factor, Adf-1, has been identified that is required *in vitro* for *Adh* gene transcription (HEBERLEIN, ENGLAND and TIJAN 1985), although it is not yet known whether this factor is encoded by a gene on *2L*.

Positive control has been hypothesized to regulate dosage compensation of the *X*-chromosome in Drosophila (SCHWARTZ 1973; MARONI and PLAUT 1973). *Trans*-acting activators of *X*-chromosome transcription could provide a system of control where *X*-linked templates compete for a limited quantity of regulatory substance. These activator molecules ideally should be autosomally encoded to maintain equivalent levels between males and females. *X*-linked gene expression would be positively correlated with the ratio of autosomes to *X* chromosomes (MARONI and LUCCHESI 1980). In a previous study, we failed to detect elevated transcription levels in *2L* trisomies, relative to diploids, from an *X*-chromosome region containing many genes (DEVLIN, GRIGLIATTI and HOLM 1984). This finding was confirmed and extended by *in vitro* transcription analysis (BHADRA and CHATTERJEE 1986). In the present work, four separate *X*-linked loci were examined in two of the four major autosomal trisomies, and we again detected no increase in *X*-linked activity. Taken together, these findings suggest that if genes encoding transcription activators exist for the *X*-linked loci examined, they may not reside on these autosomal arms (*2L* or *3L*). Alternatively, the effect of such regulatory genes may be minimized because other copies of similar genes, or of genes encoding additional components of a multi-product complex, are located elsewhere in the genome. In its simplest form, the autosomal-activator model predicts that the total amount of *X*-linked gene product synthesized per cell should be independent of the number of *X* chromosomes (MARONI and LUCCHESI 1980; WILLIAMSON and BENTLEY 1983). If the quantity of activator molecule is varied, an equivalent change in *X*-chromosome expression should be observed in males and females. It is therefore noteworthy that hyperploidy for a region of *3R* is capable of activating *X*-chromosome transcription in males but not in females (GHOSH and MUKHERJEE 1986).

Relationship with other regulatory phenomena: Dosage compensation occurs by increasing the level of *X*-linked gene expression in males rather than by decreasing the expression in females (SPRADLING and

RUBIN 1983). This regulation does not appear to be disrupted in autosomal trisomies. While several *X*-linked loci displayed one-third reductions in activity in trisomies for *2L* or for *3L*, the amount of product in males and females remained equal, hence these loci remain dosage compensated. Thus, two opposing regulatory systems—that is, repression by the autosomal duplication and hyperactivation by male-female dosage compensation—are capable of acting in concert. This result implies that these two regulatory systems operate by independent mechanisms (this is not to say that both could not affect the same process, or that both regulatory systems operate through similar mechanisms). We have previously presented evidence suggesting that *X*-linked compensation between euploid males and females and downward regulation observed in trisomic-*X* strains may occur, at least in part, by independent mechanisms (DEVLIN, HOLM and GRIGLIATTI 1985). Support for the hypothesis that male-female dosage compensation and regulation by genetic modifiers operate independently of one another can be obtained from the observations made on *X*-linked gene expression between strains. Marked differences in the expression of several *X*-linked genes exist between strains, presumably a consequence of different modifier loci present in each strain. However, despite the different levels of expression between the strains, equal amounts of activity are observed between males and females within a strain.

A large number of studies have recently reported the existence of trans-acting modifiers that alter the level at which particular enzyme loci are expressed (PIPKIN and HEWITT 1972; MCDONALD and AYALA 1978; BIJLSMA 1980; LAURIE-AHLBERG *et al.* 1980; HORI and TANDA 1981; LAURIE-AHLBERG *et al.* 1981; HORI *et al.* 1982; LAURIE-AHLBERG *et al.* 1982; WILTON *et al.* 1982; MARONI and LAURIE-AHLBERG 1983; KING and MCDONALD 1983; BEWLEY and LAURIE-AHLBERG 1984; MIYASHITA and LAURIE-AHLBERG 1984). Considerable polymorphism of these regulatory genes exists in nature, and, in combination with different allelic states of the structural gene (or closely mapping *cis*-acting regulators), large differences occur in the quantities of enzymes synthesized. *Trans*-acting modification of gene expression in trisomies and in wild strains displaying altered enzyme levels could be mediated by the same regulatory loci. In the former, the quantity of regulatory product is altered by gene dosage, while in the latter this arises from allelic variation. The differential expression of these *trans*-acting regulatory loci has the potential to control the expression of genes, or groups of genes, on a developmental or evolutionary time scale (BRITTEN and DAVIDSON 1969; WILSON 1976).

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