GENE EXPRESSION IN AND DEVELOPMENT OF TRISOMIES OF *DROSOPHILA MELANOGASTER*

by

ROBERT HARRY DEVLIN

B.Sc., University of British Columbia, 1978

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

In the Department

of

Zoology

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December, 1984

© Robert Harry Devlin, 1984

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Zoology

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date Jan 21, 1985

ABSTRACT

Drosophila melanogaster individuals trisomic for an entire chromosome arm can survive to late stages of pupal development. To examine gene expression in these hyperploids, the levels of five enzymes whose structural genes are located on the left arm of chromosome two have been examined both in aneuploid and in diploid strains. Elevated levels of enzyme activity were observed in larvae possessing small segmental duplications for these genes. However, in 2L trisomies, the three distally mapping loci showed compensated levels of expression close to that observed in the diploid strains. Analysis of electrophoretic variants revealed that for one of these compensated loci all three alleles were expressed in trisomies. Two proximally located genes displayed dose-dependent levels of enzyme activity. For most genes, autosomal compensation appears to be very discrete: either the expression of the gene is repressed or it is not. To extend these observations, and to determine if autosomal compensation was peculiar to the left arm of chromosome two, trisomies for the X, for 2R, and for 3L also were examined. Compensating and non-compensating loci were also found on 3L, whereas all loci examined in X-chromosomal trisomies were dosage compensated. This suggests that X-chromosomal and autosomal trisomies are not necessarily analagous. Dosage compensation in X-chromosomal trisomies (metafemales) may occur exclusively or partially by the mechanism that operates between euploid males and females. However, some compensation in X trisomies may occur by

regulatory controls distinct from male-female dosage compensation as indicated by the following results. The expression of LSP-1a, a gene that normally escapes complete dosage compensation in diploid males, was fully compensated in trisomic-X larvae. Possibly, compensation of this gene in these individuals was mediated by regulatory mechanisms other than those controlling male-female dosage compensation. As such, loci that normally do not reside on the X chromosome, but which have been transposed to this chromosome, might be expected to escape compensation in metafemales. This appears to be the case; an Adh gene that had been transposed from the second to the X chromosome was expressed at a similar level (per gene) in metafemales and females. In addition, a native X-chromosomal locus appeared to be compensated between males and females, but was not compensated in X-chromosomal trisomies. Thus, some X-linked loci escape regulation by dosage compensation in metafemales. It is possible that some of the regulatory systems operating in X-chromosomal and autosomal trisomies are analagous, and reflect a common form hyperploid compensation.

The level at which compensation occured was investigated by measuring the quantities of RNA produced by several genes in whole-arm trisomies. For the heat-shock gene, hsp 83, compensation for protein levels appeared to be post-transcriptionally regulated. However, measurements of RNA synthesis on salivary gland polytene chromosomes revealed that for most of the genes compensation was transcriptionally regulated.

Dosage compensation on the autosomes probably reflects the existence of a system that normally operates in diploids to control gene expression by negative regulation.

TABLE OF CONTENTS

PAGE

ABSTRACT..... ii

TABLE OF CONTENTS..... v

LIST OF TABLES..... vii

LIST OF FIGURES..... x

ACKNOWLEDGEMENTS..... xii

GENERAL INTRODUCTION..... 1

CHAPTER 1. MEASUREMENTS OF GENE PRODUCT LEVELS IN TRISOMIC-2L LARVAE

I. Introduction..... 19

II. Materials and Methods..... 20

III. Results..... 28

IV. Discussion..... 39

CHAPTER 2. MEASUREMENTS OF GENE PRODUCT LEVELS IN TRISOMIC-3 AND TRISOMIC-X LARVAE

I. Introduction..... 47

II. Materials and Methods..... 49

III. Results..... 60

IV. Discussion..... 79

CHAPTER 3. INFLUENCE OF TRISOMY ON THE EXPRESSION OF LOCI MAPPING OUTSIDE OF THE DUPLICATED REGIONS

I. Introduction..... 93

II. Materials and Methods..... 96

III. Results..... 101

IV. Discussion..... 130

CHAPTER 4. EFFECT OF EXTENSIVE ANEUPLOIDY ON VIABILITY

AND PHENOTYPE

I. Introduction..... 151

II. Materials and Methods..... 155

III. Results..... 158

IV. Discussion..... 217

CHAPTER 5. COMPENSATION IN 2L TRISOMIES IS TRANSCRIPTIONALLY REGULATED

I. Introduction..... 228

II. Materials and Methods..... 231

III. Results..... 236

IV. Discussion..... 258

CHAPTER 6. REGULATION OF DOSAGE COMPENSATION IN METAFEMALES

I. Introduction..... 265

II. Materials and Methods..... 267

III. Results..... 271

IV. Discussion..... 288

BIBLIOGRAPHY..... 293

LIST OF TABLES

TABLE		PAGE
1.	Biochemical loci examined in this study.....	27
2.	Enzyme levels for compensating loci in diploid and trisomic-2L larvae.....	30
3.	Enzyme levels for noncompensating loci in diploid and trisomic-2L larvae.....	31
4.	Enzyme levels for two unlinked loci in diploid and trisomic-2L larvae.....	32
5.	Dose dependence of enzyme levels in small segmental aneuploids in late third instar larvae.....	36
6.	Enzyme levels for compensating loci in diploid and trisomic-3L larvae.....	62
7.	Enzyme levels for noncompensating loci in diploid and trisomic-3L larvae.....	63
8.	Enzyme levels in diploid and trisomic-2R larvae.....	64
9.	Enzyme levels in males, females, and metafemales.....	69
10.	Heat-shock protein 68 and 83 synthesis in diploid and trisomic-3L salivary glands.....	73
11.	Small heat-shock protein synthesis in diploid and trisomic-3L salivary glands.....	75
12.	Heat-shock RNA 83 levels in fat bodies and salivary glands.....	77
13.	Activity of third chromosome enzyme loci in 2L trisomies.....	103
14.	Enzyme activity for a 2R-linked enzyme locus in 2L trisomies.....	104
15.	Activity of second chromosome enzyme loci in 3L trisomies.....	106
16.	Activity of third chromosome enzyme loci in 3L trisomies.....	107
17.	Activity of a 2L-linked enzyme locus in 2R trisomies.....	108
18.	Activity of second chromosome enzyme loci in metafemales.....	110

19. Activity of third chromosome enzyme loci in metafemales..... 111

20. X-linked enzyme levels in Oregon R iso-X strains..... 113

21. X-linked enzyme levels in C(2L)lt;F(2R)bw/F(2R)bw; iso-X strain..... 113

22. X-linked enzyme levels in C(3L)ru st; F(3R)e^{s}/ F(3R)e^{s}; iso-X strain..... 114

23. X-linked enzyme levels in C(2L)lt/+/F(2R)bw; iso-X trisomic-2L individuals..... 115

24. X-linked enzyme levels in C(3L)ru st/+/F(3R)e^{s}; iso-X trisomic-3L individuals..... 115

25. X-linked enzyme levels in diploid and trisomic-2L larvae..... 116

26. X-linked enzyme levels in diploid and trisomic-3L larvae..... 117

27. Quantity of LSPs in diploid and trisomic-3L hemolymph from female larvae..... 120

28. Quantity of LSPs in diploid and trisomic-2L hemolymph from female larvae..... 122

29. Quantity of LSPs in males, females, and metafemales..... 124

30. Expression of Adh from various chromosomal positions in diploid and trisomic-2L larvae..... 126

31. Summary of regulatory effects on enzyme loci in trisomies..... 132

32. Embryonic viability of whole arm trisomies..... 162

33. Inferred viability of selected aneuploids from the crosses in Table 32..... 163

34. Viability of double trisomies and triploids deficient for one autosomal arm..... 174

35. Inferred viabilities of selected aneuploids from the crosses in Table 33..... 175

36. Survival of autosomal monosomics..... 177

37. Number of sex-comb teeth in normal and sex-transformed trisomies..... 182

38. Effect of da and Fl on the embryonic viability of autosomal trisomies..... 187

39. Survival to late pupae of autosomal trisomies heterozygous for Fl and possessing different doses of da^{+}..... 190

40. Expression of dominant markers in trisomies..... 192

41. Expression of Bar variegator in trisomic-2L males..... 194

42. Expression of sc^{v2} in aneuploids for 2L and 3L..... 196

43. Enzyme levels in fat bodies from diploid and trisomic-2R female larvae..... 198

44. Live weight, protein content and DNA content in female diploid and trisomic larvae..... 212

45. Estimates of cell size in diploid and triploid, and trisomic pupae..... 216

46. ^{3}H-uridine incorporation into regions of polytene chromosomes from diploid and trisomic larvae..... 240

47. Repression of grain counts over 2L or regions of 2L on grain counts over 61A-68C..... 244

48. Amount of LSP-1b and ADH mRNAs relative to LSP-1g..... 254

49. X-linked Adh gene activity in males, females, and metafemales..... 275

50. Relative amounts of LSPs in euploid strains..... 278

51. Relative amounts of LSPs in aneuploid strains..... 279

52. Filter hybridization of LSP-1 RNA..... 281

53. Relative amounts of LSPs in L82 strain..... 284

54. Mapping $LSP\text{-}1^{L82}$ by recombination..... 286

LIST OF FIGURES

FIGURE		PAGE
1.	Mating procedure utilized to produce trisomic larvae...	22
2.	Levels of GPDH and ADH enzyme in diploids and trisomies possessing the same active alleles for *Gpdh* and *Adh*.....	34
3.	Electrophoretic analysis of GPDH in diploid and trisomic-2L larvae.....	38
4.	Summary bar graph of enzyme quantities in 2L trisomies relative to diploids.....	42
5.	Strategy employed to produce compound-free chromosomes for the third chromosome.....	51
6.	Cytological preparations of chromosome F(3R)VD1e^{s} in diploid and trisomic larvae.....	52
7.	Electrophoretic analysis of PGM in diploid and trisomic-3L pupae.....	67
8.	Autoradiograms of heat-shock protein and RNA from salivary glands.....	72
9.	Summary bar graph of enzyme quantities in 3L trisomies relative to diploids.....	82
10.	Electrophoretic analysis of larval serum proteins on cellulose-acetate strips and in SDS-polyacrylamide gels.....	100
11.	Relationship between ADH enzyme activity in diploids and 2L trisomies bearing transformed *Adh* genes.....	129
12.	Summary of the expression level of genes in X-chromosomal and autosomal trisomies.....	135
13.	Number of trisomies affecting individual loci.....	138
14.	Viability to late third-instar larvae of partial trisomies for 2L.....	160
15.	Post embryonic viabilities of the five major whole arm trisomies.....	166
16.	Viability of 2L and 3L trisomies at different temperatures.....	170
17.	Sex ratio of autosomal trisomies at different developmental stages.....	173

18. Phenotypes of trisomies..... 180

19. Sex-comb morphology of diploid and trisomic pupae either possessing sex transforming mutations or not.... 185

20. Comparison of enzyme quantites from whole larvae and from isolated fat body..... 200

21. Kinetic measurements on GPDH from diploids and 2L trisomies..... 203

22. Relationship between PGI activity and larval size in female and male 2L trisomies..... 206

23. Relationship between GPDH activity and larval size in female and male 2L trisomies..... 208

24. Relationship between ADH activity and larval size in female and male 2L trisomies..... 210

25. Electron micrographs of eye facets from diploids, triploids, and trisomies..... 214

26. Transcription autoradiogram of salivary gland polytene chromosomes from a trisomic-2L female larvae..... 238

27. Relationship between incorporation of ^{3}H-uridine into 2L vs region 61A-68C of 3L in diploids and 2L trisomies..... 243

28. Transcription in four approximately equal subdivisions of 2L in diploids and 2L trisomies. 248

29. Quantites of LSP genes and mRNA in diploids and trisomies..... 252

30. Relationship between ADH enzyme activity and ADH mRNA in three strains..... 256

31. Quantity of Malic enzyme in diploid males and females and in metafemales..... 273

32. Analysis of a mutation that reduces the amount of LSP-1a protein and RNA..... 283

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Tom Grigliatii and Dave Holm, for their support, encouragement and advice, and for calmly tolerating my idiosyncracies. Throughout the course of this study, they, and Don Sinclair, have provided a source of knowledge and wisdom for me to draw from. I must especially thank Don, Peter Cserjesi and Carey Johnson for providing day-to-day discussions about much of this research. Many others have allowed this research to unfold, noteably Karen Morin, Hugh Brock, Tom Mommson, Grant Pogson, and Ruth and Budd Devlin, for which I am most appreciative.

A special thank you is deserved by my wife, Drew Devlin. She has contributed to this work immeasureably by providing emmotional support and by helping with the production of the manuscripts. I could not have completed this work without her.

General Introduction

The number of chromosomes usually does not vary between members of the same sex within a species or between cells of the same organism. Constancy of genetic material appears to be an important prerequisite for developmental and evolutionary stability. Remarkable meiotic and mitotic machinery ensures proper disjunction of chromosomes such that equivalent karyotypes are maintained. However, because considerable karyotypic variability is observed between species, changes to genome structure and size appear to be fundamental events associated with the evolutionary process.

Several processes can introduce variation in gene content within a species; such alterations introduce new potential for genetic diversity into a population. Changes in the number of chromosome sets per nucleus is termed euploidy while changes in the number of chromosomes or parts of chromosomes is termed aneuploidy. The latter condition changes the genetic balance by adding or removing particular chromosome regions. Alterations to the dose of a structural gene is usually directly correlated with the quantity of gene product which accumulates in the cell. This appears to be true in a wide variety of organisms (Jacob and Monod, 1961; Grell, 1962; Garen and Garen, 1963; Prittard and Ramakrishnan, 1964; Stetson and Somerville, 1971; Ferguson-Smith et al., 1973; Alledice and Tedesco, 1975; Chandler and Prittard, 1975; O'Brien and McIntyre, 1976; Metzenberg and Chia, 1979; Gerbaud and Guerineau, 1980; Fasiolo et al., 1981). Thus, availability of a gene for transcription

appears to be a limiting factor controlling gene expression.

In many species, females have twice as many X chromosomes as males. This imbalance in gene content is rectified by dosage compensation, a regulatory mechanism that equalizes gene expression between individuals possessing one or two X chromosomes. Previous studies on aneuploidy in metazoans have not revealed the existence of autosomal dosage compensation. Individuals heterozygous for either a deficiency or a duplication of a particular autosomal gene produce, respectively, 0.5 and 1.5 times the amount of gene product found in normal diploid individuals.

The purpose of this study was to determine the effect of large degrees of aneuploidy on gene expression in Drosophila melanogaster. To this end, the products of several biochemical loci have been quantified in individuals that are trisomic for an entire chromosome arm and in diploid controls. These studies showed that, in trisomies, many gene products are observed at diploid quantities despite an increase in gene dosage from 2 to 3. Thus, in Drosophila, dosage compensation is not limited to the sex chromosomes, but can also occur in large autosomal aneuploids. As such, this thesis has attempted to: 1) document the extent to which autosomal compensation occurs, 2) determine at what level of gene expression it is regulated and 3) examine the relationship between autosomal and sex chromosomal dosage compensation.

Male-female dosage compensation in Drosophila has been reviewed by Lucchesi (1973; 1978; 1983), Stewart and Merriam (1980), Mukherjee (1982), and Baker and Belote (1983). The

remainder of this introduction summarizes some pertinent observations made on dosage compensation. Many aspects of this material are discussed further in relevant parts of the thesis. The reader familiar with Drosophila genetics can leave the introduction at this point and proceed directly to the experimental section of chapter one.

Naturally occurring differences in gene dosage are observed in some organisms. In mealy bugs, a difference in gene dosage exists but it is only functional: an entire chromosome set is inactivated in one sex (Chandra and Brown, 1974). Consequently, haploid cells are reduced in size, and to compensate for this difference, more cells are present per organism (Berlowitz et al., 1968). In Habrobracon (wasps), haploid individuals are fertile males while diploids are female (Whiting, 1943). Compensation for differences in gene dose in polytenic nuclei of these individuals is accomplished by allowing an extra round of DNA replication to occur in males (Rasch et al., 1977). It is not surprising that organisms have evolved systems to compensate for differences in gene dose, since the consequences of aneuploidy are often severe.

Variation in chromosome number is associated with sex determination in some organisms (Bull, 1983). Both in man and in Drosophila, the female possesses two X chromosomes, whereas the male has only one (the male also possesses a Y chromosome which in Drosophila contains a paucity of genes, and is not required for survival). In some Drosophila species, this can result in a ploidy difference for up to sixty percent of the genome (McKnight, 1939). Nontheless, the levels of gene

products produced from most loci located on the sex chromosomes are equivalent between the sexes, a phenomenon termed dosage compensation. This particular condition,both in terms of viability and gene expression, appears to defy the normal responses to aneuploidy. One can readily appreciate the importance of such a regulatory mechanism for maintaining the balance of gene expression within the cell. Presumably most metabolic processes are identical between males and females and are optimum when a specific level of product is present. Dosage compensation is found in several different phyla in the animal kingdom (Lyon, 1972; Muller et al., 1931; Casartelli, 1968; Meneely and Wood, 1984; Hodgkin, 1983; Arora and Rao, 1979; Rao and Ali, 1982) and occurs via different mechanisms.

In eutherian mammals, the mechanism of dosage compensation involves inactivation of one of the X chromosomes in the somatic cells of the females (Gartler and Riggs, 1983). Random condensation of this chromosome into a heteropycnotic element occurs during early development (Kozak, 1975) and produces a mosaic of clonally inherited tissue with one or the other of the X chromosomes inactive in gene expression. This effectively reduces the female cell to an X/O condition, and equalizes the X-linked expression between male and female cells. What controls condensation in eutherians is not known. The use of X:Autosome translocations has revealed that, in mouse, the mechanism acts subchromosomally since both pieces of such a translocation can condense (Cattanach, 1975). In man, a single inactivation centre is favoured (Gartler and Riggs, 1983). Maintenance of the inactivated state may be mediated partly by

methylation of cytosine since treatment with 5-azacytidine can reactivate genes from a condensed X chromosome (Mohandes et al., 1981). Marsupials also display dosage compensation, but a major difference from the eutherian system is that the paternal X is preferentially inactivated in females (Cooper et al., 1975). It is interesting that non-random inactivation also occurs in those eutherian embryonic cells that do not give rise to the fetus (Tagaki and Sasaki, 1975). This suggests an imprinting mechanism may exist in the male germ cells to mark X chromosomes and a countersystem may exist in the eutherian female to remove it. In mammalian spermatocytes the X chromosome is found in a completely inactive form (Lifschytz, 1974), somewhat analagous with the Barr body in the female soma. It is easy to imagine that in marsupial evolution, selection may have favored the continued repression of gene activity of this paternal X after transmission to the zygote. In eutherians, the factors responsible for condensation presumably could diffuse randomly between maternally and paternally derived X's (Chandra and Brown, 1974).

In 1922, Bridges suggested that the differences in form and function observed between males and females are the direct result of dosage differences arising from the haplo-X condition in males. The X chromosome was considered to contain an abundance of female determining modifier genes, whereas the autosomes contain a preponderance of male determining loci. As such, a cell's determined fate to become male or female would depend on the ratio of X chromosome material to that of the autosomes. In a 2X condition the balance is tipped in favor of

female while in the haplo X it is invariably male. He supported his idea with observations on whole chromosome heteroploids (Bridges, 1925). A triploid female designated 3X:3A (this denotes the number of X chromosomes and the number of autosome sets, ratio X/A = 1), was virtually identical to that of a diploid female (2X:2A, ratio = 1). 1X:3A individuals were invariably male (often termed metamales, ratio = .33) and 3X:2A individuals were female (termed metafemales, ratio = 1.5). The X:Autosome ratio appeared to control the sex determination of a cell-- .5 or below yields a male, 1 or above yields a female. Individuals possessing a 2X:3A karyotype have a ratio of .66, intermediate between the male and female level, and accordingly, have cells that are either male or female (hence their name, intersexes). It is important to note that the cells can be either male or female but not intermediate, which suggests that determination and subsequent differentiation is limited to a binary mode of action. Bridges suggested that the characteristic male phenotype displayed by haplo-X individuals was a normal consequence of changing the balance of modifiers within the genome, and was no different in principle to the characteristic phenotype displayed by other chromosomally haploid individuals (ie. haplo-4 individuals).

The majority of characteristics in the male and female organism are similar, both at the morphological and biochemical levels. Bridges suggested that nonsex-determining loci on the X chromosome had a very highly balanced set of positive and negative modifiers located on the same chromosome, and thus variation in X-chromosome number would have little effect.

Muller et al. (1931) postulated that dosage compensation is brought about by X-linked modifiers that are dose dependent and inhibitory in nature. Since the inhibitory modifiers vary directly with the ploidy of the X chromosome, males and females would display no net difference in X-linked gene expression. Muller originated the term "dosage compensation" to describe this phenomenon.

Goldschmidt (1956) hypothesized that dosage compensation need not be mediated by genetic modifiers but instead could result from developmental and physiological differences that normally exist between the sexes. Thus, modulation of X-linked gene expression would be controlled indirectly by sex-determining loci located throughout the genome.

With these hypotheses in mind, it is now pertinent to examine the experimental evidence confirming the existence of dosage compensation in Drosophila. Muller (1950) described some early work utilizing the hypomorphic eye mutation $\underline{w^m}$. This mutant contains only a small fraction of the wild-type quantity of eye pigment and enables one to visually determine the levels of pigment in a haplo-X male and diplo-X female. No difference was observed between euploid males and females. A duplication for the w^m locus doubled pigment levels in males, but resulted in only a fifty percent increase in the female. Clearly then, for each $\underline{w^m}$ allele, the male possesses twice as much pigment as does the female. These observations have been confirmed using spectrophotometric quantitation of extracted eye pigments and have been extended with measurements using wild

type alleles of the white locus (Smith and Lucchesi, 1969). Komma (1966), Seecof et al. (1969), and Bowman and Simpson (1973) demonstrated that the two X-linked enzymes G6PDH and 6PGDH displayed identical maximum reaction velocities in males and females. Observing the phenotypic end product or protein product of a gene is subject to many interactions with other loci, and could be misleading.

Dosage compensation could be mediated by processes ranging from DNA replication to post translational modifications. Mukherjee and Beerman (1965) demonstrated that total RNA synthesis on larval salivary gland polytene X chromosomes is equivalent between male and female. Other evidence also strongly suggests that the phenomenon is transcriptionally regulated (Roehrdanz and Lucchesi, 1977). Since enzyme activites appear to be dose dependent in segmental aneuploids, it seems evident that a factor limiting product levels appears to be the availability of a gene for transcription. Other intermediate processes appear not to be rate limiting. Thus, measurements of enzyme activities indirectly represent the rate of transcription of the structural gene coding for it.

Using electrophoretic variants of 6PGD, Kazazian et al. (1966) demonstrated that both X chromosomes of the female were active simultaneously and that the level of expression of each was equivalent. (This was previously suspected on genetic grounds; individuals simultaneously heterozygous for mutant and wild type alleles at two X-linked loci are phenotypically wild type.) If dosage compensation is controlled at the transcriptional level, then it is possible that either the male

X chromosome is hyperactived or that transcription from the female X's is being supressed. Offerman (1936) observed a difference in the cytological properties of the male and female polytene X chromosomes. The single male X appeared to be paler staining and swollen in thickness to almost the size of the paired autosomal arms. In contrast, the female X's appeared cytologically more like autosomal regions. It was not known whether this difference was the result of chromosomes being in an unpaired condition, or if it reflected an important aspect of male X-chromosome structure. Dobzhansky (1957) observed that in D. insularis x D. tropicalis hybrids, all chromosome arms remain in the asynapsed condition, and that the male X chromosome was invariably larger and paler staining than either the female's X chromosomes or the autosomes. Aronson et al. (1954) and Laird et al. (1980) have demonstrated that indeed the male polytene X contains only one half the DNA of the female, but that it is ten percent richer in proteins. Khesin (1973) and Leibovitch et al. (1976) found no difference in the capacity of the male or female polytene X chromosomes to bind E. coli RNA polymerase I. However, Leibovitch and Bogdanova (1983) found that the male polytene X possesses a higher affinity than the female X for exogenous histone H1 in vitro. Template activity in vitro for E. coli polymerase I is twice as high on a male X than on a female's (Khesin et al., 1974; Leibobvitch et al., 1976; Chatterjee and Mukherjee, 1981). This difference can be abolished by treating the chromosomes with a 1M salt wash prior to transcription analysis (Chatterjee et al., 1981) suggesting that a

non-covalently bound chromatin factor is responsible for hyperactivation. DNA replication also occurs at a faster rate on the male X chromosome (Berendes, 1966). Taken together, this evidence implies that a structural difference exists in the chromatin of the male X that allows an increased rate of transcription. Considering this effect is observed on fixed chromosomes and noting the above mentioned cytological characteristics, it is possible that the chromatin of the male X is less tightly compacted, making it easier for polymerase to gain access or to actively transcribe a gene. The effect may be mediated by nucleosome packing density, since a deficiency for the histone gene cluster (which presumably results in an intracellular deficiency of histone proteins) increases the rate of X-chromosome transcription in vivo (Khesin and Leibovitch, 1978). However, it should be noted that Eissenberg and Lucchesi (1983) did not detect a differential sensitivity of the X-linked heat shock genes in D. pseudoobscura to digestion by DNAse I.

The compensated response is not affected when an X-linked gene in a surrounding piece of X-chromosome is inserted into another region of the genome (Seecof et al., 1969; Tobler et al., 1971; Baillie and Chovnick, 1971; Bowman and Simpson, 1973). Similarly, a large autosomal segment transposed to the X chromosome is insensitive to the dosage compensation mechanism (Roehrdanz et al., 1977). Tobler (1975) investigated dosage compensation for the enzyme tryptophan pyrolase in stocks of flies that had been transformed with wild-type DNA. These

stocks were mutant for vermillion, the X-linked gene coding for the enyzme, but had the wild-type gene sequence inserted in a new chromosomal position (Fox et al., 1971). These flies displayed dosage compensation. More recently, P-element mediated transformation (Rubin and Spradling, 1982) has allowed a closer examination of this question. Autosomal genes transposed to the X chromosome can acquire the ability to dosage compensate (Spradling and Rubin, 1983; Scholnick et al., 1983; J. Posakony, personal communication). Hyperactivation of these genes occurs in males, although the magnitude may be somewhat less than two fold the female level of expression. Hazelrigg et al., (1984) has found that sequences mediating hyperactivation of the X-linked white gene are located within 12kb surrounding the structural gene. Mutations (Muller, 1950) and wild types genes (Roberts and Evans-Roberts, 1979; Ota et al., 1981; Brock and Roberts, 1982) are known which do not dosage compensate. Presumably these exceptions represent cases where the regulatory element has been damaged or has not been acquired during the course of evolution. Korge (1981) has demonstrated that a site regulating the ability of the X-linked gene Sgs-4 to dosage compensate is recombinationally separable from the structural gene. These results imply that compensation is propagated from many regulatory sites distributed throughout the X chromosome, and that activation is a cis-acting alteration to chromatin structure.

As previously discussed, Goldschmidt felt that dosage compensation could be mediated by the normal physiological differences between the sexes. However, Muller (1950), Komma

(1966), and Smith and Lucchesi (1968) demonstrated that the expression of the X chromosome remained unaffected by mutations which phenotypically transform females into the opposite sex, or males into intersexes. Goldschmidt (1956) rebutted this observation by claiming that these transformed individuals actually had not changed sex in a physiological sense, only morphologically. Lakhotia and Mukherjee (1969) examined transcription in female individuals possessing some haplo X cells in their salivary glands, and found that all cell types behaved autonomously. The sex of the organism had no effect on dosage compensation, and it was concluded that the regulation was a cellular phenomenon. Indeed, aneuploid Drosophila tissue culture cells with from one to six X chromosomes all express identical levels of X-linked gene product (Kakpakov et al., 1969; Gvozdev et al., 1971; Nikoshkov and Kakpakov, 1981).

Stewart and Merriam (1975), and Faizullin and Gvozdev (1973) designed experiments to detect inhibitory control loci on the X chromosome, as predicted by Muller's hypothesis. Dosage compensation was only reported with changes involving a whole X chromosome. If a fraction of the chromosome was added, expression of enzyme loci appeared to be dose dependent. Furthermore, duplication of a region outside the structural gene for an enzyme had little effect on its expression. Thus, these authors concluded that modifiers which could inhibit expression of loci in females to the level found in the male were not present on the X chromosome. However, Birchler (1980) has recently described, in abstract form only, some preliminary observations which do support Muller's hypothesis. He finds

specific regions of the X that when varied in dose, have an inverse effect on expression of loci outside the aneuploid region. A decision on whether these regions reflect actual regulatory loci awaits further experimentation.

If dosage compensation is controlled by modifiers within the X chromosome itself, altering autosomal regions should have no effect. No difference in X-linked gene product per unit of DNA is found between diploid and triploid individuals, either monitoring enzyme levels (Seecof et al., 1969; Lucchesi and Rawls, 1973a; Faizullin and Gvozdev, 1973; Maroni and Plaut, 1973a) or transcription (Maroni and Plaut, 1973b; Ananiev et al., 1974). However, triploid cells (3X:3A) are larger (Dobzhansky, 1929) and contain 1.5 times as much gene product as diploid cells (2X:2A). Autosomal triploids have been examined with from one to three X chromosomes; 3X:3A individuals have identical levels of product as 2X:3A intersexes (Seecof et al., 1969; Ananiev et al., 1974; Lucchesi and Rawls, 1973b) and both have only slightly more product than a triploid metamale (1X:3A) (Lucchesi et al., 1977). Moreover, metafemales (3X:2A) dosage compensate (Lucchesi et al., 1974; Stewart and Merriam, 1975), whereas triploids (3X:3A) (possessing the same number of X's) have a fifty percent increase in X-linked gene activity. It is clear then that the ploidy of the autosomes has a profound effect on X-linked gene expression. Thus, the level of X-linked product per cell is not dependent on the number of X chromosomes present, but rather on the ploidy of the autosomes. However, the transcriptional rate of an X-linked gene is dependent both on the number of autosome sets and on the number of other X

chromosomes within the cell. In other words, X-linked gene transcription is proportional to the ratio of autosome sets to X chromosomes. This prompted the hypothesis that X-chromosome transcription is dependent on an autosomally produced activator which exists in limited quantity within the cell (Maroni and Plaut, 1973b; Schwartz, 1973; Lucchesi, 1977). A requirement of this hypothesis is that the activator be restricted in action to X-linked genes. In a diploid male cell, the single X would be utilizing all the activator. In the female, the two X's would be in competition for the same quantity of activator and each would express at only one-half the rate of the male X. In triploids, the total amount of activator would be increased per cell by fifty percent, which produces a proportional increase in X chromosome transcription rate.

A complication exists when the X/A ratio is altered by adding an additional autosome set. Cell size is increased accordingly and the concentration of regulatory molecules (per gene) would be reduced. If concentration dependent X-linked repressors of X-chromosome transcription were regulating dosage compensation, then the expression from this chromosome would be directly correlated with nuclear volume. For example, the same amount of X-linked repressor would be produced in 2X:2A and 2X:3A individuals but since the nuclear volume is increased by 1.5 in the latter, a concomitant one-third reduction in the concentration of regulatory molecules would result. Thus, both positive and negative theories of regulation are compatible with the data obtained for X-chromosome transcription between diploids and triploids. However, Maroni and Lucchesi (1980)

tested the autosomal activation hypothesis by varying the X/A ratio with X-chromosome aneuploids. All of these individuals possessed a diploid set of autosomes. Individuals possessing two X's transcribed at .5 the rate of a male, while those possessing 1.5 X's transcribed at .66 the male rate. Thus, even in diploids (presumably with the same cell size) X-chromosome transcription was inversely dependent on the X/A ratio. Similar observations have been made in diploid females possessing additional X-chromosome fragments (Williamson and Bentley, 1983).

Baker and Ridge (1980) have demonstrated that sex differentiation in Drosophila is a cellular, rather than an organismal process. Thus, triploid intersexes (2X:3A) possess cells which are either male or female. The average expression of their X chromosomes is intermediate between male and female. If cells can transcribe their X-chromosomes only at either the male or female rate, then both high and low expressing nuclei are expected to be found. Maroni and Plaut (1973b) measured X-chromosome transcription in individual nuclei from 2X:3A intersexes, and found a unimodal distribution of activities. This suggests that sex determination and dosage compensation may be controlled by separate mechanisms. Measurements of transcription on the polytene X chromosome averages the expression of many separate genes and many copies of each gene. It is possible that individual genes on each chromosome, when subjected to intermediate levels of regulation, are expressed at either the male or female rate, the average of which is intermediate between the two. Alternatively, dosage

compensation may be a flexible phenomenon capable of multiple levels of expression, whereas the differentiated sexual phenotype is limited either to male or to female.

However, genetic analysis of the Sex lethal (Sxl) locus (Cline 1978, 1979, 1983, 1984) has revealed that dosage compensation and sex determination do have a common regulatory foundation. The expression of this gene is required in females both to prevent hyperactivation of the X chromosome (Lucchesi and Skripsky, 1981) and to maintain the female state of sexual development (Sanchez and Nothiger, 1982; 1983). Necessarily, this gene must be repressed in males; constitutive mutants at this locus are male lethals, since these individuals die if X-chromosome hyperactivation cannot occur. The signals which control the activity state of Sxl have not been identified, although autoregulation has been implied. It is clear, however, that the X/A ratio plays an important part in this process in conjunction with the action of the daughterless gene, (da). The wild type product from the da locus, in addition to nonsex-specific requirements, is needed for the expression of Sxl. Thus, daughters from da mothers die as embryos from a lack of Sxl^{+} product (Cline, 1978).

The components which are responsible, in part, for hyperactivation of the X chromosome in males have been identified at the genetic level. Several male-specific lethal mutations which reduce the expression of the X chromosome have been reported (Belote and Lucchesi, 1980a; 1980b). Presumably the wild type function of these genes is intimately involved in

allowing hyperactivation of X-linked loci. These products are required throughout male development (Belote, 1983). The absence of these products only partially rescues the female lethality of Sxl null mutations (Skripsky and Lucchesi, 1982; Cline, 1984).

CHAPTER ONE

MEASUREMENTS OF GENE PRODUCT LEVELS IN TRISOMIC-2L LARVAE

Introduction

In this chapter, the amounts of products synthesized from five genes were examined in diploid and trisomic-2L larvae. These genes were located within the duplicated arm and were expected to produce approximately 1.5 times the amount of product in trisomies as in diploids. While two of the gene products were found to be dose dependent, the other three were observed to be at diploid (compensated) levels. Subsequent experiments revealed that, for one locus, compensation operates independently of the number of other active alleles present. All three alleles of a compensated locus are expressed in each cell, and as such compensation appears to operate by reducing the level of expression of each gene by one-third.

Materials and Methods

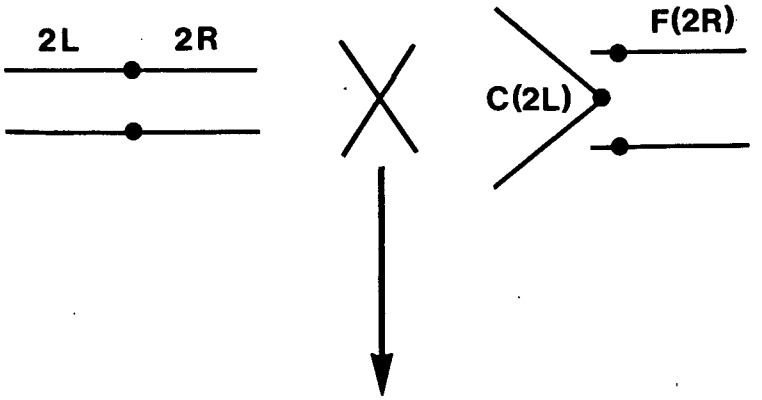
Genetic Strains and Procedures:

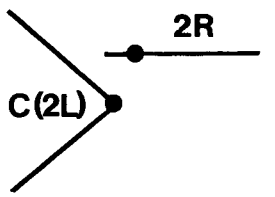
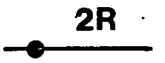
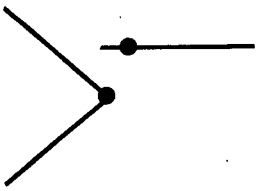
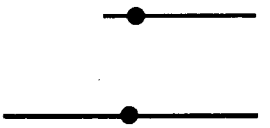
A list of biochemical loci examined in this and subsequent chapters is presented in Table 1. The following strains of *D. melanogaster* were used: (i) Oregon R, (ii) C(2L)SH1+; F(2R)bw/F(2R)bw, (iii) C(2L)VH11t; F(2R)bw/F(2R)bw, (iv) C(2L)VD4, al $Gpdh^{B}$ Bl/In(2L)Cy, al Cy $Gpdh^{A}$; F(2R)bw/F(2R)bw, (v) C(2L)P, b; C(2R)SK1A cn bw, (vi) al $Gpdh^{B}$ Bl L/In(2L+2R)Cy, al Cy $Gpdh^{A}$, (vii) Df(2L)GdhA/In(2LR)SM1, Cy lt, (viii) $Gpdh^{BO-1-4}$/In(2LR)SM1, Cy lt, (ix) b Adh^{N2} osp pr cn, (x) $Gpdh^{A}$, (xi) $Gpdh^{B}$, (xii) $Gpdh^{C}$, and (xiii) several strains with translocations between the Y and 2L chromosomes (Lindsley et al., 1972), which are listed in Table 5. Characteristics of the other mutant alleles and chromosomes have been described previously (O'Brien and MacIntyre, 1976; Lindsley and Grell, 1968; Grell, 1970; Holm, 1976).

Individuals trisomic for the left arm of chromosome 2 were produced as shown in Figure 1 (see Fitze-Earle and Holm, 1978). Genetic and cytological studies revealed that whereas monosomic progeny die during embryogenesis, a high proportion of the hybrid progeny inheriting three entire left arms of chromosome 2 survive to pharate adults. In fact, the viability of trisomic-2L and trisomic-X individuals is comparable.

Five gene-enzyme systems on chromosome 2L, one on the X chromosome, and one on chromosome 3L have been analyzed in this

Figure 1. Mating procedure utilized to produce trisomic larvae. In general a standard strain was crossed to a strain bearing a compound chromosome and two free chromosomal arms. Zygotes are formed which are trisomic or monosomic for the arm associated with the compound chromosome.



C(2L);F(2R) gametes / standard gametes		
		
	trisomy 2L	monosomy 2L

chapter. Their cytological map positions (O'Brien and MacIntyre, 1976; Voelker et al., 1979a; Voelker et al., 1979b; Woodruff and Ashburner, 1979) are listed in Table 1. To show that the activity of each enzyme normally was proportional to the number of gene copies, enzyme levels were measured in strains with small duplications (three doses) for each of the five loci studied on chromosome 2L. Segmental aneuploids were generated with the T(Y;2) chromosomes described by Lindsley et al., (1972).

Flies trisomic for chromosome 2L, but carrying either a deficiency or a null allele for a specific locus were produced by crossing C(2L)lt;F(2R)bw/F(2R)bw females to males that were heterozygous for the inverted chromosome In(2LR)SM1,Cy lt and either $Gpdh^{EO-1-4}$ (a noncomplementing null allele for Gpdh) or Df(2L)GdhA (a deficiency for the Gpdh structural gene) or to males homozygous for Adh^{N2} (a noncomplementing null allele for Adh). Larvae carrying either the null allele or deficiency were distinguished by the presence of pigment in the larval Malpighian tubules in contrast to their nonpigmented (lt/lt/lt) sibs.

A compound chromosome heterozygous for two Gpdh electrophoretic variants was constructed by mating C(2L)P,b;C(2R)SK1A,cn bw males to al $Gpdh^{B}$ Bl L/ In(2L+2R)Cy,al Cy $Gpdh^{A}$ females that had been treated with 2500 rads from a ^{60}Co source. A C(2L)VD4,al $Gpdh^{B}$ Bl/ In(2L)Cy,al Cy $Gpdh^{A}$;C(2R)SK1A,cn bw individual was recovered, and a stock was established. From a cross of

females bearing this compound 2L chromosome with C(2L)P,b; F(2R)bw/F(2R)bw males, C(2L)VD4,al Gpdh^{B} Bl/In(2L)Cy,al Cy Gpdh^{A}; F(2R)bw/F(2R)bw nondisjunctional progeny were recovered. These individuals, when crossed to flies possessing the Gpdh^{C} allele on standard second chromosomes, produce trisomies containing three different electrophoretic variants.

All strains were reared at 25 degrees C on standard cornmeal sucrose medium supplemented with the mold inhibitor Tegosept (0.22%) and with the antibiotics tetracycline and streptomycin at 10 mg/liter each.

Enzyme Assays:

All determinations of enzyme activity were performed on 50 to 150mg samples of late third instar larvae collected from the sides of uncrowded half pint culture bottles. Homogenates were prepared from 25mg of larvae per ml of 10mM phosphate buffer (pH 8.2) for all enzymes except dihydroxyphenylalanine (dopa) decarboxylase (DDC), for which 0.1 M Tris (pH 7.2) was used. After centrifugation, enzyme activity (except for DDC) was monitored by the change in absorption at 340nm by the production or utilization of NADH or NADPH; DDC activity was determined from the amount of $^{14}CO_2$ released from ^{14}C-1 dopa, corrected for spontaneous decarboxylation in the presence of heat-inactivated extract (Hodgetts and Konopka, 1973). All assays were optimized with respect to all

components. The conditions for each assay were as follows: phosphoglycerate kinase (PGK; EC 2.7.2.3), 0.1M Tris, pH 7.6/0.9mM EDTA/15mM NADH/2.2mM ATP/13mM glycerate-3-phosphate/3.2mM $MgSO_4$/2.5 units of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) per ml; a-glycerol-3-phosphate dehydrogenase (NAD) (GPDH EC 1.1.1.8), 0.1 M glycine, pH 9.5/2.5mM glycerol-3-phosphate/10mM NAD+; cytoplasmic malate dehydrogenase (cMDH; EC 1.1.1.37), 50mM phosphate, pH 7.3/0.25mM NADH/5mM oxaloacetic acid; alcohol dehydrogenase (ADH; EC 1.1.1.1), 0.1M glycine phosphate buffer, 1.7g/liter, pH 9.0/2.5 mM NAD/2% ethanol; dopa decarboxylase (DDC; EC 4.1.1.26), 0.1M Tris, pH 7.2/0.12mM pyridoxal-5'-phosphate/25 mM phenylthiourea/0.2 uCi (1 Ci=3.7 x 10^{10} becquerels) of (1-^{14}C) dopa (New England Nuclear; 3 Ci/mmol; isocitrate dehydrogenase (IDH; EC 1.1.1.42), 0.1M Tris pH 8/5mM Mg^{2+}/0.05mM NADP/0.5mM isocitrate; and 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), 0.1M Tris, pH 7.5/1.3 mM NADP/20mM $MgCl_2$/3.1mM 6-phosphogluconate. Activites are expressed as units per mg of protein in the extract, where one unit is defined as the utilization of 1 µmol of substrate per min. Protein concentration was determined (Lowry, 1952), with bovine serum albumin as the standard.

The enzyme levels expected in the absence of dosage compensation were estimated by summing the activity found in the compound-free strain plus one half of the activity found in the Oregon R strain. This corresponds to the parental contribution of chromosome 2L to the karyotype of the trisomies. The enzyme

levels expected in the presence of autosomal dosage compensation were estimated as simply two-thirds of the expected noncompensated value. Standard error estimates for these values were obtained by partitioning the variance from the two diploid strains in accordance both with their contribution to the estimated mean and with their sample sizes. Observed and expected values were compared using a t-test with correction, were necessary, for differences in sample variance as suggested by Welch (1974).

Electrophoretic Procedures

Electrophoresis of GPDH in trisomies possessing three variants was performed in a 12% (wt/vol) starch/0.1M Tris citrate gel system, pH 6.9 run at 300V (25-35 mA) for 32hr. Gels were stained for GPDH (Shaw and Prasad, 1970).

Table 1 Biochemical loci examined in this and subsequent chapters (from Treat-Clemons and Doane, 1984).

Gene	Product	Genetic Map Position
Adh	alcohol dehydrogenase	2L-50.1
Ak	arginine kinase	3L-25.2
Ald	aldolase	3R-91.5
Aldox	aldehyde oxidase	3R-57.2
Cat	catalase	3L-44.3
cMdh	cytoplasmic malate dehydrogenase	2L-37.0
Ddc	dopa-decarboxylase	2L-53.9
Est-6	esterase-6	3L-36.8
Fum	fumarase	1-19.9
Gpdh	alpha-glycerophosphate dehydrogenase	2L-20.5
Gpt	glutamate-pyrovate transaminase	1-42.6
Had	beta-hydroxyacid dehydrogenase	1-54.4
hsp 22	22 kD heat shock protein	3L-67B
hsp 23	23 kD heat shock protein	3L-67B
hsp 26	26 kD heat shock protein	3L-67B
hsp 27	27 kD heat shock protein	3L-67B
hsp 68	68 kD heat shock protein	3R-95D
hsp 70	70 kD heat shock protein	3R-87A and 87C
hsp 83	83kD heat shock protein	3L-63BC
Idh	isocitrate dehydrogenase	3L-27.1
LSP-1a	larval serum protein-1 alpha	1-39.5
LSP-1b	larval serum protein-1 beta	2L-1.9
LSP-1g	larval serum protein-1 gamma	3L--1.41
LSP-2	larval serum protein-2	3L-37
Men	malic enzyme	3R-51.7
Pgd	6-phosphogluconate dehydrogenase	1-0.63
Pgi	phosphoglucoisemerase	2R-20.5
Pgk	phosphoglycerate kinase	2L-5.9
Pgm	phosphoglucomutase	3L-43.4
Sod	superoxide dismutase	3L-34.6
Zw	glucose-6-phosphate dehydrogenase	1-62.9

RESULTS

The effect of aneuploidy for small genetic segments on enzyme activity has been extensively examined in a number of species. Indeed, changes in enzyme activity associated with aneuploids are often used to locate the structural gene of an enzyme or a dose-dependent locus regulating its expression (O'Brien and MacIntyre, 1976; Rawls and Lucchesi, 1974). However, in Drosophila the effect of autosomal trisomy on gene expression is unknown. Therefore, we examined the expression of seven gene-enzyme systems located within and outside a trisomic region, namely chromosome 2L. This was accomplished by comparing enzyme levels in diploid parental stocks and their trisomic offspring. The results for the two diploid parental stocks, Oregon R and C(2L)+;F((2R)bw/F(2R)bw and for their trisomic-2L offspring are presented in Tables 2-4. Expected enzyme levels are also presented. From previous findings with segmental aneuploids for autosomal regions, a simple prediction is that genes duplicated in trisomies would show strict dose dependence. Accordingly, the expected enzyme level would simply represent the sum of the activities of each of the three gene copies. Alternatively, a compensatory mechanism may exist for controlling the expression of the genes in this trisomic arm. The simplest prediction would be that compensation acts on all three alleles equally, resulting in a diploid level of activity, approximately two-thirds that of the noncompensated level. The enzyme activities of three loci, Pgk, Gpdh, and cMdh, are clearly at the level

expected if compensation occurs (Table 2). These loci are located in approximately the distal two-thirds of the chromosome arm. In contrast, the enzymes produced by the two proximally located loci, Adh and Ddc, show dose-dependent levels of activity, indicating an absence of dosage compensation (Table 3). ADH activity is significantly higher than expected, even for a noncompensated gene. Thus, it appears that some loci in trisomic individuals are capable of, or are directed toward compensation, whereas others are not, and this difference appears to be regionally distributed along the chromosome arm. Results from reciprocal crosses were statistically indistinguishable.

The structual genes for IDH on chromosome 3L and 6PGD on the X chromosome were monitored to observe what effect, if any, trisomy 2L might have on the activites of unlinked loci. The enzyme activities of these loci in trisomies was approximately 15% below the expected diploid level (Table 4).

A direct assessment of the effect of trisomy on the expression of a gene can be made by comparing the activity of the same two alleles in both diploid and trisomic-2L strains. This was accomplished by comparing the enzyme activity of individual loci on the same C(2L) chromosome in C(2L)-bearing diploid strains with a trisomic strain in which the standard chromosome carried either a null allele or a deficiency for the enzyme locus in question. The results are shown in Figure 2. For Gpdh, the expressions of the two active alleles is reduced by approximately one-third in the trisomic strains carrying either a null allele or a deficiency

Table 2 Enzyme levels for compensating loci in diploid and trisomic-2L larvae.

Genotype	Enzyme and chromosome position		
	Pgk 22C-23E	Gpdh 25F-26B	cMdh 30F-31D
Oregon R (diploid)	0.986 ± 0.031 (10)	0.212 ± 0.008 (14)	3.36 ± 0.056 (10)
C(2L)+;F(2R)bw/ F(2R)bw (diploid)	0.831 ± 0.019 (11)	0.132 ± 0.008 (14)	3.71 ± 0.053 (10)
C(2L)+/ +/F(2R)bw (CF♀ x OR♂) (trisomic-2L)	0.973 ± 0.049 (10)	0.154 ± 0.005 (10)	3.91 ± 0.294 (4)
C(2L)+/ +/F(2R)bw (OR♂ x CF♀) (trisomic-2L)		0.168 ± 0.019 (3)	3.73 ± 0.102 (11)
Noncompensated Estimate	1.32 ± 0.016	0.238 ± 0.006	5.39 ± 0.044
Compensated Estimate	0.883 ± 0.011	0.159 ± 0.004	3.59 ± 0.029

Enzyme activities expressed at the mean ± SE in units/mg of protein. Estimates of enzyme activities expected in trisomies for dose-dependent (noncompensated) and compensated gene expression are provided. Numbers in brackets represent the sample sizes.

Table 3 Enzyme levels for noncompensating loci in diploid and trisomic-2L larvae.

Genotype	Enzyme and chromosome position	
	Adh 35B2	Ddc 37B10-37C7
Oregon R (diploid)	67.6 ± 2.4 (14)	8.61 ± 1.02 (16)
C(2L)+;F(2R)bw/ F(2R)bw (diploid)	40.9 ± 1.7 (14)	10.9 ± 0.78 (18)
C(2L)+/ +/F(2R)bw (CF x OR) (trisomic-2L)	89.0 ± 1.9 (10)	16.7 ± 0.714 (20)
C(2L)+/ +F(2R)bw (OR x CF) (trisomic-2L	88.1 ± 1.7 (3)	
Noncompensated Estimate	74.7 ± 1.8	15.2 ± 0.552
Compensated Estimate	49.8 ± 1.20	10.1 ± 0.348

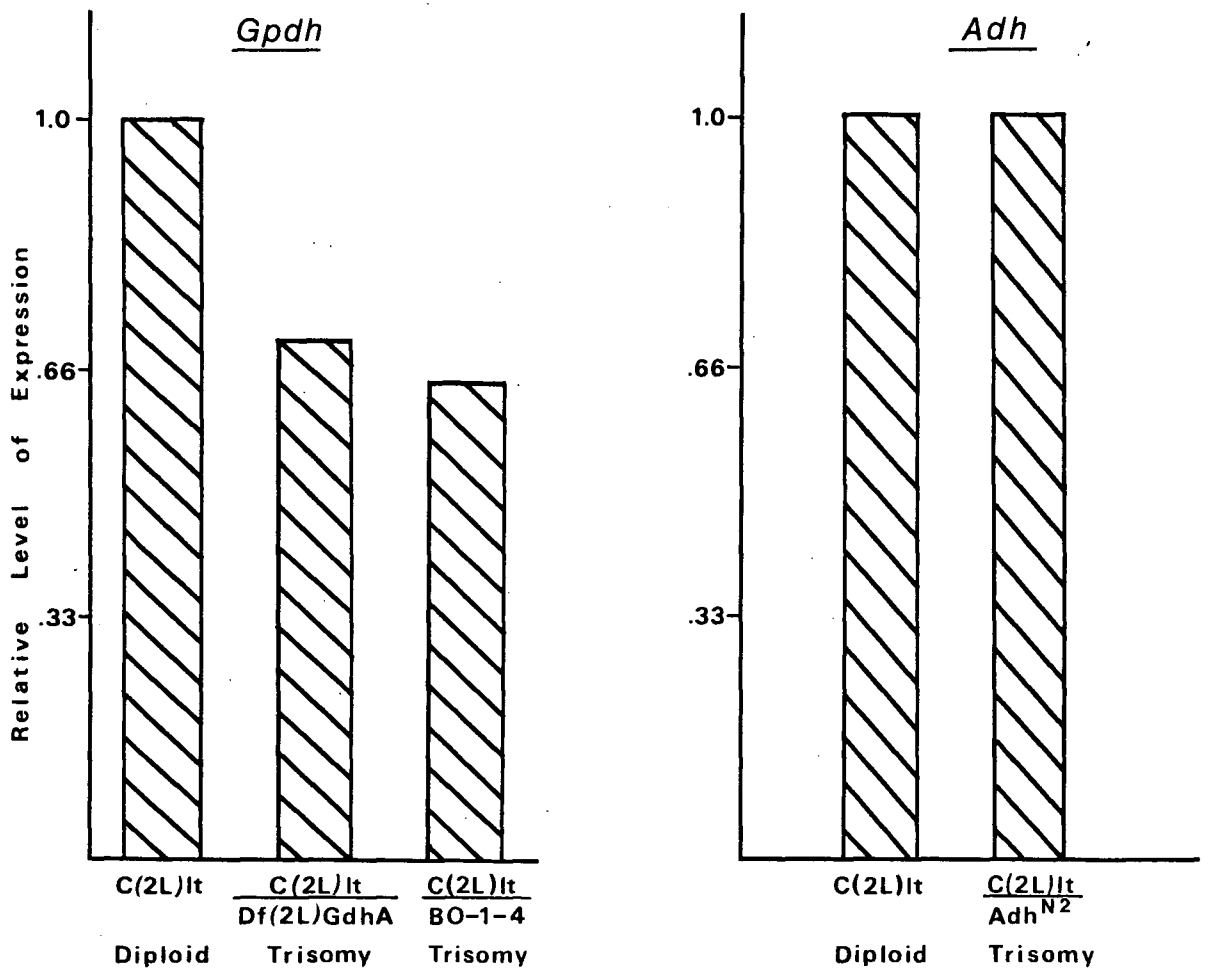
Enzyme activities expressed at the mean ± SE in units x 10^{-3} per mg of protein for Adh and in units x 10^{-4} for Ddc. Estimates of enzyme activities expected in trisomies for dose-dependent (noncompensated) and compensated gene expression are provided. Numbers in brackets represent the sample sizes.

Table 4 Enzyme levels for two unlinked loci in diploid and trisomic-2L larvae.

Genotype	Enzyme and chromosome position: Idh 66B-67C	Enzyme and chromosome position: 6Pgd 2D3-2D6
Oregon R (diploid)	110 ± 4.3 (10)	36.8 ± 1.2 (10)
C(2L)+;F(2R)bw/ F(2R)bw (diploid)	73.3 ± 2.8 (7)	54.0 ± 1.1 (10)
C(2L)+/ +/F(2R)bw (CF x OR) (trisomic-2L)	74.9 ± 1.0 (10)	40.2 ± 1.6 (10)
C(2L)+/ +/F(2R)bw (OR x CF) (trisomic-2L)	81.5 ± 4.0 (3)	
Estimated mean	91.7 ± 1.39	49.7 ± 0.576
Repressed Estimate	60.6 ± 0.93	33.1 ± 0.38

Enzyme activities expressed at the mean ± SE in units x 10^{3} per mg of protein. Estimates of enzyme activities expected in trisomies for dose-dependent (noncompensated) and compensated gene expression are provided. Numbers in brackets represent the sample sizes.

Figure 2. Expression of enzyme loci on the C(2L)1t chromosome in diploids and in trisomies possessing either a null allele or a deficiency for the loci measured. Only the genotype of the left arm of chromosome 2 is given. Activities from loci on the C(2L)1t chromosome are graphed relative to the diploid level. All standard errors were less than 5% of the mean. (Left) Gpdh. (Right) Adh.


Gpdh
Relative Level of Expression
1.0
.66
.33
C(2L)It
Diploid
C(2L)It / Df(2L)GdhA
Trisomy
C(2L)It / BO-1-4
Trisomy
Adh
1.0
.66
.33
C(2L)It
Diploid
C(2L)It / Adh^N2
Trisomy

for the structural gene. This clearly demonstrates that the expression of this gene-enzyme system is compensated in trisomic-2L individuals. In an analogous experiment involving the noncompensating locus, Adh, the expression of the two active alleles in the C(2L) chromosome was unaffected in trisomic individuals bearing an Adh null allele on the standard second chromosome. These results reveal that the response of compensating and noncompensating genes is independent of the number of active alleles.

For the observed autosomal dosage compensation to be a valid phenomenon, compensating loci must be dose dependent in diploid strains with small duplications at the same stage of development. Small duplications of the structural genes for all five enzymes were made, and enzyme assays were performed on mature third instar larvae (Table 5). The enzyme activities in these aneuploids showed dose dependence; that is, the levels in the strains with three doses were significantly higher than those of their diploid parents.

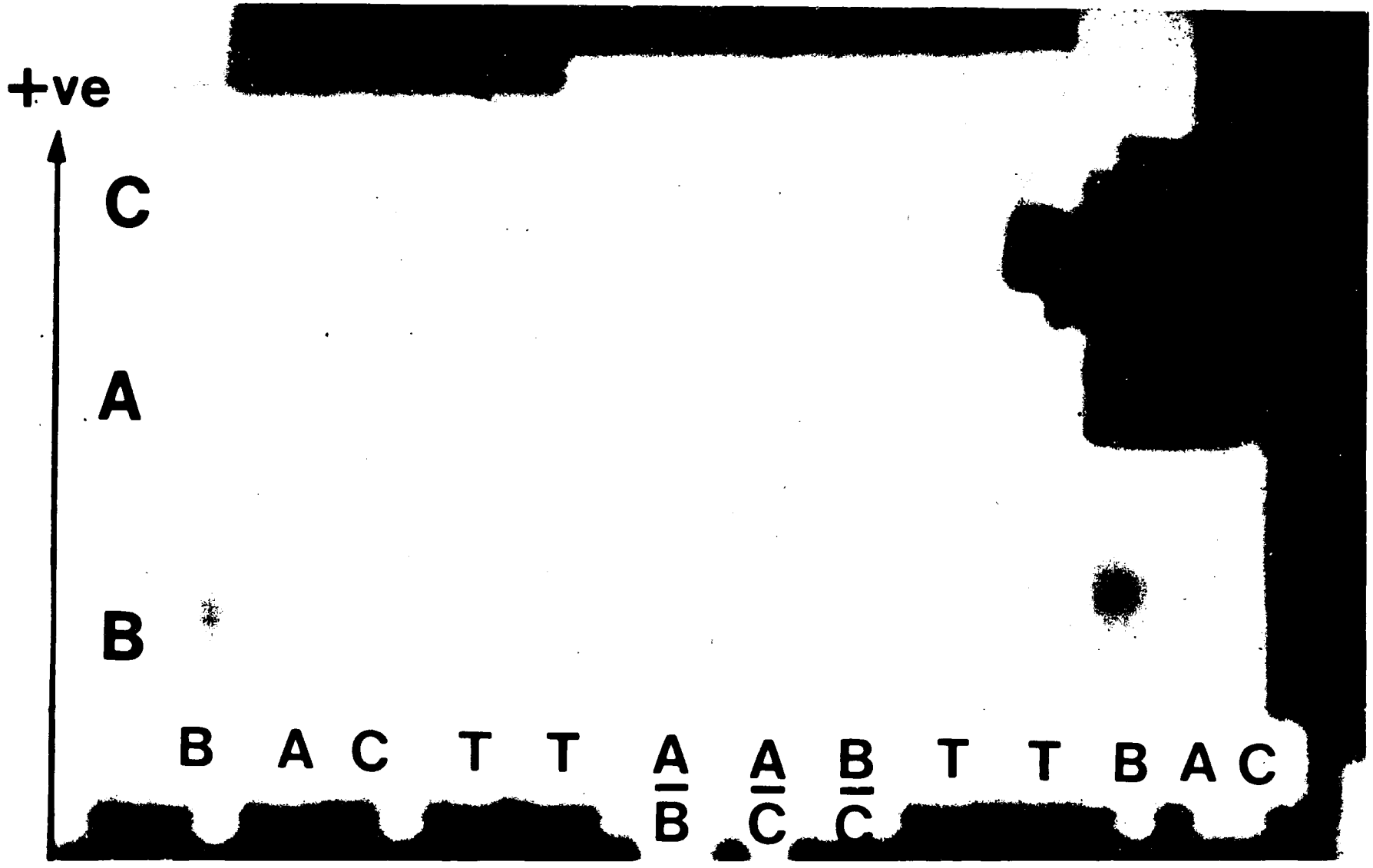
Simplistically, compensation may occur by one of two mechanisms: first, each allele may be expressed at two-thirds of the normal diploid level or, second, one allele may be completely repressed while the other two are fully active. To distinguish between these two alternatives, trisomies possessing three electrophoretic variants for Gpdh were produced. Starch gel electrophoresis of this enzyme from trisomies demonstrated that all three alleles of this compensated gene were expressed (Figure 3).

Table 5 Dose dependence of enzyme levels in small segmental aneuploids in late third instar larvae.

Gene	Duplication	Translocation	Dose	Activity	Ratio
Pgk	22D-24A	G120	2	0.559 ± 0.018 (5)	
		R136	2	0.535 ± 0.009 (7)	1.31
		DpG120-R136	3	0.714 ± 0.033 (8)	
Gpdh	25F-26B	D106	2	0.113 ± 0.008 (5)	
		G105	2	0.136 ± 0.008 (5)	1.66
		DpD106-G105	3	0.207 ± 0.007 (5)	
cMdh	30F-31EF	L52	2	1.53 ± 0.098 (3)	
		A162	2	1.44 ± 0.130 (3)	1.29
		DpL52-A162	3	1.91 ± 0.040 (5)	
Adh	34B-35D	G74	2	39.1 ± 0.67 (6)	
		P58	2	36.7 ± 0.68 (6)	1.79
		Dp G74-P58	3	68.0 ± 1.60 (6)	
Ddc	36EF-37D	D219	2	11.03 ± 0.85 (4)	
		H174	2	11.70 ± 0.76 (4)	1.41
		Dp D219-H174	3	15.98 ± 0.36 (4)	

Activity units are the same as in Tables 2 to 4. Ratio represents the enzyme levels found in duplication-bearing larvae relative to the mean of that found in their parents.

Figure 3. Electrophoretic analysis of Gpdh variants in trisomies. B, A, and C represent mobility variants of Gpdh. Lanes B, A, and C are from diploid homozygous individuals; lanes A/B, A/C and B/C are from dipoid heterozygotes; lanes designated T are from trisomies possessing all three variants produced from reciprocal crosses. The left lane of each pair of Trisomic samples were derived from trisomic offspring produced from compound-free males, and the right lane from compound-free female parents. +ve, Mobility towards anode.


+ve
C
A
B
B A C T T A/B A/C B/C T T B A C

DISCUSSION

In Drosphila, X-linked loci transcribe at different and predictable rates depending on the genotype (Lucchesi, 1977; 1983). The total X-linked transcription within a diploid nucleus is constant, regardless of the number of X chromosomes. Thus, the transcriptional rate of a single X-linked structural gene varies in direct proportion to the number of X-linked templates present in the nucleus. For example, individuals with one or with three X chromosomes transcribe X-linked loci at twice or at two-thirds the rate of diploid females, respectively. In addition, the total level of X chromosome-linked transcription is directed to match that of the autosomes and is directly dependent on their number. For example, X-linked transcription in individuals possessing two X chromosomes and three sets of autosomes (a triploid intersex) is 50% greater than normal diploid females (two X chromosomes and two sets of autosomes). The observation that individuals trisomic for the X chromosome show dosage compensation (Lucchesi et al., 1974; Stewart and Merriam, 1975) is of particular relevance to this study. Whereas diploid individuals with one, two or three X chromosomes express sex-linked traits equally, males or females having small duplications or deletions of the X chromosome genes do show dose-dependent levels of gene expression (Lucchesi, 1978; Maroni and Lucchesi, 1980).

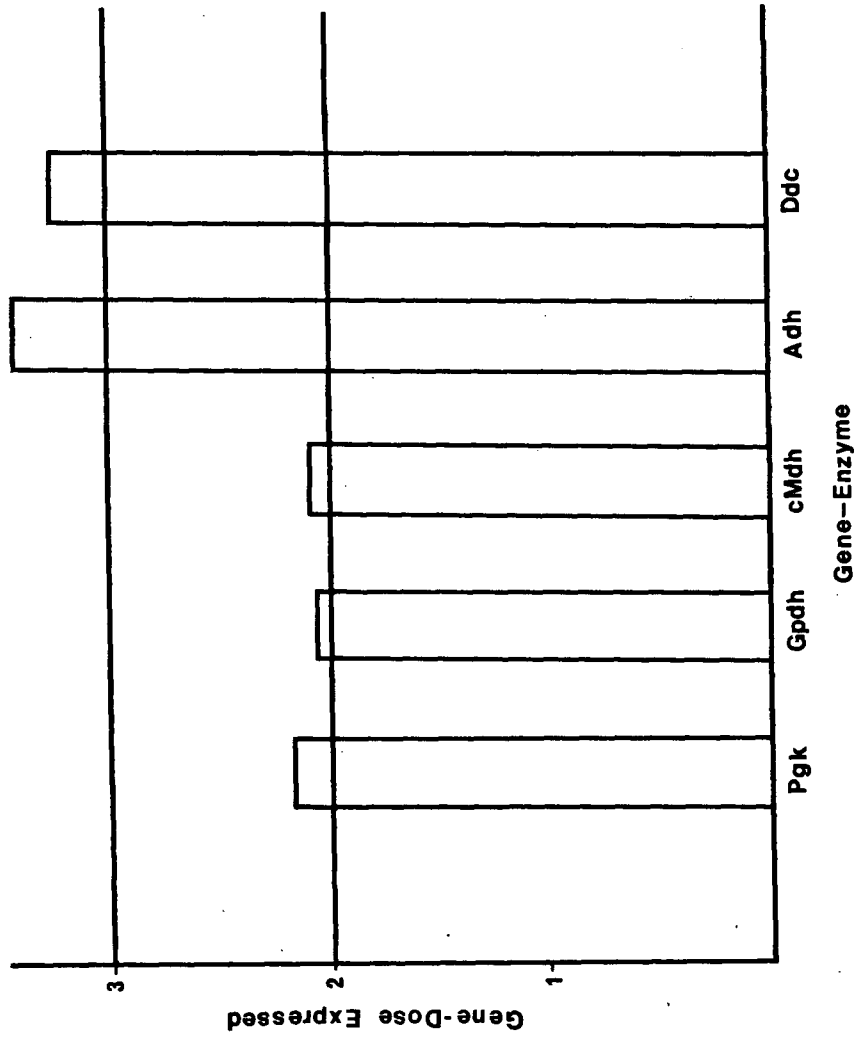
In contrast to X-linked loci, it has been observed that autosomal loci are dose dependent regardless of the chromosomal constitution. Genes in small duplicated segments of the

autosomes, like their X-linked counterparts, produce dose-dependent levels of gene product (Table 2). However, in individuals trisomic for the entire left arm of chromosome 2, we have observed that three distally mapping enzyme loci (Pgk (7.6), Gpdh (20.5), and cMdh (37.2)) show levels of activity expected of a diploid. Thus, a compensatory mechanism appears to be acting. Not all genes on the trisomic chromosome arm are compensated since two proximally located loci (Adh (50.1) and Ddc (53.9)) remain dose dependent. Superficially, autosomal dosage compensation appears to be an all-or-none phenomenon acting at the level of the individual gene.

The results of the five gene-enzyme systems studied suggest that the distal two-thirds of the chromosome compensates while the proximal one-third does not (Figure 4). One can speculate that this regionality in the compensatory response reflects either a difference in gene organization or a difference in chromosome structure capable of responding to compensation. Alternatively, this regionality may be simply an artifact of the small number of loci sampled. While two genes in D. melanogaster are not dosage compensated, they are not located in distinct regions (Roberts and Evans-Roberts, 1979a; Ota et al., 1981). In contrast, regionality for sex-linked dosage compensation has been observed in a related species, D. miranda (Strobel, et al., 1978).

To further examine the response of compensated and noncompensated loci in trisomies, the same two active alleles for both Adh and for Gpdh were measured in diploid

Figure 4. Summary bar graph of enzyme quantities in 2L trisomies relative to diploids. Level 2 gene-dose expressed represents the expected compensated level of expression; level 3 gene-dose expressed represents the expected noncompensated estimate.


Gene-Dose Expressed
3
2
1
Pgk
Gpdh
cMdh
Adh
Ddc
Gene-Enzyme

and in trisomic strains (Figure 2). In the trisomic strains, the standard chromosome possessed either a null allele or a deletion that eliminated the activity of the third allele. For Adh, the activity of each allele was identical in trisomic and diploid strains, as expected of any noncompensated locus. The identical expression of Adh alleles in trisomic and diploid strains suggests that any physiological differences between them is minimal. In contrast, the expression of each Gpdh allele in the trisomic strain was reduced by one-third relative to the expression of this same allele in the diploid strain. Analysis of trisomies possessing three electrophoretic variants for GPDH demonstrated that all three loci were expressed. The implications of these results are discussed further in chapter 2.

To examine the influence of trisomy 2L on unlinked loci, the levels of IDH from chromosome 3L and 6PGD from the X chromosome were measured. In the trisomies, each displayed a level somewhat lower than expected (approximately 15%). Whether this reflects a general effect of trisomy on nonlinked genes, differences in gender, or simply genetic variation (Laurie-Ahlberg et al., 1980; Bijlsma, 1980) (isogenic strains were not used) awaits a more extensive survey of other unlinked loci and other trisomies. Because we have not established that the decreases in IDH and 6PGD activities are a specific effect of trisomy, we hesitate to use them as internal standards. Therefore, in dealing with grossly altered genotypes such as trisomies, any changes in enzyme activity should perhaps be interpreted initially as locus specific rather than reflective

of total genomic activity.

The data raises two questions. First, by what mechanism does autosomal dosage compensation occur and, second, why should it exist for autosomal aneuploids? Two mechanisms for sex-linked dosage compensation have been hypothesized, and both are presumed to act at the level of transcription. One model is based on repression and the other is based on activation of X-linked gene expression. The first model postulates the existence of X-linked loci, themselves not dosage compensated, which modulate the activity of other X-linked genes by partially repressing transcription (Muller, 1950). As the number of X chromosomes increases, the concentration of the repressor substance increases and the amount of transcription from each X-linked template decreases (compensation). Evidence has been presented for (Birchler, 1980) and against (Stewart and Merriam, 1975) the existence of these repressor loci on the X chromosome. The second model suggests that the transcription is modulated by nonlinked loci that produce a limited amount of a diffusible activator that X-linked genes compete for the activator (Maroni and Plaut, 1973; Schwartz, 1973). Increases or decreases in the number of X chromosomes have no effect on the total amount of transcription from the X-linked genes because the amount of activator substance remains constant. Recently, evidence has been presented for the latter model of sex-linked dosage compensation (Maroni and Lucchesi, 1980; Maroni and Plaut, 1973). Either model can adequately account for the compensation we found in trisomic-2L strains. Indeed, Rawls and Lucchesi (1974) have reported that regions of chromosome 2L are capable

of reducing the activity of GPDH, indicating that negative regulatory loci may exist on chromosome 2L.

It is not surprising that a mechanism exists to compensate for the imbalance in X-linked templates which occurs between males and females in many higher organisms. However, because autosomes generally do not vary in number, it is not obvious why autosomal dosage compensation should exist. The fact that survival and compensation can occur for both X chromosomal and autosomal trisomies allows the speculation that in Drosophila, dosage compensation in these individuals simply reflects the existence of a homeostatic mechanism. Such a control mechanism would be responsible for modulating the overall level of gene expression, even in normal diploids. What is observed as compensation in trisomies may simply be this normal regulatory mechanism forced to cope with extreme perturbations within the genome.

CHAPTER TWO

MEASUREMENT OF GENE PRODUCT LEVELS IN TRISOMIC-3L AND TRISOMIC-X LARVAE

Introduction

When small duplications or deficiencies are examined, the level of most gene products varies directly with the gene dose (Grell, 1962). Many regulatory gene products may influence the accessibility of a gene to the transcriptional apparatus, which in turn appears to be an important factor influencing the final level of gene product (Chandler et al., 1983; Parker and Topol, 1984). In the previous chapter we examined the expression of several loci in Drosophila melanogaster individuals possessing various duplications for the left arm of chromosome two (Devlin et al., 1982). While all five loci examined showed elevated levels of enzyme activity in small duplications, three of these genes showed compensated (diploid) levels of enzyme activity when duplicated in whole-arm trisomies. This altered mode of gene expression could be caused by regulatory genes, which are present in the duplicated arm and control the compensated loci (Rawls and Lucchesi, 1974).

Compensation in autosomal trisomies is reminiscent of the situation observed for compensation in metafemale (trisomic-X) individuals. Dosage compensation of X-linked genes may be thought of as an evolutionary strategy required to equalize gene expression between individuals possessing different numbers of X chromosomes for sex determination (Lucchesi, 1983, Baker and Belote, 1983). On the other hand, compensation for autosomal trisomy is not expected since the number of autosomes normally

does not vary within a species. We therefore proposed that compensation of autosomal genes in whole arm trisomies may reflect the existence of processes which normally operate to control gene expression in diploids (also, see Seecof, 1961).

This chapter examines the extent to which autosomal compensation occurs, and to determine if the response is similar to that observed for X-chromosome dosage compensation. We have found that compensation of autosomal loci can also occur in trisomies for the left arm of chromosome three. Four of six loci compensate while the other two are dose dependent. In contrast, five X-linked loci were studied in metafemales and all dosage compensated. Two genes located on 3L, which do not compensate in 3L trisomies of D. melanogaster, do dosage compensate between males and females in D. pseudoobscura (Abraham and Lucchesi.1974; Pierce and Lucchesi, 1980). These observations suggest that regulation of compensation in hyperploids may differ from X chromosome dosage compensation between males and females.

MATERIALS AND METHODS

Production of Trisomies

Genetic symbols are described in Lindsley and Grell (1968), and the nomenclature of individual compound chromosomes and free arms is by the alphanumeric system suggested by Holm (1976). The stock C(3L)VG1, ru st; F(3R)VD1,e^{s}/F(3R)VD1^{s} was constructed as shown in Figure 5: C(3L)VZ1,ri; C(3R)VK1,e^{s} females were treated with 2500 rads of gamma-irradiation from a ^{60}Co source and crossed to T(3;4)e/TM3,Sb Ser males. Surviving (radius incompletus) ri males were crossed to T(3;4)e/TM3 females and individual ri lines were established. One of these lines possessed F(3R) chromosomes which were cytologically normal in that no obvious euchromatic breakpoints were detectable. While the heterochromatic breakpoints of this half translocation have not been confirmed, we can exclude on genetic grounds the left and right arms of the second, probably the right arm of the third chromosome and the left arm of the X. Thus, we are certain that no additional large duplications of euchromatic regions would be associated with this free arm (see Figure 6). C(3L)VZ1,ri was replaced with C(3L)VG1,ru st by nondisjunction. Trisomies for the left arm of chromosome three are produced by crossing C(3L)VG1,ru st; F(3R)VD1,e^{s}/F(3R)VD1,e^{s} to an Oregon R standard strain. Monosomics for 3L are poorly viable,

Figure 5. Strategy employed to produce compound-free chromosomes for the third chromosome. Wavy lines represent treatment with gamma radiation from a ^{60}Co source.
In the second genotype that was treated with radiation, the standard chromosome was marked with the mutations ri and p^{p}.
Genotypes at bottom of page are: A) C(3L)VG1,ru st;
F(3R)VD1,e^{s}/F(3R)VD1,e^{s} and
B) F(3L)VD1,ri/F(3L)VD1,ri;C(3R)VK1,e^{s}

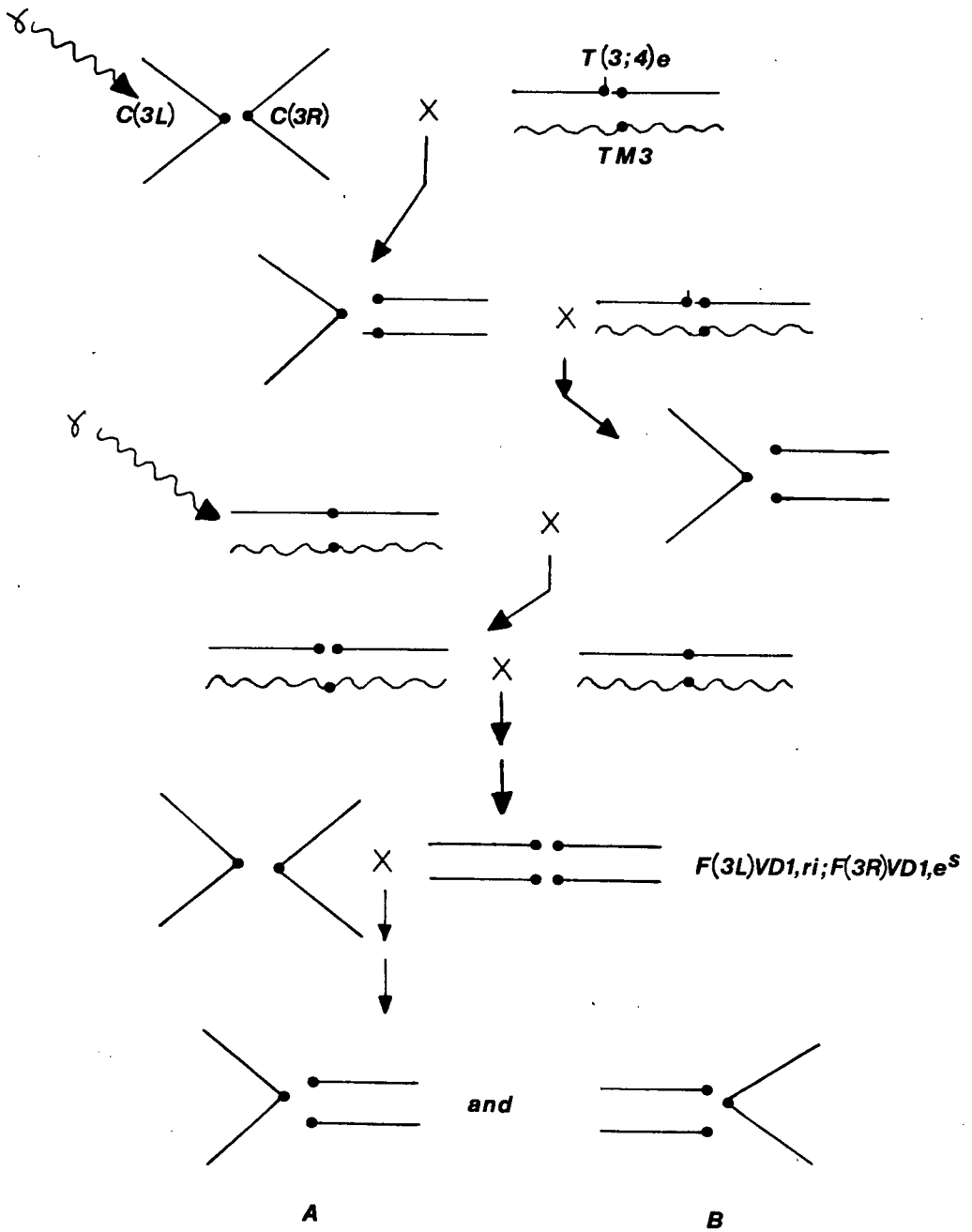

γ
C(3L)
C(3R)
X
T(3;4)e
TM3
γ
F(3L)VD1,ri;F(3R)VD1,e^s
and
A
B

Figure 6. Cytological preparations of chromosome F(3R)VD1e^{s} in diploid and trisomic larvae. Salivary gland polytene chromosome preparation from a female trisomic-3L larvae. Note the thickened 3L element, and the absence of small chromocentral duplications. B. Mitotic chromosome preparation from trisomic-3L brain tissue. Note that nine major autosomal arms, two X's and two small fourth chromosomes are present. C. Cytological verification of the of C(3L)VZ1,ri; F(3R)VD1e^{s}/F(3R)VD1e^{s} stock.

and are not recovered in populations of third instar larvae within ten days development at 25 degrees C.

The strain F(3L)VD1,ri/F(3L)VD1,ri; C(3R)VK1,e^{ss} was constructed as follows: ri pP/TM3 females were treated with 2500 rads of gamma-radiation and crossed to C(3L)VG1,ru st; F(3R)VD1,e^{ss}/F(3R)VD1,e^{ss} males and F(3L)F(3R)/TM3 heterozygous individuals were recovered. A viable combination of F(3L) and F(3R) chromosomes was established. These were crossed to C(3L)P2,ri;C(3R)VK1,e^{ss} males, from which F(3L)VD1,ri/F(3R)VD1,ri; C(3R)VK1,e^{ss} nondisjuctional individuals were recovered. By crossing these individuals either to a standard strain or to Dp(3R)173/Df(3R)a74 (a strain bearing a deficiency for 83DE; Keppy and Dennel, 1979), individuals trisomic for 3R are produced.

The stock y^2/y^2; F(2L)pr/F(2L)pr;C(2R)P,px was constructed as follows: The F(2L)pr chromosome was derived by recombination between F(2L)nub^2 b pr and a wild type F(2L)+, and the C(2R)P,px chromosome was introduced by nondisjunction. This stock, when crossed to a standard Oregon R strain, resulted in fair survival of trisomic-2R larvae. Monosomics for 2R fail to hatch.

The C(3L)VD2,Ly Pgm^2/In(3L)P,Me Pgm^4; F(3R)VD1,e^{ss}/F(3R)VD1 e^{ss} strain was constructed as follows: A Ly st

chromosome was constructed and was used to recover Ly recombinants from Ly st/Pgm^2 females. These were tested for the Pgm^2 marker and a Ly Pgm^2 chromosome was balanced over In(3L)P,Me,Pgm^4+In(3R)C,Sb. Females of this stock were irradiated and crossed to C(3L)VG1,ru st; C(3R)VD1,+ males. A C(3L)VD2,Ly Pgm^2/In(3L)P,Me Pgm^4 chromosome was recovered and crossed to C(3L)VG1,ru st; F(3R)VD1,e^s/F(3R)VD1,e^s to produce the desired stock. When this stock is crossed to Pgm^1/TM3,Sb Ser, phenotypically Sb^+ trisomic-3L pupae can be recovered which possess three different electrophoretic variants for Pgm.

Trisomies for the X-chromosome were produced by crossing C(1)RM,y pn v/O females to Oregon R males. Larvae were sexed (Kerkis, 1931), and metafemales were identified by their black mouthparts.

Strains were raised at 25 degrees C on standard cornmeal-sucrose food containing 0.22% Tegosept and 10mg/l each of streptomycin and tetracycline.

Enzyme Assays:

Wandering third instar larvae were collected from the side of 1/2 pint culture bottles, washed in distilled water and dried on a nitex screen. Larvae were homogenized at 25mg per ml of

10mM PO_4, 1mM phenylthiourea, pH 8.2, allowed to sit on ice for ten minutes, and then centrifuged at 12,000g for ten minutes at 4 degrees C. Enzymes were measured from appropriately diluted extracts to give measurements directly dependent on extract volume. Duplicate measurements were made from each extract, from which was substracted a blank value derived from an assay without substrate. The assay conditions were as follows- AK: 0.1M glycine, 0.2M KCl, 20mM $MgCl_2$, 0.2mM NADH, 10mM ATP, 2mM phosphoenol- pyruvate, 20mM arginine, 20u/ml pyruvate kinase, 20u/ml lactate dehydrogenase; CAT: 0.1M PO_4, pH 7.5, 20mM H_2O2; EST-6: 0.1M $NaPO_4$, pH 6.8, 10^{-4}M b-napthyl acetate, 10^{-4} M eserine, 5 x 10^{-5} M p-chloromercuribenzoic acid, stop reaction with 0.5% o-dianisidine + 5% SDS; FUM: 0.1M $NaPO_4$, pH 7.8, 50mM Na malate; GPT: 0.1M $NaPO_4$, pH 7.5, 1M alanine, 10u/ml lactate dehydrogenase, 0.2mM NADH, 10mM a-ketoglutarate; G6PDH: 0.1M Tris, pH 8.5, 20mM $MgCl_2$, 0.52mM NADP, 6.6mM glucose-6-phosphate; b-HAD: 0.1M Tris, pH 8.5, 30mM gluconic acid, 10mM NAD; IDH: 0.1M Tris, pH 7.5, 5mM $MgCl_2$, 50uM NADP, 2mM Na malate; 6PGD: 0.1M Tris, pH 7.5, 1.3mM NADP, 20mM $MgCl_2$, 3.1mM 6-phosphogluconate; PGI: 0.1M Tris, pH 7.5, 2mM fructose-6-phosphate, 5mM $MgCl_2$, 0.5mM NADP, 1µ/ml glucose-6-phosphate dehydrogenase; PGM: 0.1M Tris, pH 7.5, 5mM $MgCl_2$, 0.5mM NADP, 1µ/ml Glucose-6-phosphate dehydrogenase, 5mM glucose-1-phosphate; SOD: 0.1M PO_4, pH 7.8, 1mM EDTA, 1.24mg/ml cytochrome-c, 10^{-4}M hypoxanthine, 3.3u/ml xanthine oxidase. Rates were determined

by changes in absorbance at : 340nm for AK, GPT, G6PDH, b-HAD, IDH, G6PD, PGI and PGM; at 240nm for CAT; at 550nm for EST-6 and SOD; and at 250nm for FUM. For SOD, the rate of cytochrome-c reduction (measured at 550nm) before and after the addition of extract was used to calculate units of enzyme activity (McCord and Fridovitch, 1969). Activities are expressed as changes in absorbance per min per mg soluable protein in the extract. Protein was assayed by the method of Lowry et al. (1952) or Bradford (1976). Expected levels of enzyme activity in trisomies were estimated from the parental contribution of chromosome arms. Thus, an expected dose dependent level of enzyme is that activity found in the compound-free strain plus one-half the activity found in Oregon R. A compensated estimate is two-thirds the dose dependent estimate.

Enzyme Electrophoresis

Extracts of pupae were run in 89mM Tris, 89 mM borate, 1mM EDTA, pH 8.2 on Cellogel (Kalex Scientific) electrophoretic membranes, and stained for PGM activity after Shaw and Prasad (1970).

Quantitation of heat-shock protein synthesis:

For each sample, salivary glands were dissected from ten female third-instar larvae and incubated at 37 degrees C for 1

hour in Ephrussi-Beadle saline containing 1mM PO_4, pH 6.8 (PBEBS). The glands were then placed at 25 degrees C and incubated in 20µl PBEBS containing 20µCi ^{35}S-methionine (New England Nuclear 496 Ci/mmol). After a 30 minute incubation, 20µl of Laemli (1970) SDS complexing solution was added. The glands were disrupted by sonication, boiled for 10 minutes and TCA precipitable counts determined. From each sample 25,000 cpm were run on a 28cm long, 15% SDS-acrylamide gel cross-linked with 0.38% DATD, with a 3% stacking gel. After electrophoresis, the gels were prepared for fluorography (Bonner and Laskey 1974), dried and exposed to X-ray film for seven days. Autoradiograms were laid over the gels and appropriate bands were excised and counted by scintillation counting.

Measurement of heat-shock RNA.

Female, wandering, third-instar larvae were heat shocked at 37 degrees C for one hour. Fat body and salivary glands were dissected from these individuals in ice-cold 10mM PO_4, 1mM phenylthiocarbimide, pH 7.5, separated and frozen at -70 degrees C. For each sample, RNA was extracted from tissue from 25 individuals in 0.14M NaOAc, pH 4.5, 4µg/ml polyvinylsulphate, 50µg/ml heparin, 2mg/ml bentonite, 1% SDS, 0.1% diethylpyrocarbonate. After extracting once with phenol, once with phenol:chloroform:iso-amyl alcohol (50:50:1), once with chlorform: iso-amyl alcohol (50:1), the RNA was precipitated with 1/10 volume of acetate and 2.5 volumes of ethanol. The RNA

was dissolved in distilled water, made to 2% formaldehyde, denatured five minutes at 65 degrees C, cooled and run on 1% agarose gels containing formaldehyde (Rave et al., 1979). The RNA was blotted to nitrocellulose, prehybridized, and then hybridized (Thomas, 1980) simultaneously with clones for the hsp 83 gene (pPW244) and for the hsp 70 gene (pPW229) (Holmgren et al., 1979). The filters were washed for 30 min at 25 degrees C in two changes of 2 X SSC, 0.1% SDS, and for one hour at 65 degrees C in two changes of 0.1 X SSC, 0.1% SDS. The blots were exposed to X-ray film for variable periods of time to allow densitometric scanning of the bands in the linear range of the film. DNA was labelled with ^{32}P by nick translation (Rigby et al., 1977).

RESULTS

In the previous chapter we observed dosage compensation of several autosomal loci in individuals trisomic for the left arm of chromosome two. To examine this phenomenon further, enzyme levels from genes within three additional whole arm trisomies of Drosophila melanogaster have been measured.

Compensation of autosomal loci is not limited to 2L.

Compound-free strains for the third chromosome were generated in an Oregon R background as described in Materials and Methods. The survival of trisomic-3L individuals was cytologically verified (Figure 6). Their viability throughout development is comparable to that observed both for trisomic-2L and for trisomic-X individuals. In comparison trisomies for 3R (with or without a deficiency for 83DE) and for 2R survive very poorly. Since conclusions based on measurements from individuals with poor viabilities may be erroneous, an analysis of 3R was not done, and observations for 2R were limited to one locus. As in our previous study, our analysis was restricted to the third instar larval stage; trisomies for any major chromosome arm rarely survive to the adult stage.

The activity of six enzymes, whose structural genes are located on 3L are shown for individuals trisomic for 3L and for their diploid parents (Tables 6 and 7). Estimated values corresponding to a dose dependent and to a dosage compensated

(diploid) level of expression are also presented (see Materials and Methods). Four of the six loci (Idh, Ak, Sod, Pgm) had levels of activity in trisomic-3L individuals consistent with compensated levels of expression (Table 6). Two loci, Est-6 and Cat, displayed dose dependent levels of enzyme activity (Table 7). These observations are similar to those found for trisomy 2L: approximately two-thirds of the loci compensate in trisomies while the remainder are dose-dependent.

The regional distribution of compensating and non-compensating loci in the trisomic-2L condition was non-random. Genes located in the distal two-thirds of the chromosome were compensated, whereas the proximal loci were not. In the present investigation, the distribution of compensating loci appears less ordered, and a regional separation of dosage-compensated from dose-dependent loci was not observed. However, as was the case with 2L, both non-compensating loci (Est-6 and Cat) are located within the proximal one-third of the chromosome arm (although all loci measured on 3L were limited to within the proximal two-thirds of 3L). As noted above, trisomies for 2R and for 3R survive poorly, and they display phenotypes that characteristically are associated with aneuploidy. Nevertheless, we were able to measure one locus in trisomic-2R individuals. This gene (Pgi) displayed a dose-dependent level of gene product (Table 8). Curiously, it too is located in the proximal one-third of 2R.

How does dosage compensation occur at the level of the individual gene? Simplistically, all three alleles of a single,

Table 6 Enzyme levels for compensating loci in diploid and trisomic-3L larvae.

Genotype	Enzyme Locus			
	Idh	Ak	Sod	Pgm
Oregon R (diploid)	0.622	14.2	0.172	1.29
	± .022	± 0.6	± .009	± .09
	(6)	(7)	(4)	(6)
C(3L)VG1, ru st; F(3R)VD1,e^{s}/ F(3R)VD1,e^{s}	0.529	13.2	0.156	1.12
	± .022	± .04	± .006	± .11
	(6)	(6)	(4)	(6)
C(3L)VG1, ru st/ +/F(3R)VD1,e^{s} (trisomic-3L)	0.539	15.4	0.187	0.97
	± .012	± 0.6	± .007	± .11
	(6)	(7)	(4)	(6)
Estimates				
dose-dependent	0.840	20.3	0.107	1.77
	± .012	± .2	± .001	± .06
compensated	0.560	13.5	0.161	1.18
	± .008	± .1	± .002	± .04

Activities expressed as the Mean (± standard error) change in absorbance per mg protein per min. except for Sod where the activity is expressed as the concentration of extract protein required to give a 50% reduction in reaction velocity. Number in parenthesis is the sample size.

Table 7 Enzyme levels for noncompensating loci in diploid and trisomic-3L larvae.

	Enzyme Locus	
Genotype	Est-6	Cat
Oregon R (diploid)	0.663 ± .023 (10)	6.74 ± .45 (6)
C(3L)VB1, ru st; F(3R)VD1,e^{s}/ (diploid)	0.340 ± .010 (10)	6.18 ± .25 (6)
C(3L)VB1, ru st/ +/F(3R)VD1,e^{s} (trisomic-3L)	0.737 ± .002 (10)	8.61 ± .61 (6)
Estimates		
dose-dependent	0.672 ± .008	9.55 ± .17
compensated	0.448 ± .005	6.37 ± .12

Activities expressed as the Mean (± standard error) change in absorbance per mg protein per min. Number in parenthesis is the sample size.

Table 8 Enzyme levels in diploid and trisomic-2R larvae.

Genotype	PGI
Oregon R (diploid)	2.04 ± 0.08 (6)
F(2L)pr/F(2L)pr/C(2R)P, px (diploid)	2.75 ± 0.23 (6)
F(2L)pr/+/C(2R)P, px (trisomic-2R)	3.76 ± 0.19 (6)
Estimates	
dose-dependent	3.77 ± .12
compensated	2.51 ± .08

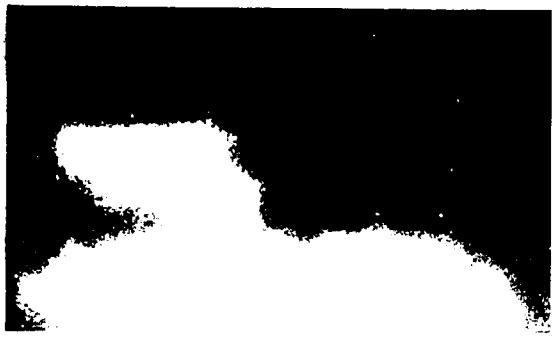
Activity expressed as in Table 6.

dosage compensated gene could be expressed at two-thirds of the diploid level. Alternatively, two of the three alleles could be expressed preferentially. The enzyme encoded by the compensating gene, Pgm, exists in a monomeric form and thus allows a simple test to distinguish between these two possibilities. Trisomic-3L individuals with different electrophoretic variants at each of the 3 Pgm cistrons were constructed. Figure 7 clearly shows that extracts from these individuals possessed all three allozymes in approximately equivalent amounts. Thus, in trisomic-3L larvae, all three genes apparently contribute equally to the total level of PGM. Since measurements of total PGM activity in these individuals showed diploid levels, one can infer that each Pgm structural gene in trisomic-3L larvae produced only two-thirds the amount of enzyme as does the same allele in a diploid.

X-linked gene activity in metafemales

For most genes, males and females produce equivalent amount of gene product. This is true for X-linked genes as well, despite the difference in X-chromosome number between the sexes. Several studies (Lucchesi et al., 1974; Stewart and Merriam, 1975; Gvozdev et al., 1983) have revealed that trisomic-X (metafemales) individuals express diploid levels of X-linked gene products. However, contradictory results also exist. Faizullin and Gvozdev (1973) have reported that two X-linked loci (6-Pgd and Zw) produce dose dependent levels of

Figure 7. Electrophoretic analysis of PGM in diploid and trisomic-3L pupae. 1. diploid, Pgm^{1}/ TM3,Sb Ser Pgm^{4} 2. trisomic-3L, C(3L)VD2,Ly Pgm^{2}//In(3L), Me Pgm^{4}/ Pgm^{1}/F(3R)VD1,e^{ss}. 3. diploid, C(3L)VD2,Ly Pgm^{2}/ In(3L)P,Me Pgm^{4}; F(3R)VD1,e^{ss}/ F(3R)VD1,e^{ss}



1 2 3

enzyme in metafemales. To examine gene expression in metafemales, we measured the activity of five X-linked gene-enzyme systems. In addition to 6-Pgd and Zw (G6PD), we have examined the expression of Fum, Gpt, Had, three loci that had not been investigated previously in metafemales. The data for males, females and metafemales are presented in Table 9. In metafemales, 6-Pgd and Zw show diploid levels of activity, although the former is slightly higher than expected. The three other loci (Fum, Gpt, Had) were also compensated in metafemales. The structural genes for these enzymes are widely distributed along much of the length of the X-chromosome. For b-HAD, despite a marked difference in activity between males and females, an appropriate compensated response was still observed. Thus, it would appear that many loci on the X-chromosome are capable of dosage compensating in metafemales, as well as in males and females.

Heat-shock genes are differentially expressed in trisomies.

The heat-shock genes comprise a group of coordinately controlled genes that are activated in response to environmental stress. After heat shock, these genes display an altered chromatin structure and transcribe at very rapid rates. The final production of heat-shock proteins results from marked alterations both in the transcriptional and translational processes. In D. pseudoobscura, at least one of

Table 9 Enzyme levels in males, females and metafemales.

Genotype	Enzyme Locus				
	Pgd	Fum	Gpt	Had	Zw
+/O (male)	0.158	0.258	1.50	0.243	0.141
	± .012	± .003	± .17	± .011	± .009
	(6)	(4)	(6)	(5)	(6)
C(1)RM, y pn v/Y (female)	0.143	0.295	1.19	0.129	0.150
	± .009	± .018	± .07	± .007	± .011
	(6)	(4)	(6)	(6)	(6)
C(1)RM, y pn v/+ (metafemale)	0.175	0.318	1.45	0.158	0.147
	± .019	± .014	± .14	± .007	± .014
	(6)	(4)	(6)	(6)	(6)
Estimates					
dose-dependent	0.222	0.424	1.94	0.251	0.221
	± .005	± .009	± .06	± .005	± .006
compensated	0.148	0.283	1.29	0.167	0.147
	± .003	± .006	± .04	± .003	± .004

Activity expressed as in Table 6.

these heat-shock genes is X-linked (hsp 83 on XR) and dosage compensates between males and females (Pierce and Lucchesi, 1980). The homologous gene in D. melanogaster, hsp 83, is found on the same chromosomal element, at cytological position 63BC on chromosome 3L. The structural genes for two heat-shock proteins, hsp 68 and 70, are located on the right arm of chromosome 3. The hsp 68 protein is encoded by a single gene located at 95D while five genes are responsible for hsp 70 production; these are located in two clusters, with two genes at 87A and three genes at 87C. To determine whether the 3L heat-shock proteins were dosage compensated in 3L trisomies, we measured the amount of each hsp synthesized in salivary glands after incubation at 37 degrees C for one hour (see Figure 8a). Since all of the hsp 68 and 70 genes are located on the right arm of chromosome three, they are found at equivalent dosages in all the strains used here. Table 10 shows the ratio of incorporation of ^{35}S-methionine into hsp 68 relative to hsp 70. Two points are apparent. First, the ratios are quite similar between all three strains examined. This result suggests that the expression of these two genes are not differentially affected by a duplication of 3L. Second, the total incorportation of ^{35}S-Met into hsp 68 is approximately one-fifth of the amount found for hsp 70. Therefore, the amount of final product is directly proportional to the number of templates for each of these heat-shock proteins. This value would be expected only if equivalent levels of transcription and similar efficiencies of translation were involved in the

Figure 8. Autoradiograms of heat-shock protein and RNA from salivary glands. A. Heat-shock proteins 1. diploid, compound-free stock 2. diploid, Oregon R 3. trisomic-3L B. Heat-shock RNA 1. trisomic-3L 2. diploid, compound-free 3. diploid, Oregon R.

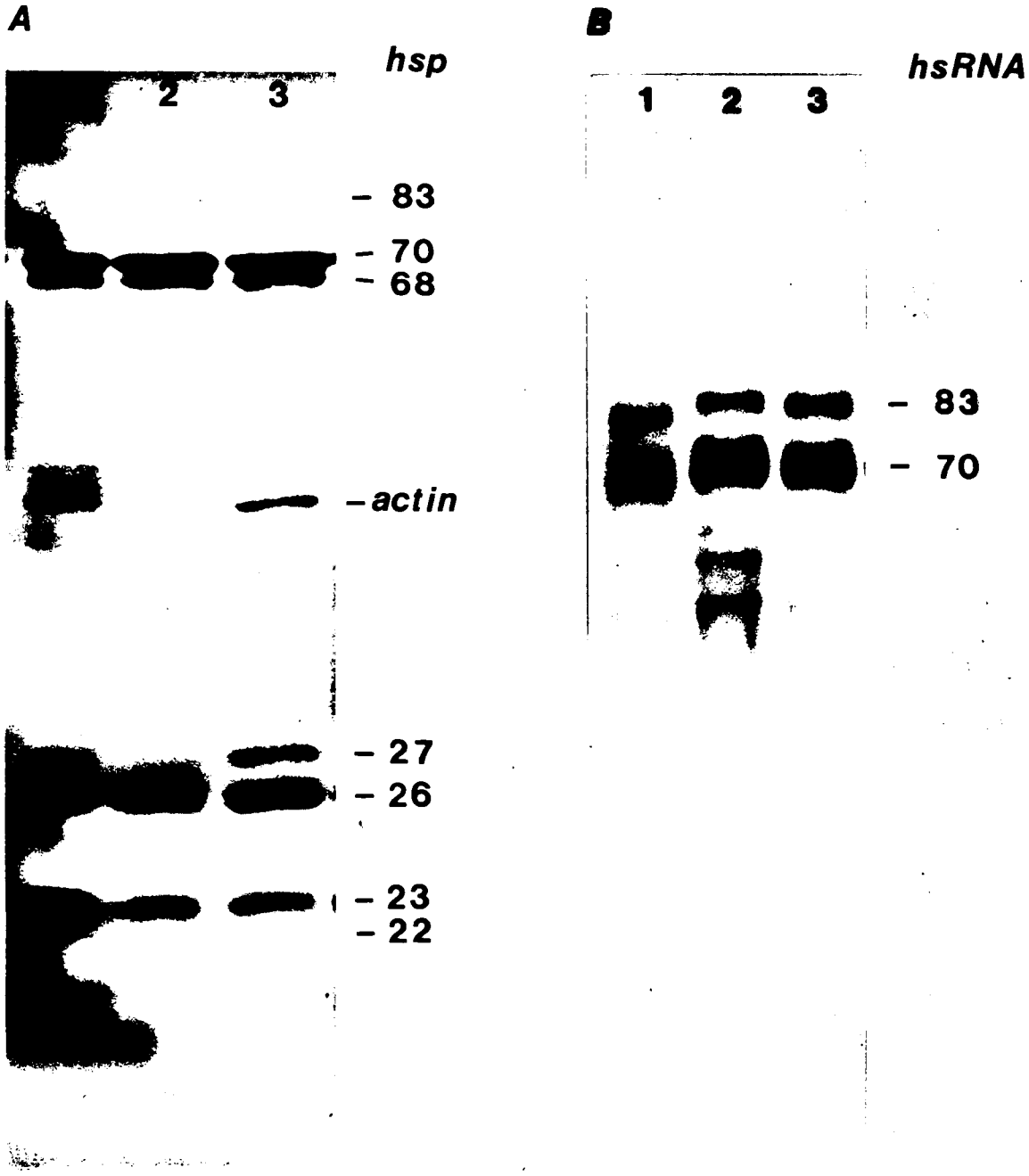

A
hsp
2
3
– 83
– 70
– 68
–actin
– 27
– 26
– 23
– 22
B
hsRNA
1
2
3
– 83
– 70

Table 10 Heat-shock protein 68 and 83 synthesis in diploid and trisomic-3L salivary glands.

Genotype	Heat Shock Protein 68	Heat Shock Protein 83
Oregon-R (diploid)	0.222 ± 0.11 (11)	0.030 ± .004 (10)
C(sl)VG1, ru st; F(3R)VD1,e^{ss}/F(3R)VD1,e^{ss} (diploid)	0.233 ± .016 (10)	0.042 ± .005 (10)
C(3L)VG1, ru st/+/ F(3R)VD1,e^{ss} (trisomic-3L)	0.224 ± .012 (14)	0.039 ± .004 (14)
Estimates		
dose-dependent		0.057 ± .003
compensated		0.038 ± .001

The incorporation into hsp 68 is expressed relative to hsp 70 alone while hsp 83 is expressed relative to the incorporation into hsps 68 plus 70. The values given are the mean ± standard error. Numbers in parentheses are the sample sizes.

production of these two heat-shock proteins. Because equivalent levels of production are observed for hsp 68 and hsp 70 between the three strains, and because a differential response was not observed within a strain, we felt that these two proteins could serve as internal standards to quantify the relative amounts of the hsps encoded by loci on 3L.

The level of another hsp is also shown in Table 10. Hsp 83 is expressed at a diploid level in trisomic-3L strains. Thus, it appears that hsp 83 production in these trisomies is compensating for the increased gene dose. Another heat-shock puff, located at 67B on 3L, contains genes coding for the four small heat-shock proteins (hsp 27, 26, 23 and 22). All four are encoded within a 12kb region with transcription oriented in both directions (Corces et al., 1980). Although tightly linked, the low molecular weight hsp genes showed a differential response to trisomy (Table 11). The amount of hsp 26 and hsp 27 formed in salivary glands was dose dependent in trisomic-3L individuals. However, hsp 23 showed an over compensated level of expression, and hsp 22 showed only partial compensation. These deviations from a discrete response for hsp 22 and 23 may have resulted from imprecise recovery of these two heat-shock proteins due to similar migration rates on the SDS gels. If the expression of hsp 22 and 23 are combined, a clearly compensated level of expression is observed. How the small hsp's show this diverse response is not known. The complexity of the heat-shock response would make any of a number of mechanisms possible.

To investigate whether the regulation of hsp production in

Table 11 Small heat-shock protein synthesis in diploid and trisomic-3L salivary glands.

Genotype	Heat Shock Protein 27	26	23	22
Oregon-R (diploid)	0.033 ± .004 (11)	0.151 ± .009 (11)	0.343 ± .054 (11)	0.064 ± .005 (9)
C(sl)V81, ru st; F(3R)VD1,e^{s}/F(3R)VD1,e^{s} (diploid)	0.061 ± .018 (8)	0.265 ± .021 (10)	0.327 ± .037 (10)	0.088 ± .003 (10)
C(3L)V81, ru st/+/ F(3R)VD1,e^{s} (trisomic-3L)	0.093 ± .026 (14)	0.316 ± .034 (14)	0.244 ± .023 (14)	0.102 ± .008 (14)
Estimates				
dose-dependent	0.078 ± .008	0.341 ± .010	0.480 ± .023	0.120 ± .002
compensated	0.052 ± .005	0.227 ± .006	0.332 ± .015	0.080 ± .001

The incorporation into hsp 68 is expressed relative to hsp 68 plus 70.

3L trisomies is mediated at the level of gene transcription we measured the levels of hsp 83 RNA isolated from salivary glands and from fat body. Northern blots of total RNA from these tissues were hybridized simultaneously to probes corresponding to the hsp 83 and the hsp 70 genes (Figure 8b), and the ratio of the two RNA levels was determined from densitometric scans of the autoradiograms. Table 12 shows these data, as well as expected compensated and dose-dependent values. It is clear that the level of hsp 83 RNA in trisomies, although somewhat lower than expected, is consistent with a dosage-dependent level of expression. Equivalent results were obtained in both trisomies examined. Thus, dose-dependent levels of hsp83 RNA were observed in trisomies while only diploid levels of the corresponding protein are found. These results imply that the hsp 83 gene is transcribed at the same rate in each strain, regardless of the number of templates, and that the production of heat-shock protein 83 in trisomies is regulated at the post-transcriptional level. It is possible that compensation for the small hsp's (23 and 22) is also regulated post-transcriptionally, although I have not attempted to determine that here.

A possible complication exists when measuring one product relative to another. If the transcription of both the hsp 83 gene and the internal standard (hsp 70) genes is affected similarly by the trisomic condition (either both increased or reduced) a dose dependent level of hsp 83 RNA synthesis would be observed. Since this experiment did not measure absolute levels of hsp 83 RNA, we cannot resolve this

Table 12 Heat-shock RNA 83 levels in fat bodies and salivary glands.

Genotype	Tissue	
	Fat Body	Salivary Gland
Oregon-R (diploid)	0.284 ± 0.39 (4)	0.314 ± .027 (4)
C(3L)VG1, ru st; F(3R)VD1,e^{s}/F(3R)VD1,e^{s} (diploid)	0.316 ± .056 (4)	0.333 ± .034 (4)
C(3L)VG1, ru st/+/ F(3R)VD1,e^{s} (trisomic-3L)	0.428 ± .062 (5)	0.457 ± .044 (6)
Estimates		
dose-dependent	0.458 ± .029	0.490 ± .018
compensated	0.305 ± .019	0.327 ± .012

hs RNA 83 levels expressed relative to hs RNA 70.
Mean ± standard error
Numbers in parentheses are the sample sizes.

difficulty. However, we can say that the transcription of the hsp 83 gene either is unaffected by the trisomic condition or responds similarly to the hsp 70 genes. That the hsp 70 genes are unaffected by trisomy for 3L is supported by the observation that the amount of hsp 68 protein relative to hsp 70 remained unchanged between diploids and trisomic-3L individuals.

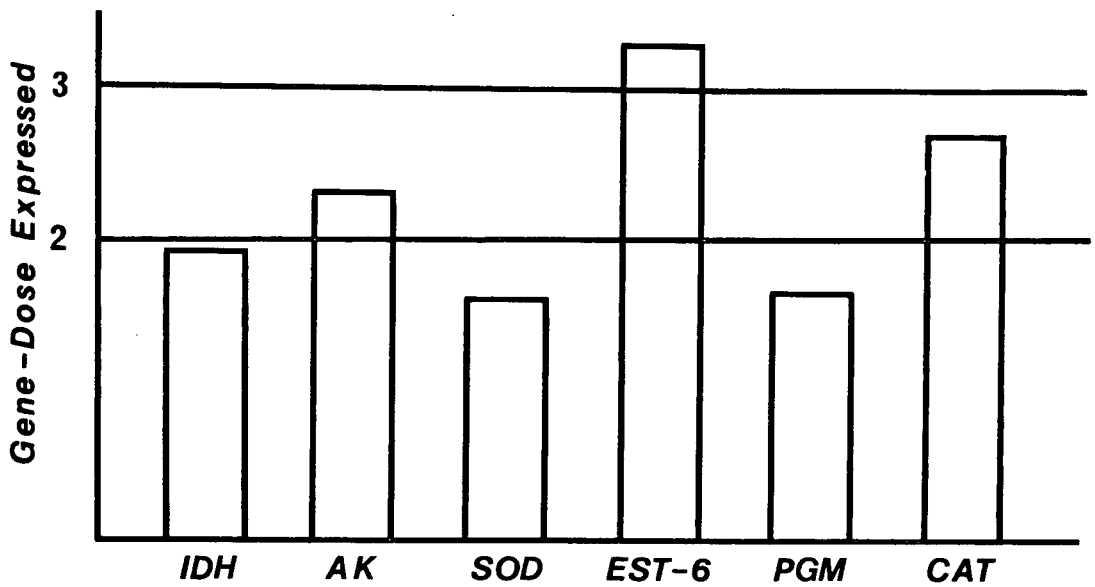
DISCUSSION

Normally, a direct correlation is observed between gene dosage and gene product in a wide variety of organisms. Investigations of enzyme levels have been made on many species that are aneuploid for whole chromosomes or chromosome arms. The difference in ploidy that exists for the sex chromosomes of birds and Lepidopterans results in a dosage dependent difference in Z-linked gene products (Johnson and Turner, 1979; Braverstock et al., 1982). Elevated levels of gene product have been observed in trisomies from several plants (McDaniel and Ramage, 1970; Nielson and Frydenberg, 1971; Carlson, 1972; Nielson and Scandalios, 1974; Smith and Conklin, 1975; Fobes, 1980; Tsaftaris et al., 1981; Birchler and Newton, 1981; McMillin and Scandalios, 1982; Wijsman and van den Berg, 1982) and animals (Marimo and Giannelli, 1975; Magenis et al., 1975; George and Francke, 1976; Kurnit, 1979; Cox et al., 1980; Epstein et al., 1982; Klose and Putz, 1983; Steinback and Benz, 1983). In fact, many of these studies have used the dosage dependent response in trisomies for gene mapping. These observations indicate that the availability of a structural gene for transcription generally sets limits on the amount of gene product synthesized within a cell. This implies that factors responsible for maintaining particular levels of gene expression are generally in excess, and that small changes in the doses of the genes they control (one to three copies) are not sufficient to change their effective concentration.

In contrast, this study has confirmed our previous findings that, in Drosophila, dosage compensation in whole arm trisomies is not restricted to the X-chromosome. Many autosomal loci display diploid levels of activity when duplicated in whole-arm trisomies. Approximately two-thirds of the loci examined in trisomic-3L (see Figure 9) or in trisomic-2L individuals show a compensated (diploid) level of expression. Independence of gene-product levels on gene dose (dosage compensation) has also been observed in lower (Osley and Hereford, 1981; Pearson et al., 1982) and higher eukaryotes (Schwartz, 1971; Carlson, 1972; Birchler, 1979; Fobes, 1980; Birchler and Newton, 1981; Devlin et al., 1982; McMillin and Scandalios, 1982).

Compensation of genes in autosomal trisomies occurs by a mechanism that operates on all three copies of a duplicated locus. This study revealed that compensated levels of PGM enzyme activity are found in trisomic-3L individuals. Using electrophoretic variants, it is clear that all three copies of this locus were transcriptionally active in trisomic individuals. Since PGM is a monomeric enzyme, it is not possible to demonstrate that all three alleles are active in a single cell in these individuals. This issue has been resolved by examining the activity of the Gpdh locus which is located on the left arm of chromosome 2 and is dosage compensated in trisomic-2L strains. GPDH is a dimeric molecule, and if all three alleles are expressed in a single cell, the ratios of material in the various bands should be predictable (1:2:4:2:1). Reanalyses of a trisomic-2L strain with three separable electrophoretic variants of Gpdh showed that all three

Figure 9. Summary bar graph of enzyme quantities in 3L trisomies relative to diploids. Gene dose expressed 2 indicates a diploid level of enzyme activity. Gene dose expressed 3 indicates a dose-dependent quantity.


Gene-Dose Expressed
3
2
IDH
AK
SOD
EST-6
PGM
CAT

structural genes were active in each cell. We also observed that, even though the expression of each Gpdh template was reduced by two thirds in trisomic individuals (compensating), the total amount of enzyme produced remained directly sensitive to changes in the Gpdh structural gene dose; ie. if deficiencies or null activity alleles were used in trisomies the amount of GPDH activity was reduced further by one third. These results eliminate the possibility that regulation occurs by feedback inhibition by product levels for this locus. Moreover, the results with the Gpdh deficiency reveal that compensation in trisomies does not arise from competition between individual copies of a compensated gene for a locus specific factor (Aragoncillo et al. 1978). The regulatory effect appears to act equally on each allele of a particular gene and independently of the others. Indeed limitations in the normal synthetic pathway are not observed in diploids; that is, the amount of GPDH enzyme is directly dependent on the structural gene dose (O'Brien and McIntyre, 1972; Bewley et al., 1974). Thus, it seems likely that compensation in whole-arm trisomies arises from alterations to transcriptional processes that normally occur in diploids.

Since one-third of the loci measured on an autosomal arm are not compensated in trisomies, the compensatory mechanism does not operate on the whole chromosome arm as a unit. In trisomies for 2L, compensating and non-compensating loci appeared to be segregated into separate regions of the chromosome and thus could be regulated as large chromosomal subunits. In contrast, in 3L trisomies compensating and

non-compensating loci were not segregated into separate regions of the chromosome. More importantly, measurements of transcription on polytene chromosomes from salivary glands of 2L trisomies indicate that, for large chromosomal regions, a regional distribution of compensating and noncompensating loci does not exist (see Chapter 5). In addition, the data on the small heat shock proteins in 3L suggest that even within a small chromosomal domain (12kb), adjacent genes may behave independently. This argues that compensating and non-compensating loci are distributed throughout the chromosome arm. Therefore no simple mechanism, such as partitioning based on chromosomal location, exists to distinguish between loci that respond to compensation and those that do not.

The mRNA for hsp 83 is found at dose dependent levels in 3L trisomies. However, since heat-shock protein 83 synthesis occurs at a diploid rate, post-transcriptional regulation is implied. Heat shock protein synthesis occurs by a well choreographed interaction of transcriptional and translational processes (Schlesinger et al., 1982). DiDominico et al.(1982a) have suggested that the production of heat shock protein may be controlled by autoregulation. This type of regulation could account for compensated levels of hsp 83 production in trisomic-3L individuals. No matter how much hsp mRNA is produced from a particular gene, the amount of protein accumulated could be restrained by autogenous (Parsons and Mackie, 1983) or independent controls. After heat shock in trisomies, hsp 83 mRNA may have been degraded more rapidly or translated less efficiently than hsp 70 RNA. For example,

DiDominico et al. (1982b) have shown that rapid cessation of hsp 70 synthesis after heat shock is accomplished by selective degradation of hsp 70 mRNA. It is therefore of some interest that dosage compensation in yeast can occur by translational control or by selective RNA degradation (Osley and Hereford, 1981; Pearson et al., 1982). While compensation for hsp 83 in trisomic-3L individuals may involve post-transcriptional regulation, this situation is probably atypical. Measurements of other mRNA levels and of transcription rates on the polytene chromosomes suggest that compensation is generally mediated at the level of transcription (see chapter five). In view of the autoregulatory nature of hsp production in Drosophila it is particularly noteworthy that the X-linked hsp 83 gene acquired the ability to dosage compensate at the transcriptional level in D. pseudoobscura (Pierce and Lucchesi, 1980). The evolutionary pressure for this may have arisen from a requirement for this protein during non-heat shock conditions. In this regard, hsp 83 can be found in the cytoplasm under normal (non-stressful) conditions (Lindquist, 1980).

What is the mechanism by which compensation is brought about in autosomal trisomies? A simple hypothesis, that was originally proposed to explain how sex-linked dosage compensation could apply to dosage compensation in autosomal trisomies. This hypothesis postulates the existance of trans-acting repressors encoded on the same linkage group as the genes whose expression they influence (Muller et al., 1931). It is well documented that changes in the dose of a regulatory gene can affect the level of expression of the gene that it

controls. For example in a systematic survey of the Drosophila genome, Rawls & Lucchesi (1974) observed that several chromosomal regions, when duplicated, repressed the activity of a gene enzyme system located on the same linkage group, and the magnitude of these changes approached 33%. It is quite conceivable that structural genes and negative trans-acting regulatory loci controlling them often would be located on the same chromosome arm. In this case, dosage compensation would be observed since the increased repressor concentration would nullify the increase in template number. In corn, this hypothesis has been particularly well tested. Birchler (1979, 1981) observed that the activity of several enzmyes were inversley related to the dose of chromosome 1L. One of these loci, Adh-1, is located within this arm and consequently produces similar levels of product in genotypes with various doses of 1L (Schwartz, 1971; Birchler, 1979). An alternative hypothesis for the regulation of dosage compensation suggests that rate-limiting activators of transcription are encoded by genes located on chromosomes (or chromosome arms) other than those whose transcription they influence (Schwartz, 1971). X-chromosome transcription appears to be continually adjustable in strains with X/A ratio between 0.33 and 1.5 (Maroni and Plaut, 1973b; Maroni and Lucchesi, 1980; Williamson and Bentley, 1983). Although these studies did not measure single gene products in single cells, they are compatible with the theory that X-linked dosage compensation results from competition for limited activators of transcription encoded on the autosomes.

The limiting activator theory appears to be less attractive

for explaining dosage compensation in autosomal trisomies because of the special contrivances required to accommodate it. For example, several genes would have to be regulated by the same activator so that dosage dependence could be observed in small segmental aneuploids. All genes that respond to a single activator must reside in the same chromosome arm for a discrete response to be observed in autosomal trisomies. Furthermore, the limiting activator theory would predict that trisomy for one arm would increase the expression of some genes located elsewhere in the genome; activation of unlinked loci is very rare in trisomies (see chapter 3).

In the previous chapter, we noted similarities between compensation observed in trisomic-X and trisomic-2L strains. It is possible that compensation in all trisomies (sex-linked and autosomal) could be controlled by similar mechanisms. However, the literature contains conflicting reports on the levels of 6-PGD and G6PDH (Faizullin and Gvozdev, 1973; Lucchesi et al., 1974; Stewart and Merriam, 1975; Williamson and Bentley, 1983; Gvozdev et al., 1983). For these reasons we examined the expression of five X-linked genes in metafemales. These included the two previously mentioned gene-enzymes and three other loci (Fum, Gpt, Had) whose expression had not been examined in metafemales. The data we obtained for metafemales is consistent with a compensated level of expression for four of five loci measured. A fifth locus, 6-Pgd, produced approximately 20% more product than was expected for a compensated response. It is noteworthy that while compensated levels of X-linked protein products are observed in metafemales,

both dose dependent and compensated levels of transcription have been observed (Ananiev et al.,1974; Lucchesi et al., 1974). These observations may be interpreted as a lack of precision in the regulatory mechanism involved. However, lack of precision is not observed between euploid males and females, and therefore, it is possible that dosage compensation in metafemales occurs by a mechanism that differs from male-female compensation. Consistent with this view, we have recently found that the expression of LSP-1a, an X-linked gene which does not completely compensate between euploid males and females, is fully compensated in metafemales (see chapter six). Clearly, a conclusion regarding the analogy between compensation in X and in autosomal hyperploids awaits resolution of the transcription rate in metafemales. It is apparent from these investigations that dosage compensation for variations in chromosome number is a complex phenomenon and that it may be influenced by several regulatory systems.

In the family Drosophilidae, dosage compensation for differences in chromosome number between the sexes is observed for at least three of the major chromosomal elements (chromosome arms). Between species, an element can exist either as an autosome or as an X-chromosome. For example, element D (after Patterson and Stone, 1952) comprises the left arm of chromosome three in D. melanogaster but is the right arm of the X-chromosome in D. pseudoobscura. Another chromosome element exists as a second X-chromosome in D. miranda, X_2. This neo-X chromosome appears to be only partially compensated between males and females (Strobel et

al, 1978; Das et al., 1982) and perhaps allows a fortuitous glimpse at an evolutionary intermediate of dosage compensation. Lucchesi (1978) and Charlesworth (1978) have discussed how dosage compensation may have arisen during the establishment of a heteromorphic pair of sex chromosomes. If chromosome arms behave inherently as independent regulatory units, then this might facilitate the acquisition of sex-linked dosage compensation when these arms become X chromsomes. If such an hypothesis were correct, then X-linked dosage compensation could be a specialized extension of autosomal compensation. Alternatively, if trisomy (X and autosomal) and male-female compensation operate as two independent mechanisms, then sex-linked dosage compensation may evolve by the acquisition of the necessary regulatory elements on the new X-chromosome.

Some light can be shed on this problem by examining the response of the same gene to alterations in ploidy when it is present on an autosome or on the X. Three of the genes which have been measured in this study (involving trisomies for element D in *D. melanogaster*) are also present on the right arm of the X in *D. pseudoobscura*. One of these genes, *Idh*, compensates both in autosomal trisomies of *D. melanogaster* and between males and females of *D. pseudoobscura* (Abraham and Lucchesi, 1975). However, two other genes, *Est-6* (*Est-5* in *D. pseudoobscura*; see Wright, 1961, and Abraham and Lucchesi, 1974) and *hsp 83* (RNA levels), do not compensate in *D. melanogaster* but do in *D. pseudoobscura* (Abraham and Lucchesi, 1975; Pierce and

Lucchesi, 1980). Since Est-6 protein and hsp 83 mRNA synthesis do not regulate in D. melanogaster trisomies, these genes probably have aquired new regulatory controls during evolution that allowed them to compensate for chromosome number in D. pseudoobscura. Therefore, the ability of a gene to dosage compensate in autosomes is not prerequisite for dosage compensation in sex chromosomes. The ability of a gene to dosage compensate on the X arises from signals distant from the structural gene (Korge, 1981), since an autosomal gene transposed onto the X-chromosome can dosage compensate (Spradling and Rubin, 1983; Scholnick et al., 1983). Presumably, sequences located near the gene are important for mediating this effect since an X-linked gene (with flanking sequences) transposed to an autosome retains its ability to dosage compensate (Hazelrigg et al., 1984). The evolving X chromosome may acquire new sequences capable of causing hyper-active transcription in males in a fashion analogous to other eukaryotic transcription enhancers. In any event, these studies on individual genes in different Drosophilid species are concordant with the notion that autosomal and male-females dosage compensation occurs by two separate mechanisms. However, they do not clarify the issue of whether or not compensation in all trisomies (autosomal and X-chromosomal) occurs by a common mechanism.

Organisms have exploited several means to modulate gene expression for variations in gene dose (Casartelli et al., 1969; Rasch et al., 1977; Fraser and Nurse, 1979; Berger, 1982; de C. Bicudo, 1983; Gartler and Riggs, 1983; Baker and Belote, 1983).

This diversity alone is testimony to the complexity of eukaryotic regulatory systems. Compensation in whole arm trisomies reveals control mechanisms which have certainly not evolved to accomodate differences in gene dosage. However, the extensive and widespread existence (see chapter three) of this response suggests that it is a fundamental property of gene control in eukaryotes. A simple interpretation is that hyperploid compensation reflects a homeostatic regulatory system for maintaining balanced rates of gene expression in diploids (Devlin et al., 1982). It would not be surprising though to find that some organisms have exploited and perfected this form of regulation to compensate for naturally occurring deviations from euploidy.

CHAPTER THREE

INFLUENCE OF TRISOMY ON THE EXPRESSION OF LOCI MAPPING OUTSIDE OF THE DUPLICATED REGION

Introduction

The synthesis of functional gene products involves a variety of interacting processes. Each reaction in a synthetic pathway has, to varying degrees, the potential to regulate the quantity or quality of the gene product. Many of these steps require the action of enzymes or cofactors coded for elsewhere in the genome. Thus, 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 (Ganschow and Schimke, 1969; Twardzik et al., 1971; Bewley and Lucchesi, 1977; Johnson et al, 1981; King and McDonald, 1983). Others do not appear to modify the final gene product (Rawls and Lucchesi, 1974a) and may therefore exert their effect at the transcriptional level. Where the quantity of a regulatory-gene product affects the kinetics of a biosynthetic step, the potential exists for end product regulation (Kacser and Burns, 1981). The mechanism by which such genes act is likely to be quite varied, and systems utilizing "positive" or "negative" control can be envisaged (Goldberger, 1979).

Regulatory sequences have been identified in eukaryotes which confer specialized enhancement of transcription in response to various physiological or developmental stimuli (Banerji et al., 1981; Dierks et al., 1981; Pelham, 1982; Banerji et al., 1983; Queen and Baltimore, 1983; Chandler et al., 1983; Gillies et al., 1983).

Cis-acting enhancement of transcription by Ty1 transposable elements in yeast has been shown to require products in trans to exert its effect (Dubois et al., 1982). In Drosophila, X-chromosome dosage compensation also involves an activation of transcription and requires trans-acting gene function (Belote and Lucchesi, 1980). Many examples of regulation of gene expression in eukaryotes involve large changes in transcriptional activity (for examples, see Ashburner and Bonner 1979, or Searle and Tata 1981), in contrast with the regulatory effects described in the previous two chapters, which were much more subtle. Reductions in gene expression on the order of one-third seem trivial compared to the dramatic alterations displayed by some genes. Perhaps more paradoxical is the observation that among strains, and among individuals of a strain, considerable variability in gene activity can be detected (see chapter 4).

The purpose of this chapter is to explore the extent to which gene repression occurs in trisomies, and to discuss the implications of this regulation for Drosophila. The previous two chapters examined the expression of genes within the duplicated arm. Here we examine the expression of genes elsewhere in the genome as well as the expression of two families of genes, one of natural evolutionary origin and the other artificially created by P-element mediated transformation (transformation was performed by J. Posakony who kindly provided the Drosophila stocks). We find that the majority of loci can be repressed by a trans-acting mechanism in at least one autosomal trisomy, while few activations of expression are

observed. Modulation can operate independently of other regulatory mechanisms (such as X-chromosome dosage compensation or developmental cues) and does not obligatorily affect similar genes in similar ways.

Materials and Methods

Genetic stocks and crosses

Trisomies were produced as described in previous chapters. For the experiments concerning X-linked enzyme activity in autosomal trisomies, and isogenic X chromosome (iso-X) from the Oregon R stock was introduced into the compound-free stocks by nondisjuncion, to yield the following stocks: iso-X/iso-X;C(2L)VH1,lt;F(2R)bw/F(2R)bw and 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 rads of gamma irradiation were mated to a compound second stock. A newly induced C(2L)b Adh^{N2} osp pr chromosome was introduced into a compound free stock by nondisjunction to produce the following stock: 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, and null mutations in the native Adh genes. From these crosses female trisomic-2L larvae were selected in which all ADH enzyme activity is encoded by the transposed gene.

Enzyme Assays:

The assays for PGK, GPDH, ADH, IDH and 6-PGD are described in chapter 1 and for AK, PGM, CAT, AO, ME, PGI, FUM, GPT and G6PD in chapter 2. The assay conditions for Aldolase (ALD) were: 0.1M Tris, pH 7.5, 0.2mM NADH, 1mM fructose-1-6-diphosphate, 1 unit glucose-6-phosphate dehydrogenase/ml, 10 units triosephosphate isomerase/ml. In the experiment involving Adh transformants, ADH enzyme activity was assayed in 0.5 M Na_2CO_3, 2mM NAD, 0.275M butan-2-ol, pH 9.0 (Sofer and Ursprung, 1968).

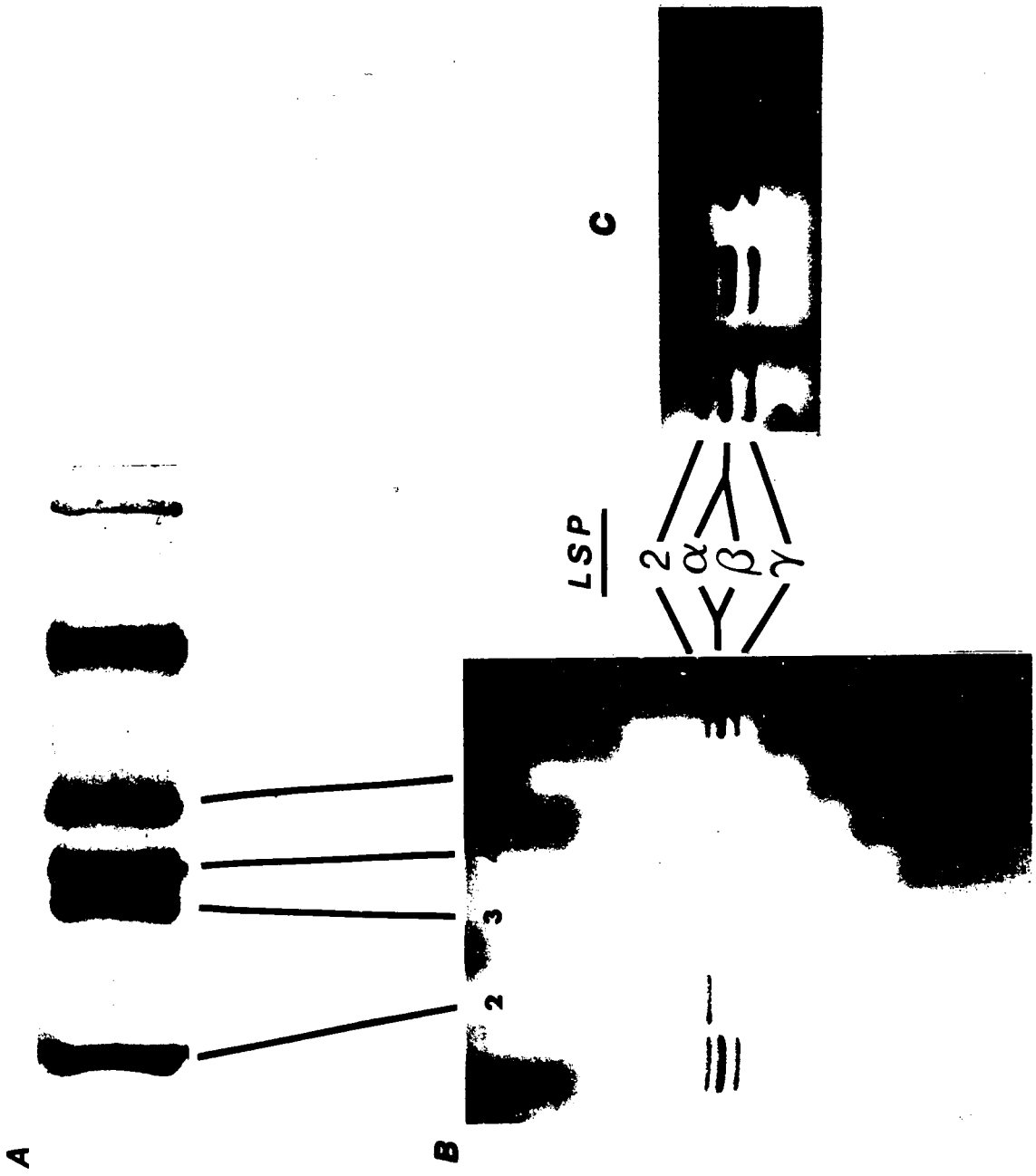
Analysis of Larval Serum Proteins.

Larval serum proteins were analysed in two electrophoretic systems. For analysis by SDS-gel electrophoresis, samples of hemolymph from wandering third-instar larvae (using females only in experiments with autosomal trisomies) were collected in 10mM PO_4, 1mM PTU, pH 7.5 to which was added an equal volume of Laemli SDS complexing buffer. After boiling for 10 minutes, the samples were run on 9% SDS gels (cross-linked with DATD) with a 3% stacking gel (Brock and Roberts, 1980). After staining with coomasie blue R250 and destaining, the gels were scanned with a Beckman DU-8 scanning spectrophotometer. The amounts of individual LSP's were expressed as the percentage of

total LSP in the sample. This analysis was successful only for measurements of LSP-2 and LSP-1g: LSP-1a and LSP-1b were not separated by this method (Figure 10a).

To resolve the a and b subunits of LSP-1, hemolymph was collect in 10mM $Na_2B_4O_7$, 1mM PTU, .01% bromophenol blue, pH 10.0 and electrophoresed on Cellogel (Kalex Scientific) cellulose-acetate strips in 50mM Na barbital, pH 9.4 for 3.5 hours (Roberts and Evans-Roberts, 1979b). The strips were stained with coomasie blue, destained and the bands of interest were excised and dissolved in glacial acetic acid for twelve hours (after Roberts and Evans-Roberts, 1979a). The optical density of the samples was measured at 595nm. To identify the particular LSP on the strips (Figure 10b), various bands were cut out and eluted into SDS-complexing buffer and run on an SDS gel (Figure 10C). The order of migration in SDS gels crosslinked with DATD is LSP-1g, LSP-1b, LSP-1a and LSP-2. On Cellogel strips, migration is LSP-2, LSP-1a, LSP-1b, and LSP-1g, although this latter protein was not reproducibly resolved and therfore was not measured in this system.

Figure 10. Electrophoretic analysis of larval serum proteins on cellulose-acetate strips and in SDS-polyacrylamide gels.
A. Separation of LSP subunits on Cellogel membranes
B. SDS-polyacrylamide gel of LSP subunits. Lanes 1 and 6 are total hemolymph samples from late third instar larvae. Lanes 2 to 5 contain LSP samples isolated from Cellogel membranes by eluting excised bands in Laemli's SDS complexing solution.
C. SDS gel of total hemolymph from: 1. Oregon R; 2. 2L trisomy; 3. compound-free diploid; 4. Oregon R


A
B
C
LSP
2
α
β
γ
3
2

RESULTS

I 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 investigation constitutes a comprehensive survey of the expression of genes outside the trisomic arms. We have examined the expression of fifteen enzyme loci in larvae trisomic for one of 2L, 2R, 3L and the X-chromosome. The results of this analysis has revealed several effects of aneuploidy on gene expression in Drosophila.

Genes outside the duplicated region can be repressed

The compensatory response observed for genes within a duplicated arm operates by reducing the transcription of each structural gene by one third (see chapter 5). Males and females possess similar levels of most gene products suggesting that variation in X-chromosome number does not affect the expression of autosomal genes. If compensation of autosomal genes occurs by a similar mechanism as that operating between males and females, then trisomy for one arm should not affect genes located elsewhere in the genome. In addition, an overall reduction in expression of all loci proportional to the size of

the duplication could arise through competition of all genes for transcriptional components that may be present in limiting quantities. The data presented in this and the next chapter suggests that both of these hypotheses are incorrect.

The activities of six enzymes in trisomic-2L larvae and their diploid parental strains are presented. These enzymes are encoded by genes located on 2R, 3L and 3R, which are diploid in all three strains. Also shown are estimates of the enzyme activity expected if: 1) no alteration of activity was occuring and 2) if gene activity was repressed by one-third. The reader is directed to Figure 12 where the expression of each gene in trisomies, relative to the expression in diploids, is graphically represented. In 2L trisomies, four of the six enzymes (AK, PGM, CAT, and MEN) were present at quantities close to that observed in the diploid controls (Table 13). 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 activations of gene expression by an autosomal trisomy that we have observed (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 (Table 14). The activity of this gene-enzyme was reduced by close to one-third in the trisomies. 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 the other autosomal arms, were

Table 13 Activity of third chromosome enzyme loci in 2L trisomies.

Genotype	Enzyme Locus				
	AK	Pgm	Cat	Aldox	Men
y/y (diploid)	10.5	1.41	7.70	9.49	.470
	± .76	± .10	± .64	± .45	± .052
	(5)	(5)	(5)	(5)	(5)
y/y;C(2L)1t, F(2R)bw/ F(2R)bw (diploid)	11.4	1.36	7.06	10.9	.395
	± .91	± .08	± .54	± .86	± .063
	(5)	(5)	(5)	(5)	(5)
y/y;C(21)1t/ +/F(2R)bw (diploid)	10.6	1.27	8.06	13.2	.432
	± .62	± .11	± .35	± .93	± .050
	(5)	(5)	(5)	(5)	(5)
Estimated Mean	10.9	1.39	7.38	10.21	.433
	± .49	± .05	± .31	± .44	± .034
	(10)	(10)	(10)	(10)	(10)
Repressed Estimate	7.27	.927	4.92	6.81	.289
	± .33	± .03	± .21	± .29	± .023
	(10)	(10)	(10)	(10)	(10)

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.
FF

Table 14 Enzyme activity for a 2R-linked enzyme locus in 2L trisomies.

Genotype	Enzyme Locus
	Pgi
F(2L)b pr/F(2R)b pr; F(2R)bw/F(2R)bw (diploid)	7.59 ± .42 (6)
C(2L)lt;F(2R)bw/ F(2R)bw (diploid)	7.25 ± .21 (6)
C(2L)lt/F(2L)b pr; F(2R)bw/F(2R)bw (trisomic-2L)	5.35 ± .17 (6)
Estimated Mean	7.42 ± .12
Repressed estimate	4.95 ± .08

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

examined. Unlike the situation observed for 2L, six of the seven gene-enzymes systems showed reduced levels of activity in 3L trisomies relative to diploids. Four of the loci (Pgk, Gpdh, Adh, and Pgi) produced approximately one-third of the enzyme activity in these trisomies than that found in diploids (Table 15). The two other loci (Men and Ald, both on 3R) displayed levels of activity intermediate between the two estimated values (Table 16). The other autosomal gene, Aldox, was unaffected by the duplication of 3L. Thus, trisomy for 3L appears to repress more loci than does trisomy for 2L, although not all loci are equally affected.

The last autosomal trisomy examined was for the right arm chromosome two. Because these aneuploids are poorly viable we were able to measure the expression of only one gene located outside of 2R. This gene, Gpdh on 2L, was repressed by one-third in this trisomic-2L strain (Table 17).

Clearly, trisomy for the major autosomal arms in D. melanogaster can have striking effects on gene expression, not only on those genes located within the duplicated arm but also on other arms or linkage groups. By far the most common effect is a reduction in activity by approximately one-third. This type of repression would result if trans-acting repressors of these genes were being duplicated by the trisomic condition.

Since Muller's 1931 proposal that X-linked repressors are responsible for X-chromosome dosage compensation, little supportive evidence has been obtained. It has been inferred that the X-chromosome bears few modifiers of autosomal gene expression because males and females express most genes equally.

Table 15 Activity of second chromosome enzyme loci in 3L trisomies.

Genotype	Pgk	aGpdh	Adh	Pgi
Oregon R (diploid)	5.17	1.64	0.388	8.05
	± .35	± .03	± .021	± .52
	(5)	(6)	(6)	(6)
C(3L)VG1,rust/ F(3R)VD1,e^{s}/ F(3R)VD1,e^{s} (diploid)	4.39	.967	.344	6.28
	± .16	± .021	± .015	± .35
	(4)	(6)	(6)	(6)
C(3L)VG1,rust/ +/F(3R)VD1,e^{s} (trisomic-3L)	2.87	.811	.235	5.31
	± .10	± .026	± .015	± .27
	(5)	(6)	(6)	(6)
Estimated Mean	4.78	1.31	.366	7.17
	± .10	± .01	± .006	± .16
Repressed Estimate	3.19	.876	.244	4.78
	± .07	± .006	± .004	± .10

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 16 Activity of third chromosome enzyme loci in 3L trisomies.

Genotype	Men	Aldox	Ald
Oregon R (diploid)	.452	5.14	1.14
	± .048	± .48	± .06
	(7)	(6)	(4)
C(3L)VG1, rust/ F(3R)VD1,e^s/ F(3R)VD1,e^s (diploid)	.364	5.93	1.25
	± .022	± .46	± .04
	(6)	(6)	(4)
C(3L)VG1, rust/ +/F(3R)VD1,e^s (trisomic-3L)	.330	5.61	.983
	± .011	± .19	± .057
	(7)	(6)	(5)
Estimated Mean	.408	5.54	1.19
	± .014	± .17	± .02
Repressed Estimate	.272	3.69	.797
	± .009	± .11	± .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 17 Activity of a 2L-linked enzyme locus in 2R trisomies.

Genotype	Enzyme Locus Gpdh
Oregon R (diploid)	.998 ± .028 (4)
F(2L)pr/F(2L)pr; C(2R)px (diploid)	.646 ± .059 (4)
F(2L)pr/+/ C(2R)px (trisomic-2R)	.639 ± .049 (6)
Expected Mean	.822 ± .016
Repressed Estimate	.548 ± .010

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

If X-chromosomal and autosomal trisomy are comparable conditions, then trisomy for the X chromosome could repress the expression of some autosomal genes. We have measured eight gene-enzyme systems with representatives from the four major autosomal arms in males, females and metafemales (Tables 18 & 19). All of these loci produced similar levels of product between males and females, despite their non-isogenic condition. In metafemales, six of these autosomal loci were unaffected (Gpdh, Adh, Idh, Pgm, Cat, Aldox). However, two loci were repressed, although only partially; Pgk and Pgi produced about 15% less enzyme in metafemales than euploid males or females. In metafemales, few autosomal loci show the specific repression (one-third) that is seen in autosomal trisomies. Therefore, it appears that the X and the autosomes do not possess equivalent regulatory effects on 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 (see chapter 2). This includes the gene encoding for the a variant of larval serum protein (LSP-1a). A related gene, LSP-1g, located on 3L, also displays a one-third reduction in expression in metafemales (see section II of this chapter).

Decreasing the X/A ratio in trisomies does not result in increased levels of X-linked gene expression

Table 18 Activity of second chromosome enzyme loci in metafemales.

Genotype	Enzyme Locus			
	Pgk	Gpdh	Adh	Pgi
Oregon R +/0 (male)	3.48 ± .23 (6)	.714 ± .043 (5)	.125 ± .006 (5)	3.89 ± .22 (6)
C(1)RM, y pn v/Y (female)	3.24 ± .15 (6)	.682 ± .053 (6)	.154 ± .012 (6)	3.57 ± .04 (6)
C(1)RM y pn v/+ (3X2A)	2.91 ± .08 (6)	.668 ± .039 (6)	.162 ± .018 (6)	3.14 ± .40 (6)
Estimated Mean	3.36 ± .07	.698 ± .017	.139 ± .004	3.73 ± .06
Repressed Estimate	2.24 ± .05	.465 ± .011	.093 ± .003	2.49 ± .04

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 19 Activity of third chromosome enzyme loci in metafemales.

Genotype	Enzyme Locus			
	Idh	Pgm	Cat	Aldox
Oregon R +/O (male)	.369 ± .013 (6)	.633 ± .049 (4)	4.13 ± .27 (6)	6.19 ± .25 (5)
C(1)RM, y pn v/Y (female)	.330 ± .014 (6)	.784 ± .115 (5)	3.91 ± .32 (6)	5.42 ± .25 (5)
C(1)RM, y pn v/+ (metafemale)	.339 ± .026 (6)	.772 ± .083 (6)	3.95 ± .38 (6)	6.17 ± .33 (5)
Estimated Mean	.349 ± .005	.709 ± .034	4.02 ± .10	5.81 ± .09
Repressed Estimate	.233 ± .003	.472 ± .023	2.68 ± .06	3.87 ± .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.

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 (2X3A) is 50% greater than in a diploid female (2X2A). Thus, this effect is consistent with the limiting activator model of dosage compensation; ie. factor(s), coded for by autosomal genes, that are specifically required for X-chromosome transcription. It is not known if entire autosome sets are required to manifest this effect. Conceivably, by increasing the autosomal content of the genome in trisomies, activation of X-linked loci (at least in males) could occur. To test this possibility, the activity of four X-linked enzymes was monitored in iso-X chromosome stocks both for trisomy 2L and for trisomy 3L. Marked variations in expression was observed from this iso-X-chromosome in the different parental stocks. (For examples, see Fum between iso-X and CF2 strains (Tables 20 & 21) or Zw between the two compound-free strains (Table 21 & 22). However, despite these differences, the activity for these enzymes were dosage compensated (Tables 21-24). It is interesting to note that the amount of GPT is significantly higher in males than females in three of the five stocks.

Male and female data were pooled for the analysis of expression between diploids and trisomies. When a comparison is made between genotypes of the pooled male and female data (Tables 25 & 26), two observations can be made. First, both autosomal trisomies failed to activate any of the X-linked loci

Table 20 X-linked enzyme levels in Oregon R iso-X strain.

	Enzyme Locus			
Genotype	Pgd	Fum	Gpt	Zw
female	.163 ± .008	.263 ± .022	1.23 ± .05	.143 ± .005
	(7)	(7)	(7)	(7)
male	.153 ± .007	.253 ± .053	1.15 ± .05	.118 ± .009
	(7)	(7)	(7)	(13)
male/ female ratio	1.12	.962	.935	.826

Table 21 X-linked enzyme levels in C(2L)lt;F(2R)bw/F(2R)bw; iso-X strain.

	Enzyme Locus			
Genotype	Pgd	Fum	Gpt	Zw
male	.182 ± .008	.156 ± .006	1.01 ± .03	.162 ± .009
	(8)	(8)	(8)	(8)
female	.185 ± .009	.177 ± .008	1.22 ± .04	.171 ± .006
	(8)	(8)	(8)	(8)
male/ female ratio	1.02	1.13	1.21	1.06

Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parenthese are the sample sizes.

Table 22 X-linked enzyme levels in C(3L)ru st;F(3R)e^{s}/F(3R)e^{s};iso-X strain.

	Enzyme Locus			
Genotype	Pgd	Fum	Gpt	Zw
female	.198 ± .005 (7)	.232 ± .006 (7)	1.39 ± .04 (7)	.224 ± .010 (7)
male	.185 ± .006 (7)	.249 ± .013 (7)	1.53 ± .04 (7)	.225 ± .013 (7)
male/female ratio	.934	1.07	1.10	1.00

Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parenthese are the sample sizes.

Table 23 X-linked enzyme levels in C(2L)lt/+/F(2R)bw; iso-X trisomic-2L individuals.

	Enzyme Locus			
Genotype	Pgd	Fum	Gpt	Zw
female	.128 ± .004 (8)	.225 ± .009 (8)	1.04 ± .03 (8)	.082 ± .002 (8)
male	.119 ± .004 (8)	.232 ± .009 (8)	1.19 ± .04 (8)	.091 ± .003 (8)
male/ female ratio	.929	1.03	1.14	1.11

Table 24 X-linked enzyme levels in C(3L)ru st/+/F(3R)e^{8}; iso-X trisomic-3L individuals.

	Enzyme Locus			
Genotype	Pgd	Fum	Gpt	Zw
male	.179 ± .006 (10)	.194 ± .011 (10)	.844 ± .064 (10)	.153 ± .007 (10)
female	.139 ± .006 (8)	.174 ± .018 (8)	.816 ± .067 (8)	.134 ± .007 (8)
male/ female ratio	.776	.897	.967	.876

Enzyme activity is expressed as the change in absorbance per min per mg of protein. Numbers in parenthese are the sample sizes.

Table 25 X-linked enzyme levels in diploid and trisomic-2L larvae.

Genotype	Enzyme Locus			
	Pgd	Fum	Gpt	Zw
iso-X Oregon R (diploid)	.158 ± .003 (14)	.258 ± .014 (14)	1.19 ± .02 (14)	.131 ± .003 (26)
iso-X; C(2L)lt F(2R)bw/ F(2R)bw (diploid)	.184 ± .003 (16)	.167 ± .003 (16)	1.12 ± .01 (16)	.167 ± .003 (16)
iso-X; C(2L)lt/ +/F(2R)bw (trisomic-2L)	.124 ± .001 (16)	.229 ± .003 (16)	1.12 ± .01 (16)	.087 ± .001 (16)
Estimated Mean	.171 ± .001	.213 ± .003	1.16 ± .01	.149 ± .001
Repressed Estimate	.114 ± .001	.142 ± .002	.770 ± .003	.099 ± .001

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

Table 26 X-linked enzyme levels in diploid and trisomic-3L larvae.

Genotype	Enzyme Locus			
	Pgd	Fum	Gpt	Zw
iso-X Oregon R (diploid)	.158 ± .003 (14)	.258 ± .014 (14)	1.19 ± .019 (14)	.131 ± .003 (26)
iso-X; C(3L)ru st; F(3R)e^{ss}/ F(3R)e^{ss} (diploid)	.192 ± .002 (14)	.241 ± .004 (14)	1.46 ± .014 (14)	.225 ± .004 (14)
iso-X; C(3L)ru st/ +/F(3R)e^{ss} (trisomic-3L)	.161 ± .002 (18)	.185 ± .005 (18)	.856 ± .024 (18)	.145 ± .003 (18)
Estimated Mean	.175 ± .001	.249 ± .004	1.33 ± .01	.178 ± .001
Repressed Estimate	.122 ± .001	.166 ± .003	.883 ± .004	.119 ± .001

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

examined. Such a finding suggests that if loci encoding transcription activators exist for the X-linked loci examined, they either do not reside on these autosomal arms or if they do, many other copies are also present elsewhere in the genome. Second, all four loci were 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 similar way as do autosomal loci.

This experiment had initially been performed with Basc X chromosomes rather than iso-X chromosomes from the Oregon R stock. Essentially the same results were observed for 2L trisomies as those reported here; however, 3L trisomies homozygous for the Basc chromosome were so extremely inviable that further analysis was precluded.

II. 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. Based either on gene function or on chromosomal position, no simple rule can be applied to group genes into those which are unaffected, activated, or repressed. However, some property of each gene or condition of its expression must render it susceptible to regulation in trisomies. To address this question, the expression of a group of genes which have only recently diverged

(Brock and Roberts, 1983) has been examined. The larval serum proteins are coded for 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. This latter group of genes is of interest. While each gene in this group is similar in sequence composition (Smith et al., 1981), all of them are located on different chromosome arms (LSP-1a on the X, LSP-1b on 2L, and LSP-1g on 3L).

Measurements of LSP's were made from samples of hemolymph collected from wandering third-instar larvae (Figure 10). A absolute quantitation of these proteins (per larvae or per mg live weight) proved difficult owing to technical problems involved in quantitatively extracting hemolymph. As an alternative, the amount of each LSP-1 was measured relative to the others, and to LSP-2 to provide an internal standard for measurement. Therefore,it was importnat to examine the expression of LSP-2 in trisomies relative to diploids. The absolute amounts of this protein were determined from hemolymph samples taken from trisomic-3L larvae and their diploid parental stains and is shown in column 5 of Table 27. Elevated levels of this protein were observed in trisomies (about 40% over the expected compensated level) suggesting that LSP-2 gene expression is unaffected by the duplication. The level of this protein relative to the LSP-1 proteins is also in agreement; the ratios LSP-2/LSP-1a and LSP-2/LSP-1b suggest that LSP-2 is dose dependent in 3L trisomies and that the expression of LSP-1a and LSP-1b genes are unaffected. Relative to LSP-1g, equivalent

Table 27 Quantity of LSPs in diploid and trisomic-3L hemolymph from female larvae.

Genotype	LSP Ratio 2/1a	LSP Ratio 2/1b	LSP Ratio 1g/2	total LSP-2 protein
iso-X Oregon R (diploid)	1.88 ± .11 (8)	1.35 ± .09 (8)	1.03 ± .09 (11)	0.739 ± .052 (8)
iso-X; C(3L)ru st; F(3R)e^{s}/F(3R)e^{s} (diploid)	1.59 ± .08 (8)	1.54 ± .09 (8)	0.905 ± .09 (11)	1.01 ± .07 (8)
iso-X; C(3L)ru st/ +/F(3R)e^{s} (trisomic-3L)	2.65 ± .08 (8)	2.65 ± .16 (8)	1.07 ± .09 (16)	1.29 ± .03 (8)
Dose-dependent Estimate	2.53 ± .05	2.22 ± .05		1.38 ± .04
Compensated Estimate	1.69 ± .03	1.48 ± .03		0.92 ± .03

Measurements in right-most column are in units of absorbance by Coomasie Blue at 595nm.

amounts of LSP-2 are observed in the two diploid and in the trisomic strain. Since LSP-1g and LSP-2 are both located on 3L, we can deduce that LSP-1g protein levels are also elevated in trisomic-3L larvae. Thus, in conclusion, the expression of the four LSP genes occurs at the same rate in 3L trisomies as in diploids.

The quantities of the three LSP-1 subunits produced in trisomic-2L larvae are shown in Table 28. The amount of LSP-1g relative to LSP-2 was the same in these trisomies as in their diploid parents. Since both of these genes are on 3L, and 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-1b gene is duplicated by the trisomic condition yet an eqivalent amount of protein is observed as in diploids. Thus, this gene is compensating in these trisomies for its increased gene dose by producing one-third less product per template. The other LSP-1 examined (LSP-1a 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 of aneuploidy: two of the LSP-1 genes appear to down-regulate while the other is unaffected. It is noteworthy that the two genes which responded similarly are more closely related to each other than they are to the gene that failed to respond (Smith et al, 1981). While this seemed like a possible explanation, the expression of these genes in trisomies for the X chromosome suggests otherwise. The expression of LSP-1 genes in X-chromosomal trisomies will be discussed in more detail in chapter 6. In summary, those

Table 28 Quantity of LSPs in diploid and trisomic-2L hemolymph from female larvae.

	LSP Ratio		
Genotype	1b/2	1a/2	1g/2
Oregon R (diploid)	.937	.646	1.03
	± .029	± .046	± .03
	(8)	(8)	(16)
C(2L)lt; F(2R)bw/ F(2R)bw (diploid)	.833	.664	.827
	± .053	± .023	± .067
	(9)	(9)	(6)
C(2L)lt/ +/F(2R)bw (trisomic-2L)	.813	.424	1.02
	± .024	± .022	± .17
	(9)	(9)	(6)
Dose dependent Estimate	1.30	.655	.929
	± .03	± .012	± .032
Repressed Estimate	.868	.436	.619
	± .020	± .008	± .021

experiments showed that the X-linked LSP-1a gene was compensated in metafemales despite the inability of this gene to completely dosage compensate between males and females. The expression of the closely related gene on 2L, LSP1-b, was not affected in trisomic-X larvae. However, Table 29 shows that LSP-1g protein levels were reduced dramatically in metafemales, even beyond the level expected for a discrete one-third reduction. This is the only autosomal gene examined to date whose product is markedly reduced in quantity in metafemales from the level found in diploids. The quantities of LSP-2, relative to the LSP-1 subunits, are similar in metafemales, females and males. Thus, duplication of the X chromosome does not appear to alter the expression of all LSP's in similar ways. Indeed, the two genes that do respond similarly (LSP-1a and LSP1-g) 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. However, the converse is obviously not true since many different loci can show the same response in trisomies.

Adh transformants in 2L trisomies

The preceding section revealed that very similar genes can respond differently to the same whole-arm trisomic condition. Two explanations for this result seem plausible. It is possible that members of the LSP gene family, while similar at the

Table 29 Quantity of LSPs in males, females and metafemales.

genotype	LSP Ratio	
	LSP-1b/LSP-2	LSP-1g/LSP-2
Oregon R +/O (male)	1.06 ± .07 (8)	1.04 ± .15 (9)
C(1)RM,y pn v/Y (female)	0.756 ± .046 (8)	1.04 ± .08 (9)
C(1)RM,y pn v/+ (metafemale)	0.913 ± .088 (7)	0.521 ± .080 (9)
Estimated Mean	0.908 ± .021	1.04 ± .04
Repressed Estimate	0.606 ± .014	0.693 ± .030

structural gene level, could have very different regulatory sequences. Alternatively, the LSP-1 genes may be controlled by similar 5' sequences but local differences in the regulation of chromatin domains could affect the expression of these three genes. An altered mode of gene expression could arise in trisomies depending on whether or not the duplicated arm encoded a regulatory gene capable of modifying the chromatin domain containing the gene being examined. To investigate this possibility we have constructed six trisomic-2L strains which possess functional Adh genes in different chromosomal locations. The diploid strains possessing transposed copies of Adh were synthesized and provided by Drs. J. Posakony and D. Goldberg. There were two objectives to the present experiment: first, to see if the expression of the Adh gene could be repressed with it residing in non-native chromosomal positions and second, if not repressed, to determine if the Adh gene, when located in different chromosomal positions, maintains the same level of expression between diploids and trisomies. To accomplish this, the expression of single Adh structural genes from wild-type (the strain D. Goldberg used to originally clone Adh; Goldberg, 1980) and five transformed strains were examined in diploids and in 2L trisomies. As shown in Chapter 1, the expression of the Adh gene was activated in 2L trisomies; ie., ADH enzyme activity was 19% higher than a dose-dependent estimate. The data in column 2 of Table 30 shows that the expression of the single Adh genes varies considerably in different chromosomal positions. No apparent correlation appears to exist

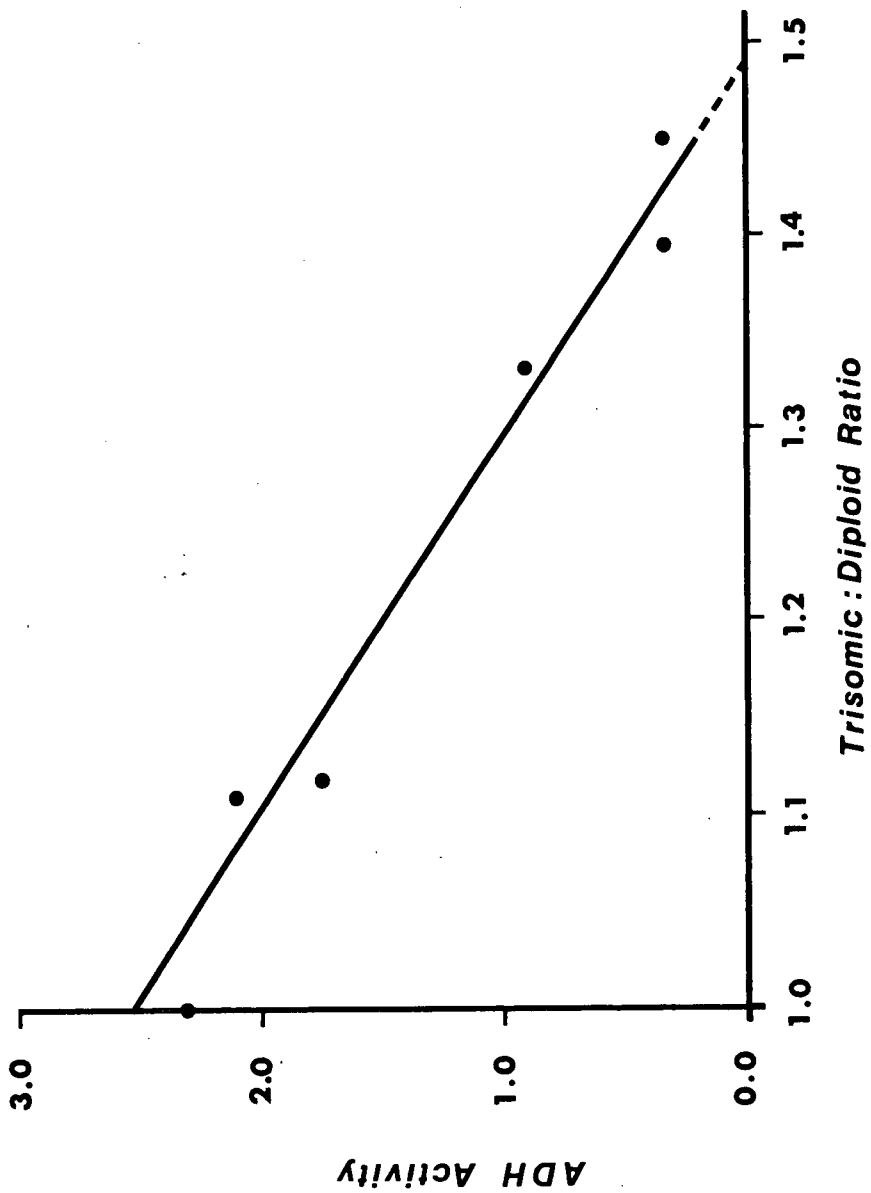
Table 30 Expression of Adh from various chromosomal positions in diploid and trisomic-2L larvae.

strain	chromosomal position	karyotypic condition: diploid	karyotypic condition: trisomic-2L	ratio
w;Adh	35B	2.09 ± .16 (4)	2.36 ± .22 (6)	1.13
tAP-1	19E	1.75 ± .10 (4)	2.02 ± .17 (6)	1.15
tAP-3	36A	0.893 ± .08 (4)	1.19 ± .02 (6)	1.33
tAP-5	17C	2.29 ± .11 (4)	2.29 ± .18 (6)	1.00
tAP-14	89A	0.339 ± .043 (4)	0.493 ± .034 (6)	1.45
tAP-17	12A	0.333 ± .016 (4)	0.463 ± .029 (6)	1.39

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

between gene expression and the chromosome arm on which Adh resides. However, the two strains which display the lowest activity (about 15% wild type) are both transpositions of only 4.8kb of Adh region DNA. The activity of these same genes in the trisomic-2L condition is also shown in Table 30. Generally, the genes expressed similarly in trisomies as 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 diploid levels. The expression of the Adh^F gene in these experiments again showed that a duplication of 2L results in an activation of this gene. The other Adh transformants were also activated in trisomies to varying extents. Figure 11 depicts the relationship between the amount of enzyme present in diploids versus 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 basal 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 a near zero level could be activated 50% by the duplication of 2L.

Figure 11. Relationship between ADH enzyme activity in diploids and 2L trisomies bearing transformed Adh genes.
The ADH enzyme activity 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= -.979, b= -.195, X intercept = 1.49.


ADH Activity
3.0
2.0
1.0
0.0
1.0
1.1
1.2
1.3
1.4
1.5
Trisomic : Diploid Ratio

Discussion

In this chapter an examination has been made of how wide spread effects of trisomy are, in terms of numbers and types of genes that are affected. The extent of these effects are summarized in Figure 12. Two points are immediately obvious. First, not all genes in a given trisomic condition are affected in a similar way. If general physiological disturbances were responsible for altered modes of gene expression (eg. reduced polymerase, ribosomes etc.) then all gene products might be expected to be affected to a similar degree. Since distinct effects were observed for some loci but not others, suggests that discrete regulatory mechanisms operate to modify gene expression in trisomies. Second, a particular gene can be affected differently by the three trisomies. For example, whereas Cat is unaffected, Zw is repressed in all three trisomies. In contrast, Gpdh is repressed by one-third in 2L and 3L trisomies, but not in metafemales. The individuality of the enzyme profiles for each trisomy again argues that discrete regulatory mechanisms are responsible for the observed differences.

The most common regulatory effect observed in trisomies is a one-third repression of gene expression relative to diploids. Reductions in the amounts of gene products in trisomies has 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 and Newton, 1981) and mammals (Klose and

Putz, 1983). Table 31 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 gene resides on the duplicated arm, and are expressed in a dose-dependent manner. A large number of loci show this type of regulation; of thirteen loci that were measured in all three trisomies, only three (23%) were not repressed by one-third in any of the trisomies. Since approximately 40% of the genome was not duplicated in any of the three trisomies, it seems likely that these loci are not exceptional and that negative regulators of their expression may be located on either 2R or 3R. A simple prediction would be that about 4300 bands (3 trisomies x 1000 bands/arm divided by .70) would need to be examined in the trisomic condition to repress all loci that were examined.

Thus, it would appear that most of the enzyme-loci measured in these studies are under the control of at least one negative regulatory gene. As such, gene expression in Drosophila is controlled in part by the quantities of repressors which 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. Although some of these loci are dispensible under laboratory conditions (Voelker et al., 1980) it is doubtful that flies bearing null activity mutants would be sufficiently fit to survive in the wild. For example, homozygous Gpdh null mutants can be maintained in the laboratory but they are incapable of flight owing to the

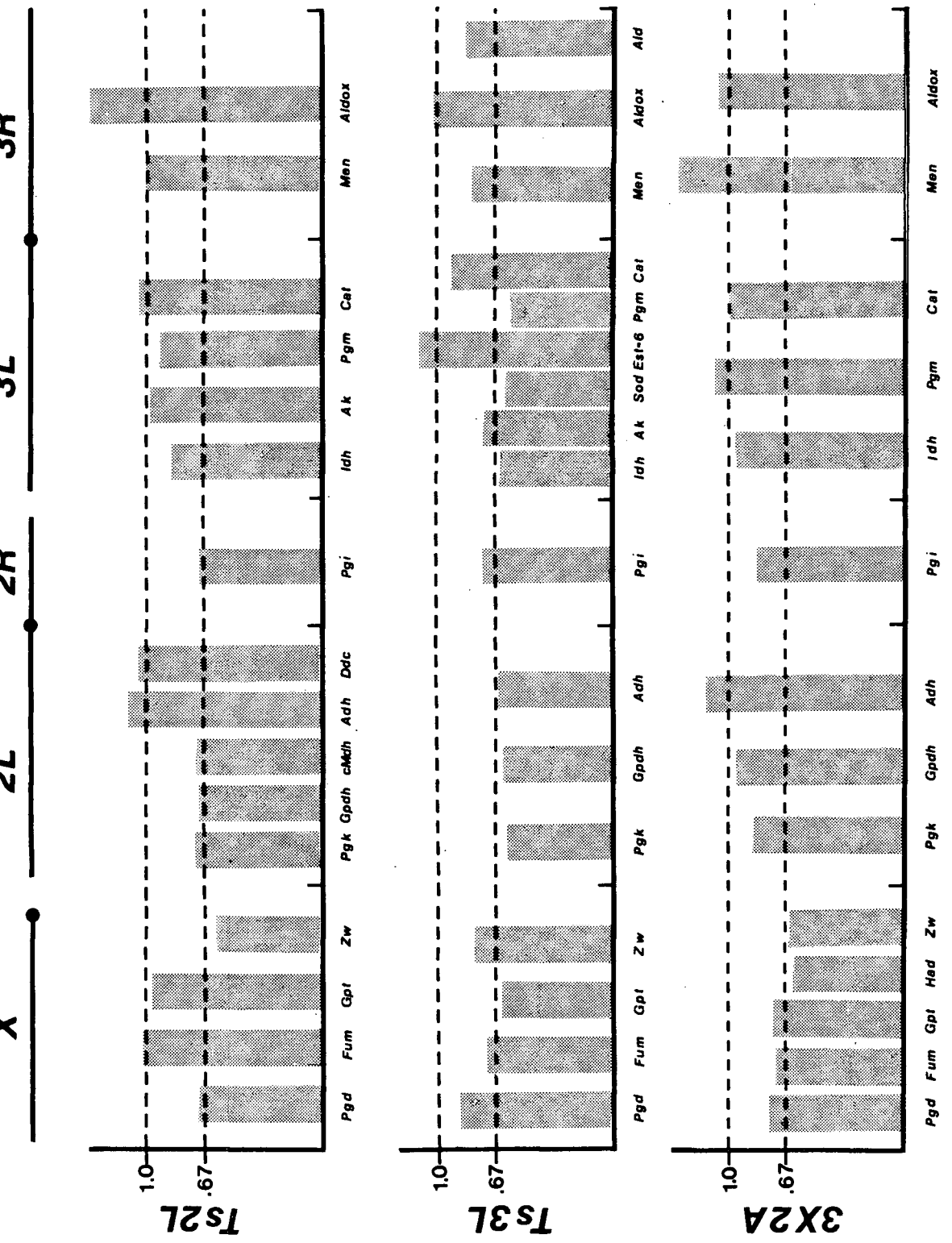
Table 31 Summary of regulatory effects on enzyme loci in trisomies.

Regulatory Effect	Trisomic Chromosome 2L	3L	X	total
unaffected	8	3	6	17
activated	1	0	1	2
partially repressed	1	3	3	7
repressed by one third	6	11	4	21
number fully repressed on same chromosome arm	3(50%)	4(36%)	4(100%)	11
total examined	16	17	14	47

inefficient shuttling of NADH equivalents across the mitochondrial membrane. Many of the enzymes shown in Figure 12 show a characteristic exponential increase in activity as larval development proceeds and then reductions of activity at the onset of pupariation (O'Brien and McIntyre, 1976). The amount of each gene product is roughly correlated with body weight during periods of high metabolic activity (larval growth) and thus could be considered "housekeeping" enzymes. It is likely that this set of genes is highly biased for historical reasons (studies on metabolic enzymes are among the oldest and their loci are among the first to be mapped by electrophoresis). Thus, negative repression of the type observed here may be more common for housekeeping genes than it is for other types. However, several genes measured in this study are inducible, or are developmentally regulated and also appear to be negatively regulated. Thus, modulation of gene expression by negative repressors may also operate independently of developmental cues and in concert with other regulatory mechanisms. Indeed, systems of positive and negative control could be operating simultaneously and the expression of complex loci such as scute or bithorax may also be repressible by concentration dependent modifiers (Lewis, 1978; Botas et al., 1982).

The 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 quite possible that several regulatory loci are duplicated in each trisomy. Figure

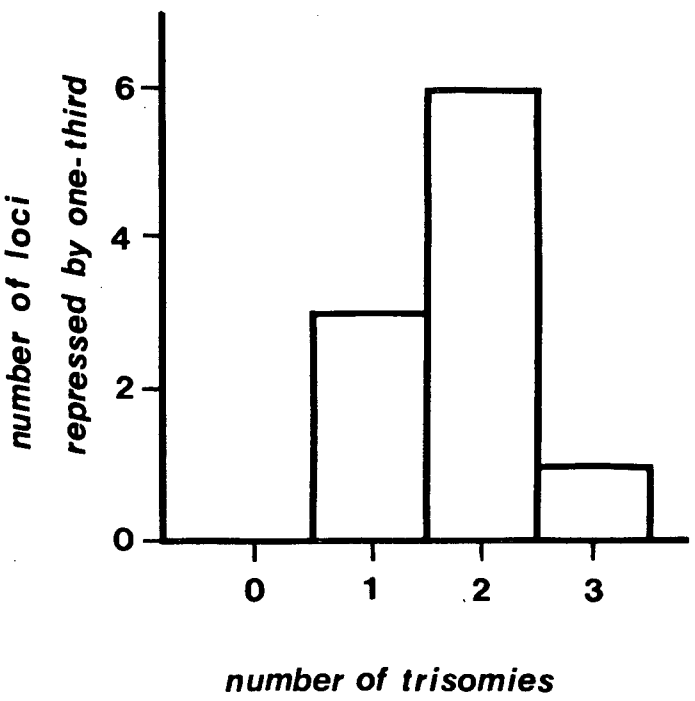
Figure 12. Summary of the expression level of genes in X-chromosomal and autosomal trisomies. On each X axis, a value of 1.0 represents the diploid level of expression per gene.


X
2L
2R
3L
3R
Ts2L
Ts3L
3X2A
1.0
.67
Pgd Fum Gpi Zw Pgk Gpdh cMdh Adh Ddc Pgi Idh Ak Pgm Cat Men Aldox
Pgd Fum Gpi Zw Pgk Gpdh Adh Pgi Idh Ak Sod Est-6 Pgm Cat Men Aldox Ald
Pgd Fum Gpi Had Zw Pgk Gpdh Adh Pgi Idh Pgm Cat Men Aldox

13 shows the distribution of the number of loci which are affected by different numbers of trisomies. If the regulatory loci were extremely common then we might expect most loci to be affected by all trisomies. However, a mean number of 1.38 trisomies affect each locus for the three aneuploid conditions tested. If the whole genome were surveyed an average of 2.3 trisomies would be expected to affect each locus. Since there must be at least one regulatory locus 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, we can conclude that each regulatory gene affects the expression of more than one genetic locus. At this point, however, we cannot distinguish between many regulators operating on a few genes each or a few regulators each operating on many loci.

Few loci appear to be activated in the trisomic conditon. Table 31 shows that of the 47 gene-trisomy combinations examined only two resulted in levels of product per template significantly above diploid. One of these, Men, will be discussed in detail in chapter 6. The other locus, Aldox, belongs to a family of genes which code for enzymes that bind molybdenum as a cofactor. Several trans-acting genetic loci have been identified (mal, cin, lxd, and lpo) that are required for the production of normal quantities of these enzymes (see Bentley and Williamson, 1979) and additional loci may exist. If these or other loci synthesize

Figure 13. Number of trisomies affecting individual loci.
Mean number of trisomies affecting an individual gene = 1.38.


number of loci
repressed by one-third
6
4
2
0
0
1
2
3
number of trisomies

products at levels that limit the rate of synthesis of AO, then changes in their concentration would regulate the quantity of this enzyme. If one of these genes is located on 2L and if it is expressed at a dose-dependent level, increased quantities of AO synthesis would result. While several different loci exist which produce enzymes capable of converting acetaldehyde to acetic acid, (Dickinson and Gaughan, 1981) strains bearing null mutations for AO (Dickinson, 1970) possess less than 2% of the wild-type activity. Therefore the over production of AO in 2L trisomies is certainly a regulatory effect and does not arise from the duplication of a second AO locus. Such regulatory genes could conceivably act at any step from the establishing the correct chromatin structure for Aldox expression to the final maturation of the enzyme molecule.

There are relatively few other cases of trans-activation of gene expression by gene duplication (Williamson and Bentley, 1983), although several reports of increased levels of unlinked enzymes in human trisomy 21 have appeared (Mellman et at., 1964; Mattei, 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, and more likely, loci that are capable of regulating other genes in a positive and directly correlated fashion are extremely rare. This would imply that most of the components required for biosythesis 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 produce levels of product that are directly dependent on their dose. Thus, components involved in gene expression appear not to be rate limiting and, as such, suggests that modulations (not induction or inactivations) of gene activity may not be controlled positively, but rather are mediated by changes in quantities of negative regulators.

The expression of Adh in various chromosome positions has provided evidence that positive effectors of gene modulation do exist, but that their level of synthesis is 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. This may arise because the chromatin configuration into which Adh has inserted is not as ideal as the native position (ie phasing, degree of condensation). It is noteworthy, in this regard, that the two transformants displaying the lowest level of expression possess the least amount of flanking Adh region DNA (J. Posakony, personal communication). Perhaps these particular Adh genes are not as well buffered from local perturbations as are the larger insertions. In trisomies for 2L, the expression of Adh is activated by an amount that is dependent on the 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 subjected to activity increases as high as 45% in 2L trisomies. The relationship between level of expression and the amount of activation suggests that a gene which is only slightly

active would be activated by 50%. These results indicate that a locus exists on 2L which positively regulates Adh. When this gene is duplicated in trisomies, 1.5 times more regulator is produced than in diploids. When the Adh gene is fully active (ie. in its native chromosomal position) the activator is incapable of increasing the expression any further because an upper limit may exist for Adh expression. However, when the expression level is low the activator can increase the expression of this gene. Only at very low expression levels can the activator act in a concentration dependent way; at intermediate levels of expression, activation does not vary directly with the quantity of activator. This suggests that the two processes affecting the expression of Adh are, to some extent, competitive. For example, chromosomal position could alter chromatin condensation, while the activator could affect the stability of RNA polymerase binding. Both processes could alter the rate of transcription initiation. This is reminiscent of an experiment described by Pelham and Bienz (1982). In monitoring the expression of the herpes thymidine kinase gene in COS cells, these authors observed that TK activity could be activated by placing either a viral enhancer or a heat shock promoter in the vicinity of the tk gene (and growing the cells at 42 degrees C). However, if the heat-shock promoter was added to the TK-enhancer element construct, no further activation of expression was observed. The two positive regulatory effects were not additive and it appeared that TK gene expression had an upper limit under these conditions.

The fact that the three LSP-1 genes can respond

differently in the different trisomies studied suggests that the regulators do not operate on a component of gene expression that is common to all three genes. These genes are very similar in sequence composition and are coordinately regulated at the RNA level (Powell et al; 1984). In addition, the studies on Adh have shown that expression of this gene varies in different chromosomal positions. A possible mechanism by which regulators could operate is through alterations of the local chromatin configuration. Recently, several diverse DNA sequence elements have been described which are capable of affecting the level of expression of genes located in cis over a range of several kb of DNA. These elements entitled enhancers, were first discovered as viral promoter elements but have since been discovered as native structures in the mammalian genome. Some of these enhancers apparently require trans-acting genetic function to operate (see introduction). While the wild-type function of these elements is to activate transcription, and they are themselves under positive regulation, evidence does exist for trans-acting repression of transcription. The cassette storage genes involved in mating type switching in Sacchromyces cerivisae are negatively controlled by the wild-type function of SIR loci (silent information repressors) (Nasmyth, 1982). Enhancer elements appear to act more as switches, either eliminating transcription or greatly increasing it. Similar elements conceivably exist which respond to diffusible regulatory signals in a concentration dependent manner. Studies on hormone activation of gene expression have shown that the level of gene activity varies with the dose of

the inducing agent. Hormone-receptor mediated induction of transcription occurs by the binding of this complex to specific DNA sequences (Chandler et al., 1983). The finding that most genes in Drosophila are under a form of negative control allows the possibility that gene modulation during development could be controlled by varying the level of expression of repressor coding loci. As discussed previously, it is likely that more than one gene is controlled by a given regulatory locus. If this is the case, then regulation of gene batteries in a coordinate manner is quite conceivable (see Davidson and Britten, 1979).

Positive control has been hypothesized to regulate dosage compensation of the X-chromosome in Drosophila (Schwartz, 1973; Maroni and Plaut, 1973b). Evidence to support this has been summarized in previous chapters. Trans-acting, limiting activators of X-chromosome transcription could provide a system of control where X-linked templates compete for the regulatory substance. These activator molecules should be autosomally encoded to maintain equivalent levels between males and females. In surveying four loci in two of the four major autosomal trisomies, we detected no increase in X-linked activity. Autosomal trisomies change the X/A ratio in females from 1.0 to 0.91 and in males from 0.5 to 0.45. While these changes are small, the aneuploid condition still provides a valid assay system for changes in the X/A ratio by modifying the autosomal content, rather than the X-chromosome content (Maroni and Lucchesi, 1980). For example, it is conceivable that only one or a few autosomally encoded activator genes are responsible for

controlling the level of X-linked transcription. Activations of X-linked enzymes were not observed in 2L or in 3L trisomies. Therefore, if only a few positive activator genes exist, they must reside on the other chromosome arms. Alternatively, such activator genes could be reiterated and distributed throughout the genome. Rawls and Lucchesi (1974a) examined the activity of an X-linked enzyme locus in strains of flies bearing different duplications for much of the genome. They did not observe any one particular region that could activate the expression of this gene. This finding suggested that either the regulatory loci may be redundant or they do not exist. In the present study, approximately half of the autosomal genome was duplicated in two different aneuploids. If these activator loci are redundant, they do not appear to reside on 2L or 3L.

Recent evidence suggests that dosage compensation occurs by increasing the level of expression in males rather than by decreasing the expression in females (Spradling and Rubin, 1983). This regulation does not appear to be disrupted in trisomies. Further, several X-linked loci displayed one-third reductions in activity in trisomies for 2L or for 3L, yet these loci are dosage compensated between males and females. Thus, two opposing regulatory systems are capable of acting in concert--that is, repression by the autosomal duplication and hyperactivation by male-female dosage compensation. 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, ie. RNA polymerase initiation or elongation).

A parallel conclusion can be obtained for the observations made on X-linked gene expression between strains. Marked differences in the expression of several X-linked genes exist between strains. Presumably this is a consequence of different modifier loci present in each strain. However, despite the different levels of expression between the strains, equal levels of activity are observed between males and females. This argues that male-female dosage compensation and regulation by genetic modifiers operate by independent processes.

Genetic modifiers are known to have pronounced effects on gene expression. Selection for individuals displaying the extremes of a phenotype from wild-type strains can arise through differential accumulation and loss of modifiers. The potential adaptive significance of such changes has been recognized for many years and successfully demonstrated in the laboratory (McDonald et al., 1978). A large number of studies have recently reported the existence of trans-acting modifiers which alter the expression level of a particular enzyme locus (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 in the allelic condition of these regulators exists in nature, and in combination with different allelic states of the structural gene (or closely mapping cis regulators) large differences occur for the quantities of enzymes synthesized

(over tenfold for Adh, Laurie-Ahlberg et al., 1980). Several of these studies have shown that the modifiers can map to regions distant from the structural gene and that they affect the number of enzyme molecules synthesized. As such, these modifier loci may encode diffusible regulators of gene transcription. Presumably, altered levels of structural gene expression arise from different quantities of regulator being synthesized from distinct alleles of the modifier gene.

Variations in the dose of these modifier genes could also affect the quantity of regulatory product. Trans-acting modification of gene expression in trisomies and in 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 regulatory loci has the potential to regulate the expression of genes or groups of genes, on a developmental or evolutionary time scale (Britten and Davidson, 1969; Wilson, 1976; Davidson and Britten, 1979).

Coordinate regulation of gene expression allows functionally related products to be synthesized together at appropriate times. Presumably, it is a common occurance in the complexity of differentiation and development. The X-chromosome of Drosophila is an extraordinary example of a group of functionally unrelated genes that are modulated by a common mechanism. Dosage compensation operates as a consequence of evolutionary constraints which are applied to the X-chromosome (monosomy versus disomy for sex determination purposes) rather

than as a system which coordinately regulates different gene products that are functionally or developmentally related. 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. There would be little selective pressure for regulatory genes and the loci they control to be on the same chromosome arm. Clearly, pericentric inversions, translocations and transpositions, over a long evolutionary history, would disperse such regulatory loci. Thus, we observe that of the loci which show repression in autosomal trisomies, less than half are located on the aneuploid arm. However, all of the enzyme-loci that were repressed in X-chromosomal trisomies were located on this arm. It seems paradoxical that autosomal trisomies can repress the expression both of X-linked and of autosomal loci whereas X-chromosomal trisomies affect X-linked genes almost exclusively (an exception should be recalled; one autosomal gene, LSP-1g, was repressed in metafemales). Two possible simple explanations for this enigma seem plausible. In evolutionary terms, it may not be advantageous to have regulatory genes (whose product levels have adaptive significance) on a chromosome which normally varies in dose. While this should present no 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 in some cell types (Maroni et al., 1974). Furthermore, the selective pressure for these modifier loci to acquire dosage compensation may be low because their effects may

be buffered by other autosomally encoded regulators which act on the same genes. Alternatively, modifiers which regulate autosomal genes may be equally abundant on the X chromosome as on the autosomes. In X-chromosomal trisomies, unlike their autosomal counterparts, elevated quantities of regulatory gene product may not be synthesized because the majority of these genes are themselves dosage compensated. 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. The few activations of gene expression observed in trisomies offers indirect evidence to suggest that negative regulatory genes do not control other negative regulators. The types of regulatory mechanisms that may operate in metafemales will be discussed further in chapter 6.

Previous reports (Rawls and Lucchesi, 1974a, 1974b) have detected the existence of trans-acting regulatory effects which appear to modify the expression of particular genes. Recently, Birchler (personal communication) has made an extensive survey of the Drosophila genome for dosage-sensitive modifiers of Adh expression, and Maroni and Laurie-Ahlberg (1983) have analyzed several genetic modifier loci which affect the quantity of ADH produced. A combination of these two approaches, as well as a detailed analysis of individual regulatory loci, should inform us of the complexity of trans-acting gene control. Regulatory elements that allow a particular gene to respond to trans-acting modifiers may be found by standard in vitro modification of the gene and its flanking sequences, followed by

P-element mediated transformation and analysis of the response to specific genetic conditions. It will be particularly interesting to know whether the control elements are integral parts of the immediate 5' promoter, or whether they are more remote and propogate their effect over relatively long distances. In this regard, it would be interesting to know whether the transposed Adh genes examined in 2L trisomies in this chapter can be repressed by trisomy for 3L as is the Adh gene in its native position.

CHAPTER FOUR

EFFECT OF EXTENSIVE ANEUPLOIDY ON VIABILITY AND PHENOTYPE

Introduction

Aneuploids in Drosophila and other eukaryotes are less viable than their diploid counterparts. The parallel observation that the karyotype of most species, and most cell types within an individual are invariant, strongly suggests that a balanced genomic condition is a necessary prerequisite for fitness. In those cases where natural variations in gene dosage exist, a mechanism often operates to minimize deleterious consequences (Lucchesi, 1978).

The physiological effects of aneuploidy are variable. For example, primary trisomics for any of the chromosomes in Datura produce characteristic changes from the diploid phenotype (Blakeslee, 1922). In maize, however, only two of the ten possible trisomics are morphologically distinguishable from diploids (Rhoades and McClintock, 1935). Other species are affected little by aneuploidy for modest amounts (about 5%) of the genome. However, many of the organisms which are resistant to changes in chromosome number are polyploid (Khush, 1973) and consequently the overall change to gene balance is less. In contrast, changes in number of chromosome sets is quite well tolerated in many species. The plant kingdom is a particularly rich source of auto and allopolyploids, perhaps because these organisms can reproduce vegetatively and are succeptible to hybridization (Stebbins, 1966).

In Drosophila, the diploid and triploid condition have been verified cytologically (Bridges, 1921) and genetic evidence exists for the survival of haploid and tetraploid females

(Morgan, 1924; Cline, 1983). The work by Lindsley et al. (1972) and Stewart and Merriam (1973) has provided quantitative observations on the extent to which adult Drosophila can withstand aneuploidy, and estimates of the number of loci which are haplo or triplo abnormal. They found that only one region of the genome must be present in two doses in diploids for viability, and that all other autosomal regions could be duplicated with little effect. The X chromosome has two hyperploid sensitive regions (Bx and Co) which cause phenotypic changes and one region which is lethal when duplicated in males. The X chromosome and the autosomes contain approximately 58 regions which, when they are present in a single dose, are lethal or have a phenotypic effect. Many of these loci fall into a class of mutations called Minutes which are characterized by abnormal bristle morphology and delayed growth. Hyperploidy is better tolerated than hypoploidy. While adult hyperploids duplicated for approximately 10% of the genome are recovered with modest frequency, hypoploids involving more than 3% (and commonly not more than 1%) of the genome are not recovered.

The physiological disruptions associated with aneuploidy appear to arise from reduced cell viability. Ripoll (1980) induced mosaic patches of aneuploid tissue in an otherwise diploid individual and found that the aneuploid cells were at a competitive disadvantage. It is reasonable to assume that aneuploidy exerts its effects by disrupting the balance of gene expression within the cell that in turn affects organismal development. A study comparing the anatomy of the various trisomics of Datura revealed that different effects were

correlated with different aneuploid regions (Sinnot et al., 1934). This conclusion is confirmed by examining the parallel phenotypes displayed by primary and tertiary trisomics. Thus, all of the primary trisomics of Datura are morphologically distinct (Blakeslee, 1922) and the phenotypic modifications observed in aneuploids reflect the action of genes contained in these regions. In Nicotiana (Goodspeed and Avery, 1939) and Datura (Avery et al., 1959) different characteristics may be affected in similar ways; for example, if leaves are elongated in a particular trisomic then stem, flowers and seeds will also be elongated. These observations suggest that, in addition to reduced cell viability, disruptions can also occur in processes determining positional information, morphogenesis or differentiation.

Bridges (1922) suggested that the morphological differences observed between diploids and aneuploids in Drosophila arise from the relative genetic imbalance. Thus, individuals that are haploid for the fourth chromosome display characteristic cuticular differences from the diploid. Individuals haploid for the X chromosome are also phenotypically distinct from diplo-X individuals (males and females), and Bridges argued that this was caused by the quantitative difference in the activity of genes on this chromosome. While the phenotype of males and females is undoubtedly the consequence of the X-chromosome dosage, it is unlikely that the difference in product levels for sex-determining genes encoded on this chromosome is solely responsible. Indeed, autosomal loci are known that can alter or reverse the sex of an individual (Lindsley and Grell, 1968). It

is probably the functional state of these and other genes that X-chromosome ploidy influences (Baker and Ridge, 1980).

In this chapter, we examine the pre-adult viabilities of individuals possessing grossly abnormal amounts of chromosomal material. The results suggest that inviability is observed at all stages of development, but that the embryonic stage is least sensitive and the pupal stage is most sensitive. Phenotypic effects include disruptions in morphological characteristics and perhaps sexuality. In one case, evidence of a cryptic regulatory effect is observed in trisomic-2L females which are heterozygous for a mutation in a gene which regulates both sex determination and dosage compensation.

Materials and Methods

Aneuploids possessing different sized duplications of chromosome-2L were synthesized with the T(Y;2) chromosomes described by Lindsley et al. (1972). Crossing T(Y;2)B251/In(2LR)SM1,Cy females to males carrying different T(Y;2)s with breakpoints in various positions on 2L produces individuals which possess a duplication of 2L from the heterochromatin to the breakpoint of the translocation. Selection of appropriate T(Y;2)s (all broken in the long arm of the Y chromosome) allowed accurate identification of these aneuploids in the larval stages (yellow mouthparts) relative to the diploid controls (black mouthparts).

For viability and development studies, eggs were collected over a two hour period after the females had been actively laying many eggs. (For the experiment designed to measure the sex ratio of trisomies, eggs were collected over a twenty-four hour period.) Hatching was monitored thirty hours after egg lay. Scoring of larval phenotypes and sex was determined every twelve hours at the wandering third instar larval stage, or as first instars if yellow was serving as the marker and subsequent development was unsure.

Post-embryonic viability of trisomies was estimated by collecting several hundred larvae at different stages of development and allowing them to pupate. The differences in pupation frequencies were assumed to arise from lethality. Pupal development was scored directly.

2L trisomies bearing a deficiency for the Gpdh region

(for kinetic analysis of GPDH enzyme) were produced by crossing Y/Y;C(2L)VH1,lt; F(2R)bw/F(2R)bw females to Df(2L)GdhA/Bc Elp males. Bc^{+} progeny were selected at the wandering larval stage. Enzyme was extracted from these individuals and compared to that of their euploid, compound-free parents.

To determine what effect organism size had on enzyme activity, individual wandering third-instar larvae were collected, sexed and rinsed in distilled water, and then dried and weighed. Each larva was homogenized in 200µl of 10mM PO_4, 1mM PTU, pH 7.5. The extract was spun for 1 minute in an Eppendorf microfuge, and the supernatent was assayed for ADH, GPDH, and PGI activities.

Fat bodies were dissected from female trisomic-2L larvae or their diploid parents, and stored frozen in groups of fifty per genotype. These samples were thawed, sonicated and centrifuged; the supernatent was assayed for PGK, GPDH, ADH, PGI, CAT and AO enzyme activities.

To measure the extent of variegation of the Bar gene, the size of the pupal or adult eye was measured by tracing the eye with the aid of a micrometer eyepiece. The tracings were cut out and weighed to provide an estimate size.

The quantity of DNA was measured by the diphenylamine method described by Burton (1968). Protein measurements were as described in previous chapters. Cell size was estimated by photographing adult or pupal eyes in a scanning electron microscope (Cambridge Stereoscan 250-T). A tracing outlining a group of ten facets from each eye was cut out and weighed.

Comparison of these values between eyes from diploid and triploid strains suggested that this method was reliably quantitative.

Results

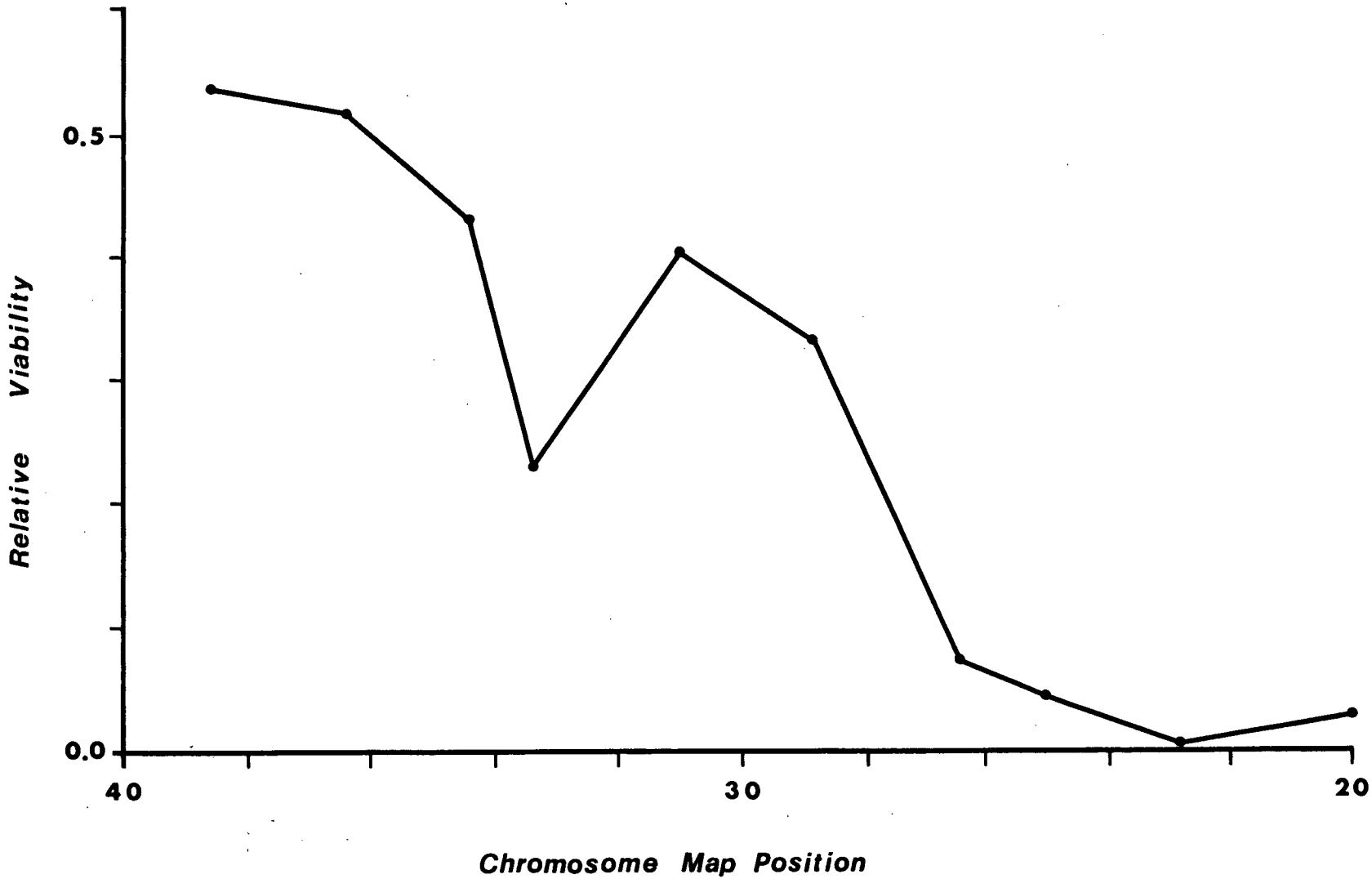
Effect of extensive aneuploidy on viability and development.

The cumulative effects of increasing the size of segmental aneuploidy are well documented for adult viability in Drosophila (Lindsley et al., 1972). Whether this is the case for larval development (where growth occurs largely by increases in cell size rather than cell number) is unknown. Therefore, the viabilities were examined of individuals possessing various sized duplications for the left arm of chromosome two through larval development. Figure 14 shows that viability is inversely correlated with the extent of aneuploidy, and that duplications which exceed more than one-half the chromosome arm are affected most severely. Similar effects and conclusions were reached by Lindsley et al (1972) when they examined adult viability. Taken together, these results suggest that aneuploid inviability arises from effects on physiological processes that are common to all stages of development.

We have screened over 450,000 monosomic-2L or trisomic-2L and 200,000 monosomic-3L or trisomic-3L zygotes for EMS induced mutations that would allow such individuals to survive to adult. None were recovered suggesting that lethality of these individuals does not arise from the excess of gene product encoded by a single gene.

Compound chromosomes allow simple manipulation of large regions of the Drosophila genome. By crossing various combinations of stocks bearing compound or compound-free

Figure 14. Viability to late third-instar larvae of partial trisomies for 2L. Viability measured relative to diploid controls produced in the same crosses. Sample sizes are between 119 and 301 for each point. Translocations used were: B110, A62, G74, B92, L52, A111, H121, H116, R136 (Lindsley et al., 1972). Duplications extend from the proximal region of 2L (chromosome position 40) out to the more distal chromosomal positions, which are indicated on the X axis.


0.5
0.0
Relative Viability
40
30
20
Chromosome Map Position

chromosomes it is possible to vary the dosage (from one to three) of any chromosome arm independently of the dosage of any other arm. The crosses used in this investigation are presented in Table 32. Since several aneuploid types are produced from some crosses, the viability of particular aneuploid classes are presented in Table 33. Aneuploid classes are often inferred; for example, monosomic progeny have very poor viability and hatching frequency. Therefore, it has been assumed that all individuals monosomic for one autosomal arm behave similarly, independent of the ploidy of any other arm. Similarly, all whole arm trisomies can develop as far as the larval stage. Therefore, when crosses are made where the frequency of egg hatch is reduced, it is assumed that the surviving larvae represent the combination of aneuploid conditions which are most viable by themselves (ie. double trisomics have been assumed to survive better than double monosomics). These considerations are not trivial since balanced aneuploids possessing reduced or increased numbers of chromosome sets (ie. haploids, triploids, tetraploids) have been described.

Cross 1 shows that even a standard wild-type strain can display a reduced frequency of egg hatch. This presumably arises from embryonic lethality as well as from deposition of unfertilized eggs. Crosses 2 to 5 examine the four possible autosomal compound-free stains. These lines display egg-hatch frequencies of about 50% and suggest that all possess similar meiotic properties; in both sexes, the two free arms segregate and the compound moves at random. If the compound paired with one of the free arms, the hatch would be about 25%; if the

Table 32 Embryonic viability of whole arm trisomies.

Exp.	Parent		Eggs	First Instars	hatch
	Female	Male			
1	Oregon R	Oregon R	384	364	.924
2	y/y;C(2L)lt; F(2R)bw/F(2R)bw	y/y;C(2L)lt; F(2R)bw/F(2R)bw	1812	897	.495
3	y2/y2;F(2L)pr/ F(2L)pr;C(2R)px	y2/y;F(2L)pr/ F(2L)pr;C(2R)px	1513	782	.517
4	C(3L)ru st; F(3R)e^{s}/F(3R)e^{s}	C(3L)ru st; F(3R)e^{s}/F(34)e^{s}	1222	555	.454
5	F(3L)ri/ F(3L)ri;C(3R)e^{s}	F(3L)ri/ F(3L)ri;C(3R)e^{s}	1279	619	.484
6	Oregon R	C(4R)Pl+	112	101	.901
7	C(1)RMy pn v/O	Oregon R	992	693	.699
8	C(2L)+; F(2R)bw/F(2R)bw	Oregon R	495	232	.469
9	Oregon R	C(3L)ru st; F(3R)e^{s}/F(3R)e^{s}	1282	583	.455
10	F(3L)pr/F(2L)pr; C(2R)px;y/y	Oregon R	1141	522	.457
11	F(3L)ri/F(3L)ri; C(3R)e^{s}	Oregon R	1929	307	.159
12	C(3R)e^{s}; F(3L)ri/F(3L)ri	X Y/O; Dp Tpl/Df Tpl	1472	245	.166
13	C(2L)VH1,lt; C(2R)VHS7,Pin	C(2L)VH1,lt; (2R)VHS7,Pin	1010	289	.286
14	C(3L)SH2+; C(3R)SH19+	C(3L)SH2+; C(3R)SH19+	2467	645	.261
15	y2/y2;C(2R)px; F(2L)pr/F(2L)pr	C(2L)lt; C(2R)Pin+	1369	287	.209
16	F(3L)ri/F(3L)ri C(3R)e^{s}	C(3L)+;C(3R)+	815	200	.245
17	C(3L)ru st; F(3R)VD1e^{s}/ F(3R)VD1e^{s}	C(3L)+;C(3R)+	2157	262	.121

Table 33 Inferred viability of selected aneuploids from the crosses in Table 32.

Exp.	karyotypic class	% viability	latest stage of development
1	diploid	92.4	adult
2	diploid	99.0	adult
3	diploid	103.4	adult
4	diploid	90.8	adult
5	diploid	96.8	adult
6	Ts4	90.1	adult
7	TsX	93.1	adult
8	Ts2L	93.7	adult
9	Ts3L	91.0	adult
10	Ts2R	91.4	pigmented pupae
11	Ts3R	31.8	second instar
12	Ts3R/Df(3R)Tpl	66.6	tan pupae
13	diploid	1.00	adult
14	diploid	1.00	adult
15	Ts2R	83.9	third instar 1
16	Ts3L	98.2	pigmented pupae 2
17	Ts3R	48.6	first instar 3

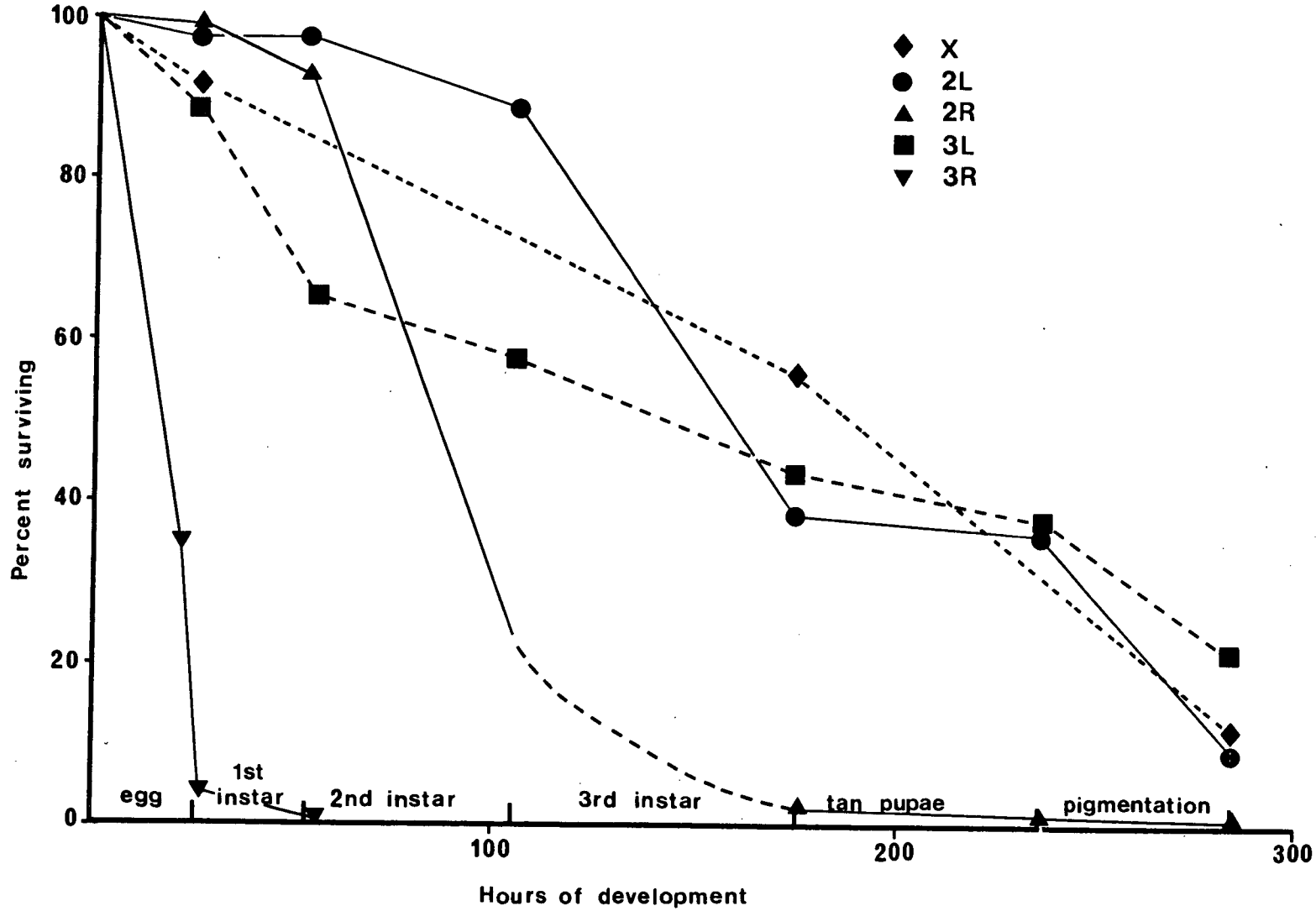
Nondisjunctional adults also recovered: 1) 16; 2) 9; 3) 7
Ts= Trisomy

trivalent moved randomly, a hatch of less than 20% would be expected. Thus the primary meiotic products from these compound-free lines are gametes which are either nullosomic or disomic for the arm corresponding to the compound chromosome. Crosses 13 to 17 demonstrate that few nondisjunctional gametes are produced by the compound-free strains and that they are suitable for the present investigations.

Crosses 6 to 12 examine the embryonic viabilities of whole-arm trisomies (Tables 32 and 33). It is clear that all but one chromosome arm has little effect on embryonic viability when present in the trisomic condition. Trisomies for the right arm of chromosome three survive very poorly. This may arise from two causes: 1) this arm is the largest of the five major chromosome arms examined, and 2) it bears the haplo-triplo lethal locus in its proximal region (chromosome region 83DE). Cross 12 shows that the viability of individuals with a deficiency for the triplo-lethal region, but are otherwise trisomic for 3R, have enchanced viabilities relative to complete 3R trisomies. However, the viability of these individuals was lower than that observed for other trisomies suggesting that size and/or other triplo inviable loci may exist on 3R.

Figure 15 shows the post embryonic viabilities of the five major trisomies of Drosophila. As was discussed in the previous section, the egg stage is particularly refractory to the detrimental effects of trisomy. However, as larval development proceeds, trisomies begin to die and continue to do so until late stages of pupation. Trisomies for 2L, 3L and the X chromosome can survive to the adult stage. For 2L trisomies

Figure 15. Post embryonic viabilities of the five major whole arm trisomies. Developmental times are shown for 2L trisomies. The viability of trisomic-2R second and third instar larvae was not measured. However, observations suggest that few individuals progress past the second larval molt.


100
80
60
40
20
0
Percent surviving
X
2L
2R
3L
3R
egg
1st instar
2nd instar
3rd instar
tan pupae
pigmentation
100
200
300
Hours of development

grown at 18 degrees C, this frequency was estimated to be about one per 2000 zygotes. The viability to adult stage for 3L trisomies was not calculated, but is also extremely low. The adult viability of metafemales varies considerably (Brehme, 1937; Frost, 1960; Rolfes and Hollander, 1961). For the strain used in this study, adult viability was less than one in 200. However, for another genotype, C(1)RM,YS,In(1)EN,y/In(1)sc$^{y^2}$, the adult viability exceeded 5%. Thus, X-chromosomal trisomies have viabilities similar to 2L and 3L trisomies through pre-imaginal development and pupation, but show somewhat better survival through the final stages of imago development.

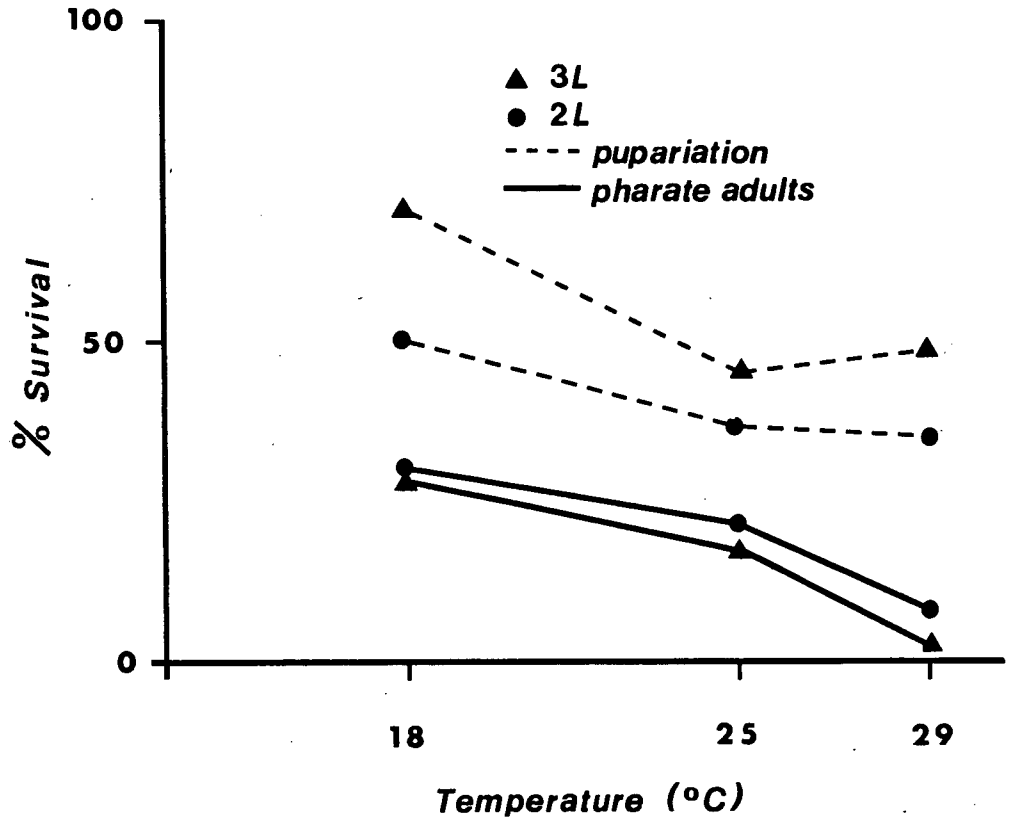
The developmental rate was measured for 2L and 3L trisomies and for their diploid parent strains. The rate at which development proceeds for the trisomic-2L strain is plotted on the X axis (Figure 15). A comparison of the developmental rates of Oregon R and 2L trisomies suggests that trisomies possess retarded development relative to diploids. The delay is linear throughout development (r=.996) suggesting that the trisomic condition affects most developmental stages similarly. The data were collected on the first individuals to reach a particular developmental stage and thus likely represents a bias in favour of the healthier individuals in the population. Indeed, the delay in development experienced by the average trisomic individual is likely to be much larger since the range for the population was far greater in the trisomic strains than in diploids. For example, late third instar larvae were observed for as long as 270 hours for 2L trisomies and 290 hours for 3L

trisomies, while in the diploid strain the last larvae had pupated 180 hours post egg deposition.

Dobzhansky (1928) reported that the viability of metafemales can be increased by growth at low temperature. Indeed, the viability of trisomies both for the X and for the autosomes is negatively related to temperature. Figure 16 shows that survival through the larval period is increased by maintaining the cultures at 18 degrees C. Surivival through the pupal stage may also be increased slightly by growth at low temperature; high temperature markedly reduces the number of trisomies that survive to late stages of development. Temperature can have marked effects on the expression of many genes (Suzuki et al, 1976) and can result in elevated levels of gene product (unless the particular gene produces a temperature-sensitive abnormal product). Many products are synthesized at elevated levels in trisomies by genes present on the duplicated arms. Therefore, high temperature may accentuate this imbalance.

Elevated temperature also increases the level of expression of genes subjected to position-effect variegation (Spofford, 1976). Moreover, at least one suppressor of variegation, Y^{bb}Su(Var)-5, has been suggested to cause elevated levels of transcription (Clark and Kiefer, 1977). However, this suppressor, and two enhancers of variegation (En(Var)5 and En(Var)7), did not affect the viability of 2L trisomies. Similarly, deficiencies for large heterochromatic blocks suppress variegation, but Df(2R)MS210, which removes most of the heterochromatin of

Figure 16. Viability of 2L and 3L trisomies at different temperatures.


100
50
0
% Survival
▲ 3L
● 2L
---- pupariation
—— pharate adults
18
25
29
Temperature (°C)

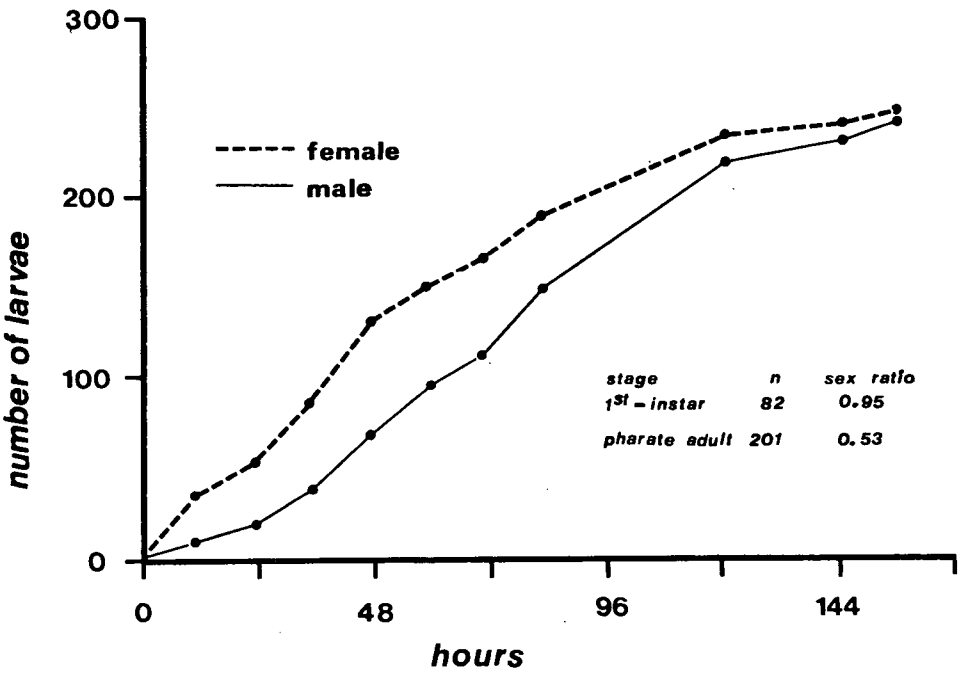
2L, did not reduce the viability of 2L trisomies. Of some interest, however, is that 2L trisomies which possess a deficiency of the 39DE region of 2L were found to survive to the adult stage at a frequency of about 1%. This chromosome region contains the histone gene cluster, and a deletion of this region has been shown to suppress position effect variegation (Khesin and Leibovitch, 1978; Moore et al., 1979). It is possible that removal of histone gene templates causes an elevation in the overall level of gene expression. Since this might be expected to reduce the viability of 2L trisomies, an alternative explanation is warranted. The deficiency used (Df(2L)161, Wright, et al., 1976) is large and trisomies produced with this chromosome are not as severely aneuploid as are whole-arm trisomies.

The viabilities of male and female trisomies for 2L and 3L are shown in Figure 17. While trisomic male larvae develop slower than do females, their viability to the larval pupal boundary is similar to that of their sisters. However, males do not survive as well as females through pupal development (see table inserts in Figures 17a and b). This may indicate that the haplo-X condition is not equivalent to that of the diploid female; it may be less able to accommodate the additional physiological stress caused by aneuploidy.

If X-chromosome dosage compensation is operating fully in metafemales then these individuals might be expected to better withstand additional degrees of aneuploidy than autosomal trisomies. This was tested by examining the viability of double trisomies (Tables 34 & 35). Two points are striking. First,

Figure 17. Sex ratio of autosomal trisomies at different developmental stages. A. Trisomy 2L B. Trisomy 3L. Zero time on the X axis represents the time when the first larvae began to pupate. Larvae were sexed and counted as they developed to the wandering larval stage of development. The table inserts in each figure show the sex ratios of trisomies at the first instar larval stage in A., and at the pharate adult stage in A. and in B.

A


300
200
100
0
number of larvae
female
male
stage n sex ratio
1st - instar 82 0.95
pharate adult 201 0.53
0
48
96
144
hours

B

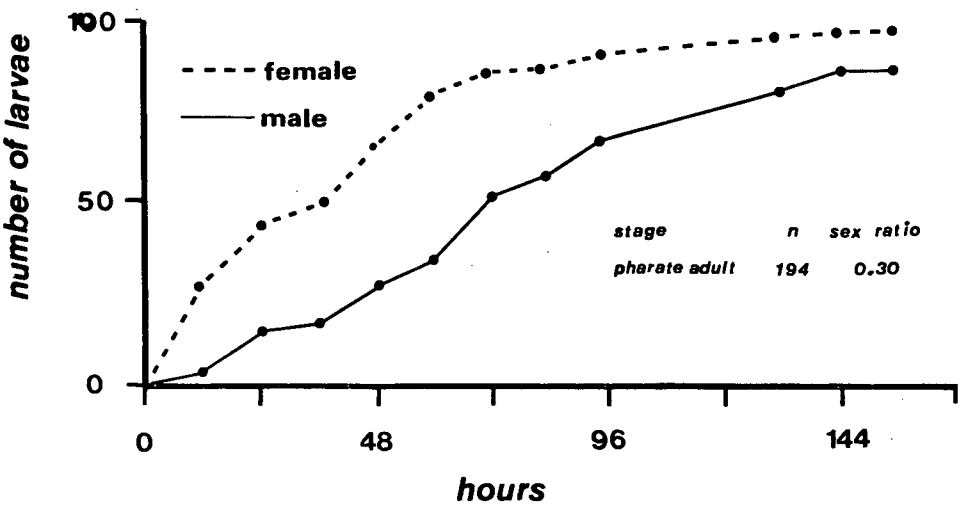

100
50
0
number of larvae
female
male
stage n sex ratio
pharate adult 194 0.30
0
48
96
144
hours

Table 34 Viability of double trisomies and triploids deficient for one autosomal arm.

Exp.	Parent: Female	Parent: Male	eggs	first instars	hatch
1	Oregon R	C(2L)lt;C(2R)Pin+	1834	165	.089
2	Oregon R	C(3L)+;C(3R)+	1999	249	.125
3	y2/y2;F(2L)pr/ F(2L)pr;C(2R)px	F(3L)ri/F(31)ri; C(3R)e	1053	3	.003
4	y2/y2;F(2L)pr/ F(2L)pr;C(2R)px	C(3L)ru st; F(3R)e^{s}/F(3R)e^{s}	2394	67	.028
5	y/y;C(2L)lt; F(2R)bw/F(2R)bw	F(3L)ri/F(3L)ri; C(3R)e^{s}	1512	66	.044
6	y/y;C(2L)lt; F(2R)bw/F(2R)bw	C(3L)ru st; F(3R)e^{s}/F(3R)e^{s}	1517	55	.036
7	C(1)RMy v pn/O	C(3L)ru st; F(3R)e^{s}/F(3R)e^{s}	2044	467	.228
8	C(1)RMy v pn/O	C(2L)lt;F(2R)bw/ F(2R)bw;y/y	2141	558	.261
9	C(1)RMy v pn/O	F(2L)pr/F(2L)pr; C(2R)px;y2/y	981	183	.186
10	C(1)RMy v pn/O	F(3L)ri/F(3L)ri; C(3R)e^{s}	666	10	.015
11	C(3L)ru st; F(3R)e^{s}/F(3R)e^{s}	C(2L)lt;C(2R)Pin+	2485	39	.016
12	F(3L)ri/F(3L)ri; C(3R)e^{s}	C(2L)lt;C(2R)Pin+	2486	52	.021
13	y/y;C(2L)lt; F(2R)bw/F(2R)bw	C(3L)+;C(3R)+	1510	31	.021
14	y^{2}/y^{2};F(2L)pr/ F(2L)pr;C(2R)px	C(3L)+;C(3R)+	7895	129	.016
15	C(1)RM,y v pn/y; F(2L)pr/F(2L)pr; C(2R)px	C(3L)P2,ri; C(3R)VMK1,e^{s}	1040	2	.0019

Table 35 Inferred viabilities of selected aneuploids from the crosses in Table 34.

Exp.	karyotypic class	% viability	latest stage of development
1	Ts2L;Ts2R	35.6	first instar
2	Ts3L;Ts3R	50.0	first instar
3	Ts2R;Ts3R	1.14	first instar
4	Ts2R;Ts3L	11.2	second instar
5	Ts2L;Ts3R	17.5	early third instar
6	Ts2L;Ts3L	14.5	second instar*
7	TsX;Ts3L	0	egg
8	TsX;Ts2L	8.8	
9	TsX;Ts2R	0	egg
10	TsX;Ts3R	0	egg
11	2X;3N-3R	12.6	second instar
12	2X;3N-3L	16.7	third instar
13	2X;3N-2R	16.4	third instar
14	2X;3N-2L	13.1	first instar
15	3X;3N-2L	0	egg

* confirmed cytologically; one late second instar larvae contained ten large chromosome arms, two fourth chromosomes, and an X and a Y chromosome. Ts= Trisomy. 3N = triploid for autosomal arms except for those indicated as missing by a - sign.

the embryonic viability of double trisomies for both arms of a single chromosome (ie Ts2L; Ts2R in cross 1 or Ts3L; Ts3R in cross 2) survive much better than do double trisomies involving one arm each from the second and third chromosomes (crosses 3 to 6). Second, double trisomies involving the X chromosome and one autosomal arm (crosses 7 to 10) survive worse, on average, than the double autosomal trisomies. This includes several of the combinations that involve 3R, the largest chromosome arm (crosses 2 and 5). Thus, the X chromosome does not appear to be compatible with additional aneuploidy any more than any of the other chromosome arms.

Previous chapters revealed that many genes are capable of being dosage compensated in autosomal trisomies. This apparently occurs by reducing the level of expression of these genes by a form of negative regulation arising from the hyperploid condition. A corollary to these observations might be that positive regulation would occur in the hypoploid condition. Monosomy for the X chromosome is a viable karyotypic condition because dosage compensation rectifies the genic imbalance. However, monosomies for autosomal arms do not survive well (Table 36), suggesting that hyperactivation does not arise as a consequence of the haploid condition (see also Dobzhansky, 1957). It should be noted that a high proportion of these autosomal monosomics developed through the egg stage, often forming unhatched larvae. These individuals hatched only occasionally, and as late as 48 hours after fertilization. It is possible that many of the genes in the monosomic arm are indeed expressed at diploid levels in monosomics (compensated)

Table 36 Survival of autosomal monosomics.

Parent		monosomic produced	# of 48 hr old eggs	# with mouth parts	% unhatched monosomic larvae
female	male				
y/y;C(2L)lt; F(2R)bw/ F(2R)bw	Oregon R	2L	100	85	85.0
C(3L)ru st; F(2R)e^{s}/ F(2R)e^{s}	Oregon R	3L	502	451	89.8

but the inviability arises as a consequence of a few unregulated genes. The twofold difference in product level between monosomics and diploids for these genes may be sufficient for this effect. To reduce this factor to 1.5, triploids were produced which were deficient for one autosomal arm (Tables 34 & 35). These individuals survived better than did the diploid monosomics, but still possessed limited developmental potential relative to trisomies. These triploid individuals possessed only two X chromosomes. If X-chromosome dosage compensation was incomplete in these individuals (see Baker and Belote, 1983) it may have contributed to their reduced viability. To test this, two crosses were made that should have yielded triploid females deficient for only one autosomal arm. However, in cross 15 of Table 34 and in the cross C(1)RM+/y^2; C(2L)ho/+/C(2R)px; C(3L)ri/+/C(3R)cu triploid females by C(2L)P,b; F(2R)bw/F(2R)bw males no such individuals were recovered. These experiments suggest that, at best, limited potential exists for complete hypoploid compensation at the chromosome arm level in Drosophila.

Phenotype

Aneuploid individuals are phenotypically distinct from diploids (see Figure 18). The alterations may be very subtle; for example missing, duplicated or mispositioned hairs between eye facets (Figure 25). The previous section of this chapter revealed that all trisomics display common developmental

Figure 18. Phenotypes of trisomies.
A. SDS-polyacrylamide gels of protein samples extracted from pharate adults: left, Oregon R; middle, 2L trisomy; right, 3L trisomy. Whereas quantitative differences in band intensities are apparent, no bands were completely lost in trisomies, nor were any unique bands observed.
B. A late hatching larvae presumed to be monosomic for the left arm of chromosome two. Development of spiracles, trachea, mandibular hooks, and denticle belts were noted in these individuals.
C. top left and right, trisomic-2L females; bottom left, diploid female; bottom right, diploid male.
D. Salivary gland polytene chromosomes from a trisomic-3R larvae (rarely surviving), presumed to carry a deletion for the haplo-triplo lethal region. Chromosome 3R can be seen as the thick arm joining the chromocentre at six o'clock.

A
B
C
D

abnormalities such as slow growth and lethality. Adult autosomal and X-chromosomal aneuploids share phenotypic disruptions as well: gnarled hind legs and misshapen abdomens.

The sexual phenotype of Drosophila is correlated with the ratio of X chromosomes to the number of autosome sets. When this ratio is 0.5 or less, male development occurs, and when it is equal to or exceeds 1.0, female development results. Flies with an X/A ratio of .66 (ie. 2X3A, triploid intersexes) display sexual characteristics of males and females. Autosomal trisomies possess a large change in the autosomal content of an otherwise diploid genome. In females trisomic for 2L or for 3L the X/A ratio is approximately .89 and yet no effect on female development is obvious. This suggests that the threshold for male development lies somewhere between an X/A ratio of .66 and .89. An examination of the late-pupal trisomic males revealed that, while 2L trisomies appeared normal, 3L trisomies possessed rather rudimentary testes which failed to elongate in many individuals (2L and 3L trisomic testes both possessed bundles of approximately sixty-four sperm with normal head morphology). Poor development of male sexual characteristics was not expected since the X/A ratio in these individuals was approximately .44, which is lower than the ratio required for complete male development.

The sex-comb teeth present on the forelegs of males provide a convenient marker for examining sexual development. For example, while diploid males possess about ten sex-comb teeth on each leg (see genotype 1 in Table 37), females have none (genotype 2). A mutation which shifts development towards the

Table 37 Number of sex-comb teeth in normal and sex-transformed trisomies.

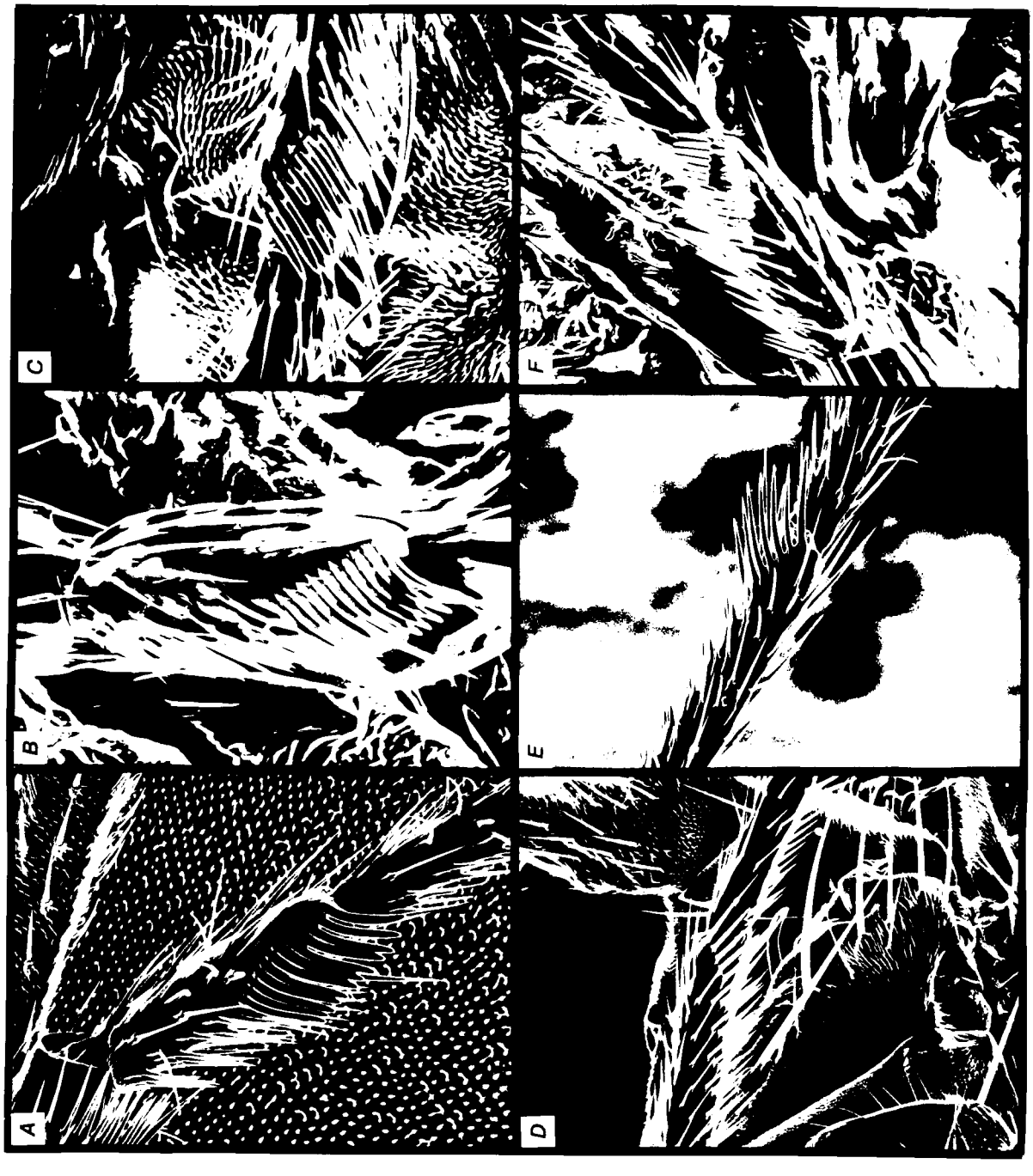
	Genotype	# of teeth	% gap	n	foreleg phenotype
1	X/Y Oregon R	10.2 ± 0.2	0.0	22	male
2	XX Oregon R	0.0 ± 0.0		10	female
3	X/Y;C(2L)1t/+/ F(2R)bw	11.8 ± .01	10.0	30	male
4	X/X;C(2L)1t/+/ F(2R)bw	0.0 ± 0.0		10	female
5	X/Y;C(3L)ru st/ +/F(3R)VD1e^{s}	6.9 ± 0.1	3.3	30	reduced male
6	X/X;C(3L)ru st/ +/F(3R)VD1e^{s}	0.0 ± 0.0		10	female
7	X/Y;Mas/+	10.7 ± 0.2	0.0	22	male
8	X/X;Mas/+	7.0 ± 0.1	0.0	30	intersexual
9	X/Y;C(2L)1t/+/ F(2R)bw;Mas/+	11.8 ± 0.2	6.7	23	male
10	X/X;C(2L)1t/+/ F(2R)bw;Mas/+	9.0 ± 0.2	16.7	27	intersexual
11	X/Y;C(3L)ru st/ Mas/F(3R)VD1e^{s}	6.8 ± 0.3	25.0	12	reduced male
12	X/X;C(3L)ru st/ Mas/F(3R)VD1e^{s}	3.9 ± 0.2	0.0	30	intersexual
13	X/Y;Bs dsxpP/ dsx pP	4.8 ± 0.1		26	intersexual
14	X/X;Bs dsxpP/ dsx pP	5.1 ± 0.1		22	intersexual
15	X/Y;Bs C(3L)ru st/ dsx pP/ F(3R)VD11,dsxpP	4.5 ± 0.2		10	intersexual (1)
16	X/X;Bs (C(3L)ru st/ dsx pP/ F(3R)VD11,dsxpP	5.4 ± 0.3		8	intersexual

(1) basitarsis one/half normal length with gnarled appearance

male mode of expression (Masculinizer, Mas) causes an intermediate number of sex-comb teeth to develop in the female (genotype 8). However, the structure of these teeth are intermediate between those observed in the male and the corresponding female bristles (Figure 19 E). Furthermore, a mutation which shifts both male and female to an intersexual state reduces the number of teeth in males and increases them in females (genotype 13 and 14). The morphology of the bristles in both sexes are similar, and are of intermediate sexual characteristic (Figure 19).

The phenotype of the sex-combs in trisomies for 2L and for 3L are different from each other and from diploids (genotype 3). Table 37 shows that trisomic-2L males possess on average slightly more teeth than diploid males. While morphologically these teeth are the same as diploids (Figures 19A and B), the development of the comb appears to be disrupted slightly since a gap is often observed between two rows of teeth (Figure 19F). Apparently it is not simply the X/A ratio which determines the sexual phenotype since in males trisomic for 3L the number of sex-comb teeth is dramatically reduced (genotype 5, Table 37). The reduction in sex-comb tooth number is consistent with the poor testicular development observed in these individuals. These results suggest that the duplication of 3L partially shifts the development of these individuals towards female. To test this possibility further we examined the interaction between a sex transforming locus and the trisomic condition. The trisomic-2L condition increased the number of sex-comb teeth in males and females bearing the Mas mutation (compare

Figure 19. Sex-comb morphology of diploid and trisomic pupae either possessing sex transforming mutations or not. A. Trisomic-2L male B. Oregon R diploid male C. Trisomic-3L male D. diploid dsx pP female E. diploid Mas/ Ly st female F. Trisomic-2L male with comb-gap phenotype.


A
B
C
D
E
F

genotypes 7 and 8 versus 9 and 10). A similar result was obtained in wild-type trisomies and therefore suggests that duplication of 2L promotes male development slightly. The trisomic-3L condition again appeared to promote female development; reduction in sex-comb tooth number is observed in trisomic-3L males or females bearing the Mas mutation (genotype 7 and 8 versus 11 and 12). Another mutation (allelic to Mas), dsx, prevented the duplicaton of 3L to affect sex-comb tooth number in males and femles (genotypes 13 and 14 versus 15 and 16). This mutation is the null condition of dsx locus, whereas Mas is neomorphic, suggesting that the modificatin of sex-comb phenotype requires the activity of the dsx wild-type product.

It is entirely possible that the modifications to sexual development observed in 3L trisomies arises from disruptions to processes which are not directly involved in sex differentiation. The expression of an X-linked gene, Sxl (Sex-lethal), has been shown to be required for female development to proceed (Sanchez and Nothinger, 1982). The expression of Sxl in female zygotes is dependent on the activity of the da (daughterless) locus during oogenesis. As such, da, a sex-specific maternal-effect lethal, is required for female development. Experiment I (see Table 38) demonstrates that the effect of da was unchanged in 2L trisomies from that observed in diploids; females did not hatch from the egg whereas males had excellent viability. However, experiment II shows that this was not the case for 3L trisomies. The trisomic-3L females derived from da mothers are still

Table 38 Effect of da and Fl on the embryonic viability of autosomal trisomies.

Parents			First-instar larvae			
Female	Male	# of eggs	y+ female	%	y male	%
I						
y/y;da/da	C(2L)lt; F(2R)bw/F(2R)bw	733	0	0	170	93
II						
y/y;da/da	C(3L)ru st; F(3R)e^{s}/F(3R)e^{s}	491	0	0	25	20
III						
y/y;C(2L)lt; F(2R)bw/ F(2R)bw	Fl oc ptg v/Y	510	12	9	100	78

completely lethal as expected, but in addition, the viability of their brothers was also severely reduced. These results suggest that a duplication of 3L makes males succeptible to the sex-specific killing action of da.

One of the primary roles of the da+ gene product is to allow expression of the Sxl locus in females. As mentioned previously, Sxl+ is required for the expression of female characteristics. Sxl+ gene product, however, is also required in females to prevent hyperactivation of X-chromosome transcription (Cline, 1978). Necessarily, the Sxl gene must not be active in males, since hyperactivity of the X-chromosome is required for dosage compensation and male viability (constitutive mutants for the Sxl locus are therefore male lethal and da insensitive). What determines the mode of Sxl expression in the two sexes is not known.

The relationship between X-chromosomal and autosomal ploidy in the control of dosage compensation probably arises from the interaction between specific genes in these two regions. This possibility was tested by examining the viability of females that were heterozygous for the female lethal allele of Sxl (Fl) and trisomic either for 2L or for 3L. The rational for this experiment is as follows. Females require the Sxl+ product to prevent X-chromosome hyperaction and absence of this product is essential in males. Thus, reduced quantities of Sxl+ product are required to allow hyperactivation to occur. This interpretation is supported by the observation that some complementing mutations

at the Sxl locus allow partial survival of females. Somehow, the quantity of Sxl^+ product must be monitored to manifest this regulation. It seems logical that such a gene product should be encoded by an autosomal locus. The ratio of Sxl^+ product to the autosomal gene product could be reduced to one-third the ratio observed in wild-type females by duplicating this locus and at the same time the Sxl^+ product is reduced (by making the females heterozygous). This level could be below the threshold required to elicit hyperactivation. The following experiments suggest that this might be the case. Trisomic-3L females heterozygous for Fl are perfectly viable. However trisomic-2L females of the same Sxl constitution have drastically reduced viability; they are about 10% as viable as wild type through to the first instar larval stage of development (cross III, Table 38). Thus, it would appear that a locus exists on 2L which interacts with Sxl and results in reduced viability. Since da is on 2L, the inviability may have arisen as a consequence of elevated levels of da^+ product. To test this, 2L trisomies were produced which possessed only two copies of the wild type allele of this gene. Experiment I in Table 39 shows that the viability of these females (+ class) is not rescued relative to the females possessing three copies of da^+ (Gla class). Control crosses suggest that males, 3L trisomies and diploids are unaffected (Experiments I, II and III in Table 39). These data imply that a locus on 2L other than da is fundamentally involved with Sxl in determining the state

Table 39 Survival to late pupae of autosomal trisomies heterozygous for Fl and possessing different doses of da+.

I Parents		Trisomic-2L Progeny				
		male		female		female/ male ratio
Female	Male	y;Gla	y	Gla	+	
y/y;C(2L)lt; F(2R)bw/ F(2R)bw	Fl oc ptg v/Y da/Gla	45	78	1	0	.008

II Parents		Trisomic-3L Progeny				
		male		female		female/ male ratio
female	male	Gla	+	Gla	+	
C(3L)ru st; F(3R)e^{s}/ F(3R)e^{s}	Fl oc ptg v/Y da/Gla	7	2	15	3	2.0

III Parents		Diploid Progeny				
		male		female		female/ male ratio
female	male	Gla	+	Gla	+	
+/+ (Canton S)	Fl oc ptg v/Y da/Gla	115	105	101	73	.79

of dosage compensation of the X-chromosome. Whether this locus regulates the expression of Sxl gene or interacts with its gene product is not known.

III General Physiological Considerations

Expression of dominant mutations and genes subjected to position-effect variegation.

The phenotype of strains with low or new activity mutations can be used to infer the expression level of the gene (Muller, 1950). In addition, such mutations can also be used to monitor alterations to general metabolic processes (Sinclair et al., 1984). As such, the expression of several markers, which had phenotypes which can be observed in late pupae, were examined (Table 40). The dominant phenotype was suppressed in trisomies for three mutations, all of which arise as a consequence of haplo-insufficiency. All mutations which have new functions (neomorphs) were unaffected in trisomies, although the expression of one wild-type allele (ru+) may have been reduced. Thus, a general effect which alters the expression of most genes in a similar way was not observed.

Genes juxtaposed to heterochromatin are subject to variegated expression. A potent modifier of position-effect variegation is heterochromatin. Removal of large blocks of heterochromatin (such as the Y chromosome) has long been known to enhance the variegated phenotype (ie. reduce the number of

Table 40 Expression of dominant markers in trisomies.

Dominant Mutation	Location	type1	trisomy examined	Expression
S	2L-1.3	haplo insufficient	Ts2L	fully suppressed (2)
Sp	2L-22.0	neomorph	Ts2L	unaffected
Tft	2L-53.2	neomorph	Ts2L	unaffected
Fl	2L-54.8	neomorph	Ts2L	unaffected (3)
L	2R-72.0	neomorph	Ts2L	unaffected
Gla	2L or 2R	neomorph	Ts2L	unaffected
ru+	3L-0.0	wild type	Ts3L	reduced
R	3L-1.4	haplo insufficient	Ts3L	suppressed
Ly	3L-40.5	haplo insufficient	Ts3L	fully suppressed (3)
D	3L-40.7	neomorph	Ts3L	variable (3,4)
Gl	3L-41.4	neomorph	Ts3L	unaffected
Sb	3R-58.2	neomorph	Ts3L	unaffected (2)

1. Some neomorphs may be antimorphic (Lindsley et al., 1972); 2. expression recessive in triploids; 3. expression dominant in triploids; 4. in one cross allula were present in D bearing pupae, in another they were absent.

cells in which the gene is active). Conversely, the addition of heterochromatin to the genome tends to decrease the influence of the position effect. A theory has been proposed which suggests that the addition of chromatin to the genome causes an increase in gene expression by binding inactivating (heterochromatin promoting) factors (Zuckerkandl, 1974). Autosomal trisomies possess, as a minimum, one additional block of heterochromatin (corresponding to the duplicated arm) in addition to a 10% increase in total chromatin. To monitor what effect this condition may have on variegating genes, the expression of the Bar gene when it is inserted into the Y chromosome was examined.. In diploids this rearrangement (B^{m^v}) reduces the size of the eye relative to diploids (compare 1 and 3, Table 41). However, trisomic males possessing this Y chromosome have eye sizes similar to controls with a normal Y chromosome (compare 2 and 4). This presumably arises because the B^m gene is largely inactivated in the developing eye. Thus, it would appear that duplications for the left arm of chromosome two strongly enhance positon-effect variegation. This observation was examined more thoroughly using a rearrangement (In(1)sc^{v2}) which variegates both for the achaete (ac) and for the scute (sc) genes (Table 42). The wild type functions of these genes are required for normal bristle and hair development. The sc^{v2} mutation reduces the number of hairs present on the abdominal tergites (ac^+ expression) and the number of thoracic dorsocentral and scutellar bristles (sc^+ expression) (compare genotypes 1, 8 and 9

Table 41 Expression of Bar variegator in trisomic-2L males.

	Genotype	Eye Size(um^2)	n	trisomy/diploid
1	Oregon R (diploid)	424 ± 5.4	20	
				0.84
2	C(2L)+/+/F(2R)bw (trisomic-2L)	354 ± 13.9	20	
3	y sc/ $B^{sv}Y$ (diploid)	148 ± 14.7	50	
				2.23
4	C(2L)+/+/F(2R)bw; +/$B^{sv}Y$ (trisomic-2L)	330 ± 7.9	50	

Because B^{s} is a dominant mutation, increased eye size arises from a reduced level of expression.

versus 2 and 6, Table 42). Removal of the Y chromosome decreases the expression of these genes (genotype 3) consistent with the classic response for variegating genes. In contrast, the trisomic-3L condition supresses variegation (genotype 4). Strong enhancement of variegaton was again observed in whole-arm trisomies for 2L (genotype 5) and in individuals which possess proximal duplications for this arm (Dp(2L) 38C-40 for genotype 9 and Dp(2L) 36F-40 for genotype 10) The proximal region of 2L contains the histone-gene cluster, a deficiency for which suppresses variegation (Khesin and Leibovitch, 1978; Moore et al., 1979; Moore et al., 1983). The levels of enhancement by the proximal duplications are not as strong as the whole arm trisomies. It is quite likely that specific genes are located elsewhere on 2L which are capable of enhancing variegation when duplicated. This has been recently verified (Don Sinclair personal communication) and is consistent with the finding that a large number of suppressors of variegation were induced by ethyl-methane sulfonate (which may be amorphic in nature) that map to the central region of this arm (Sinclair et al., 1983b). Interestingly, of over fifty suppressor of variegation mutations isolated, only a few map to the left arm of chromosome three (Sinclair, personal communication). This may account for the lack of enhancement observed in 3L trisomies. These results suggest that modifications to the expression of variegated genes in trisomies does not arise as a consequence of altered chromatin content in the nucleus, but rather from increased quantities of specific gene products synthesized from these duplicated arms.

Table 42 Expression of sc^{v2} in aneuploids for 2L and 3L.

#	genotype	n	tergite 3	tergite 2	dorso-centrals	scutellar
1	Oregon R	10	119.7 ± 3.0	104.5 ± 2.4	4 ± 0	4 ± 0
2	V2/Y	25	42.8 ± 1.86	41.5 ± 1.84	1.2 ± .16	3.80 ± .38
3	V2/O	25	12.4 ± 1.36	13.5 ± 1.22	0.84 ± .15	2.44 ± .19
4	V2/Y C(3L)ru st/ +/F(3R)e^{ss}	26	69.5 ± 2.04	56.1 ± 2.02	2.92 ± .14	2.96 ± .17
5	V2/Y C(2L)lt/+/ F(2R)bw	25	1.48 ± .40	2.68 ± .82	1.36 ± .19	1.60 ± .82
6	C(2L)SH1lt; F(2R)bw/ F(2R)bw/Y	50	44.8 ± 1.36	37.6 ± 1.23	3.16 ± .14	2.82 ± .16
7	C(2L)lt; F(2R)bw/ T(Y,2)B110RBS V2/Y	25	19.2 ± 1.5	15.3 ± 1.2	3.08 ± .73	1.48 ± .25
8	+/Y;C(2L)+; F(2R)bw/ F(2R)bw	10	113.4 ± 4.5	96.4 ± 1.9	4.0 ± 0	4.0 ± 0
9	+/Y,C(2L)lt; F(2R)bw/ T(Y,2)B110RBS	10	115.8 ± 3.2	101.4 ± 2.4	3.8 ± .10	4.0 ± 0
10	V2/YC(2L)lt; F(2R)bw; T(2;3)VZ1R/ F(3R)VD1e^{ss}	18	10.8 ± 1.2	6.61 ± .88	3.17 ± .17	1.5 ± .35

Values given are means ± standard errors.

Repression of enzyme activity is a cellular phenomenon.

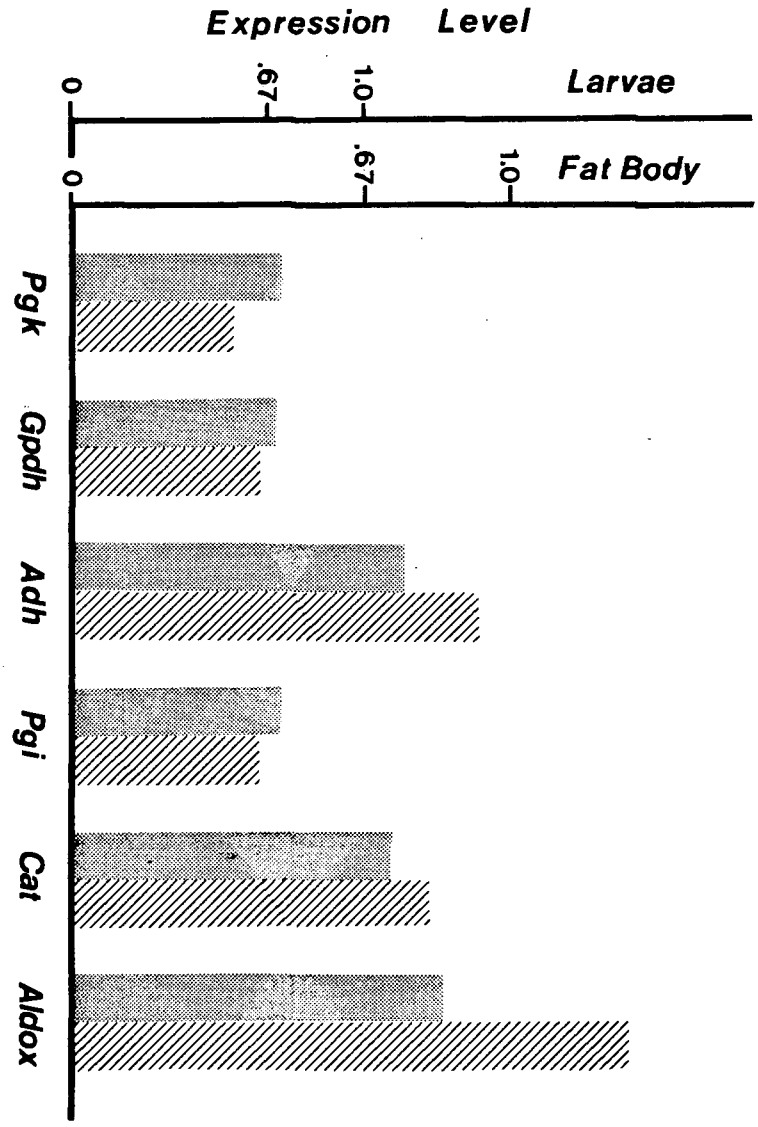
Compensation of loci in autosomal trisomies could result at the organismal level from alterations in tissue quantity or changes in tissue specificity of gene expression. Such effects should not be observed in specific tissues. Several enzymes, which all display characteristic responses in trisomic-2L third instar larvae, were measured in extracts made from fat bodies dissected from trisomies and diploids. This tissue is one of the major producers of the enzymes listed in Table 43. A similar pattern is observed in measurements made from fat bodies dissected from larvae of the same developmental stage (Table 43). Of the genes located on 2L, only ADH showed dose-dependent levels of activity. The activities of PGK and a-GPDH were clearly not dose-dependent, and in fact were lower than expected for a compensated level of expression. Of the tested loci not located on 2L, Pgi was repressed, Aldox was activated and Cat remained unaffected. Thus, the pattern of enzyme activity observed in fat body was very similar to that seen in whole larvae (Figure 20). However, the overall level of all enzymes (both on a live weight and mg protein basis) in trisomic-2L fat body preparations were lower than expected. This may have resulted from less fat body being recovered from these individuals, since this tissue is much more fragile in trisomies than in diploids. This cannot account for the compensated levels of activity found in larval extracts. If it had, we would not have observed dose dependent values for enzymes such as ADH, which is largely produced in the fat body.

Table 43 Enzyme levels in fat bodies from diploid and trisomic-2L female larvae.

	Enzyme Locus					
Genotype	Pgk	aGpdh	Adh	Pgi	Cat	Aldox
Oregon R (diploid)	1.84 ± .23 (11)	1.49 ± .17 (11)	.324 ± .031 (11)	3.93 ± .23 (11)	3.19 ± .27 (11)	1.36 ± .09 (11)
y/y;C(2L)lt/ F(2R)bw/ F(2R)bw (diploid)	1.84 ± .09 (11)	1.56 ± .09 (11)	.212 ± .009 (11)	5.57 ± .46 (11)	3.96 ± .39 (11)	2.49 ± .37 (11)
y/+;C(2L)lt/ +/F(2R)bw (trisomic-2L)	.995 ± .056 (11)	.991 ± .031 (11)	.348 ± .019 (11)	2.12 ± .13 (11)	2.89 ± .20 (11)	2.49 ± .20 (11)
Estimate	2.76 ± .07	2.31 ± .06	.374 ± .009	4.75 ± .13	3.58 ± .12	1.93 ± .09
Repressed	1.84 ± .05	1.54 ± .01	.249 ± .006	3.16 ± .09	2.38 ± .08	1.28 ± .06

Enzyme activity is expressed as the change in absorbance per min per mg of protein.

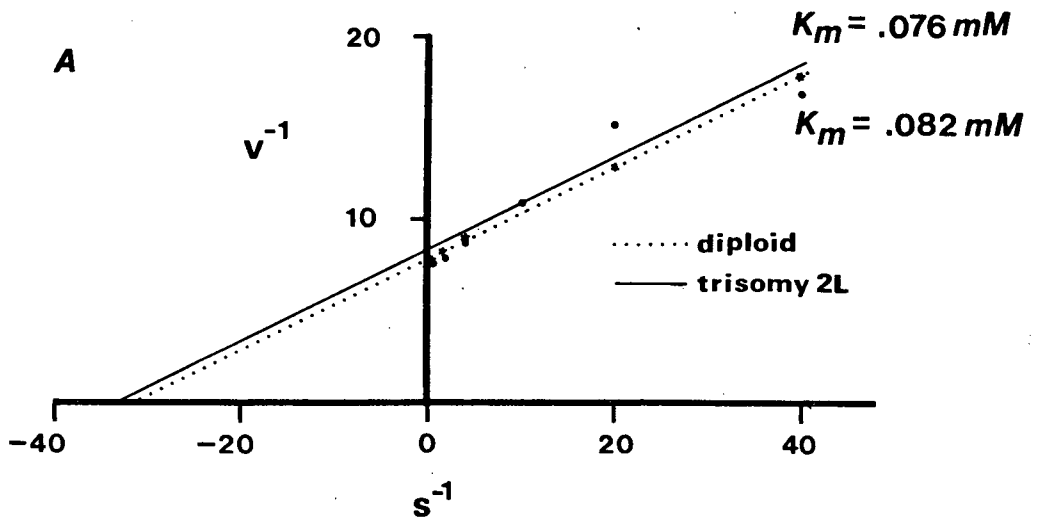
Figure 20. Comparison of enzyme quantites (in 2L trisomies relative to diploids) from whole larvae and from isolated fat bodies. Left bar for each enzyme represents data from whole larvae, right bar for fat bodies. Expression level of 1.0 represents the diploid amount per gene.

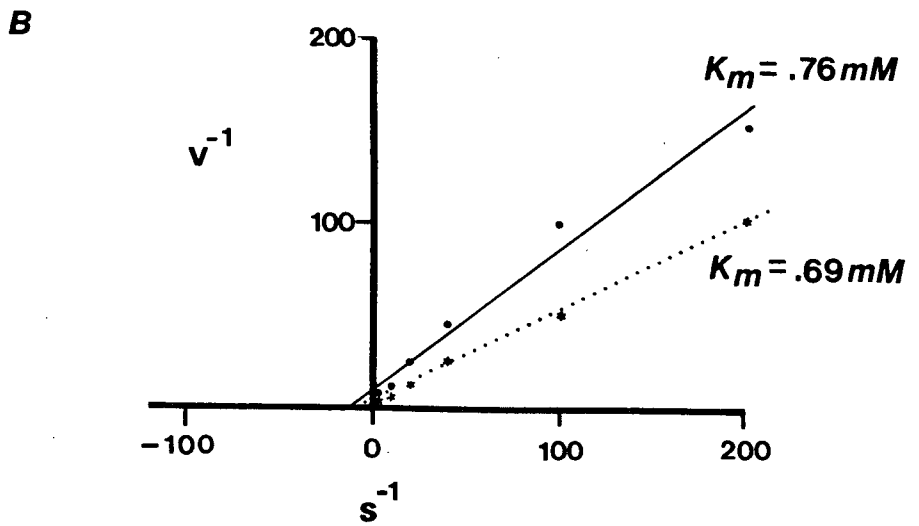

Expression Level
0
.67
1.0
Larvae
0
.67
1.0
Fat Body
Pgk
Gpdh
Adh
Pgi
Cat
Aldox

Since both dose-dependent and repressed loci (based on larval measurements) were affected in similar ways in extracts from the fat-body, the overall reduction of activity in fat-body extracts was probably experimental in origin. However, the inconsistency between the results obtained for a specific tissue and for the whole organism is a serious discrepency that warrants further investigation.

Modification to the enzyme molecules which would account for reduced catalytic activity in the extracts have not been detected. Trisomies for 2L were produced which contained a deficiency for the Gpdh locus and thus possessed only the same two alleles for this gene as the parental compound-free strain. Enzyme was extracted from late third-instar larvae from both genotypes and kinetic aspects were examined for the substrates a-glycerophosphate and NAD. The Km's for these two substrates (Figure 21) were not sufficiently different to suggest that postranslational modifications of this enzyme are occurring. In addition, the electrophoretic properties of this enzyme do not appear to be altered substantially (Chapter 1). These results differ from those of Rawls and Lucchesi (1974b). These authors found that a duplication for region 21A to 25CD (the distal quarter of 2L) significantly reduced the apparent Km of GPDH for a-glycerophosphate. Two explanations seem possible. The present study was not as thorough as that performed by Rawls and Lucchesi, and the results could be fortuitous. Alternatively, the measurements made by Rawls and Lucchesi were made on enzymes from adult flies, whereas those in this study were obtained from larval enzymes. Perhaps different regulatory

Figure 21. Kinetic mmeasurements on GPDH from diploids and 2L trisomies. Lineweaver-Burke plots for A. NAD and B. alpha-glycerophosphate.


A
20
10
v^{-1}
K_m = .076 mM
K_m = .082 mM
diploid
trisomy 2L
−40
−20
0
20
40
s^{-1}

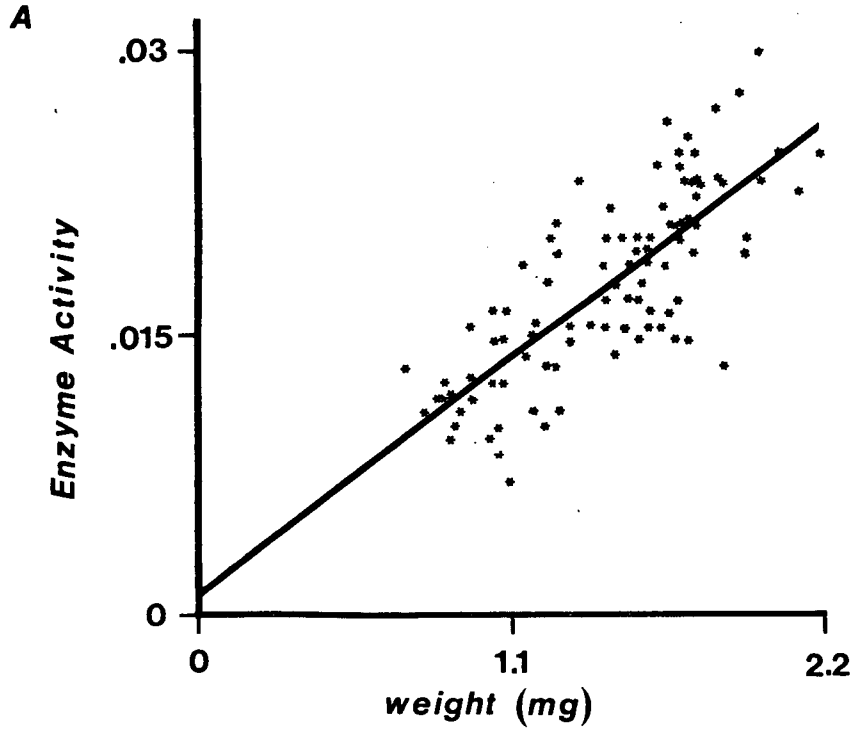

B
200
100
v^{-1}
K_m = .76 mM
K_m = .69 mM
−100
0
100
200
s^{-1}

affects are operating between these two developmental stages.

Modulations of enzyme activities are not correlated with changes in organism or cell size, nor with changes in total protein or DNA levels.

Several observations suggest that repression of activity is not the result of general physiological disruptions. First, the response is fairly discrete. If a locus is producing significantly less product in a trisomy, then in over 85% of the cases the reduction is not significantly different from one-third. If modulations in enzyme activity in trisomies were brought about by disruptions in internal metabolic processes (such as proteolysis), then such discrete effects and locus specificity would not be expected. Trisomic third instar larvae are some 10-15% smaller than their diploid parental larvae (Table 44). It has been reported previously that some enzymes show a non-linear relationship of activity with changes in the size of the adult fly (Clarke et al., 1979). To test whether such an effect is responsible for the reduced levels observed in trisomies, the activities of three loci (two which are modulated (*Gpdh* and *Pgi*) and one which is not *Adh*) were examined in extracts from single larvae trisomic for 2L. The regression lines obtained from these tests are shown in Figures 22-24. The enzyme activities of the two modulated loci (*Gpdh*, *Pgi*) show a relationship that is closely proportional to size. Estimates of this data suggest that the

Figure 22. Relationship between PGI activity and larval size in female (A) and male (B) 2L trisomies. Enzyme activity units are the change in absorbance at 340nm per min per 10ul of extract.


A
.03
.015
0
Enzyme Activity
0
1.1
2.2
weight (mg)

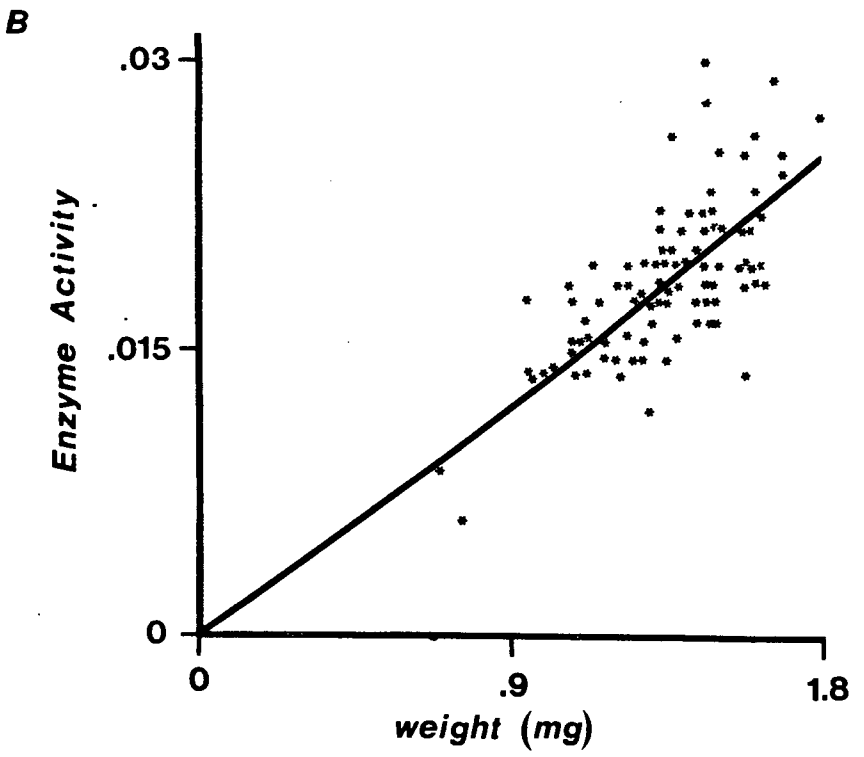
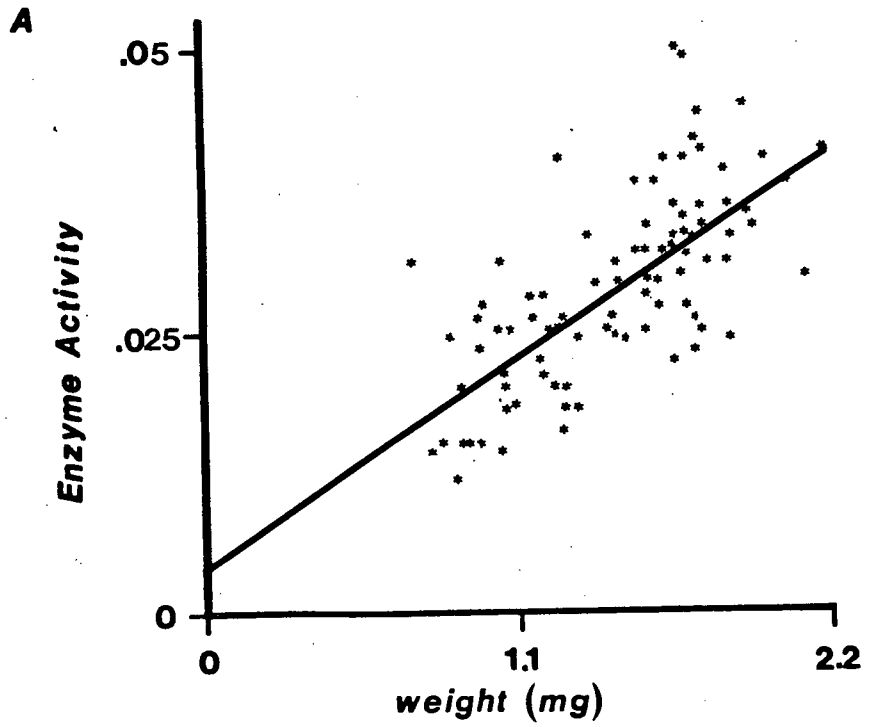

B
.03
.015
0
Enzyme Activity
0
.9
1.8
weight (mg)

Figure 23. Relationship between GPDH activity and larval size in female (A) and male (B) 2L trisomies. Enzyme activity units are the change in absorbance at 340nm per min per 50ul of extract.


A
.05
.025
0
Enzyme Activity
0
1.1
2.2
weight (mg)

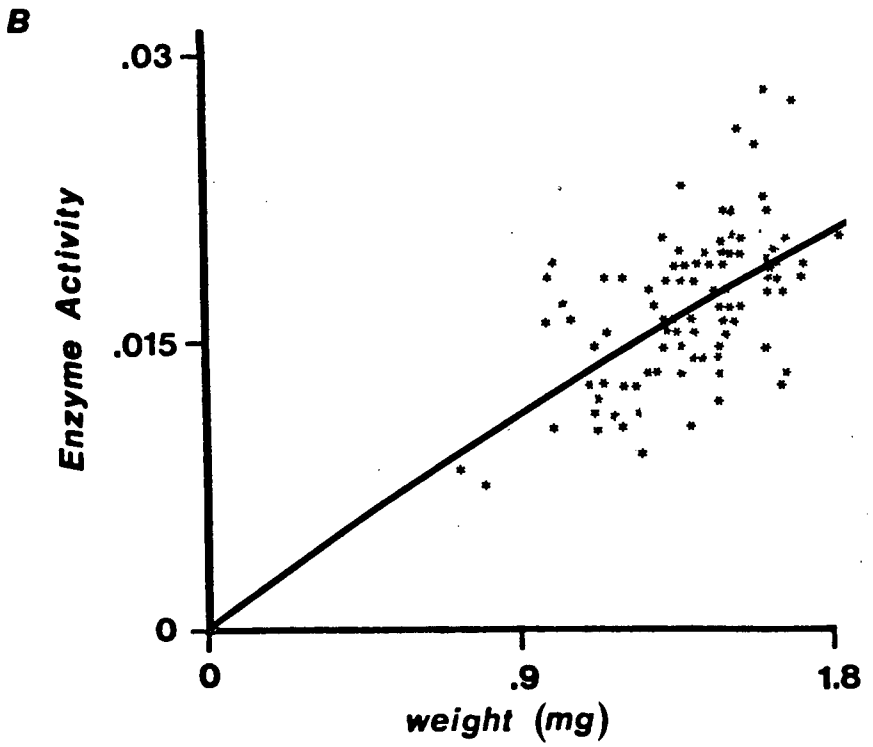
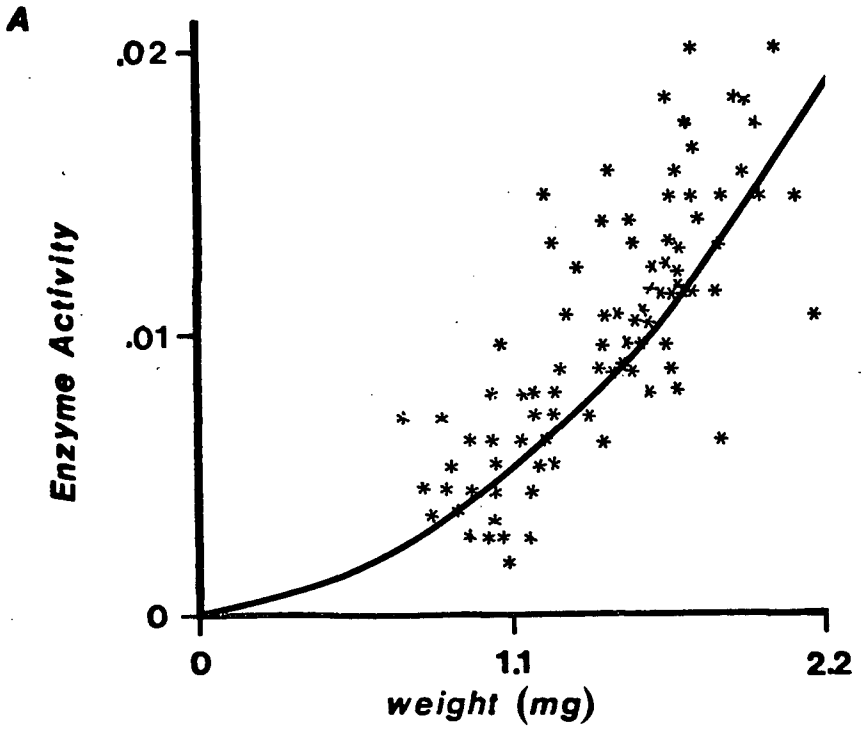
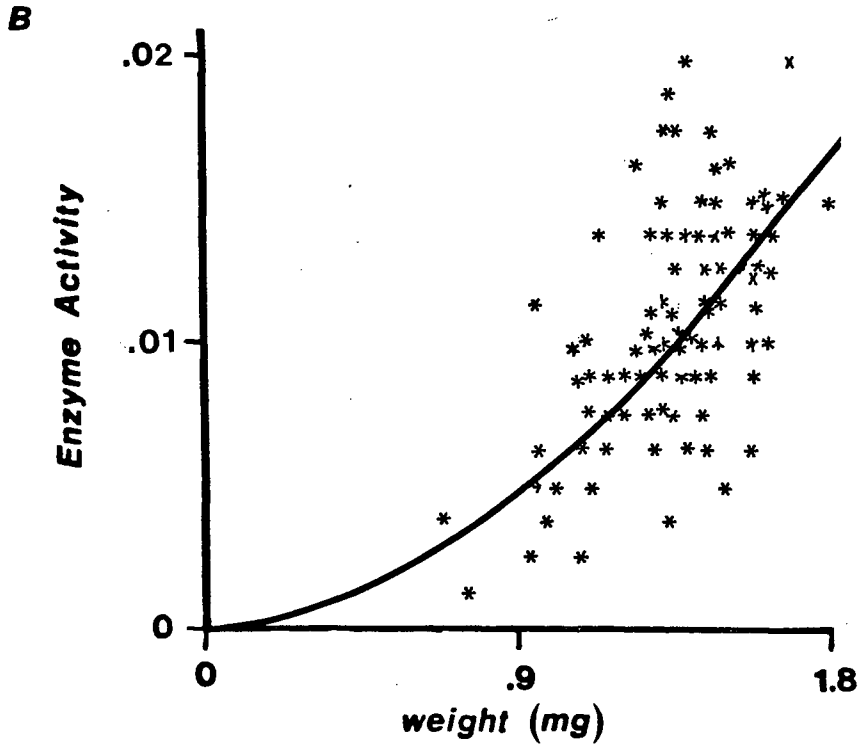

B
.03
.015
0
Enzyme Activity
0
.9
1.8
weight (mg)

Figure 24. Relationship between ADH activity and larval size in female (A) and male (B) 2L trisomies. Enzyme activity units: change in absorbance at 340nm per min per 50ul of extract.


A
.02
.01
0
Enzyme Activity
0
1.1
2.2
weight (mg)


B
.02
.01
0
Enzyme Activity
0
.9
1.8
weight (mg)

differences in activity (per unit weight) resulting from differences in larval size between diploids and trisomies are less than 2% for both enzymes. The other enzyme (Adh) is clearly non-linear and shows an activity curve whose slope increases with increasing size of the larvae. Interpolation of this data suggests that the size difference of trisomies should result in an ADH activity approximately 9% lower than the expected noncompensated level. ADH activity, however, was found at dose dependent levels in these individuals. Clearly these relationships are not striking enough to result in the observed changes in enzyme activity in trisomies.

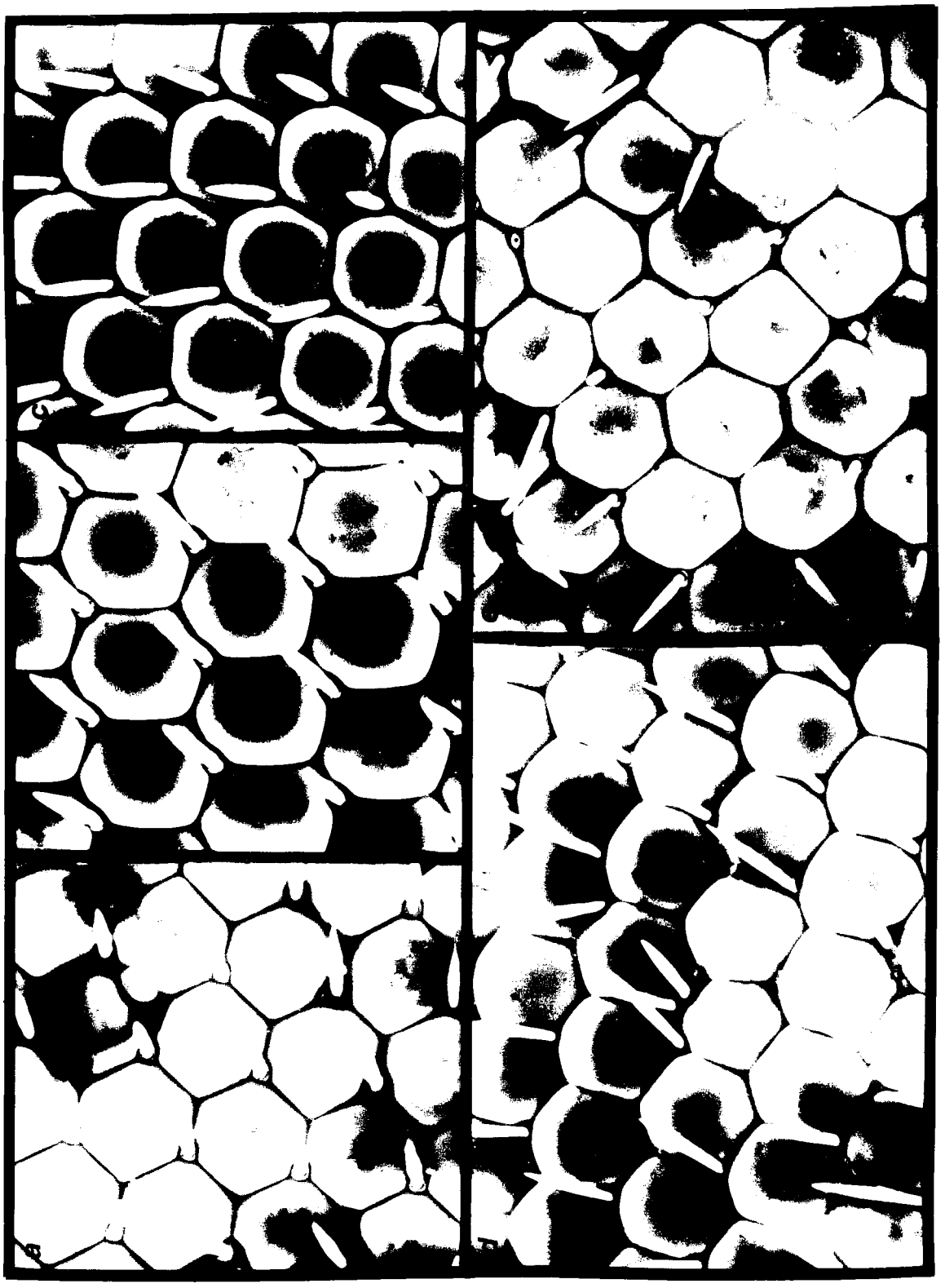
Table 44 gives values for protein and DNA content in trisomies and diploids. Although levels of DNA and protein are reduced on a live weight basis, DNA per mg soluble protein was not different between the strains. Changes in cell number or in levels of polytenization could account for reduced levels of DNA. However the magnitude of the change suggests that this could not be responsible for the observed changes in enzyme activity. Of course, changes in polyteny of individual genes would not be detected by such crude measurements as these. In chapter 5 a more critical test of this possiblity is described.

Alteration of cellular volume could change the concentration of regulatory molecules and result in altered levels of gene expression. As an estimate of cell size we measured the size of eye facets in electron micrographs of pharate adults (Figure 25). The ratio of facet size in adult triploid females to diploid females is 1.47 (Table 45), and is consistent with the observation that cell size is proportional

Table 44 Live weight, protein content and DNA content in female diploid and trisomic larvae.

Genotype	size (mg)	ug protein/ mg live weight	ug DNA/ mg live weight	ug DNA/ mg protein
Oregon R (diploid)	1.78 ± .05 (10)	97.6 ± 2.29 (7)	.347 ± .012 (5)	3.56
C(2L)lt; F(2R)bw/ F(2R)bw/ (diploid)	1.90 ± .05 (10)	106 ± 1.8 (8)	.319 ± .020 (3)	3.01
C(2L)lt/+/ F(2R)bw (trisomic-2L)	1.59 ± .09 (10)	99.1 ± 1.3 (8)	.349 .013 (3)	3.52
C(3L)ru st; F(3R)VD1e^{s} F(3R)VD1e^{s} (diploid)	1.89 ± .02 (10)	90.9 ± 1.6 (8)	.306 ± .012 (3)	3.37
C(3L)ru st/ +/ F(3R)VD1e^{s} (trisomic-3L)	1.69 ± .07 (10)	86.2 ± 1.9 (10)	.289 ± .007 (3)	3.35

Figure 25. Electron micrographs of eye facets from diploids, triploids, and trisomies. a. diploid adult b. triploid adult c. diploid pupae d. trisomic-2L pupae e. trisomic-3L pupae.



to the level of ploidy in many organisms. Both trisomies examined had estimated cell sizes only 10% lower than diploids, and the ratio of cell size to organism size was remarkably similar in all the genotypes (Table 45). The extra DNA content (approximately 10% in each trisomy) did not result in an increase in cell size. It would appear that relative to diploids, trisomic individuals are smaller, and have less soluble protein, because their cells are smaller.

Table 45 Estimates of cell size in diploid and triploid, and trisomic pupae.

female genotype	stage	size (arbitrary units)	(n)	size relative to Oregon R	cell size / larvae size
Oregon R (diploid)	adult	167 ± 5	10	1.00	
C(1)RMy w^{ma} m/ Basc 3N (triploid)	adult	246 ± 7	12	1.47	
Oregon R (diploid)	pupae	180 ± 3	10	1.00	101
y/y;C(2L)lt; F(2R)bw/F(2R)bw (diploid)	pupae	199 ± 3	10	1.10	105
y/+;C(2L)lt/ +/F(2R)bw (trisomic-2L)	pupae	167 ± 6	10	.931	106
C(3L)SH2+; F(3R)VD1e^{B}/ F(3R)VD1e^{B} (diploid)	pupae	194 ± 5	12	1.08	103
C(3L)SH2+/+/ F(3R)VD1e^{B} (trisomic-3L)	pupae	171 ± 5	12	.953	102

Discussion

This chapter has explored the effects of aneuploidy for large regions of the genome on viability and phenotype of pre-adult Drosophila. The conclusions derived from this study are consistent with the body of literature that previously has examined this problem in adult Drosophila as well as other species. Since we also have examined the levels of several gene products in some of these aneuploids (see previous chapters), it is possible to speculate on the genetic basis of these syndromes.

The larger the degree of aneuploidy, the greater will be the disruption observed. As increased numbers of loci are duplicated, more gene products are synthesized at elevated (and thus unbalanced) levels relative to the majority of loci in the genome. As this condition is exaggerated, increased disruptions to physiological processes ensue and viability and phenotype are altered. While disruptions to physiological and developmental pathways are undoubtedly the cause of inviability, the results presented in the previous chapter suggest that it is not increased product levels alone that are responsible. The most notable effect, particularly in 3L trisomies, is that many product levels are present at less than diploid levels. That most genes are recessive suggests that half the normal amount of product is sufficient to maintain the wild-type phenotype. However, as suggested by Muller (1950) and elaborated further by Kacser and Burns (1980), a physiologically significant difference exists between diploid and one-half diploid levels of

gene product. Muller noted that considerable variability exists in the expression of particular genes. We have also presented data consistent with this view. At a discrete developmental stage, large variations in the amount of gene product can exist for organisms of similar size (see Figure 24 for example). If these variations were sufficiently severe, then some individuals with half the synthetic potential for a particular gene could have product levels so low that normal processes may become limited. Thus, quantities of gene product are present that, while usually in excess, can act as a buffer against natural variation in gene expression. This suggests that the diploid level of gene product is adaptively significant and probably represents the optimum. Reductions from this level, even as little as one-third, may be important. Thus, the regulatory system controlling compensation of autosomal genes may not be responsible for the survival of these large aneuploids. In fact, such negative regulation, when operating on genes outside the trisomic arm, may be partially responsible for the inviability. The overall survival and phenotype of an aneuploid individual would depend on what proportion of the linked genes are compensated versus a combination of the number that are linked and remain dose-dependent, plus the number that are unlinked and have reduced expression. This conclusion has also been derived from similar studies on aneuploids of corn (Birchler and Newton, 1982).

The ability to tolerate aneuploidy varies with developmental stage. Many of the gross aneuploids observed in this study (such as double trisomies) were able to develop through

embryogenesis and emerge from the egg, but soon became moribund and died (see also Wurgler, 1972). Similarly, autosomal monosomics were able to develop to late stages of organogenesis and occasionally to first instar larvae. (This is in contrast to the conclusion drawn by Herskowitz and Schalet, 1957). Thus, the processes involved in early development in Drosophila appear to be relatively insensitive to aneuploidy. Maternally synthesized products deposited in the egg during oogenesis may be able to temporarily buffer development against abnormal gene expression. This conclusion has also been drawn from the observations on the viability of Drosophila possessing large deletions (Garcia-Bellido et al., 1983). Larval development proceeds largely by amitotic growth except for the imaginal discs which are not required during this period (Shearn et al., 1971) and, as such, aneuploid lethality during this period probably arises from metabolic disruptions. However, it is during pupal development that trisomies become most sensitive to elevated temperature and when males are less viable than females. These conditions perhaps overtax an already strained system and reduce viability further. However, it is possible that lethality in pupal development is also caused by specific developmental defects. Pupal development involves cell division and complex morphogenetic movements.

The studies that examined the viabilities of double trisomies revealed that the condition involving the X plus an autosome were no more viable than double autosomal trisomies. In addition, autosomal trisomies which were also trisomic for the X-chromosome survived poorly compared to autosomal trisomic

males and females. This suggested that, despite the existence of X-chromosome dosage compensation between haplo, diplo and triplo-X diploids, the X-chromosomal trisomic condition to a large degree paralleled the autosomal trisomic conditions. This possibility is dealt with further in chapter six. However, whereas pre-imaginal viability is comparable, the viability of metafemales from pupation to adult is better than for autosomal trisomies. Two reasons may account for this. First, some genes remain dose dependent in autosomal trisomies; on the X chromosome such genes may be compensated by the male-female dosage compensation mechanism. It appears that dosage compensation operates throughout development and is particularly important during the pupal period. This is suggested from studies which examine the viability of male cells produced in females by somatic crossing over. These cells have poor viability in larvae and are inviable in pupae (Sanchez and Nohtinger, 1982). Thus, dosage compensation of X-linked genes during pupal development may enhance the viability of metafemales relative to autosomal trisomies. Second, metafemales may survive better than autosomal trisomies because most of the loci that are repressed in the trisomic-X condition are on the X chromosome. In contrast, autosomal trisomies affect loci throughout the genome (Figure 12). Thus, the net imbalance of gene products in X-chromosomal trisomies is much less than that found in their autosomal counterparts.

This study has revealed that monosomies for all four major autosomal arms die as embryos or first instar larvae. Thus, these individuals are incapable of fully compensating the

expression of all of their haploid genes back to the diploid level. This is clearly not the case for the X chromosome. However, a series of mutations are known (male-specific lethals) which prevent the X-chromosome from being hyperactivated in males. This condition renders these individuals functionally as well as karyotypically monosomic for this chromosome. The viability of these individuals is considerably better (up to the pupal stage) than for autosomal monosomics (see Belote, 1983). Perhaps the male specific lethal mutations are not abolishing all compensatory activity in males. Indeed, the levels of several X-linked enzymes in these mutant males are about sixty percent of the diploid female level (Belote and Lucchesi, 1980). It is also possible that monosomy for the X chromosome does not affect the expression of unlinked genes as much as autosomal monosomics. This possibility can be inferred only from the lesser ability of the X chromosome, when it is trisomic, to affect unlinked loci (relative to the autosomal trisomies).

The observation that trisomic-3L males may possess some female characteristics whereas trisomic-2L males do not, suggests that factors determining sexuality are not evenly distributed throughout the genome. The balance theory of sex determination suggests that male determining and female determining genes exist and that the relative distribution of these factors affects the sexual phenotype of the zygote. In species with sex chromosomes, the expression of one sexual phenotype may be determined by one of the sex chromosomes (eg, maleness by the mammalian Y chromosome) and determinants for the other sex presumably are located on the autosomes. In

Drosophila, male determinants are hypothesized to exist on the autosomes because genotypes possessing two X chromosomes are shifted towards maleness when an additional autosome set is present in triploids. In Drosophila, triploid intersexes possessing additional pieces of X chromosomes have been examined (Dobzhansky and Schultz, 1931; Patterson et al.,1937; Pipkin, 1940) and as a general trend, most X-chromosome fragments were capable of shifting the sexual phenotype towards femaleness. Whether this arises from an increase in the number of female-determining genes or from enhanced viability of cells that are set at the female rather than the male mode, is not known (Baker and Belote, 1983). In diploids, large duplications of autosomal material did not influence the sex of adult flies (Patterson et al., 1937). In intersexes, extensive studies by Pipkin (1947; 1959; 1960) revealed that duplication of autosomal regions did not shift development towards male phenotypes. Both sets of experiments are consistent with the hypothesis that only female determinants exist, and that these are located exclusively on the X chromosome. However, the phenotype of individuals possessing a duplication for the left arm of chromosome three suggests that female determinants may also exist on the autosomes. It is possible that in Drosophila sex is not determined by a balance between male and female determinants but, instead, it is controlled by the relative amounts of female determinants alone. Thus males, with only one X-chromosome, would have less female determinants than females with two X chromosomes. Triploid intersexes may be shifted towards male development, not because they have an additional

set of autosomal determinants, but because the concentration of female determinants has been reduced one-third by the increase in cell size. It is important to note that 3L has become an X chromosome in other Drosophila species. If autosomal regions possessed male determinants, then when this chromosome became a sex chromosome (by translocation to the existing X chromosome) the individuals receiving a single dose (males) would have their sex determining gene balance shifted towards femaleness. Alternatively, if this chromosome arm possessed female determining genes, then maintenance of correct sexuality with the original X and Y chromosomes would be facilitated by a reduced ability of this element to promote female development when hemizygous.

That trisomic-3L males are sensitized to the lethal action of the mutation daughterless again suggests that they are more female-like than diploid males. The lethal effect of da on females presumably results from lack of expression of the Sxl locus (Cline, 1983; Skripsky and Lucchesi, 1982). However, since the Sxl locus normally is not expressed in haplo-X individuals, the inability of this locus to be activated in zygotes from da mothers would be inconsequential. Shifting to femaleness would only strengthen the above controls, and diploid and trisomic-3L males should have similar viabilities. Mosaic analysis has shown that cells in which the Sxl gene is removed by somatic crossing over develop phenotypically as male. From these experiments, it has been interpreted that the Sxl^{+} product is required to sustain female development, as well as regulating dosage

compensation. Since X-linked genes are dosage compensated in trisomic-3L males it follows that the Sxl locus is not active. Thus, the absence of Sxl^+ product could reduce viability if the physiology of these individuals is partly female.

Cline (1983; 1984) has suggested that the interactions between da and Sxl do not arise from discrete alterations in the expression states of these genes. Indeed, specific effects appear to depend on thresholds of gene product levels. During the course of these studies we noted that trisomic-2L females heterozygous for Fl (a female-specific lethal allele of Sxl) die early during development. This result suggests that the level of Sxl product is measured relative to another gene product which acts as a reference. If, as Cline proposes, Sxl is not transcribed in males the ratio of Sxl^+ product to the reference product would be zero. In a female this ratio would be a characteristic of the transcription rate of the two genes. The present data would suggest that this ratio needs only to fall to one-third of that found in the diploid female to elicit the male mode of dosage compensation and/or sexual development. It is likely that activation of dosage compensation is responsible for the observed lethality, since mutations are known which transform females into healthy (albeit sterile) males (Lindsley and Grell, 1968; Cline, 1984).

Two simple possibilities for this regulation seem likely. First, a locus on 2L could code for a gene product that is used to determine the number of autosome sets present. 1.5 times

more product would be produced in 2L trisomies if this gene is not compensated. Alternatively, a regulatory locus on this arm may repress the expression of the Sxl gene in a way analagous to repression of the enzyme loci shown in Figure 12. By either mechanism, Sxl^{+} product relative to the autosomal standard in Fl/+ heterozygotes would be reduced to one-third. However, in the second possibility, the autosomal reference genes need not reside on 2L. Diploid females that are heterozygous for Fl are viable, suggesting that the threshold ratio for activation of dosage compensation lies between 0.5 and 0.33. However, it is also possible that dosage compensation may be activated in some cells of Fl/+ heterozygotes, but these cells normally die, and are not observed. However, in the absence of the wild-type products of male specific lethal loci (required for hyperactivation) these cells may survive and develop as male tissue (Skripsky and Lucchesi, 1982). It is noteworthy that metamales (1X:3A), which have one Sxl gene per three autosome sets, are also capable of hyperactivating their X-chromosomes (Lucchesi, et al; 1977; Cline, 1983).

Since polyploid cells are larger than diploids, possibly the synthetic activity of most genes has not been altered. This has been demonstrated in yeast (Cifferi et al., 1969), in plants (DeMaggio and Lambrukos, 1974; Roose and Gottlieb, 1980; Timko et al., 1980), in Drosophila (Lucchesi and Rawls, 1973) and in mammals (Priest and Priest, 1969; Westerveld et al., 1972; Chang et al., 1983). However, we did not observe an increased cell size in autosomal trisomics suggesting that this relationship

may not hold for fractions of a genome. Exceptions do exist where the expression of various genes are either increased (Timko et al., 1980) or decreased (Audit et al., 1976; Timko et al., 1980) with increasing ploidy. It is possible that effects arising from increased quantities of regulatory molecules could account for examples of modified gene expression in a polyploid series. These would have to act in a concentration-independent fashion since the nuclear volume usually varies directly with DNA content. In trisomies, alterations to cell size, organism size, protein and DNA content can not by themselves account for the observed changes in gene product levels. General modifiers of gene expression also do not appear to be responsible. The next chapter investigates the level at which autosomal trisomies exert their effect on gene expression.

CHAPTER FIVE

COMPENSATION IN 2L TRISOMIES IS TRANSCRIPTIONALLY REGULATED

Introduction

Dosage compensation for X-linked genes in Drosophila is one of the better described examples of modulation of gene expression in eukaryotes. Diploid individuals possessing from one to three X-chromosomes transcribe this chromosome at different rates relative to the autosomes (reviewed by Baker and Belote, 1983). The total amount of X-chromosome transcription in each strain remains constant; hence, equivalent levels of gene products accumulate in the different genotypes. Of particular interest to the present study is the observation that individuals trisomic for the X-chromosome display diploid levels of gene products (Lucchesi et al., 1974; Stewart and Merriam, 1975). While sex-linked dosage compensation operates at the level of transcription (Mukherjee and Beerman, 1965), the molecular mechanisms involved remain obscure. A fundamental, yet unresolved, question is whether transcription rates for X-linked templates are determined at the male or at the female rate, or whether a continuous adjustment of transcription rate is possible in response to a varied amount of X-chromatin present in the nucleus. In metafemales (3X2A), the former possibility would predict noncompensated levels of transcription, while the latter would result in compensated levels. Data supporting both of these possibilities has been presented (Ananiev et al.,1974; Lucchesi et al.,1974; Gvozdev et al.,1983). Therefore, this leaves open the question of whether

or not dosage compensation in these hyperploids occurs by mechanisms in addition to those which mediate dosage compensation between males and females. The relationship, if any, between dosage compensation in autosomal trisomies and male-female and/or metafemale dosage compensation is also uncertain.

Compensation of genes not linked to sex chromosomes is not limited to Drosophila. In Zea mays, an aneuploid series for chromosome one has little effect on ADH enzyme activity, despite the location of the Adh-1 gene in the left arm of this chromosome (Birchler, 1979; op. cit. for related references). Several examples of dosage compensation have also been described in yeast strains with multiple copies of genes present on plasmids (Faye et al., 1981; Osley and Hereford,1981; Pearson et al.,1982). None of these regulatory effects in yeast are transcriptionally mediated; instead, control appears to be operating at the level of translation, RNA stability, or terminal product maturation. The maintenance of normal levels of product in these aneuploids may arise from limitations present in their normal synthetic pathways.

The level at which compensation occurs in autosomal trisomies of Drosophila is generally unknown. In a previous chapter, hsp 83 compensation was found to be post-transcriptionally regulated. Aneuploidy might be expected to have a variety of physiological effects on gene expression. However, since individual loci behave discretely (either they compensate or they show dose dependence), the modulations of gene product levels may arise from control mechanisms operating

in normal diploids. The work described in this chapter was undertaken to determine whether: (1) dosage compensation in autosomal trisomies is regulated at the level of transcription, (2) the ability to undergo autosomal dosage compensation is regionally distributed along the chromosome, and (3) autosomal compensation operates independently at each genetic locus.

Autoradiography was utilized to measure transcription on the polytene chromosomes. We have found that ^{3}H-uridine incorportation on 2L is approximately 15% higher in 2L trisomies than it is in normal diploids suggesting that many, but not all, loci are compensated. In addition, mRNA produced from a compensating gene (LSP-1b) and a noncompensating gene (Adh) was measured by filter hybridization. The data revealed that compensation of LSP-1b occurs prior to translation. Taken together, these experiments suggest that when compensation for a gene is observed in autosomal trisomies, the level of regulation is generally transcriptional.

Materials and Methods

Drosophila stocks, crosses and culture conditions

Two diploid strains of *Drosophila melanogaster* were used to generate trisomies for the left arm of chromsome two (Fitz-Earle and Holm, 1978). A cross between standard males (Oregon R) and

Y/Y;C(2L)VH2,lt;F(2R)bw/F(2R)bw

females was used routinely. Monosomic-2L progeny from this cross die during embryogenesis, while trisomies for 2L develop with some delay relative to euploid controls. For all experiments, sampling of larvae was restricted to those third instars that were actively wandering and within 12 hours of pupation. To maintain an otherwise diploid genome, only female larvae were used in these experiments. All strains were reared at 25 degrees C on standard cornmeal-sucrose medium supplemented with the mold inhibitor Tegosept (0.22%) and the antibiotics tetracycline and streptomycin at 10mg/liter each.

Genetic markers and chromosome rearrangements not cited in the text are described in Lindsley and Grell (1967) and Grell (1970).

Transcription Autoradiography

Methods of Maroni and Plaut (1973) were modified for this study. Salivary glands were dissected from wandering third-instar larvae in Ephrussi-Beadle saline containing 1mM PO_4, pH 6.8. Glands were incubated for five minutes in 25ul of the same saline which contained 0.1µCi of ^{3}H-uridine (45.9 Ci/mmole, New England Nuclear). Glands were then fixed in aceto-methanol (1:3), rinsed in 50% acetic acid, and squashed by standard technique. After freezing the slide on dry ice, the coverslip was removed and the preparation was plunged into 100% ethanol. Slides were postfixed in ethanol-acetic acid (3:1) for 10 minutes and stored in 95% ethanol for 8 to 12 hours. They were then air dried, coated with Kodak NTB2 emulsion (diluted 1:1 with distilled water), exposed for two weeks, developed for three minutes in Kodak D-19 at 19 degrees C and stained in 0.1% toluidine blue. Grains were counted over the left arm of chromosome two, within region 1A-3C of the X-chromosome, and region 61A-68C of the third chromosome.

RNA Isolation

Fat bodies were dissected from third instar larvae in 10mM PO_4, 1mM phenylthiourea, pH 7.2. Total RNA was extracted by a modification of the method used by Brock and Roberts (1982). The extraction buffer contained 0.14M Na acetate, pH 4.5, 1% SDS, 4µg/ml polyvinylsulphate, 50µg/ml heparin, 2mg/ml bentonite, and 0.1% diethylpyrocarbonate. After extracting twice with redistilled phenol, with

phenol:chloroform:iso-amyl alcohol (50:50:1) and with chloroform:isoamyl alcohol (50:1), the RNA was precipitated in 70% ethanol. The samples were washed in 0.14M Na acetate, pH 4.5 and reprecipitated with ethanol. The nucleic acids were resuspended in distilled water and quantified spechtrophotometrically at 260nm.

Filter Hybridization

A 4ug aliquot of RNA was denatured at 80 degrees C in 2% formaldehyde for 5 minutes, cooled, and spotted onto nitrocellulose filters according to the method of Thomas (1980). Three filters were assayed and averaged for each extract. Prehybridization and hybridization were as described (Thomas, 1980), and washing was carried out in 0.1 x SSC, 0.1% SDS at 68 C. The removal of hybridized probes from the filters was performed in 0.05 x wash buffer (Thomas,1980) at 70 degrees C; removal was over 95% complete within three hours. The same filters were used for subsequent hybridization assays using the following probes: 1) SAC-1, a plasmid containing the D. melanogaster Adh gene (Goldberg, 1980), 2) pR^{d}LSP1g:1, a plasmid containing the 5' coding sequence of the LSP-1g gene (Smith et al., 1981), and 3) pLSP1b:B2.1, a plasmid containing the majority of the LSP-1b coding sequence. This latter plasmid was constructed by subcloning the 2.1 kb Bam HI fragment of pR^{b}LSP1b:1 (Smith, 1981) into plasmid pUC12 (Vieira and Messing, 1982) by

standard procedures (Maniatis et al., 1982). This fragment contains only LSP-1b coding sequence. This was necessary because a small nuclear RNA gene is present within sequences 3' to the LSP-1b structural gene in pR^{b}LSP-1b:1 (McClelland et al., 1981). Although the amount of this snRNA transcript relative to LSP-1b RNA is very low, ambiguity might arise from its presence. Control experiments showed that hybridizable counts were linearly related to the amount of RNA loaded onto the filters over the range found in these experiments. The ratio of LSP-1b to LSP-1g counts remained constant over this range, and over 95% of the counts were RNAse sensitive. Plasmids were purified and nick translated to specific activities of approximately 2 x 10^{8} cpm/ug (Maniatis et al., 1982). An estimated noncompensated value was determined by summing the LSP-1b/LSP-1g ratio from the diploid strain with 1/2 that ratio from the Oregon R strain. The compensated estimate is 2/3 of the noncompensated estimate.

Northern Blot Hybridization

For northern blots, 5ug of total RNA was denatured at 65 degrees C for 5 minutes in 1 x gel buffer containing 50% formamide, pH 8.0. The RNA was separated on 1% agarose gels containing 20mM boric acid, 1mM EDTA and 3% formaldehyde (Rave et al., 1979) and then blotted to nitrocellulose as described by Thomas (1980). The filter was hybridized to nick-translated pLSP-1b:H2.1.

Southern Blot Analysis

DNA was isolated from late third instar larvae in 100mM Tris, 100mM EDTA, 1% SDS, pH 8.0 and extracted twice with phenol, once with phenol:chloroform:isoamyl alcohol (50:50:1) and then with chloroform:isoamyl alcohol (50:1). Nucleic acids were precipitated with one-tenth volume of 3M Na acetate and 2.5 volumes of ethanol. After collecting the nucleic acids by centrifugation, 0.3ml of a 200µg/ml solution of boiled pancreatic RNAse was added and the mixture was incubated at 37 degrees C for 3 hours. The DNA was extracted with the organic solvents as described above, and reprecipitated. 10µg of the ethanol-precipitated DNA was resuspended in 10mM Tris, 1mM EDTA, pH 7.5 and dialyzed by filter floatation (Marusyk and Sergeant, 1980) against the same buffer for 30 minutes. The DNA was digested with Hind III, run on 1.2% agarose gels and blotted to nitrocellulose as described by Southern (1975).

Results

Transcription Autoradiography

Dosage compensation of X-linked genes in trisomic-X individuals has been well documented (Lucchesi et al., 1974; Stewart and Merriam, 1975). Compensation of gene products in autosomal trisomies has also been observed (Devlin et al., 1982), although the level at which such regulation occurs remains unknown. To determine if transcriptional regulation is responsible for compensation in autosomal trisomies, autoradiography was employed to measure the incorporation of ^{3}H-uridine into polytene chromosomes. This method has been successfully used to demonstrate that X-chromosome dosage compensation is transcriptionally regulated both in diploids and in triploids possessing from one to three X chromosomes (Mukherjee and Beerman, 1965; see Lucchesi, 1983 and Belote and Baker, 1983 for a review).

Transcription was examined in two genotypes. The diploid control strain contained a C(2L) and two F(2R) chromosomes. Individuals trisomic for the entire left arm of chromosome two were produced by crossing the former strain to a wild-type (Oregon R) strain carrying standard chromosomes. At the larval-pupal boundary, trisomic-2L individuals are about half as viable as, and have taken 50% longer to develop than, diploids. A transcription autoradiograph of a salivary gland nucleus from a trisomic-2L female is shown in Figure 26. It is apparent that grains are distributed predominantly over

Figure 26. Transcription autoradiogram of salivary gland polytene chromosomes from a trisomic-2L female larvae. Chromosome 2L is the thick chromosome arm at the top of the nucleus.

euchromatic regions of the chromosomes and over the nucleolus. Chromosome 2L is thicker than the other arms, and close examination reveals the presence of three synapsed homologues. The X chromosome in metafemales (3X2A) is also thicker than it is in diploid females. Table 46 shows that the mean number of grains observed over the entire length of the left arm of chromosome two from trisomic-2L individuals is similar, though consistently higher, than those of the diploid controls. By using internal controls, varibility between nuclei arising from differences in label incorporation can be minimized. The region 61A-68C on the left arm of chromosome three, which was diploid in all strains involved, was used as an internal standard. When normalized in this manner, the amount of uridine incorporated over 2L in the trisomic-2L strain was, again, slightly higher than that found in the diploids. This result is consistent with the interpretation that many loci in these autosomal trisomies are compensated at the transcriptional level. Comparisons between trisomic and diploid strains in which transcription on 2L is expressed as a fraction of the total euchromatic transcription have been made and corroborate these findings (column 4, Table 46). In the diploid strain this fraction, 0.174, corresponds closely to the proportion of polytene bands present in 2L (804/5059=.159, Lefevre, 1976). If all genes were being transcribed at equivilant rates in diploids and trisomies, then a trisomic/diploid ratio close to 1.5 would be expected. However, in all three measurements, the ratios of uridine incorporation between trisomies and diploids do not differ statistically, although the amount of incorporation is

Table 46 ^{3}H-uridine incorporation into regions of polytene chromosomes from diploid and trisomic larvae.

Genotype	Mean grains on 2L	Grain Ratios		
		2L/61A-68C	2L/total*	1A-3C/61A-68C
C(2L)lt; F(2R)bw/ F(2R)bw (diploid)	83.9 ± 12.5 (28)	1.85 ± 0.09 (27)	0.174 ± 0.012 (9)	0.300 ± 0.022 (11)
C(2L)lt/+/ F(2R)bw (trisomic-2L)	96.4 ± 8.6 (43)	2.08 ± 0.09 (37)	0.200 ± 0.007 (23)	0.289 ± 0.031 (19)
Ratio	1.16	1.12	1.15	0.963

Mean ± SE.
* total grains over all euchromatic chromosome regions

consistently 12 to 16% higher in the trisomic strain. These observations are consistent with the findings of our previous study (Devlin et al., 1982) and suggest that some loci remain dose-dependent in these aneuploids. Of the five enzyme loci measured, three showed completely compensated levels of gene product, and two were fully dose dependent. This proportion of dose-dependent loci, if representitive of all genes on 2L, would predict a rate of transcription in trisomies of approximately 1.2 times the diploid level. Studies on 3L trisomies have given results consistent with this finding.

The different rates of transcription on 2L can be demonstrated by plotting the number of grains on 2L relative to those on the 61A-68C region of 3L. These data are shown in Figure 27. A polytene chromosome comprised of three synapsed homologues will absorb more beta-particles than a chromosome containing two (see Maroni et al., 1974). We did not attempt to determine a correction factor for autosomal trisomies. However, this differential self-absorption has been estimated at 10% for metafemales relative to diploid females (Lucchesi et al., 1974). The dotted line in Figure 27 shows a slope which is 1.35 times the diploid level (1.5 minus 10%) and represents an approximation of the slope, corrected for self-absorption, expected from a trisomy when no compensation occurs. Clearly, the regression lines of the trisomy and diploid strains have comparable slopes (see Table 47). Thus, the amount of RNA synthesis on 2L is nearly equivilant in the two strains even though the trisomic-2L strain has 50% more 2L template.

Our previous studies on 2L revealed an apparent regional

Figure 27. Relationship between labelling on 2L versus labelling on region 61A-68C of 3L. The linear regression for the diploid strain is represented by the solid line and for the trisomic-2L strain by the dashed line. The dotted line represents an expected value if no compensation were occurring (1.35 x diploid). t2L: trisomy 2L.

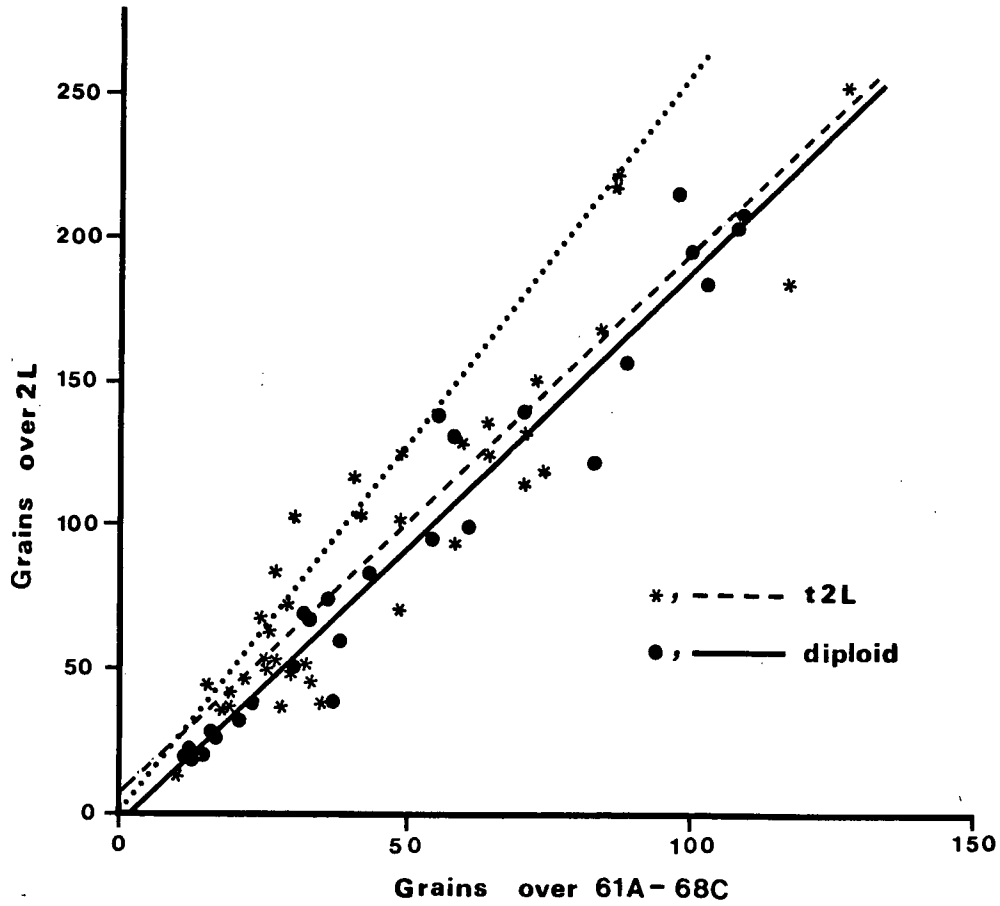

250
200
150
100
50
0
Grains over 2L
0
50
100
150
Grains over 61A-68C
*, ---- t2L
●, —— diploid

Table 47 Regression of grain counts over 2L or regions of 2L on grain counts over 61A-68C.

Genotype	Regression Parameter	Chromosome Region				
		2L	21A-24F	25A-30F	31A-36BC	36BC-40
diploid	b	1.92	0.438	0.521	0.482	0.455
	s	0.06	0.032	0.038	0.035	0.029
	a	-3.2	2.8	-2.9	-2.8	2.2
	r	0.969	0.819	0.818	0.821	0.855
	n	27	21	21	20	20
trisomic -2L	b	1.89	0.431	0.538	0.417	0.403
	s	0.07	0.025	0.025	0.029	0.032
	a	7.3	5.8	4.2	2.2	3.9
	r	0.919	0.839	0.824	0.776	0.749
	n	37	29	29	29	29
	trisomic / diploid	0.980	0.984	1.03	0.865	0.886

b= regression coefficient; s = standard error of b;
a= y intercept; r= correlation coefficient; n= number of nuclei examined.

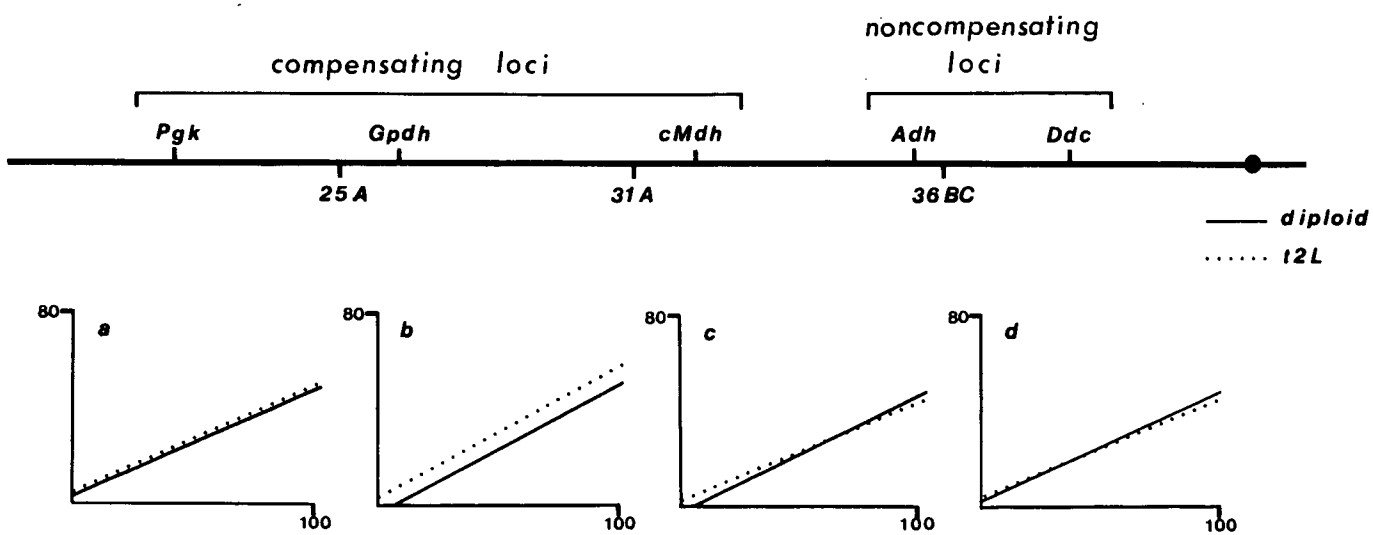
distribution of compensating and non-compensating loci. The three loci that were expressed at a compensated (diploid) level were located in the distal two-thirds of the chromosome arm, while the two loci which produced dose-dependent levels of enzyme were in the proximal portion of 2L. A regional distribution of compensating and noncompensating loci has been observed on the X_2 element in D. miranda (Strobel et al., 1978). Das et al. (1982) have shown that, within this arm, discrete regions of compensating and noncompensating loci exist. Thus it seemed possible that the compensatory response in trisomies was regionally distributed within the chromosome arm. In a similar study, involving trisomies for 3L, we did not observe a regional distribution of compensating and noncompensating loci. Nevertheless, a regulatory system peculiar to the 2L arm could be responsible for a chromosomal level of control. Compensation in autosomal trisomies may have been acting on chromosome regions rather than on individual genes. If large regions of the chromosome differ in their ability to undergo compensation in 2L trisomies, then these differences should be detectable by measurements of transcription over discrete regions. We examined four approximately equal subdivisions of 2L in the transcription autoradiographs. Two of the regions, 21A-24F and 25A-30F, include loci that showed a compensated response in our previous study (Pgk and Gpdh). The region 31A-36B includes one fully compensated gene, cMdh, and one dose dependent gene, Adh. The region 36C-40A includes the dose-dependent locus, Ddc. The amount of ^{3}H-uridine

incorporated into each region (normalized to the internal standard) was measured in the trisomic-2L and diploid strains. Regression analysis of these data are presented in Table 47. The results of this analysis are graphically represented in Figure 28. From the ratios of the slopes of incorporation in the trisomic-2L and diploid strains it is clear that each of these chromosomal regions is compensated at the transcriptional level. Furthermore, no major differences exist between the responses of different chromosomal regions within a strain. These results suggest that, at this level of resolution, a regional distribution of compensating and non-compensating loci does not exist on 2L. This technique averages the transcription of a large number of loci in each region, and, therefore, we suspect that the regionality observed arises as an artefact of the small number of loci sampled on this chromosome.

X-chromosome Transcription in Autosomal Trisomies

A striking correlation exists between the activity of the X chromosome and the number of autosome sets (Lucchesi, 1977). This relationship has been detected in comparisons of gene expression between diploids and triploids where the presence of an extra autosome set in the latter results in a 50% increase in transcription from X-linked templates (Maroni and Plaut, 1973). Furthermore, it appears that the ratio of X chromosomes to sets of autosomes is crucial, since dosage compensation can accommodate variable quantities of X-chromosome material as well

Figure 28. Relationship between labelling within four separate intervals of 2L and labelling within region 61A-68C of 3L. The solid line represents the regression for the diploid strain and the dotted line regression for the trisomic strain. For all four graphs, grain number over region 61A-68C is represented on the X axis; the Y axis depicts grain numbers over region 21A-24F in a), 25A-30F in b), 31A-36BC in c) and 36BC-40 in d). Shown above the graphs are the locations of the five enzyme loci measured in a previous study (Devlin et al., 1982). t2L= trisomy 2L.


compensating loci
noncompensating loci
Pgk
Gpdh
cMdh
Adh
Ddc
25A
31A
36BC
diploid
12L
80
a
100
80
b
100
80
c
100
80
d
100

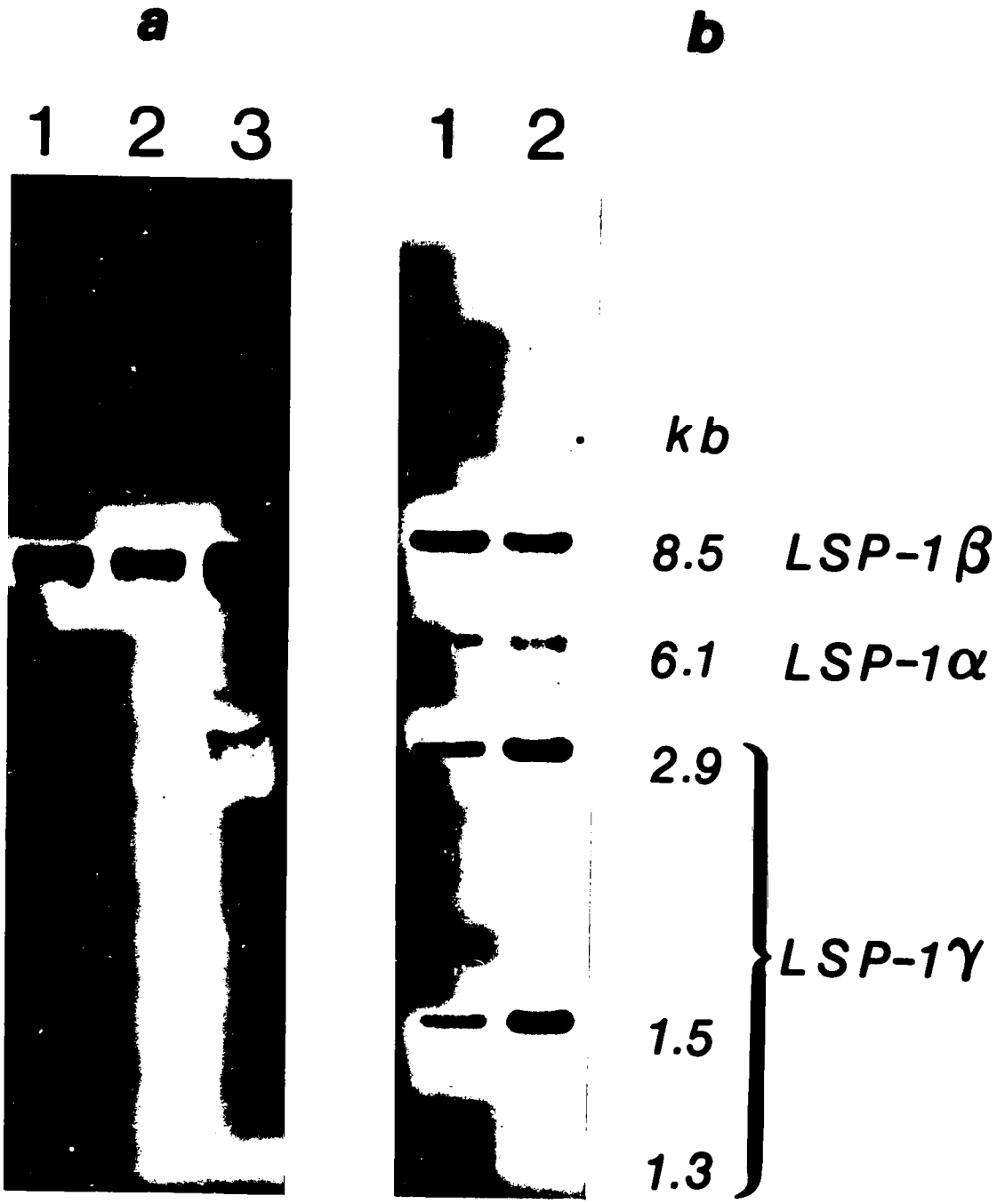
(Maroni and Lucchesi, 1980; Williamson and Bentley, 1983). To examine whether partial increases in an autosome set would alter the X/A ratio sufficiently to affect dosage compensation on the X, we compared X-linked transcription in the trisomic-2L and diploid strains. Transcription was measured by the incorporation of uridine into region 1A-3C at the tip of the X chromosome. The entries in column 5 of Table 46 express this incorporation relative to the control region. If all autosomal regions are equally capable of activating X-linked transcription, then a 10% increase would be expected in trisomic-2L individuals (in accordance with the increase in autosomal material, 8898/8094 bands). Alternatively, if autosomal loci responsible for activating X-chromosome transcription are not randomly distributed, then a much larger increase may be expected. However, we detected no increase in X-linked transcription in this experiment.

Individual Genes

The autoradiographic experiments support the concept that compensation in trisomic-2L individuals occurs at the transcriptional level. Clearly, however, individual genes could be transcribed at various rates, either under or overcompensated, with the average over large regions of the chromosome appearing as compensation. Previous studies with enzyme loci have revealed that individual genes behave discretely (Devlin et al., 1982). The reliability of inferring

gene expression by measuring the levels of terminal protein products is subject to complications arising from post-transcriptional processes. To examine further the response of individual loci in trisomies, measurements have been made for quantities of mRNA produced from two genes for which data on protein levels are available. One gene, LSP-1b (2-1.9, Roberts and Evans-Roberts, 1979a), is known to produce compensated levels of protein in trisomic-2L hemolymph samples (see chapter 3) and the other, Adh(2-50.1), displays dose dependent levels of activity. RNA extracted from wandering third instar larvae fat body was bound to nitrocellulose filters and sequentially probed with cloned DNA complimentary to LSP-1b, LSP-1g and Adh mRNAs. The LSP-1g gene, located on chromosome 3 (Roberts and Evans-Roberts, 1979a), was used as an internal control in the present investigation. A similar method has been used previously to demonstrate the lack of dosage compensation for LSP-1a RNA levels between males and females of several Drosophila species (Brock and Roberts, 1982). The LSP-1a structural gene has approximately 90% sequence homology with LSP-1b as well as LSP-1g (Smith et al., 1981) and considerable cross hybridization is expected. Figure 29b shows a Southern blot of Hind III digested genomic DNA from larvae of trisomic-2L and trisomic-3L strains that was probed simultaneously with LSP-1b and LSP-1g sequences. The major bands corresponding to these genes (Smith et al., 1981) are labelled, and when the two strains are compared a distinct difference in the band ratios is observed. Since much

Figure 29. Quantites of LSP genes and mRNA in diploids and trisomies. a) Northern blot of total RNA from 1) diploid compound free, 2) 2L trisomy and 3) Oregon R probed with LSP-1b sequences. b) Southern blot of Hind III digested genomic DNA from 1) trisomic-2L larvae and 2) trisomic-3L larvae probed simultaneously with LSP-1b and LSP-1g sequences.


a
b
1 2 3
1 2
kb
8.5
LSP-1β
6.1
LSP-1α
2.9
LSP-1γ
1.5
1.3

of the larval tissue is polytenized, this result suggests that under-replication of specific gene sequences is not occuring in the trisomic arms. This interpretation could only be inferred from the thicker chromosome arm observed in polytene chromosome preparations. In addition to the LSP-1b and LSP-1g restriction fragments, a 6.1 kb band is observed corresponding to cross hybridization of both probes to LSP-1a sequences. The degree of cross hybridization is not sufficient to affect our interpretation of the results given in Table 48, where the levels of LSP-1b RNA are expressed relative to LSP-1g. The level of LSP-1b RNA appears to be equivalent in diploid and in trisomic-2L individuals. How the modulation of LSP-1b RNA levels in trisomies is achieved is unknown. Superficially, the compensation of LSP-1b appears discordant with the observation that the X-linked gene, LSP-1a, does not dosage compensate between normal males and females (Roberts and Evans-Roberts, 1979b; Brock and Roberts, 1982). This result implies that the regulatory processes that allow compensation in trisomies may differ from those that operate to allow compensation between males and females.

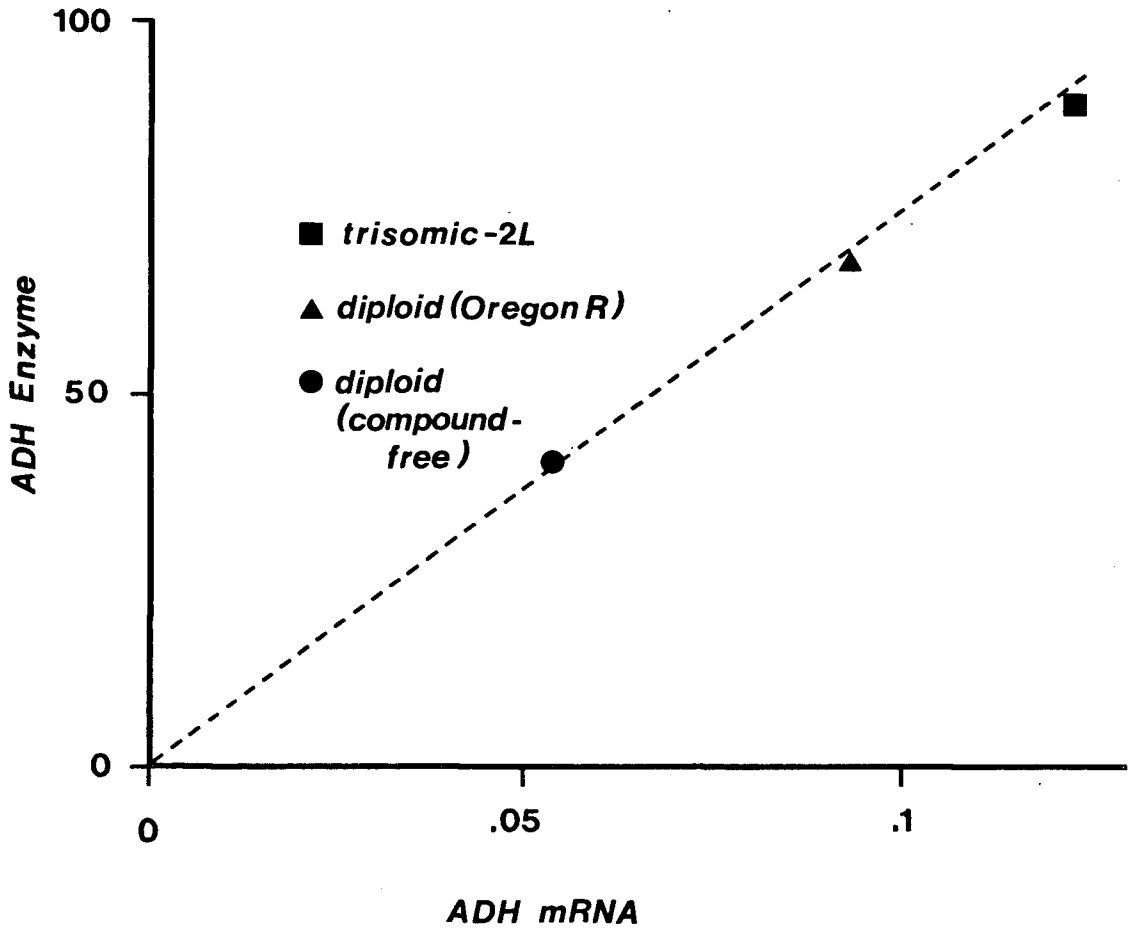
The levels of Adh mRNA seen in the two diploid strains differ considerably (Table 48). However, there is a good correlation between the amount of ADH mRNA and the level of enzyme activity in each strain (Figure 30). The reason for differences in Adh expression between these strains is unknown, but an extensive analysis of linked and unlinked modifiers of ADH enzyme levels has been reported recently

Table 48 Amount of LSP-1b and ADH mRNAs relative to LSP-1g.

Genotype	Ratio* LSP-1b/LSP-1g	Ratio* ADH/LSP-1g	LSP-1g cpm/filter
Oregon R (diploid)	1.90 ± 0.03 (3)	0.137 ± 0.013 (3)	2167 ± 197 (3)
C(2L)lt; F(2R)bw/ F(2R)bw (diploid)	1.85 ± 0.19 (3)	0.079 ± 0.021 (3)	1377 ± 152 (3)
C(2L)lt/+/ F(2R)bw (trisomic-2L)	1.78 ± 0.03 (3)	0.181 ± 0.050 (3)	599 ± 96 (3)
Noncomp. Estimate	2.81 ± .09	0.149 ± 011	
Comp. Estimate	1.87 ± 06	0.099 ± 007	

comp.= compensated; noncomp.= noncompensated. Mean ± SE
* corrected for different probe specific activities

Figure 30. Relationship between ADH enzyme activity and ADH mRNA in three strains. ADH enzyme activities are from Table 3; ADH RNA levels are from this chapter and are measured relative to LSP-1g RNA.


100
50
0
0
.05
.1
ADH Enzyme
ADH mRNA
trisomic-2L
diploid (Oregon R)
diploid (compound-free)

(Maroni and Laurie-Ahlberg, 1983). In trisomic-2L larvae, ADH enzyme activity was 19% higher than the expected dose-dependent value (Devlin et al., 1982). It is clear that the ADH mRNA levels show a similar relationship: trisomic-2L values are 21% higher than expected. The present data strongly support the results obtained with protein measurements. More importantly, these data suggest that autosomal dosage compensation acts independently for each gene at the level of transcription.

Of some concern is the difference in hybridizable counts observed between the three strains (Table 48). LSP-1g RNA is about 3.6 times less prevalent (relative to total RNA) in trisomic-2L than in Oregon R individuals, and the comppound 2L, free 2R diploid strain is intermediate. To confirm this result, three new RNA extracts for each genotype were run on Northern blots and probed with LSP-1b DNA. It is clear from Figure 29 that this difference in RNA levels is reproducible. Whether this difference arises from a biological phenomenon or a technical problem (ie. differential extractability of RNA in trisomies) is not known. It is noteworthy that the lowest RNA level is associated with the strain displaying the greatest delay in development. If mRNA quantities were the same in diploids and trisomies and translation occurred at the same rate, then the extended development time observed in trisomies could result in elevated levels of proteins in these individuals. Thus, it may be that reduced quantities of mRNA in trisomies may be a developmental strategy arising as a secondary consequence of slow growth.

Discussion

In Drosophila, when an autosomal gene is duplicated by a small segmental aneuploid, an increase in gene product is usually observed. In contrast to small duplications, the levels of some gene products in autosomal whole-arm trisomies are not dose-dependent (Devlin et al., 1982). These individuals show developmental delay and reduction in viability similar to that observed for metafemales. Levels of enzymes that accumulate under such conditions might appear compensated for various reasons (i.e. alterations in developmental cues, post-transcriptional modulations etc.). The results in this chapter reveal that most of the modulation of gene expression occurs at the level of transcription. Autoradiographic measurements suggest that in the trisomic-2L strain, approximately two-thirds of the loci on 2L are transcribed at one-third less than the diploid rate. The amounts of mRNA produced from two different genes located on 2L were consistent with the levels of their terminal protein products. LSP-1b produced compensated levels of mRNA, while Adh mRNA levels were dose-dependent. For Adh, where considerable differences in the levels of gene product exist between genotypes, a good correlation existed between the amount of mRNA and the amount of enzyme produced in each strain. Although this study did not attempt to measure the specific transcription rate of any one gene, the results of the autoradiographic experiments, together with the LSP-1b RNA determinations, suggest that compensation for differences in

gene dosage in autosomal trisomies can occur at the transcriptional level.

In trisomies for the X chromosome, measurement of X-linked enzyme activities has demonstrated the occurance of dosage compensation at the protein level (Lucchesi et at., 1974; Stewart and Merriam, 1975; Gvozdev et al.,1983; see Faizullin and Gvozdev, 1973 for an exception). However, measurements using autoradiography to determine if compensation in this genotype is occurring at the transcriptional level have produced conflicting results. Compensated levels of transcription have been observed in trisomic-X females (Lucchesi et al., 1974), suggesting that X-chromosome dosage compensation operates at X/A ratios above 1.0. Such a result implies that the regulatory system(s) which mediate dosage compensation for sex-linked genes possess considerable flexibility in their modulatory capabilities. Alternatively, a dose-dependent level of transcription products in metafemales (as observed by Ananiev et al., 1974 and Gvozdev et al., 1983) suggests that X-chromosome transcription rate is set at either the male or the female level, and that compensation at the enzyme level is brought about by post-transcriptional controls. That dosage compensation is determined at the male or female level is a possibility dealt with at length in the reviews by Baker and Belote (1983) and Lucchesi (1983). Dose-dependent levels of transcription in metafemales would suggest that regulatory controls similar to those mediating transcriptional compensation in autosomal trisomies do not exist for the X chromosome.

Measurement of transcription in larvae with varying degrees

of segmental aneuploidy for the X chromosome (Maroni and Lucchesi, 1980) have supported the hypothesis that X-chromosome dosage compensation is mediated by the competition of X-linked templates for a limiting substance that functions as an activator of X-linked transcription (Maroni and Plaut, 1973; Schwartz, 1973). Such a regulatory system would imply that the X-chromosome transcription rate should be fully adjustable over a range of X/A ratios. If this system operates in males and females, it most likely operates in metafemales. Experiments capable of detecting autosomal regulatory gene(s) involved in the production of limiting quantities of a transcription factor have been unsuccessful (see Rawls and Lucchesi, 1974). It is possible that the regulatory factors are produced by many genes distributed throughout the genome. If this were the case, a significant proportion of the genome may have to be duplicated before an effect could be detected. While autosomal trisomies alter the X/A ratio by about 10%, the measurements in this study detected no increase in X-chromosome transcription in trisomic-2L females relative to the diploid control. Indeed, X-linked enzyme loci also appear not to be activated in trisomic-2L or trisomic-3L individuals (see chapter 3). It is equally possible that regulation is carried out by one or a few genes. If this is the case, then such genes do not appear to be located on 2L. The resolution of this problem requires a more complete analysis of flies possessing large and small autosomal duplications.

The cytological manifestation of dosage compensation has been well documented. While the X-chromosomes in females are of

similar staining intensity and thickness as autosomes, the single X in males is more diffuse and appears somewhat "puffed" (Dobzhansky, 1957). This puffed condition is correlated with an increased level of transcription by E. coli RNA polymerase in vitro (Khesin and Leibovitch, 1974; Chatterjee and Mukherjee, 1981) and suggests that an altered chromatin packing may exist. In trisomic-X individuals the polytene X chromosome is 35-40% wider than in diploid females. Nevertheless, both produce the same amount of X-linked gene product per cell. Clearly, the structure of the X chromosome in metafemales does not reflect the reduced level of transcription per homologue (see next chapter for further discussion). Trisomic-2L individuals have a proportionally thickened 2L chromosome yet, many of the genes on this arm are transcriptionally repressed. If dosage compensation regulates gene expression in trisomies (for the X or for the autosomes) by altering chromatin packing, then, at least for downward regulation, this phenomenon is not observable at the cytological level. It is possible that chromosome compaction greater than that observed in diploid females is unsuitable for correct functioning of the transcription apparatus.

Alternatively, the mechanism of dosage compensation in trisomies may arise from a combination of regulatory controls not responsible for X-chromosome regulation in males and females. Such control systems may operate independently of the state of chromosome compaction. In view of the similarities in development and gene expression displayed by X-chromosome and autosomal trisomies, dosage compensation on the X chromosome and

on the autosomes may have a common regulatory foundation. Dosage compensation in whole-arm trisomies may simply reflect, or have arisen from, systems involved in maintaining transcriptional homeostasis. It is also possible that X-chromosome dosage compensation in males and females reflects a more highly evolved form of such controls. Muller et al. (1931) originally proposed that dosage compensation may be controlled by X-linked regulatory genes which act in a dose dependent manner to repress the expression of other X-linked genes. While the existence of these modifiers has not been established by experimental means, reports of some chromosomal regions capable of altering the expression of specific genes have been made (see Muller, 1950; Birchler,1980). Chromosomal regions capable of modifying the expression of autosomal genes in Drosophila have been described previously (O'Brien and Gethman, 1973; Rawls and Lucchesi, 1974; Williamson and Bentley, 1983). As discussed in previous chapters, compensation in autosomal trisomies could be regulated by loci, themselves present within the duplication, which are capable of repressing the transcription of other genes. The results of Rawls and Lucchesi (1974), (but not O'Brien and Gethman, 1973) suggest that a duplication of chromosome region 21A-25D, on chromosome 2L, is capable of repressing the activity of GPDH by almost one-third. However, no single subdivision of this region appeared completely responsible for the change, but rather all regions had a small effect. This result implies that several loci, or perhaps specific chromosome regions, exert an effect on the expression of Gpdh. The effects are not additive, however, and a

maximum reduction of one-third is observed.

The mechanism behind transcriptional compensation in trisomies is unknown. Individual genes behave discretely suggesting that regulation operates independently at each locus. The previous chapters have revealed that, in trisomies, all three copies of a given gene are transcriptionally active, and that the expression of a structural gene which is compensated in trisomies is unaffected by the number of other copies present (Devlin et al., 1982). Such a response eliminates regulatory systems dependent on feedback control of product levels, or on competition of the three genes of a particular locus for a factor required exclusively for their transciption. The regionality of the response observed for enzyme loci along the length of 2L was not supported by the present study. However, if compensation in hyperploids operates over small chromosome regions in a fashion similar to that observed for dosage compensation on the X-chromosome in males (Spradling and Rubin, 1983; Roehrdanz and Lucchesi, 1977), then such an effect would escape detection in the present study.

CHAPTER SIX

REGULATION OF DOSAGE COMPENSATION IN METAFEMALES

Introduction

Dosage compensation for differences in sex chromosome number occurs in Drosophila by transcriptional modulation (Mukherjee and Beerman, 1965). Varying the amount of X-chromosome material with a constant autosomal background does not affect the total amount of transcription from this chromosome (Maroni and Lucchesi, 1980). Consequently, X-linked genes appear to "compete" for a factor produced by the autosomes. Males, with one-half the number of X-linked genes as females, transcribe their X chomosomes at twice the rate, and triploid intersexes (2X:3A), with an intermediate X/A ratio, express their X chromosomes on average at intermediate levels (Maroni and Plaut, 1973). That transcription of X-linked genes is inversely correlated with the X to autosome ratio in a variety of aneuploids implies that dosage compensation is a single, continuously adjustable regulatory system. However, recent genetic studies suggest that the mechanism for dosage compensation may be bifunctional: hyperactivation in males and prevention of activation in females (Cline, 1978; Belote and Lucchesi, 1980; Skripskey & Lucchesi, 1982).

Individuals trisomic for the X chromosome (metafemales) produce approximately the same amount of X-linked gene products as euploid males and females and thus also demonstrate the capacity to dosage compensate. Simplistically, this reduction in the amount of product formed from the X-linked templates could be accomplished by one of two mechanisms. Compensation in metafemales (3X:2A) may reflect an extension of the regulatory

mechanisms that are responsible for dosage compensation between males and females. Alternatively, male-female dosage compensation may only be capable of setting the transcription of X-linked genes at either the male or female level. Individuals trisomic for the X chromosome (X/A ratio = 1.5) would be set at the female mode of expression and compensation in these hyperploids must then arise as a consequence of additional regulatory controls.

Several studies have revealed that compensated (diploid) levels of X-linked enzymes are produced in trisomic-X strains. However, dosage compensation in whole-arm trisomies is not limited to the X chromosome. Many of the loci duplicated in strains trisomic either for 2L or for 3L make the same amount of product as found in euploids. Dosage compensation for autosomal genes in trisomies can be explained by the presence of trans-acting regulators encoded within the duplicated arm (Rawls and Lucchesi, 1974a). Such a theory was originally proposed by Muller et al. (1931) to account for dosage compensation on the X chromosome. The parallelisms between compensation in autosomal and X-chromosomal trisomies raises the question of whether the two phenomenon could be controlled by similar mechanisms. In this chapter, the activity of several X-linked genes in euploid males and females, and in metafemales were examined. Their expression is consistent with the hypothesis that compensation in trisomic-X strains can occur by mechanisms independent of, and in addition to, male-female dosage compensation.

Materials and Methods

Genetic stocks and crosses

In general, metafemales were produced by crossing an attached-X strain marked with yellow (y) to a standard male strain and they were selected on the basis of gonad size (Kerkis, 1931) and colour of mouthparts (y^+).
The wild-type strains were Oregon R and Canton S; the attached-X strains used were C(1)RM, y pn v/O, C(1)RM, y w/O and C(1)RM, YS, In (1)EN, y/O. Genetic markers are described in Lindsley and Grell (1968). In the experiments involving Adh (Table 49), metafemales were generated by crossing C(1)RM y pn v/Y;b Adh^{N2} pr cn females to males possessing an Adh gene on their X chromosome and null alleles on the second chromosome. The male strains used in these crosses were tAP-1/Y;b Adh^{fn23} pr cn and tAP-17/Y;b Adh^{fn4} pr cn. tAP-1 is an insertion of the Adh (11.8kb) structural gene region into 19E of the X chromosome (Goldberg et al., 1983). tAP-17 is a 4.8kb insertion of Adh into 12A of the X chromosome (J. Posakony, personal communication).

The males and females listed in experiment I of Table 50 were derived from Oregon R stock cultures. The genotypes listed for experiment I in Table 51 and for the experiment in Table 52 were produced by mating Oregon R males with C(1)RM, y pn v/O females. The genotypes shown for experiment III in

Table 51 were derived from crosses between Canton S males and C(1)RM, YS. In(1)EN, y/O females. The genotypes listed for experiment IV in Table 51 represent progeny derived from crosses between Oregon R males and C(1)RM, y w/O females. The genotypes listed in each set of experiments were siblings; thus, they have the same autosomal background.

A duplication of LSP-1a was constructed from T(1;3)v^{+74c}. This rearrangement has the 9E3-4 to 11B12 region of the X chromosome inserted into the heterochromatin of the third chromosome. This translocation was balanced over In(3LR)TM6B,Tb (provided by Dr. L. Craymer) and the deletion bearing X chromosome replaced with a wild-type X. When this stock (Dp(1;3)v^{+74c}/TM6B,Tb) is crossed to C(1)RM y pn v/O females, female larvae can be recovered which are either diploid (phenotypically Tubby, Tb) or are duplicated for LSP1-a (wild-type). The response of the LSP-1a duplication to a supressor of variegation was examined. Males from a stock bearing low producing alleles of LSP-1a and the LSP1-a duplication balanced over TM6B,Tb were crossed to females possessing an attached-X chromosome and Su(Var)A154 balanced over TM6B,Tb (y cv v LSP-1a^{L-82}/Y; Dp(1;3)v^{+74c}/TM6B,Tb males x C(1)DX,yf/Y;Su(Var)A154/TM6B,Tb) females. Wild-type (non Tubby) male larvae were selected for analysis.

Enzyme Assays:

Enzyme assays were performed on extracts of wandering third instar larvae which were homogenized at 25mg live weight per ml of 10mM $NaPO_4$, 1mM phenylthiourea, pH 7.5. For malic enzyme (ME; EC. No. 1.1.1.40) the assay conditions were: 0.1M Tris, pH 7.5, 1mM $MnCl_2$, 2mM NADP, 2mM Na malate. Alcohol dehydrogenase (ADH:E.C. No. 1.1.1.1) assay conditions were: 50mM Na_2CO_3, pH 9.0, 2mM NAD, 0.275M butan-2-ol. Activity has been expressed as the mean $\pm$ standard error.

Larval Serum Protein Analysis

Larval serum proteins (LSPs) from late third instar heamolymph samples were separated by electrophoresis on Cellogel membranes (Kalex Scientific), stained with Coomasie Blue R-250, eluted in glacial acetic acid and quantified spectrophotometrically at 595nm (modified from the method of Roberts and Evans-Roberts, 1979a). Amounts of LSP-1a are expressed as a fraction of LSP-1b or LSP-2.

Quantitation of LSP-1 RNA

LSP RNA was measured in males, females and metafemales by

filter hybridization (Thomas, 1980) using the methods described in chapter five. Total RNA was bound to nitrocellulose and probed sequentially with the following nick translated DNAs (Rigby et al., 1977): pR*LSP1a:1, a plasmid containing the entire coding sequence for LSP-1a (Smith et al., 1981) and pLSP1b:B2.1, a plasmid containing the majority of the LSP-1b gene (Devlin et al., 1984).

Analysis of LSP-1 RNA on Northern blots was performed by procedures described in chapter five. RNA for the measurements was extracted either from Oregon R (wild-type) or from LSP-1a^{L-B2} (low producing variant). Filters were first hybridized to ^{32}P-labelled LSP-1a DNA and autoradiograms were produced. This probe was removed, and the filters were hybridized with ^{32}P-labelled LSP-1b DNA and re-exposed to X-ray film. Comparison of these two autoradiograms provides an estimate of the quantity of LSP-1a RNA relative to LSP-1b RNA.

The genomic restriction pattern of the LSP-1a^{L-B2} allele was examined following the prodecures described in chapter five. Southern blots were probed with ^{32}P-labelled plasmid pLSP-1aB2.9, a 2.9kb subclone with a portion of the 3' coding and flanking sequences of LSP-1a inserted into plasmic pUC12.

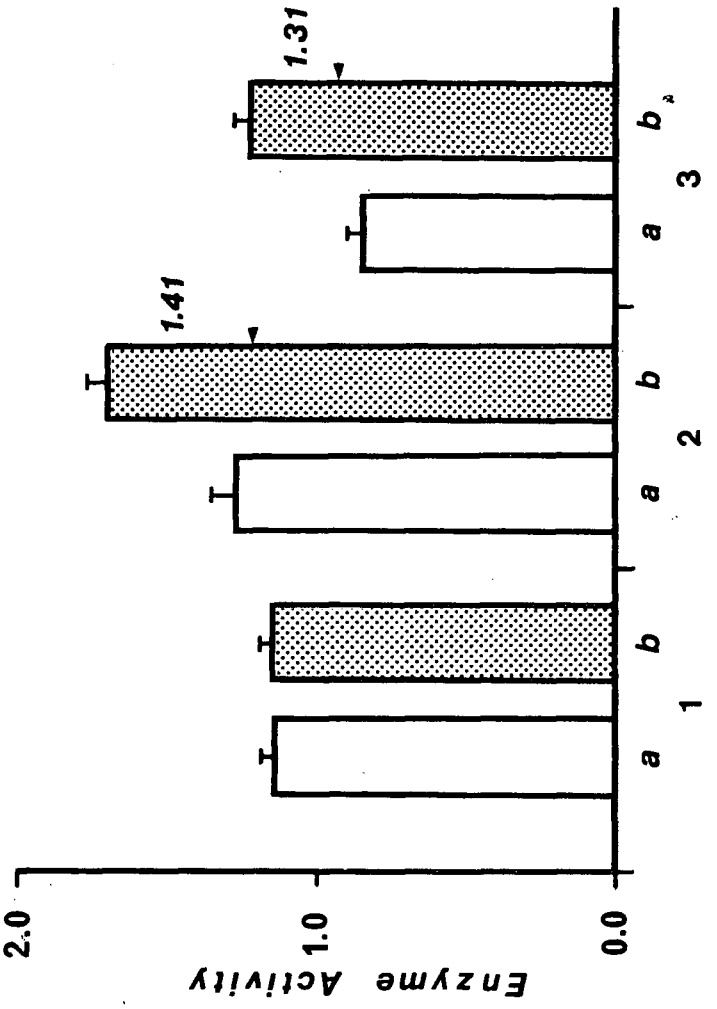
Results

The hypothesis that compensation in trisomic-X strains occurs by a mechanism separate from male-female dosage compensation makes two predictions. First, the expression of some X-linked genes, while compensated between males and females, could be dose dependent in metafemales. Second, genes failing to dosage compensate in euploid males and females, might compensate in metafemales.

Some genes are dose dependent in trisomic-X strains.

Although the structural gene for malic enzyme is autosomal (Men, 3-51.7, Voelker et al., 1981), Williamson and Bentley (1983) have described an X-chromosome region (8D10-12 to 9A1-2) that, when dulicated, increases malic enzyme activity by approximately 40%. This region apparently contains a regulatory gene that activates the Men locus. Malic enzyme activity was examined in males, females and two different trisomic-X strains. No difference in malic enzyme activity was observed between males and females, and therefore this locus appears to be dosage compensated (Figure 31). In contrast, the amount of ME activity in both trisomic-X strains was 30-40% higher than their euploid male and female counterparts. The similar effects obtained for a duplication of the region and for a whole arm trisomy suggests that the expression of the Men regulatory locus is not compensated in metafemales. Each template appears

Figure 31. Quantity of Malic enzyme in diploid males and females and in metafemales. 1. a) males and b) females from Oregon R strain; 2. a) females and b) metafemales (3X2A) from the cross Oregon R male X C(1)RM, y w/O female; 3. a) females and b) metafemales from the cross Oregon R male X C(1)RM, y pn v/O. The arrows (<) indicate the exptected level of activity if complete dosage compensation were occuring (calculated from the expected contribution of activity from the paternal X chromosome). The numbers to the right of bars 2b and 3b represent the respective ratios of enzyme activity observed to that expected if dosage compensation were complete. Enzyme activity units are the change in absorbance at 340nm/min/ml of extract. Standard error bars are shown. $n \geq 5$ extracts for all genotypes.


Enzyme Activity
2.0
1.0
0.0
1.31
1.41
a
b
a
b
a
b
1
2
3

to be expressed at the same rate as in diploid females.

To investigate this question further, we have used several lines of D. melanogaster in which the Adh structural gene is inserted into the X chromosome by transformation (Goldberg et al., 1983). The structural gene for Adh in D. melanogaster is normally located at band 35B2-3 on the left arm of chromosome two (Woodruff and Ashburner, 1979). Previous experiments have shown that: 1) the Adh gene does not dosage compensate in trisomic-2L individuals, and 2) trisomy for the X chromosome does not affect Adh gene expression when it is in its normal chromosomal position. We measured the amount of ADH produced from these transposed genes in euploid males and females and in trisomic-X females. All genotypes used in these experiments contained null alleles for the native Adh genes on the second chromosome. Therefore, the ADH activity observed represents expression from the X-linked Adh genes only. The results are shown in Table 49. In the last column, the activity per Adh gene is expressed relative to the activity found in males. The X-linked Adh gene in strain tAP-17 is able to dosage compensate partially in euploid males while strain tAP-1 does not compensate. For both transposed genes examined, the ADH activity per gene was the same in metafemales and diploid females. Thus, while the Adh gene in the tAP-17 strain acquired the ability to dosage compensate for differences in template number between males and females, it escapes compensation in trisomic-X individuals. This is not expected if the mechanism regulating dosage compensation in normal males and

Table 49 X-linked Adh gene activity in males, females, and metafemales.

Exp.	Genotype	Sex	ADH Activity1		ADH Activity per gene2
	tAP-1/Y	male	1.41 ± .06	(6)	1.00
I	tAP-1/tAP-1	female	2.49 ± .14	(6)	0.88
	tAP-1/Y	male	1.41 ± .06	(6)	1.00
	tAP-1/ C(1)RM,y pn v	meta-female	1.34 ± .08	(6)	0.95
	C(1)RM,y pn v/Y	female3	0.01	(2)	
II	tAP-17/Y	male	0.367 ± .011	(14)	1.00
	tAP-17/tAP-17	female	0.518 ± .016	(14)	0.71
	tAP-17/Y	male	0.500 ± .018	(8)	1.00
	tAP-17/ C(1)RM,y pn v	meta-female	0.373 ± .021	(9)	0.75
	C(1)RM,y pn v/Y	female3	0.008	(2)	

1. ADH activity expressed as the change in absorbance at 340nm per min per mg live weight. Numbers in parentheses are the number of extracts.

2. ADH activity is expressed per gene copy relative to the activity found in males. If complete dosage compensation occurred then the ADH activity per gene in females should be 0.5 that of males; if no compensation occurred then it should be 1.0

3. The absence of ADH activity in this group of females demonstrates that the Adh-P element construct has not transposed to the autosomes during the course of these experiments.

females also functions to reduce transcription in metafemales.

The two genes discussed above escape dosage compensation in metafemales. Their expression appears to be set at the female level. From these results we can conclude that compensation in metafemales is not an obligatory condition for all genes on the X chromosome. In contrast to the two genes discussed above, the majority of loci previously examined in metafemales show dosage-compensated levels of expression (Lucchesi et al., 1974; Stewart and Merriam, 1975; Williamson and Bentley, 1983; Gvozdev et al., 1983). This apparent contradiction is resolved if one assumes that loci which are compensated in metafemales are set at the euploid female level of expression, under the control of male-female dosage compensation, and that additional regulatory controls operate to repress their expression further. In this model, the ME activator and Adh loci escape repression by the second regulatory mechanism in metafemales and thus produce dose-dependent levels of product. If a second regulatory mechanism acts to repress X-linked gene expression in metafemales, then one might expect that some loci, which are not compensated in euploid males (activation), could be compensated in metafemales (downward regulation).

Compensation in metafemales can occur independent of male-female dosage compensation.

We have examined the expression of LSP-1a, a gene that normally is not dosage compensated between males and

females (Roberts and Evans-Roberts, 1979a). If male-female dosage compensation operates to reduce transcription in metafemales, then this gene should escape regulation, as it does in males, and produce a larger amount of LSP-1a than that found in diploid females. Alternatively, if compensation in trisomic strains operates by a mechanism independent of the male-female dosage compensation mechanism, then the amount of transcription from the LSP-1a gene could be compensated in trisomic-X strains. The amount of LSP-1a produced in strains trisomic for the X chromosome was compared with the amount found in diploid males and females. The amount of LSP-1b and LSP-2 were used as internal standards since their structural genes are located on the second and third chromosomes respectively, and remain diploid in all strains examined. Experiments I & II (Table 50) confirm the previous observation that the LSP-1a gene escapes normal dosage compensation in males. However, the results in Experiments I to III in Table 51 show that approximately equal amounts of LSP-1a are produced in metafemales and females (see columns a/b and a/2). Therefore, while the LSP-1a gene does not fully dosage compensate between euploid males and female, it is fully compensated in metafemales. The data suggest that regulatory controls other than those responsible for male-female dosage compensation are operating to repress the expression of LSP-1a in metafemales.

Translational control affects LSP synthesis during normal larval development (Powell et al., 1984) and could be responsible for LSP-1a compensation in metafemales (which

Table 50 Relative amounts of LSPs in euploid strains.

Exp.	Genotype	Sex	LSP-1a/ LSP-1b	LSP-1a/ LSP-2
I	+/Y (Oregon R)	male	0.446 ± .034 (10)	0.429 ± .032 (10)
	+/+ (Oregon R)	female	0.743 ± .070 (11)	0.630 ± .052 (11)
	male/female ratio		0.60	0.68
II	iso-x	male	0.578 ± .033 (6)	0.508 ± .015 (6)
	iso-x	female	0.749 ± .038 (6)	0.794 ± .045 (6)
	male/female ratio		0.77	0.64

Quantities of LSPs are expressed as the mean (± standard error). Numbers in parenthesis are the sample sizes.

Table 51 Relative amounts of LSPs in aneuploid strains.

Exp.	Genotype	Sex	LSP-1a / LSP-1b	LSP-1a / LSP-2
I	+/O (Oregon R)	male	0.482 ± .016 (8)	0.521 ± .034 (8)
	C(1)RM, y pn v/Y	female	0.827 ± .042 (8)	0.647 ± .024 (8)
	C(1)RM, y pn v/+	3X2A	0.861 ± .106 (7)	0.770 ± .099 (7)
	Compensated Estimate		.873 ± .022	.779 ± ..021
II	+/O (Canton S)	male	0.405 ± .017 (12)	0.537 ± .025 (12)
	C(1)RM.YS, In(1)EN, y/Y	female	0.373 ± .017 (10)	0.679 ± .046 (12)
	C(1)RM.YS, In(1)EN, y/+	3X2A	0.489 ± .023 (12)	0.852 ± .059 (12)
	Compensated Estimate		0.519 ± 019	0.811 ± .026
III	+/O (Oregon R)	male	0.467 ± .025 (8)	0.441 ± .030 (8)
	C(1)RM, y w/Y	female	0.757 ± .027 (8)	0.402 ± .038 (8)
	C(1)RM, y, w/+	3X2A	0.728 ± .064 (8)	0.394 ± .043 (8)
	Compensated Estimate		0.816 ± 018	0.562 ± .024
IV	Dp(1;3)LSP-1a/ +;C(1)RM, y pn v/Y	female	0.995 ± .063 (3)	1.13 ± 0.11 (3)
	TM6B,Tb/+; C(1)RM, y pn v/Y	female	0.824 ± .019 (3)	0.831 ± .049 (3)
	Ratio		1.21	1.36

Quantities of LSPs are expressed as the mean (± standard error). Numbers in parenthesis are the sample sizes.

display an altered developmental rate). To determine the level at which LSP-1a compensation occurs in metafemales we have measured amounts of mRNA from this gene relative to LSP-1b mRNA (Table 52). Again, the amount of LSP-1a mRNA in males is approximately two-thirds that found in females, while metafemales produced a fully compensated level. This data suggest that compensation of LSP-1a in metafemales is controlled prior to translation.

The incomplete dosage compensation of LSP-1a in males may arise because this gene normally is very rapidly transcribed. The regulatory sequences capable of eliciting hyperaction to most X-linked genes may not be capable of activating the transcription of LSP-1a further, ie. the transcription unit is saturated. This may be analagous to the situation described in chapter three where Adh expression could only be activated in 2L trisomies if the gene was partially repressed by residing in a foreign chromosomal position. A new free-arm stock for the third chromosome, induced by gamma radiation, was fortuitously included in experiments in which the expression of LSP-1a was examined. This stock appeared to have reduced levels of LSP-1a, and upon subsequent testing, an X chromosome was isolated which possessed an allele of LSP-1a that produced significantly less product than wild-type (Figure 32a). This mutation was called LSP-1a^{L82}. Measurements of total protein suggest that this allele produces approximately ten percent of the diploid amount of LSP-1a (compare Experiment I, Table 53 to Experiment I & II, Table 50). This mutation was

Table 52 Filter hybridization of LSP-1 RNA.

Genotype	Sex	XLSP-1a cpm	LSP-1a/LSP-1b
+/O (Oregon R)	male	3726	1.83 ± .12 (3)
C(1)RM,y pn v/Y	female	3112	2.67 ± .35 (4)
C(1)RM,y pn v/+	3X2A	2319	3.06 ± .02 (4)
Compensated Estimate			3.00 ± .014

Values represent the mean ± standard error. Samples sizes are in parentheses.

Figure 32. Analysis of a mutation that reduces the amount of LSP-1a protein and RNA.
A. LSP's separated on Cellogel membranes. Left, Oregon R; right, LSP-1^{L82}. B. Northern blot analysis of RNA from Oregon R (lanes 1 and 3) and from LSP-1^{L82} (lanes 2 and 4). The left autoradiogram is from a filter hybridized to LSP-1a DNA. The right autoradiogram is from the same filter which had been washed free of the LSP-1a probe, and then rehybridized with LSP-1b DNA. The amount of LSP-1a RNA (relative to LSP-1b RNA) is much lower in the LSP-1^{L82} strain than in the wild-type Oregon R strain. C. Restriction enzyme digestion of DNA isolated from Oregon R (lanes 1, 4 and 7), from LSP-1^{L82} (lanes 2, 5 and 8), and from Canton S (lanes 3 and 9). Lane 6 contained incompletely digested DNA. The enzymes used were EcoRI (lanes 1, 2 and 3), Hind III (lanes 4 and 5), and Bam HI (lanes 7, 8 and 9).

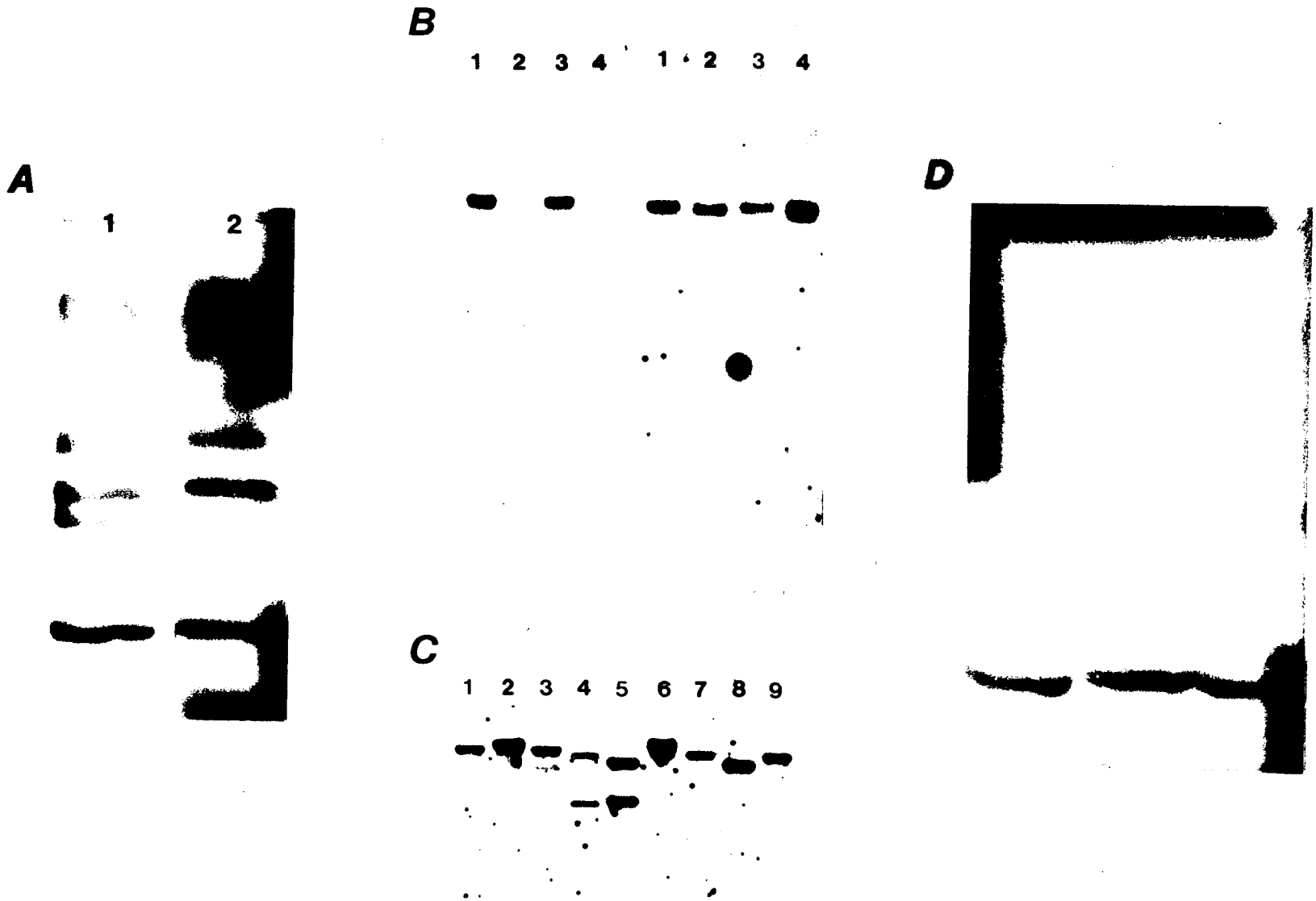

A
1
2
B
1 2 3 4 1 2 3 4
C
1 2 3 4 5 6 7 8 9
D

Table 53 Relative amounts of LSPs in L82 strain.

Exp.	Quantitation	Sex	LSP-1a / LSP-1b	LSP-1a / LSP-2
I	Total Protein Levels	male	0.064 ± .010 (6)	0.053 ± .009 (6)
		female	0.080 ± .008 (5)	0.068 ± .009 (5)
	male/female ratio		0.78	0.79
II	^{35}S-Met Pulse Labelling	male	0.295 ± .033 (6)	
		female	0.362 ± .057 (6)	
	male/female ratio		0.81	

Quantities of LSPs are expressed as the mean (± standard error). Numbers in parenthesis are the sample sizes.

mapped by recombination to 39.8 on the X chromosome (Table 54) in close agreement with the position (39.5) of LSP-1a as mapped by electrophoretic variants (Roberts and Evan-Roberts, 1979b). Further characterization showed that this gene had an abnormal genomic DNA restriction pattern (Figure 32c). The quantitiy of mRNA produced from this mutation (measured relative to LSP-1b) was significantly reduced from the diploid level (Figure 32b), presumably as a consequence of the DNA rearrangement. A comparison of LSP-1a quantities between males and females from this mutant revealed that dosage compensation remained incomplete. Measurements of LSP-1a synthesis in this stock by pulse labelling with ^{35}S-methionine confirmed this observation (Experiment II, Table 53), although the levels of LSP-1a synthesis relative to LSP-1b were higher than would be predicted from the quantities of LSP protein. Analysis of this mutant has revealed that even when the LSP-1a gene is not expressed at its maximum rate it still does not respond fully to hyperactivation by dosage compensation in males.

To demonstrate that LSP-1a levels can be raised above the diploid level, larvae with three copies of this structural gene were produced. The hyperploid segregant of T(1;3)V^{+74c} is an insertion of the X chromosome (9E3-4 to 11B12) into the heterochromatin of the third chromosome. Females duplicated for this region show elevated levels of LSP-1a relative to controls, although somewhat less than expected (Experiments IV, Table 51). The reduced level of expression of this duplicated LSP-1a gene may have arisen from position-effect variegation. This was tested by

Table 54 Mapping LSP-$1a^{182}$ by recombination.

recombinant chromosome	n	LSP-1a allele 182	LSP-1a allele +	recombinants between v and LSP-1a
v g f	12	0	12	
y cv	17	17	0	
g f	19	7	12	12
y cv v	16	9	7	9
	35			21

Table lists F_2 progeny from a cross between y cv v f females and LSP-$1a^{182}$ males.
Map distance from v to g is 11.4. Map distance from v to LSP-1a182 is 21/35 x 11.4 = 6.84. Map position of v is 33.0. Therefore map position of LSP-$1a^{182}$ is 33.0 + 6.84 = 39.8.

introducing this duplication into a stock bearing the LSP-1a^{L-E22} allele. Figure 32d shows that the duplicated LSP-1a gene is indeed synthesizing less product than a single gene in an euploid male. The duplicated gene is activated by Su(Var)A154 (a mutation which strongly supressess variegation; Don Sinclair, personal communication) (Figure 32d) indicating that it is being inactivated in its heterochromatic position. Thus, we can conclude that only a partial elevation in LSP-1a was observed in the duplication stock because the LSP-1a gene in this duplication was only partially active (or active in only a proportion of cells). More importantly, dose dependent increases of LSP-1a are observed in female larvae. Therefore, the compensation observed in metafemales probably did not arise from an inability to accumulate more LSP-1a.

Discussion

The similarity between compensation in autosomal trisomies and metafemales makes it tempting to speculate that compensation in all trisomies occurs via similar mechanisms. Compensation in autosomal trisomies is largely transcriptionally regulated (Devlin et al., 1984) and thus parallels the observation of Lucchesi et al. (1974) that X-chromosomal trisomies produce diploid levels of X-linked transcripts. If a single mechanism exists for decreasing transcription rates from those templates duplicated in all trisomies, then for X-chromosome trisomies this mechanism must work in concert with the male-female dosage compensation mechanism. When the ratio of X chromosomes to complete sets of autosomes is 1.5, the male-female dosage compensation mechanism could set transcription rates at the diploid female level and the hyperploid compensation mechanism could operate to repress X-linked product formation by an additional one-third (for reviews, and further discussion, see Lucchesi, 1983, and Baker and Belote, 1983).

The data presented in this chapter disclose the possibility that compensation in X-chromosomal trisomies occurs by a mechanism separable from male-female compensation. For example, while the Adh gene inserted into the X chromosome can acquire male-female dosage compensation, it fails to compensate both in autosomal and in X-chromosomal trisomies. Furthermore, the LSP-1a gene on the X chromosome escapes compensation between males and females, but it is fully compensated in X trisomies. The related gene LSP-1b, which is located on

the left arm of chromosome two, is also capable of compensation when this arm is trisomic (Devlin, et al., 1984). Cytological observations also suggest that two separate compensatory mechanisms are operating in metafemales. The single X chromosome in euploid males is "puffed" (Dobzhansky, 1957) and is the same size as the paired X homologues in euploid females. Indeed, the X chromosome in males possessing mutations which prevent dosage compensation is reduced to the size of a single X homologue in females (Belote and Lucchesi, 1980). Thus, hyperactivation of X-linked genes in males is correlated with increased chromosome width. In contrast, the three paired X homologues in metafemales are not diminished in size; that is, this synapsed unit is approximately 1.5 times the cross-sectional area of its diploid female counterpart. Nevertheless, the amount of product synthesized from the X chromosome in metafemales is reduced to the diploid level (compensated). Although intriguing, these observations do not constitute proof of two separate compensatory mechanisms.

The observation that the LSP-1a gene is not subject to male-female dosage compensation, but is compensated in trisomic-X individuals, supports the notion that the compensatory responses occur by separate processes. However, the observation that LSP-1a is compensated in metafemales could be fortuitous. It could be argued that transcription from the LSP-1a template occurs at the maximum rate possible. As such, this rate could not be increased in males but could be reduced in X-chromosomal trisomies. However, during the course of these investigations an LSP-1a variant allele was

discovered that produces less than ten percent of the normal amount of LSP-1a mRNA and protein. This LSP-1a low variant behaves in a manner similar to the wild-type allele in that it escapes full dosage compensation between males and females. In this case, the transcription unit would appear not to be saturated, yet complete hyperactivation still does not occur in these males.

It should be noted that the loci which escape modulation in metafemales are peculiar in that either they are regulatory loci (for Men), or they have recently arrived on the X chromosome (Adh transformants). In evolutionary time, it appears that even LSP-1a is a recent arrival on the X chromosome (Brock and Roberts, 1983). Nonetheless, the results presented in this chapter comply with the interpretation that some compensation in metafemales is accomplished by two separable mechanisms. Such mechanisms need not be mutually exclusive. It is possible that in aneuploids with X/A ratios between 1.0 and 1.5, some compensation may be brought about by the male-female compensation mechanism. Indeed, Williamson and Bentley (1983) have shown that in individuals with X/A ratios above 1.0 the expression of two X-linked genes (Zw and 6-Pgd) are affected by variations in X-chromosome content. Such an effect is consistent with male-female dosage compensation operating in metafemales. It is not known if components that mediate upward regulation in males, for example the products of male specific lethal genes (Belote and Lucchesi, 1980), are also required for downward regulation in metafemales. The issue of exactly how compensation is brought about is

further complicated by studies that report dose-dependent levels of X-linked transcription products in metafemales (Ananiev et al., 1974; Gvozdev et al., 1983). This situation would demand that dosage compensation in these individuals is post-transcriptionally regulated. Our measurements of LSP-1a mRNA in X trisomies suggest that regulation for this gene occurs at transcription.

If compensation in metafemales occurs by a mechanism distinct from male-female compensation then, an extension of this hypothesis suggests that dosage compensation in diploid males and females may be determined only at either the male or the female rate. Thus, euploid male-female dosage compensation may operate by a switching mechanism. For each cell, or perhaps each gene, hyperactivation is either on or off. This interpretation must be fitted to the evidence previously cited which suggests that dosage compensation may be continuously adjustable. As mentioned in the general introduction, continously adjustable rates of X-chromosome transcription might be interpreted from data examining the expression of a single gene in many cells (for example the amount of enzyme per organism) or the expression of many genes within a single cell (as for transcription on the polytene chromosomes). Clearly, measurement of the transcription rate of a single gene in individual cells is required. While this has been accomplished for a heat-shock gene in euploid D. pseudoobscura (Pierce and Lucchesi, 1980), producing individuals in this species that have abnormal X/A ratios is difficult. The advent of P-element mediated transformation in D.

melanogaster makes construction of appropriate strains a reality.

BIBLIOGRAPHY

Abraham I, Lucchesi JC (1974) Dosage Compensation of genes of the left and right arms of the X chromosome of Drosophila pseudoobscura and willistoni. Genetics 78:1119-1126

Allerdice PW, Tedesco TA (1975) Localization of human gene for galactose-1-phosphate-uridyltransferase. Lancet 2:39

Ananiev EV, Faizullin Lz, Gvozdev VA (1974) The role of genetic balance in control of transcription rate of the X-chromosome of Drosophila melanogaster. Chromosoma 45:193-201

Aragoncillo C, Rodrigues-Loperena MA, Salcedo G, Carbonero P (1978) Influence of homoeologous chromosomes on gene-dosage effect in allohexaploid wheat (Triticum aestivum L.). Proc. Natl. Acad. Sci. USA 75:1446-1450

Aronson JF, Rudkin GT, Schultz J (1954) A comparison of giant X chromosomes in male and females Drosophila melanogaster by cytophotometry in the ultraviolet. J. Histo. Cyto. Chem. 2:458-459

Arora P, Rao SRV (1979) Insect sex chromosomes. IV. DNA replication in the chromosomes of Gryllotalpa fossor Cytobios 26:45-55.

Ashburner M, Bonner JJ (1979) The induction of gene activity in Drosophila by heat shock. Cell 17:241-245

Audit I, Deparis P, Flavin M, Rosa R (1976) Erythrocity enzyme activities in diploid and triploid salamanders (Pleurodeles waltlii) of both sexes. Biochem. Genet. 14:759-769

Avery AG, Satina S, Rietsema J (1959) "Blakeslee: The genus Datura," The Ronald Press, New York

Baillie D, Chovnick A (1971) Studies on the gentic control of tryptophane pyrolase in Drosophila melanogaster Mol. Gen. Genet. 112:341-353

Baker BS, Ridge KA (1980) Sex and the single cell: I on the action of major loci affecting sex determination in Drosophila melanogaster. Genetics 94:383-423

Baker BS, Belote JM (1983) Sex determination and dosage compensation in Drosophila melanogaster. Ann. Rev. Genet. 17:345-393

Banerji J, Rusconi S, Schaffner W (1981) Expression of a b-globin gene is enchanced by remote SV40 in DNA sequences. Cell 27:299-308

Banerji J, Olson L, Schaffner W (1983) A lymphocyte-specific

cellular enhancer is located downstream of the joining region of immunoglobulin heavy-chain genes. Cell 33, 729-240

Bedo DG, (1982) Differential sex chromosome replication and dosage compensation in polytene Trichogen cells of Lucilia cuprina (Diptera:Calliphoridae). Chromosoma 87:21-32

Belote JM, Lucchesi JC (1980a) Control of X chromosome transcription by the maleless gene in Drosophila. Nature 285:573-575

Belote JM (1983) Male-specific lethal mutations of Drosophila melanogaster II. Parameters of gene action during male development. Genetics 105:881-896

Belote JM, Lucchesi JC (1980b) Male-specific lethal mutations of Drosophila melanogaster. Genetics96:165-186

Bentley MM, Williamson JH (1979) The control of aldehyde oxidase and xanthine dehydrogenase activities by the cinnamon gene in Drosophila melanogaster. Can. J. Genet. Cytol. 21:457-4711

Berendes HD, (1966) Differential Relication of male and female X-chromosomes in Drosophila. Chromosoma 20:32-43

Berger JD (1982) Effects of gene dosage on protein synthesis rate in Paramecium tetraaurelia Exp. Cell Res. 141:261-275

Berlowitz L, Loewus MW, Pallotta D (1968) Heterochromatin and genetic activity in mealy bugs.I. Compensation for inactive chromatin by increase in cell number. Genetics 60:93-99

Bewley GC, Laurie-Ahlberg CC (1984) Genetic variation affecting the expression of catalase in Drosophila melanogaster: correlations with rates of enzyme synthesis and degredation. Genetics 106:435-448

Bewley GC, Lucchesi JC (1977) Origin of a-Glycerophosphate Degydrogenase Isozymes in Drosophila melanogaster and their functional relationship in the a-Glycerophosphate cycle. Biochem. Genet. 15:235-251

Bewley GC, Rawls J, Lucchesi JC (1974) a-Glycerophosphate dehydrogenase in Drosophila melanogaster: Kinetic differences and developmental differentiaion of the larval and adult isozymes. J. Insect Physiol.:20-153-165

Bijlsma R (1980) Polymorphism at the G6pd and 6Pgd loci in Drosophila melanogaster. IV Gentic factors modifying enzyme activity. Biochem. Genet. 18:669-715

Birchler JA (1979) A study of enzyme activities in a dosage series of the long arm of chromosome one in maize. Genetics

92:1211-1229

Birchler JA (1980) A unifying genetic principle for dosage compensation, aneuploid inviability and sexual dimorphisms in Drosophila. Genetics 94:s9

Birchler JA (1981) The genetic basis of dosage compensation of alcohol dehydrogenase-1 in maize. Genetics 97:625-637

Birchler JA, Newton KJ (1981) Modulation of protein levels in chromosomal dosage series of maize: the biochemical basis of aneuploid syndromes. Genetics 99:247-266

Blakeslee AF (1922) Variations in Datura due to the changes in chromosome number. Amer. Natur. 56:16-31

Bonner WM, Laskey RA (1974) A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46:83-88

Botas J, del Prado JM, Garcia-Bellido A (1982) Gene-dose titration analysis in Drosophila. EMBO J. 1:307-310

Bowman J, Simpson JR (1973) Gene modulation in Drosophila: Dosage compensation of Pgd+ and Zw+ genes. Biochem. Genet. 10:319-331

Bradford MM (1976) Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. Anal. Biochem. 72:248-254

Braverstock PR, Adams M, Polkinghorne RW, Gelder M (1982) A sex-linked enzyme in birds- Z-chromosome conservation but no dosage compensation. Nature 296:763-766

Brehme KS (1937) Effects of the triplo-X condition on the development in Drosophila melanogaster. Proc. Soc. Exp. Biol. & Med. 37:578-580

Bridges CB (1921) Triploid intersexes in Drosophila melanogaster. Science 54:252-254

Bridges CB (1922) The origin of variations in sexual and sex-linked characters. The Am. Naturalist 56:51-63

Bridges CB (1925) Sex in relation to chromosomes and genes. Am. Naturalist 59:127-137

Britten RJ, Davidson EH (1969) Gene regulation for higher cells: a theory. Science 165:349-357

Brock HW, Roberts DB (1980) Comparison of the larval serum proteins of Drosophila melanogaster using one and two-dimensional peptide mapping. Eur. J. Biochem. 106:129-135

Brock HW, Roberts DB (1982) The LSP1-a gene is not dosage

compensated in the Drosophila melanogaster species subgroup. Biochem. Genet. 20:287-295

Brock H.W. Roberts DB (1983) Location of the LSP-1 genes in Drosophila species by in situ hybridization. Genetics 103:75-92

Bull JJ (1983) Evolution of sex determining mechanisms. Benjamin/Cummings Publishing Company, Inc., Calf.

Burton K (1968) Determination of DNA concentration with diphenylamine. In: Methods in Enzymology, Vol. XII. Grossman L, Moldave K (eds). pp 163-166

Carlson PS (1972) Locating genetic loci with aneuploids. Mol. Gen. Genet. 114:273-280

Casartelli C, Basile R, Dos Santos EP (1969) DNA and RNA snythesis in Rhynchosciara angelae. Nonata and Pavan (1951) and the problem of dosage compensation. Caryologia 22:203-211

Cattanach BM (1975) Control of chromosome inactivation. Ann. Rev. Genet. 9:1-18

Chandler M, Prittard RH (1975) The effect of gene concentration and relative gene dosage on gene output in Escherichia coli. Mol. Gen. Genet. 138:127-141

Chandler VL, Maler BA, Yamanoto KR (1983) DNA sequences bound specifically by glucocorticoid receptor in vitro render a heterologous promoter hormone responsive in vivo. Cell 33:489-499

Chandra HS, Brown S (1974) Regulation of S-chromosome inactivation in mammals. Genetics 78:343-349

Chang PL, Georgiadis N, Joubert GI, Davidson RG (1983) Gene dosage effects in human diploid and tetraploid fibroblasts. Exp. Cell. Res. 145:277-284

Charlesworth B (1978) Model for evolution of Y chromosomes and dosage compensation. Proc. Natl. Acad. Sci. USA 1978:5618-5622

Chatterjee RN, Mukherjee AS (1981) Chromosomal basis of dosage compensation in DRosophila X. Assessment of hyperactivity of the male X in situ. J. Cell. Sci. 47:295-309

Chatterjee RN, Dube DK, Mukherjee AS (1981) In situ transcription analysis of chromatin template activity of the X-chromosome of Drosophila following high molar NaCl treatment. Chromosoma 82:515-523

Cifferi O, Sora S, Tiboni O (1969) Efffect of gene dosage on tryptophane synthetase activity in Saccaromyces cerevisiae. Genetics 61:567-576

Clarke B, Camfield RG, Galvin AM, Pitts CR (1979) Environmental factors affecting the quantity of alcohol dehydrogenase in Drosophila melanogaster. Nature 280:517-518

Clarke SH, Kiefer B (1977) Genetic modulation of RNA metabolism in Drosophila.II. Coordinate rate change in 4S, 5S and poly-A associated RNA synthesis. Genetics 86:800-811

Cline TW (1978) Two closely linked mutations in Drosophila melanogaster that are opposite sexes and interact with daughterless. Genetics90:683-698

Cline TW (1979) A male-specific lethal mutation in Drosophila that transforms sex. Dev. Biol. 72:266-275

Cline TW (1980) Maternal and zygotic sex-specific gene interactions in Drosophila melanogaster. Genetics 96:903-926

Cline TW (1983) Interaction between Daughterless and Sex-lethal in triploids: A lethal sex-transforming maternal effect linking sex determination and dosage compensation in Drosophila melanogaster. Dev. Biol. 95:260-274

Cline TW (1984) Autoregulatory functioning of a Drosophila gene product that establishes and maintains the sexually determined state. Genetics 107:231-277

Cooper DW, vandeBerg JL, Sharman GB, Poole WE (1971) Phosphoglycerate kinase polymorphism in kangaroos provides further evidence for paternal X inactivation. Nature New Biology 230:155-157

Corces V, Holmgren R, Freund R, Morimoto R, Meselson M (1980) Four heat shock proteins of Drosophila melanogaster coded within a 12kb region in chromosome subdivision 67B. Proc. Natl. Acad. Sci. USA 77:5390-5393

Cox DR, Tucker GC, Epstein CJ (1980) Mouse trisomy 16 as an animal model of human trisomy 21. AM. J. Hum. Genet. 32:66A

Das M, Mutsuddi D, Duttagupta AK, Mukherjee AS (1982) Segmental heterogeneity in replication and transcription of the X2 chromosome of Drosophila miranda and conservativeness in the evolution of dosage compensation. Chromosoma 87:373-388

Davidson EH, Britten RJ (1979) Regulation of gene expression: possible role of repetitive sequences. Science 204:1052-1059

de C. Bicudo HEM (1983) Evidence for gene amplification operating in dosage compensation of Drosophila arizonensis. Biologisches Zentralblatt Band 102:45-55

DeMaggio AE Lambrukos J, Schrom M, Stetler DA (1974) Polyploidy

and gene dosage effects on peroxidase activity in ferns.Biochem. Genet. 12:429-440

Devlin RH, Holm DG, Grigliatti TA (1982) Autsomal dosage compensation in Drosophila melanogaster strains trisomic for the left arm of chromosome 2. Proc. Natl. Acad. Sci. USA 79:1200-1204

Devlin RH, Grigliatti TA, Holm DG (1984) Dosage compensation is transcriptionally regulated in autosomal trisomies of Drosophila. Chromosoma:in press

Dickinson WJ (1970) The genetics of aldehyde oxidase in Drosophila melanogaster. Genetics 66:487-496

Dickinson WJ, Gaughan S (1981) Aldehyde oxidases of Drosophila: Contributions of several enzymes to observed activity patterns. Biochem. Genet. 19:567-583

DiDominico BJ, Bugaisky GE, Lindquist S (1982a) The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. Cell 31:593-603

DiDominico BJ, Bugaisky GE, Lindquist S (1982b) Heat shock and recovery are mediated by different translocational mechanisms. Proc. Natl. Acad. Sci. USA 79:6181-6185

Dierks P, van Ooyen A, Mantei N, Weissman C (1981) DNA sequences preceding the rabbit b-globin gene are required for formation in mouse L cells of b-globin mRNA with the correct 5' terminus. Proc. Natl. Acad. Sci. USA 78:1411-1415

Dobzhansky T (1928) The effect of temperature on the viability of superfemales in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 14:671-675

Dobzhansky and Schultz (1934) The distribution of sex-factors in the X-chromosome of Drosophila melanogaster. J. of Genetics 28:349-386

Dobzhansky T (1957) The X-chromosome in the larval salivary glands of hybrids Drosophila insularis x Drosophila tropicalis. Chromosoma 8:691-698

Dubois E, Jacobs E, Jauniaux J (1982) Expression of the ROAM mutations in Sacchromyces cerevisiae: involvement of trans-acting regulatory elements and relation with the Ty1 transcription. EMBO J. 1:1133-1139

Eissenberg JC, Lucchesi JC (1983) Chromatin structure and transcriptional activity of an X-linked heat shock gene in Drosophila pseudoobscura. J. Biol. Chem. 258:13986-13991

Epstein CJ, McManus NH, Epstein LB (1982) Direct Evidence that the gene product of the human chromosome 21 locus, IFRC, is the

inteferon-a receptor. Bioch. Bioph. Res. Comm. 107:1060-1066

Faizullin LZ, Gvozdev VA (1973) Dosage compensation of sex-linked genes in Drosophila melanogaster. Mol. Gen. Genet. 126:233-245

Fasiolo F, Bonnet F, LaCroute F (1981) Cloning of the yeast methionyl-tRNA synthetase gene. J. Biol. Chem. 56:2324-2328

Faye G, Leung DM, Tatchell K, Hall BC, Smith M (1981) Delection mapping of sequences essential for invivo transcription of the iso-1 cytochrome C gene. Proc. Natl. Acad. Sci. USA 78:33358-2262

Ferguson-Smith MA, Newman BF, Ellis PM, Thomson DMG (1973) Assignment by deletion of human red cell acid phosphatase gene locus to the short arm of chromosome 2. Nature New. Biol. 243:271-273

Fitz-Earle M, Holm DG (1978) Exploring the potential of compound;free-arm combinations of chromosome 2 in Drosophila melanogaster for insect control and the survival to pupae of whole-arm trisomies. Genetics 89:499-510

Fobes JF (1980) Trisomic analysis of isozymic loci in tomato species: segregation and dosage effects. Biochem. Genet. 18:401-421

Fox AS, Yoon SB, Gelbart WM (1971) DNA-induced transformation in Drosophila: genetic analysis of transformed stocks. Proc. Natl. Acad. Sci. USA 68:342-346

Fraser RSS, Nurse P (1979) Altered patterns of ribonucleic acid synthesis during the cell cycle: a mechanism compensating for variation in gene concentration. J. Cell. Sci. 35:25-40

Frost JN (1960) The occurrence of partialy fertile triploid metafemales in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 46:47-50

Ganschow RE, Schimke RT (1969) Independent gentic control of the catalytic activity and the rate of degredation of catalase in mice. J. Biol. Chem. 244:449-458

Garcia-Bellido A, del Prado MJ, Botas J (1983) The effect of aneuploidy on embryonic development in Drosophila melanogaster. Mol. Gen. Genet. 192:253-263

Garen A, Garen S (1963) Complementation in vivo between structural mutants of alkaline phosphatase from E. coli. J. Mol. Biol. 7:13-22

Gartler SM, Riggs AD (1983) Mammalian X-chromosome inactivation. Ann. Rev. Genet. 17:155-190

George DL, Francke U (1976) Gene Dose Effect: Regional mapping of human nucleoside phosphorylase on chromosome 14. Science 194:851-852

Gerbaud C, Guerineau M (1980) 2um plasmid copy number in different yeast strains and repartition of endogenous and 2µym chimeric plasmids in transformed strains. Curr. Genet. 1:219-228

Gillies SD, Morrison SL, Oi VT, Tonegawa S (1983) A tissue-specific enhancer element is located in the major intron of a rearranged immunoglobulin gene. Cell 33:717-728

Goldberger RF (1979) Biological regulation and development. Plenum Press

Goldberg DA (1980) Isolation and partial charactrization of the Drosophila alcohol dehydrogenase gene. Proc. Natl. Acad. Sci. USA 77:5794-5798

Goldberg DA, Posakony JW, Maniatis T (1983) Correct development expression of a cloned alcohol dehydrogenase gene transduced into the Drosophila germ line. Cell 34:59-73

Goldschmidt RB, (1954) Different philosophies of genetics. Science 119:703-710

Goodspeed TH, Avery P (1939) Trisomic and other types in Nicotiana sylverstris. J. Genet. 38:381-458

Grell EH (1962) The dose effect of ma-1 and ry on xanthine dehydrogenase activity in Drosophila melanogaster Z. Vererbungsl. 93:371-377

Grell EH (1970) Distribution pairings: mechanism for segregation of compound autosomal chromosomes in oocytes of Drosophila melanogaster. Genetics 65:65-74

Gullov K, Friis J (1980) Chromosomal proteins in S. cerevisiae II. Chromosomal dosage effect on the cellular and nuclear content of nonhistone proteins. Current Genetics 2:75-78

Gvozdev VA, Birstein VJ, Kakpakov VT, Polukarova LG (1971) Activity of sex-linked genes in cultivated in vitra sublines of Drosophila melanogaster embryonic cells. Soviet J. Develop. Biol. 2:243-248

Gvozdev VA, Leibovitch BA, Ananiev EV (1983) Gene dosage compensation in the X chromosome of Drosophila melanogaster: transcription levels in metafemales and metamealles and the amount of 6-phosphogluconate deghdrogenase in metafemales. Dros. Info. Serv. 58:48-49

Hazelrigg T, Levis R, Rubin GM (1984) Transformation of white locus DNA in Drosophila: Dosage compensation, zeste interraction

and position effects. Cell 36:469-481

Herskowitz IH, Schalet A (1957) Induced changes in the female germ cells of Drosophila. V. The contribution of half translocation and nondisjunction to the dominant lethality induced by X-raying oocytes. Genetics 42:649-660

Hodgetts RB, Konopka RJ (1975) Tyrosine and catecholamine metabolism in wild-type Drosophila melanogaster and a mutant, ebony. J. Insect Physiol. 19:1211-1220

Hodgkin J (1983) X chromosome dosage and gene expression in Caenorhabditis elegans: Two unusual dumpy genes. Mol. Gen. Genet. 192:452-458

Holm DG (1976) Compound Autosomes. In Ashburner M, Novitski E (eds): The genetics and biology of Drosophila, Vol. 1b:529-561. New York, Academic Press

Holmgren R, Livak K, Morimoto R, Freund R, Meselson M (1979) Studies of cloned sequences from four Drosophila heat shock loci. Cell 18:1359-1370

Hori SH, Tanda S (1981) Genetic variation in the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in Drosophila melanogaster. Evidence for an autosomal modifier system. Jpn. J. Genet. 56:257-277

Hori SH, Tanda S, Fukazawa K, Hanaoka T (1982) Further studies on the modifier gene system regulating activities of X-linked enzymes in Drosophila melanogaster. Jpn. J. Genet. 57:535-550

Jacob F, Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3:318-356

Johnson MS, Turner JRG (1979) Absence of dosage compensation for a sex-linked enzyme in butterflies (Heliconius). Heredity 43:71-77

Johnson G, Finnerty V, Hartl D (1981) Post-translational modification of Xanthine dehydrogenase in a natural population of Drosophila melanogaster. Genetics 98:817-831

Kacser H, Burns JA (1981) The molecular basis of dominance. Genetics 98:639-666

Kakpakov VT, Gvozdev VA, Platove TP, Polukarova LG (1969) In vitro establishment of embryonic cell lines of Drosophila melanogaster. Soviet Gentics 5:1647-1655

Kazazian HH, Young WJ, Childs B (1965) X-linked 6-phosphogluconate dehydrogenase in Drosophila: subunit associations. Science 150:1601-1602

Keppy DD, Dennel RE (1979) A mutational analysis of the

triplo-lethal region of Drosophila melanogaster. Genetics 91:421-441

Kerkis J (1931) The growth of the gonads in Drosophila melanogaster. Genetics 16:212-224

Khesin RB (1973) Binding of thymus histone F1 and E. coli RNA polymerase to DNA of polytene chromosomes of Drosophila. Chromosoma 44:255-264

Khesin RB, Leibovitch BA (1974) Synthesis of RNA by Escherichia coli RNA polymerase on the chromosomes of D. melanogaster Chromosoma 46:161-172

Khesin RB, Leibovitch BA (1978) Influence of deficiency of the histone gene-containing 38B-40 region of X-chromosome template activity and the white gene position effect variegation in D. melanogaster.Mol. Gen. Genet. 162:323-328

Khush GS (1973) Cytogentics of aneuploids. Academic Press, London

King JJ, McDonald JF (1983) Genetic localization and biochemical characterization of a trans-acting regulatory effect in Drosophila melanogaster. Genetics 105:55-69

Klose J, Putz B (1983) Analysis of two-dimensional protein patterns from mouse embryos with different trisomies. Proc. Natl. Acad. Sci. USA 80:3753-3757

Komma DJ (1966) Effect of sex transformation genes of glucose-6-phosphate dehydrogenase activity in Drosophila melanogaster. Genetics 54:497-503

Korge G (1981) Genetic analysis of the larval secretion gene Sgs-4 and its regulatory chromosome sites of Drosophila melanogaster. Chromosoma 84:373-390

Kozak LP (1975) Evidence for dosage compensation of an X-linked gene in the 6-day embryo of the mouse. Dev. Biol. 45:65-73

Kurnit DM (1979) Down syndrome: Gene dosage at the transcriptional level in skin fibroblasts. Proc. Natl. Acad. Sci. USA 76:2372-2375

Laemli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685

Laird CD, Ashburner M, Wilkinson C (1980) Relationship between relative dry mass and average band width in regions of polytene chromosomes of Drosophila. Chromosoma 76:175-189

Lakhotia SC, Mukherjee AS (1969) Chromosomal basis of dosage compensation in Drosohila. I. Cellular autonomy of hyperactivity of the male X-chromosome in sailivary glands and sex

differentiation. Genet. Res. 14:137-150

Laurie-Alberg CC, Maroni G, Bewley GC, Lucchesi JC, Weir BS (1980) Quantitative genetic variation of enzyme activities in natural populations of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 77:1073-1077

Laurie-Alberg CC, Williamson JH, Cochrane BJ, Wilton AN, Chasalow FI (1981) Autosomal factors with correlated effects on the activites of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases in Drosophila melanogaster. Genetics 99:127-150

Laurie-Alberg CC, Wilton AN, Curtsinger JW, Emigh TH (1982) Naturally occurring enzyme activity variation in Drosophila melanogaster. Genetics 102:191-206

Lefevre G Jr (1976) A photographic representation and interpretation of the polytene chromosomes of Drosophila melanogaster salivary glands. In: The Genetics and Biology of Drosophila vol. 1a. Ashburner M, Novitski E (eds). Academic Press, New York, pp 31-66

Leibovitch BA, Bogdanova ES (1983) Binding of Mouse and Drosophila HI histones to the chromosomes and DNA. Mol. Biol. 17:162-171

Leibovitch BA, Belyaeva ES, Zhimulev IF, Khesin RB (1976) Comparison of in vivo and in vitro RNA synthesis on polytene chromosomes of Drosophila. Chromosoma 54:349-362

Lewis EB (1978) A gene controlling segmentation in Drosophila. Nature 276:565-570

Lifschytz E, Lindsley EL (1972) The role of X-chromosome inactivation during spermatogenesis. Proc. Natl. Acad. Sci. USA 69:182-186

Lindquist S (1980) Varying patterns of protein synthesis in Drosophila during heat shock: Implications for regulation. Dev. Biol. 77:463-479

Lindsley DL, Grell EH (1968) Genetic variations in Drosophila melanogaster. Carnegie Inst. of Wash. Publ. 647

Lindsley DL, Sandler L, Baker BS, Carpenter ATC, Dennell RE, Hall JC, Jacobs PA, Miklos GLG, Davis BK, Gethmann RC, Hardy RW, Hessler A, Miller SM, Nozawa H, Parry DM, Gould-Somero M (1972) Segemental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics 71:157-184

Lowry OH, Rosebrough NJ, Farr AL, Randall RA (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275

Lucchesi JC (1973) Dosage compensation in Drosophila. Ann. Rev. Genet. 1:225-237

Lucchesi JC (1977) Dosage compensation: transcriptional level regulation of X-linked genes in Drosophila. Am. Zool. 17:685-693

Lucchesi JC (1978) Gene dosage compensation and the evolution of sex chromosomes. Science 202:711-716

Lucchesi JC, (1983) The relationship between gene dosage, gene expression and sex in Drosophila. Dev. Genet. 3:275-282

Lucchesi JC, Rawls JM (1973a) Regulation of gene function: A comparison of enzyme activity levels in relation to gene dosage in diploids and triploids of Drosophila melanogaster. Biochem. Genet. 9:41-51

Lucchesi JC, Rawls JM (1973b) Regulation of gene function: A comparison of X-linked enzyme activity levels in normal and intersexual triploids of Drosophila melanogaster. Genetics 73:459-464

Lucchesi JC, Skripsky T (1981) The link between dosage compensation and sex differentiation in D. melanogaster. Chromosoma 82:217-227

Lucchesi JC, Belote JM, Maroni G (1977)X-linked gene activity in metamales. (XY;3A) of Drosophila. Chromosoma 65:1-7

Lucchesi JC, Rawls JM, Maroni G (1974) Gene dosage compensation in metafemales (3X;2A) of Drosophila. Nature 248:564-567

Lyon MF (1972) X-chromosome inactivation and developmental patterns in mammals. Biol. Review 47:1-35

Magenis RE, Koler RD, Lovrien E, Bibley RH, DuVal MC, Overton KM (1975) Gene dosage: Evidence for assignment of erythrocyte acid phosphatase locus to chromosome 2. Proc. Natl. Acad. Sci. USA 72:4526-4530

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: A laboratory manual. Cold Spring Harbor, N.Y.

Marimo B, Giannelli F (1975) Gene dosage effect in human trisomy-16. Nature256:204-206

Maroni G, Plaut W (1973a) Dosage compensation in Drosophila melanogaster triploids. II. Glucose-6-phosphate dehydrogenase activity. Genetics 74:331-342

Maroni and Plaut (1973b) Dosage compensation in Drosophila melanogaster triploids. I. Autoradiographic study. Chromosoma 40:361-377

Maroni G, Kaplan R, Plaut W (1974) RNA synthesis in Drosophila melanogaster polytene chromosomes.

Chromosoma 47:203-212

Maroni G, Lucchesi JC (1980) X-chromosome transcription in Drosophila. Chromosoma 77:253-261

Maroni G, Laurie-Ahlberg CC (1983) Genetic control of ADH expression in Drosophila melanogaster. Genetics 105:921-933

Marusyk R, Sergeant A (1980) A simple method for dialysis of small-volume samples. Analyt. Bioch. 105:403-404

Mattei JF, Baeteman MA, Baret A, Ardissone JP, Rebuffel P, Giraud F (1982) Erythrocyte superoxide dismutase and redox enzymes in trisomy 21. Acta Paediatr. Scand. 71:589-591

McClelland A, Smith DF, Glover DM (1981) Short intervening sequences close to the 5' ends of the three Drosophila larval serum protein genes. J. Mol. Biol. 153:257-272

McCord JM, Fridovitch I (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244:6049-6055

McDaniel RG, Ramage RT (1970) Genetics of a primary trisomic series in barley: identification by protein electrophoresis. Can. J. Genet. Cytol. 12:490-495

McDonald JF, Chambers GK, David J, Ayala FJ (1977) Adaptive response due to changes in gene regulation: A study with Drosophila. Proc. Natl. Acad. Sci. USA 74:4562-4566

McDonald JF, Ayala FJ (1978) Genetic and biochemical basis of enzyme activity variation in natural populations. I. Alcohol dehydrogenase in Drosophila melanogaster. Genetics 89:371-388

McKnight RH (1939) The sex-determining mechanism of D. miranda. Genetics 24:180-201

McMillin DE, Scandalios JG (1982) Genetic, immunological, and gene dosage studies of mitochondrial and cytosolic MDH variants in maize. J. of Heredity 73:177-182

Mellman WJ, Oski RA, Tedesco TA, Maciera-Coelho A, Harris H (1964) Leucocyte enzymes in Down's syndrome. Lancet 2:674-675

Metzenberg RL, Chia W (1979) Genetic control of phosphorus assimilation in Neurosporra crasse: Dose-dependent dominance and recessiveness in constituative mutants. Genetics 93:625-643

Miyashita N, Laurie-Ahlberg CC (1984) Genetical analysis of chromosomal interaction effects on the activities of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases in Drosophila melanogaster. Genetics 106:655-668

Mohandes T, Sparkes RS, Shapiro LJ (1981) Reactivation of an inactive human X chromosome: Evidence for X inactivation by DNA methylation. Science 211:393-396

Moore GD, Procunier, JD, Cross DP, Grigliatti TA (1979) Histone gene dificiencies and position-effect variegation in Drosophila. Nature 282:312-314

Moore GD, Sinclair DA, Grigliatti TA(1983) Histone gene multiplicity and position effect variegation in Drosophila melanogaster. Genetics 105:327-344

Morgan LV (1924) Polyploidy in D. melanogaster with two attached X chromosomes. Genetics 10:148-178

Mukherjee AS (1982) Dosage compensation as a model system for genetic regulation in eukaryotes. Current Sci. 51:205-212

Mukherjee AS, Beerman W (1965) Synthesis of ribonucleic acid by the X-chromosome of Drosophila melanogaster and the problem of dosage compensation. Nature 207:785-786

Muller HJ (1950) Evidence of precision of gentic adaptation. Harvey Lecture Series XLIII:165-229

Muller HJ, League BB, Offermann CA (1931) Effects of dosage changes of sex-linked genes, and the compensatory effects of other gene differences between male and female. Anat. Rec. 51 (Suppl.), 110

Naysmith KA (1982) The regulation of yeast mating type chromatin structure by SIR: an action at a distance affecting both transcription and transposition. Cell 30:567-578

Nielson G, Scandalios JG (1974) Chromosomal location by use of trisomics and new alleles of an endopeptidase in Zea mays.Genetics 77:679-686

Nikoshkov AB, Kakpakov VT (1981) Dosage compensation of sex-linked genes in established cell lines of Drosophila melanogaster. Dros. Inf. Serv. 56:103-104

O'Brien SJ, Gethman RC (1973) Segmental aneuploidy as a probe for structural genes in Drosophila: mitochondrial membrane enzymes. Genetics 75:155-167

O'Brien SJ, McIntyre RJ (1972) The a-glycerophosphate inDrosophila melanogaster. II. Genetic aspects. Genetics 71:127-138

O'Brien SJ, McIntyre R (1976) Genetics and Biochemistry of enzymes and specific proteins of Drosophila. In: Wright T, Ashburner M (eds) The genetics and biology of Drosophila. vol. 2b. Academic Press, New York, pp 395-551

Offerman CA (1936) Branched chromosomes as symmetrical duplications. J. Genet. 32:103-116

Osley MA, Hereford LM (1981) Yeast histone genes show dosage compensation. Cell 29:347-355

Ota T. Fukunaga A, Kawabe M, Oishi (1981) Interactions between sex-transformation mutants of Drosophila melanogaster. I. Hemolymph vitellogenins and gonad morphology. Genetics 99:429-441

Paigen K (1979) Acid hydrolases as models of genetic control. Ann. Rev. Genetics 13:417-466

Paigen K (1980) Temporal genes and other developmental regulators in mammals. The Molecular Genetics of Development. Academic Press. pp. 419-470

Parker CS, Topol J (1984) A Drosophila RNA polymerase II transcription factor binds to the regulatory site of an hsp 70 gene. Cell 37:273-283

Parsons GD, Mackie GA (1983) Expression of the gene for ribosomal protein s20: Effects of gene dosage. J. of Bact. 154:152-160

Patterson JT, Stone W, Bedichek S (1937) Further studies on X chromosome balance in Drosophila. Genetics 22:407-426

Patterson JT, Stone W (1952) Evolution in the genus Drosophila. MacMillan Co., New York.

Pearson NJ, Fried HM, Warner JR (1982) Yeast use translational control to compensate for extra copies of a ribosomal protein gene. Cell 29:347-355

Pellham HRB (1982) A regulatory upstream promoter element in the Drosophila hsp 70 heat-shock gene. Cell 30:517-528

Pellham HRB, Bienz M (1982) A snythetic heat-shock promoter element confers heat-inducibility on the herpes simplex virus thymidine kinase gene. EMBO J. 1:1473-1477

Pierce DA, Lucchesi JC (1980) Dosage compensation of X-linked heat-shock puffs in D. pseudoobscura. Chromosoma 76:245-254

Pipkin SB (1940) Multiple sex genes in the X chromosome of Drosophila melanogaster. Univ of Texas Publ. 4032:126-156

Pipkin SB (1947) A search for sex genes in the second chromosome of Drosophila melanogaster using the triploid method. Genetics 32:592-607

Pipkin SB (1959) Sex balance in Drosophila

melanogaster: Aneuploidy of short regions of chromosome 3, using the triploid method. Univ. of Texas Publ. 5914:69-88

Pipkin SB (1960) Sex balance in Drosophila melanogaster: Aneuploidy of long regions of chromosome 3, using the triploid method. Genetics 45:1205-1216

Pipkin SB (1961) Sex combs in unbalanced male forms of Drosophila melanogaster. J of Heredity 52:256-259

Pipkin SB, Hewitt NE (1972) Effect of gene dosage on level of alcohol dehydrogenase in Drosophila. J. of Heredity 63:331-336

Powell D, Sato JD, Brock HW, Roberts DB (1984) Regulation of synthesis of the larval serum proteins in Drosophila melanogaster. Dev. Biol. 102:206-215

Priest RE, Priest JH (1969) Diploid and tetraploid clonal cells in culture: Gene ploidy and synthesis of collagen. Biochem. Genet. 3:371-382

Prittard J, Ramkrishnan T (1964) Gene transfer by F' strains of Escherichia coli. IV. Effects of a chromosomal deletion on chromosome transfer. J.Bacteriology 88:367-373

Queen C, Baltimore D (1983) Immunoglobulin gene transcription is activated by down stream sequence elements. Cell 33:741-748

Rao SRV, Ali S (1982) Insect sex chromosomes. VI. A presumptive hyperactivation of the male X chromosome in Acheta domesticus (L.). Chromosoma 86:325-339

Rasch EM, Cassidy JD, King RC (1977) Evidence for dosage compensation in Parthenogenetic Hymenoptera. Chromosoma 59:323-340

Rave N, Crkvenjakov R, Boedtker N (1979) Identification of procolagen mRNAs transferred to diazobenzyloxymethyl paper from formaldehyde-agarose gels. Nucl. Acids. Res. 6:3359-3367

Rawls JM, Lucchesi JC (1974a) Regulation of enzyme activites in Drosophila. I. The detection of regulatory loci by gene dosage responses. Genet. Res. 24:59-72

Rawls JM, Lucchesi JC (1974b) Regulation of enzyme activites in Drosophila. II. Characterization of enzyme responses in aneuploid flies. Genet. Res. 24:73-80

Rhoades MM, McClintock B (1935) The cytogenetics of maize. Bot. Rev. 1:292-325

Rigby PWJ, Diekmann M, Rhodes C, Berg P (1977) Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. 113:237-251

Ripoll R. (1980) Effect of terminal hyperploidy on epidermal

cell viability in Drosophila melanogaster. Genetics 94:135-152

Roberts DB, Evans-Roberts S (1979a) The X-linked a-chain gene of Drosophila LSP-1 does not show dosage compensation. Nature 280:691-692

Roberts DB, Evans-Roberts S (1979b) The gentic and cytogentic localization of the 3 structural genes coding for the major protein of Drosophla larval serum. Genetics 93:663-697

Roehrdanz RC, Lucchesi JC (1977) Transcriptional controls and dosage compensation in Drosophila melanogaster. Nature 269:243-245

Roehrdanz RC, Kitchens JM, Lucchesi JC (1977) Lack of dosage compensation for an autosomal gene relocated to the X-chromosome in Drosophila melanogaster. Genetics 85:489-496

Rolfes G, Hollander WF (1961) Viability and reproductivity of superfemales in Drosophila. J. of Heredity. 52:61-66

Roose ML, Gotlieb LD (1980) Biochemical properties and level of expression of alcohol dehydrogenases in the allotetraploid plant Tragopogon miscellus and its diploid progenitors. Biochem. Genet. 18:1065-1085

Rubin GM, Spradling AC (1982) Genetic transformation of Drosophila with transposable element vectors. Science 218:348-353

Sanchez L, Nothiger R (1982) Clonal analysis of sex-lethal, a gene needed for female sexual development in Drosophila melanogaster. Wilhelm Roux's Archives 191:211-214

Sanchez L, Nothiger R (1983) Sex determination and dosage compensation in Drosophila melanogaster: production of male clones in XX females. EMBO J. 2:485-491

Schlesinger MJ, Ashburner M, Tissieres A editors (1982) Heat Shock, from bacteria to man. Cold Spring Harbor, N.Y.: Cold Shpring Harbor Laboratory 1982.

Scholnick SB, Morgan BA, Hirsh J (1983) The cloned dopa decarboxylase gene is developmentally regulated when reintegrated into the Drosophila genome. Cell 34:37-45

Schwartz D (1971) Genetic control of alcohol dehydrogenase- A competition model for regulation of gene action. Genetics 67:411-425

Schwartz D (1973) The application of the maize-derived gene competition model to the problem of dosage compensation in Drosophila. Genetics 75:639-641

Searle PF, Tata JR (1981) Vitellogenin gene expression in male

Xenopus hepatocytes during primary and secondary stimulation with estrogen in cell cultures. Natl. Inst. for Med. Res. Mill Hill, London

Seecof RL (1961) Gene dosage and enzyme activities in Drosophila melanogaster. Genetics 46:605-614

Seecof RL, Kaplan WD, Futch DG (1969) Dosage compensation for enzyme activites in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 62:528-535

Shaw CR, Prsad R (1970) Starch gell electrophoresis of enzymes- A compilation of recipes. Biochem. Genet. 4:297-320

Shearn A, Rice, T, Garen A, Gehring W (1971) Imaginal disc abnormalities in lethal mutants of Drosophila. Proc. Natl. Acad. Sci. USA 68:2594-2598

Sinclair DAR, Grigliatti TA, Kaufman TC (1984) Effects of a temperature-sensitive Minute mutation on gene expression in Drosophila melanogaster. Genet. Res., Camb. 43:257-275

Sinclair DAR, Mottus RC, Grigliatti TA (1983) Genes which suppress position-effect variegation in Drosophila melanogaster are clustered. Mol. Gen. Genet. 191:326-333

Sinnott EW, Houghtaling H, Blakeslee AF (1934) The comparative anatomy of extra chromosomal types in Datura stramonium. Carnegie Inst. Wash. Publ. 451

Skripsky T, Lucchesi JC (1982) Intersexuality resulting from the interaction of sex-specific lethal mutations in Drosophila melanogaster. Dev. Biol. 94:153-162

Smith DF, McClelland A, White BN, Addison CF, Glover DM (1981) The molecular cloning of a dispersed set of developmentally regulated genes which encode the major larval serum protein of D. melanogaster. Cell 23:441-449

Smith HH, Conklin ME (1975) Effects of gene dosage on peroxidase isozymes in Datura stramonium trisomics. Isozymes Vol. 3: Developmental Biology, Markert CL ed., Academic Press, New York pp 603-618

Smith PD, Lucchesi JC (1969) The role of sexuality in dosage compensation in Drosophila. Genetics 61:607-618

Sofer W, Ursprung H (1968) Drosophila alcohol dehydrogenase. Purification and partial characterization. J. Biol. Chem. 243:3110-3115

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol 98:503-517

Spofford JB (1976) Position effect variegation in Drosophila. In: Ashburner M, Novitski E (eds) The genetics and biology of Drosophila, vol 1C. Academic Press, New York, pp 955-1018

Spradling AC, Rubin GM (1983) The effect of chromosomal position on th expression of the Drosophila XDH gene. Cell 34:47-57

Stebbins GL (1966) Processes of organic evolution. Prentice Hall, New Jersey

Steinback P, Benz R (1983) Demonstration of gene dosage effects of AK$_3$ and GALT in fibroblasts from a fetus with 9p trisomy. Human Genet. 63:290-291

Stern C (1960) Dosage compensation- Development of a concept and new facts. Can. J. Genet. Cytol. 2:105-118

Stetson, H, Somerville RL (1971) Expression of the tryptophan operon in merodiploids of Escherichia coli. I. Gene dosage gene position, and marker effects. Mol. Gen. Genetics 3:342-351

Stewart B, Merriam J (1973) Segmental aneuploidy of the X chromosome. Dros. Inf. Serv. 50:167-170

Stewart BR, Merriam JR (1975) Regulation of gene activity by dosage compensation at the chromosomal level in Drosophila. Genetics 79:635-647

Strobel E, Pelling C, Arnheim N (1978) Incomplete dosage compensation in an evolving Drosophila sex chromosome. Proc. Natl. Acad. Sci. USA 75:931-935

Suzuki DT, Kaufman T, Falk D and the UBC Drosophila Research Group (1976) Conditionally expressed mutatations in Drosophila melanogaster. In: The Genetics and Biology of Drosophila (eds) Ashburner M, Novitski E, pp 207-263

Tagaki N, Sasaki M (1975) Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. Nature 256:640-642

Thomas PS (1980) Hybridization of dentured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA 77:5201-5205

Timko MP, Vasconselos AC, Fairbrothers DE (1980) Euploidy in Ricinus. I. Euploidy and gene dosage effects on cellular proteins. Biochem. Genet. 18:171-183

Tobler JE (1975) Dosage compensation and ontogenic expression of suppressed and transformed vermilion flies in Drosophila. Biochem. Genet. 13:29-43

Tobler J, Bowman JT, Simmons, JR (1971) Gene modification in Drosophila: dosage compensation and relocated v$^+$ genes.

Biochem. Genet. 5:111-117

Treat-Clemons LG, Doane WW (1984) Biochemical loci of the "Fruit Fly" (Drosophila melanogaster). Dros. Info. Serv. 60:17-42

Tsaftaris AS, Scandalios JG, McMillin DE (1981) Gene dosage effects on catalase expression in maize. J. of Heredity 72:11-14

Twardzik DR, Grell EH, Jacobson KB (1971) Mechanism of supression in Drosophila: A change in tyrosine tyrosine transfer RNA. J. Mol. Biol. 57:231-245

Voelker RA, Langley CH, Brown AJL, Ohnishi S, Dickson B, Montgomery E, Smith SC (1980) Enzyme null alleles in natural populations of Drosophila melanogaster: frequencies in a North Carolina population. Proc. Natl. Acad. Sci. USA 77:1091-1095

Voelker RA, Ohnishi S, Langley CH (1979) Genetic and cytogenetic studies of the malate dehydrogenaes of Drosophila melanogaster. Biochem. Genet. 17:947-956

Voelker RA, Ohnishi S, Langley CH, Gavsz J, Gyurkovics H (1983) Genetic and cytogenetic studies of malic enzyme in Drosophila melanogaster. Biochem. Genet. 19:525-534

Welch DL (1951) On the comparison of several mean values: An alternative approach. Biometrika 38:330-336

Westerveld A, Visser RPLS, Freeke MA, Bootsma D (1972) Evidence for linkage of 3-phosphoglycerate kinase, hypoxanthine-guanine-phosphoribosyl transferase, and glucose-6-phosphate dehydrogenase loci in Chines hamster cells studied by using a relationship between gene multiplicity and enzyme activity. Biochem. Genet. 7:33-40

Whiting PW (1943) Multiple alleles in complementary sex determination of Habrobracon. Genetics 28:365-382

Wijsman HJN, van denBerg BM (1982) Location of structural genes for glucose phosphate isomerase and for leucyl aminopeptidase on chromosome VII of Petunia. Theor. Appl. Genet. 63:283-287

Wilson AC (1976) Gene regulation in evolution. In: Molecular Evolution. (ed) Ayala FJ, Sinauer Assoc. Inc., Sunderland, M.A. pp 225-234

Wilton AN, Laurie-Ahlberrg CC, Emigh TH, Curtsinger JW (1982) Naturally occurring enzyme activity variation in Drosophila melanogaster. II. Relationship among enzymes. Genetics 102:207-221

Woodruff RC, Ashburner M (1979) The genetics of a small autosomal region of Drosophila melanogaster containing the structural gene for alcohol dehydrogenase. I.

Characterization of deficiencies and mapping of Adh and Visible Mutations. Genetics 92:117-132

Wright TRF, (1963) The genetics of an esterase in Drosophila melanogaster. Genetics 48:787-802

Wright TRF, Hodgetts RB, Sherald AF (1976) The genetics of dopa decarboxylase in Drosophila melanogaster. I. Isolation and characterization of deficiencies of the structural locus and the a-methyl dopa hypersensitive locus. Genetics 84:267-285

Wurgler FE, Ruch P, Graf U (1972) X-ray-induced loss of the maternal second chromosome in inseminated eggs of Drosophila melanogaster. Mutation Res. 15:31-40

Zuckerkandl E, (1974) Recherches sur les proprietes et l'activite biologique de la chromatine. Biochimie 56:937-954