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Regulation of enzyme activities in *Drosophila*

I. The detection of regulatory loci by gene dosage responses

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SUMMARY

In order to detect regulatory genetic sites in the autosomes of *Drosophila melanogaster*, the levels of X-linked glucose-6-phosphate dehydrogenase and autosomally linked α-glycerophosphate and isocitrate dehydrogenases have been monitored in extracts of flies aneuploid for regions of chromosomes II and III. In addition to expected structural gene dosage responses of α-GPDH and IDH, flies hyperploid for several autosome regions were found to display altered levels of one or more of the enzymes studied. While IDH activity was increased in flies hyperploid for segments of both chromosomes II and III, α-GPDH activity was decreased in specific hyperploids for chromosome II regions only. The latter group of segmental aneuploids were normal with respect to levels of chromosome II-linked alcohol dehydrogenase. To test if the observed responses were due to dosage changes of discrete genes lying within the larger effective segments, flies aneuploid for subdivisions of the chromosome segments 21A–25CD, 35A–40, and 70CD–71B were assayed. For two of these large segments so analysed, the apparent effects were attributable to specific small subdivisions, suggesting the presence of discrete regulatory sites within the latter. For the 35A–40 region the α-GPDH effect observed for subdivisions was not sufficient to account for the large α-GPDH decrease seen in flies hyperploid for the large, inclusive region. These observations are discussed with respect to the possible bases of effect of regulatory elements on enzyme activity.

I. INTRODUCTION

The genetic analysis of biochemical regulation involves the study of the structural genes of particular enzymes as well as genes described as regulators in that at least one function of their products is the regulation of the enzymes under consideration. Such regulation may occur at any of the several steps in enzyme synthesis or degradation or in the inhibition or activation of enzyme activity (Wyngaarden, 1970). Genetic elucidation of the existence and *modus operandi* of putative regulatory genes usually relies on the recovery of mutants in which the enzyme activity is altered but the genetic lesion maps to a site other than the

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enzyme structural gene; such was the method of identification of the *lac* repressor locus in *Escherichia coli* (Jacob & Monod, 1961). Selection of such mutants can be an efficient process in bacteria and fungi where objective screenings of large numbers of mutagenized genomes is facilitated by prototrophy and rapid generation times. Due to relatively long generation times and complex nutritional requirements, however, the study of gene regulation in complex, multicellular organisms is far less advanced than that of procaryotes and fungi.

For a variety of organisms, duplication or deletion of a segment of the genome containing a given structural gene are known to elevate or depress the levels of the latter's protein product (Grell, 1962, 1969; Ciferri, Sora & Tiboni, 1969; Seecof, Kaplan & Futch, 1969; Nielson & Frydenberg, 1971). By analogy with these structural gene systems, the intracellular concentration of the product of a regulatory gene is probably related to the number of copies of the gene present in the cell. Thus, if the expression of a structural gene is sensitive to the level of regulatory product, the amount of enzyme present in the cell should be affected by changes in dosage of the regulatory gene (Yielding, 1967; Cove, 1969). Therefore, as an alternative to searching for regulatory genes by attempting to mutate them, it should be possible to detect such genes by the effect of their dosage on the activity of enzymes whose structural genes are known and kept constant.

Whereas gene mutations can result in a wide range of phenotypes, varying from extreme hyperactivity of the gene to total abolishment of gene function, gene dosage manipulations are more limited in their potential effect. That is, in comparison to normal diploid levels, one can expect 50% more product in trisomics and 50% less product in monosomics. Therefore, only cases in which enzyme activity levels are sensitive to 50% changes in regulatory gene product will allow detection of enzyme regulatory genes via dosage changes. Furthermore, predictions of the ultimate enzyme response to changing levels of regulator substance must recognize that the regulatory system may involve either enhancement or depression of enzyme activity levels. If enzyme expression is fully proportional to regulatory gene dosage, one can predict that, in any individual trisomic for a regulatory gene, a 50% increase in enzyme levels will result if the regulator serves to enhance enzyme activity, whereas a one-third decrease will result if the regulation is negative in nature.

We have attempted to detect and localize regulatory genes within the genome of *Drosophila melanogaster* by seeking chromosome segments, which when aneuploid, effect changes in the activities of specific enzymes for which structural genes have been mapped elsewhere in the genome. To this end, flies segmentally trisomic for regions encompassing 80% of the euchromatic length of chromosome II and 60% of chromosome III were constructed. The following enzyme activities were measured in these flies: glucose-6-phosphate dehydrogenase (G6PD, E.C.1.1.1.49), α-glycerophosphate dehydrogenase (α-GPDH, E.C. 1.1.1.8), and NADP-dependent isocitrate dehydrogenase (IDH, E.C.1.1.1.42). Presumptive structural genes for these enzymes, detected by allelic electrophoretic variation, are located on the *X* chromosome and chromosomes II and III, respectively (Young, Porter

& Childs, 1964; Grell, 1967; O'Brien & MacIntyre, 1968; Fox, 1971). These enzymes were selected since their known structural gene loci represent each of the three major linkage groups in this species and thus allow observation of the linkage relationships existing between effective aneuploid segments and affected loci.

Significant effects of specific segments were classified into small changes (equal to or less than 10% of the control values) and large changes (equal to or greater than 25% of the control values), and were further characterized. In some instances, effective segments were tested in single dose (segmental monosomy); the level of alcohol dehydrogenase (ADH, E.C. 1.1.1.1) was monitored in a number of segmental trisomics; lastly, further subdivision of some effective segments was attempted by synthesizing and testing flies hyperploid for smaller portions of the segments.

While this paper relates the results of experiments designed to genetically characterize putative regulatory sites, studies describing the biochemical nature of the specific enzyme responses obtained in aneuploid flies are the topic of an accompanying paper (Rawls & Lucchesi, 1974).

2. MATERIALS AND METHODS

A number of enzymes exhibit reduced activity levels in certain portions of the fly. This is particularly true, for example, of maturing oocytes and associated ovarian tissues (Steele, Young & Childs, 1969). In order to avoid the possible bias introduced by any influence of aneuploidy on the relative distribution of ovarian tissues among experimental and control flies, only male flies were studied and compared in the following experiments.

(i) *Genetic stocks and crosses*

Wild-type flies were from a Samarkand strain. Flies segmentally aneuploid for regions of chromosomes II and III were produced using reciprocal and insertional translocations.

A few crosses employed reciprocal translations of chromosomes III and IV, i.e. *T(3;4)*, according to the method of Patterson, Brown & Stone (1940). Males heterozygous for two different *T(3;4)*'s were mated to females homozygous for recessive markers which also marked the two elements of one of the translocations. Among the progeny are flies bearing one (hypoploid), two (control), and three (hyperploid) doses of the chromosome III region delimited by the *T(3;4)* breakpoints. These F_1 types are distinguishable because of recessive markers borne by the translocation fragments.

A series of translocations of autosomes with the *Y* chromosome, synthesized by Lindsley *et al.* (1972), were the rearrangements most commonly used for the production of aneuploid flies. Matings in which both parents were heterozygous for different reciprocal *T(Y;A)*'s yielded flies hypoploid, euploid or hyperploid for the autosome segment delimited by the breakpoints of the two translocations.

Flies heterozygous for reciprocal $T(Y;A)$'s were mated to non-translocated individuals to produce sons hyperploid and euploid for terminal autosome segments.

All reciprocal $T(Y;A)$'s have their Y centromere-bearing fragment marked by either of the dominant markers y^+ or B^S while the autosome centromere-bearing element is reciprocally marked; the phenotypic distinction of euploid and aneuploid F_1 males is, therefore, possible when two parental translocations bear opposite markers. This was the case in all crosses except for the cross yielding hyperploids for the 66B-67C segment where differential marking of balancer chromosomes was used to identify the various progeny types. Since the fidelity of the marker systems used to distinguish aneuploid and control flies is dependent upon regular segregation of homologous centromeres, the latter was investigated by mating translocation flies with normal flies. The data demonstrated that the predominant segregation patterns in $T(Y;A)$ heterozygotes are alternate and adjacent-1, while only a negligible fraction of the total gametes result from adjacent-2 or other meiotic patterns. These conclusions are concordant with those of Lindsley *et al.* (1972).

Flies aneuploid or euploid for interstitial autosome segments were sometimes produced by mating males heterozygous for insertional $T(Y;A)$'s to either normal females or females whose X chromosomes were attached to a single kinetochore ($X{\cdot}X$). The presence of the autosomal segment was detected by the masking of appropriate recessive mutations.

(ii) *Fly culture and sample collection procedures*

Flies were cultured at 25° in uncrowded half-pint bottles on medium containing cornmeal, molasses, brewer's yeast and agar, tegosept-M and proprionic acid to retard mould growth, and supplemented with live yeast. Adults were collected from cultures at 3-day intervals and aged an additional 2 days on standard medium less live yeast; therefore, all samples homogenized for enzyme assays consisted of mixtures of adults ranging in age from 2 to 5 days post-emergence.

(iii) *Extract preparation*

For the extraction of enzyme from adult samples, 35–150 mg of lightly etherized flies were counted, weighed, and ground in Dounce glass tissue grinders fitted with loose pestles. For samples to be assayed for G6PD, reverse α-GPDH, and IDH activities, adults were homogenized in glass-distilled water at a ratio of 20 mg live weight per ml. Samples to be assayed for forward α-GPDH and ADH activities were ground at 10 mg of adults per ml of 0·1 M potassium phosphate buffer (pH 7·1). Twenty minutes after homogenization, all samples were centrifuged for 40 min at 12000g in a Sorvall RC2-B refrigerated centrifuge. The resultant clear supernatant was drawn and used as an enzyme source. All preparative steps were carried out at 0–5°.

(iv) *Enzyme assays*

Dehydrogenase activities were monitored as described by Lucchesi & Rawls (1973). Changes in NADH or NADPH levels were observed spectrophotometrically at 340 nm. One unit of enzyme activity is an amount of enzyme reducing one μmole of NAD^+ or $NADP^+$ (or oxidizing one μmole of NADH) per minute at 29°.

Substrate solutions used in the assay of G6PD, reverse α-GPDH, and IDH are given in Lucchesi & Rawls (1973). For the forward α-GPDH reaction (i.e. reduction of dihydroxyacetone phosphate), the assay solution was 0·1 M potassium phosphate, pH 6·5, 1·0 mM dihydroxyacetone phosphate, and 0·20 mM NADH. ADH was assayed using the substrate systems of Jacobson, Murphy & Hartman (1970), that is, 0·1 M glycine-NaOH, pH 9·5, 0·13 M sec-butyl alcohol, and 1·0 mM NAD^+.

Statistical analysis of enzyme activity values was performed by comparing each aneuploid mean and its standard deviation to the mean and standard deviation values of its control using Student's *t* test.

(v) *Protein determinations*

Protein determinations were performed on 0·05 ml aliquots of fly extract using the procedure of Lowry *et al.* (1951). Bovine serum albumin served as a standard.

3. RESULTS

When reciprocal translocations are used to produce flies aneuploid for regions of chromosome II and III, the aneuploid genome is also duplicated or deficient for chromosome IV or Y segments. To assess possible effects of imbalance of these chromosomes on the activities of enzymes used, flies carrying varying numbers of

Table 1. *Enzyme activities in flies aneuploid for chromosome IV and the Y chromosome*

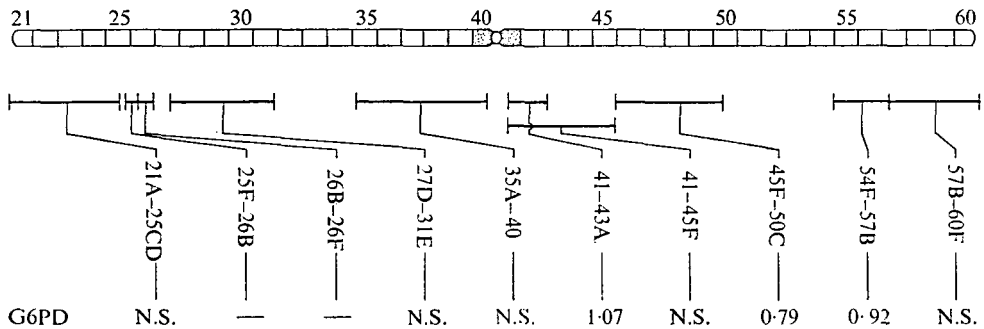
Experiment	Genotype	Enzyme activities: G6PD	Enzyme activities: α-GPDH (reverse reaction)	Enzyme activities: IDH	*N*
A. Chromosome IV aneuploids†					
	diplo-IV ($ci^D/+$)	12·5 ± 0·4	187 ± 6	37·9 ± 1·1	6
	triplo-IV ($C(4)RM/+$)	12·8 ± 0·1	206 ± 5**	35·9 ± 0·2	5
B. *Y* chromosome aneuploids‡					
	haplo-Y ($X{\cdot}Y/O$)	9·7 ± 0·4	129 ± 4	34·7 ± 0·8	6
	diplo-Y ($X{\cdot}Y/y^+Y^{BS}$)	11·2 ± 0·3**	143 ± 4*	33·5 ± 1·0	6

Values are expressed as mean units per mg protein × 10^2 ± S.E. *N* is the number of separate determinations per mean value. Asterisks denote hyperploid values differing significantly from the control value at the 5% (*) and 1% (**) levels of significance.

† Produced by mating $C(4)RM/ci^D$ males to wild-type females.

‡ Produced by mating homozygous $Y^SX{\cdot}Y^L$, *In(1)EN*, *y* females to either $Y^SX{\cdot}Y^L$, *In(1)EN*, *y*/*O* males or to $Y^SX{\cdot}Y^L$, *In(1)EN*, y/y^+Y^{BS} males.

these chromosomes were constructed, and the results are presented in Table 1. Flies disomic and trisomic for chromosome IV, but bearing otherwise diploid male genomes, did not differ with respect to G6PD and IDH activities; flies with three copies of chromosome IV displayed a significantly higher α-GPDH level of small magnitude (10 % increase). Poor viability of haplo-IV flies precluded measurement of their enzyme levels. Males having an extra Y chromosome exhibited increased



	21A–25CD	25F–26B	26B–26F	27D–31E	35A–40	41–43A	41–45F	45F–50C	54F–57B	57B–60F
G6PD	N.S.	—	—	N.S.	N.S.	1·07	N.S.	0·79	0·92	N.S.
α-GPDH	0·66	1·41	N.S.	0·75	0·73	0·89	0·79	N.S.	N.S.	0·70
IDH	N.S.	—	—	1·27	1·08	1·17	N.S.	N.S.	N.S.	N.S.

Fig. 1. Chromosome II regions studied. Region designations (i.e. 21 through 60) refer to the cytogenetic map of Bridges (1935). N.S. denotes trisomic and control fly mean enzyme activities not differing significantly ($P > 0{\cdot}05$) while significantly different values are listed as the ratio of trisomic fly activity to control fly activity.

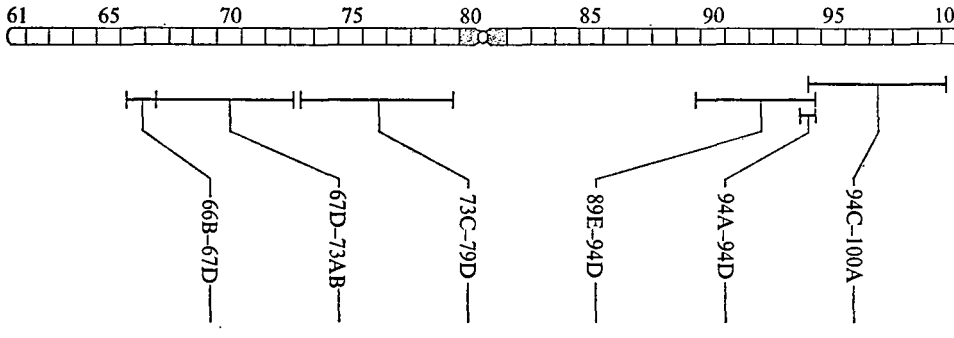
G6PD (15 % increase) and α-GPDH (11 % increase) activities, while IDH was unaffected ($P > 0{\cdot}3$). Note that the flies of experiments A and B in Table 1 differed by the presence or absence of an entire chromosome IV or Y and that the inequality with respect to the amount of these chromosomes present in the aneuploid and control flies used in this study is much smaller. Furthermore, as will be documented below, numerous autosomal aneuploids containing imbalances in their Y chromosome constitutions altogether fail to show any enzyme differences. For these reasons the effects of chromosome IV and Y aneuploidy will be disregarded in evaluating the properties of chromosome II and III aneuploids.

Viability is typically decreased in proportion to size of the aneuploid segment (Lindsley *et al.* 1972; Patterson *et al.* 1940). The aneuploid flies described in Figs. 1 and 2 varied extensively in viability, measured as the ratio of surviving adult aneuploid males to euploid males in each cross: 0·02 for 94C-100A hyperploids; 0·13 for 45F–50C hyperploids; over 0·25 for all others.

In the initial stages of this research, hyperploids for each of sixteen segments of the *D. melanogaster* genome were produced and assayed for G6PD, α-GPDH, and IDH activities. These segments collectively include about 80 and 60 % of the

euchromatic lengths of chromosome II and III, respectively, as measured on the salivary gland chromosome map of Bridges (1935). The sizes and locations of these segments as well as the departures of hyperploid enzyme levels from their controls are presented in Figs. 1 and 2.

Twelve of the sixteen hyperploids studied were found to display significantly ($P < 0{\cdot}05$) altered activity of at least one of the three enzymes monitored. Among these, two hyperploids (54F–57B and 94C–100A) exhibited responses varying by less than 10 % from control levels; these were not studied further and will not be considered beyond this point. Table 2 contains the specific activity values derived for the remaining ten hyperploid genotypes.



	66B–67D	67D–73AB	73C–79D	89E–94D	94A–94D	94C–100A
G6PD	—	N.S.	N.S.	N.S.	N.S.	1·08
α-GPDH	—	N.S.	N.S.	N.S.	N.S.	N.S.
IDH	1·53	1·26	N.S.	N.S.	N.S.	N.S.

Fig. 2. Chromosome III regions studied. Region designations (i.e. 61 through 100) refer to the cytogenetic map of Bridges (1935). N.S. denotes trisomic and control fly mean enzyme activities not differing significantly ($P > 0{\cdot}05$) while significantly different values are listed as the ratio of trisomic fly activity to control fly activity.

Two of the genotypes studied contained three doses of segments bearing a known structural gene for one of the monitored enzymes. A structural gene for α-GPDH, *Gdh*$^+$, has been localized within the chromosome II segment 25E–26B by Grell (1967). In the present study, aneuploids of the 25F–26B region displayed clear proportionality between *Gdh*$^+$ dose and reverse α-GPDH levels (Table 2). Flies hyperploid for the adjacent region, 26B–26F, give no α-GPDH response, thus aneuploidy of chromosome II segments *per se* does not affect α-GPDH levels. The other structural gene is for the enzyme IDH. On the basis of 149 chromosomes recombinant for the genetic interval between the chromosome III recessive markers *h* (3–26·5) and *th* (3–43·2), Fox (1971) placed the *Idh-NADP* (3–27·1) locus near and to the right of *h* since only five of 149 chromosomes recombinant for the *h* (3–26·5) and *th* (3–43·2) markers were also recombinant for *h* and *Idh-NADP*.

In the current study, triplication of either of the segments delimited cytogenetically as 66B–67C or 70CD–71B produced increases in IDH activity. Although both segments are in the *h* to *th* interval, by virtue of the relative locations of *h*, *th*, 66B–67C, and 70CD–71B on the cytogenetic map (Fig. 3), it is concluded that the

Table 2. *Aneuploid synthesis and enzyme levels*

Aneuploid segment	Parental translocations ♀♀	Parental translocations ♂♂	Enzyme activities: G6PD	Enzyme activities: α-GPDH (reverse reaction)	Enzyme activities: IDH	*N*
21A–25CD	*T(Y;2)D6*	+	9·0 ± 0·5	105 ± 3**	31·5 ± 0·9	6
			(9·8 ± 0·3)	(159 ± 2)	(30·5 ± 0·8)	6
25F–26B	*T(Y;2)D106*	*T(Y;2)G105*	—	189 ± 4**	—	—
				(133 ± 8)		
	T(Y;2)G105	*T(Y;2)D106*	—	60 ± 2†**	—	—
				(180 ± 5)		
27D–31E	+	*T(Y;2)B231*	12·6 ± 0·4	105 ± 4**	36·9 ± 0·8**	5
			(11·3 ± 0·4)	(140 ± 5)	(29·1 ± 0·5)	6
35A–40	+	*T(Y;2)J54*	11·4 ± 0·3	107 ± 2**	26·9 ± 0·5*	6
			(11·1 ± 0·2)	(148 ± 4)	(25·0 ± 0·4)	6
41–45F	*T(Y;2)B177*	*T(Y;2)L23*	9·1 ± 0·2	102 ± 2**	35·3 ± 0·8	6
			(8·6 ± 0·2)	(129 ± 4)	(33·4 ± 0·7)	6
41–43A	+	*T(Y;2)L12*	11·2 ± 0·2*	135 ± 2**	31·7 ± 0·8**	6
			(10·5 ± 0·2)	(152 ± 2)	(27·0 ± 0·4)	6
			13·9 ± 0·3†	206 ± 6†**	38·8 ± 1·1†**	6
			(16·6 ± 0·2)	(186 ± 4)	(44·0 ± 1·2)	6
45F–50C	*T(Y;2)L23*	*T(Y;2)L110*	6·5 ± 0·4**	122 ± 1	35·2 ± 0·6	3
			(8·3 ± 0·4)	(130 ± 4)	(32·9 ± 0·4)	3
57B–60F	*T(Y;2)L107*	+	9·7 ± 0·4	106 ± 2**	34·5 ± 0·6	5
			(10·0 ± 0·3)	(152 ± 3)	(35·2 ± 0·7)	5
66B–67C	*T(Y;3)G122*	*T(Y;3)J94*	—	—	43·8 ± 2·9**	6
					(28·6 ± 1·6)	6
67D–73AB	*T(Y;3)B96*	*T(Y;3)J150*	15·3 ± 0·3	191 ± 7	43·3 ± 1·1**	4
			(16·2 ± 0·5)	(212 ± 11)	(34·4 ± 1·9)	4

All translocations are described by Lindsley *et al.* (1972). Enzyme activities are expressed as mean units per mg protein × 10^2 ± S.E. Control values (from euploid sibs) are in parentheses. *N* is the number of separate determinations per mean value. Asterisks denote aneuploid values differing significantly from the control value at the 5% (*) and 1% (**) levels of significance.

† Hypoploid value; all other aneuploids are hyperploid.

IDH structural gene described by Fox (1971) lies within the 66B–67C segment and that the IDH increase accompanying three doses of the latter represents a structural gene dosage response. Steward & Merriam (1972) have also reported an increase in IDH in flies containing three doses of the 66B–67C segment.

The presence of an additional structural gene for Idh-NADP in segment 67D–73AB cannot be ruled out since this region's only effect in aneuploids was an

alteration of IDH activity. This segment was genetically dissected and the effective region delimited as 70CD–71B (Fig. 3). Flies hyperploid for this small segment showed a 38 % increase in IDH levels while hypoploids (segmental monosomics) displayed a reduction in this enzyme activity. The failure of these flies to display levels of IDH precisely proportional to the dosage of the aneuploid segment is not inconsistent with the presence, therein, of a structural gene; as long, as its product is not rate-limiting in the process of assembling the enzyme.

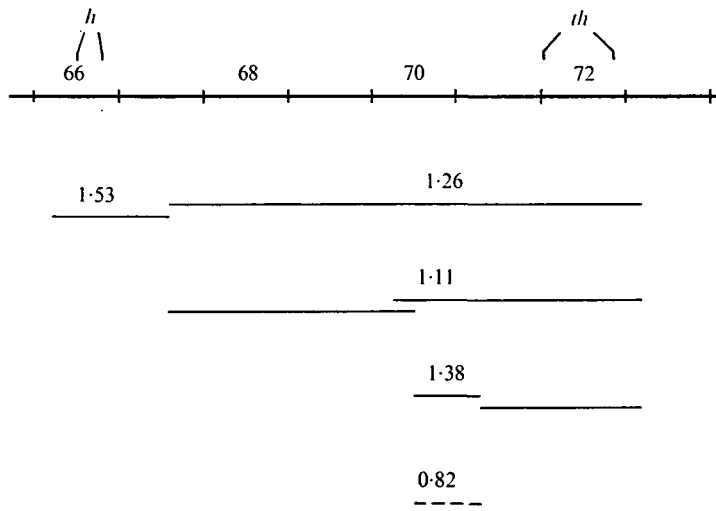


Fig. 3. Enzyme activity changes in flies aneuploid for subdivisions of the 67D–73AB region. Neither G6PD nor α-GPDH were altered in any of these aneuploids. Segmentally aneuploid flies displaying significantly ($P \leqslant 0{\cdot}05$) deviant IDH levels are represented by the ratio of aneuploid fly enzyme activity to control fly activity. Dashed line represents monosomic genotype while all other aneuploids were trisomic.

The results of hyperploidy for the remaining seven autosomal segments, all located in chromosome II, will be considered individually.

(i) *21A–25CD*

In flies hyperploid for this chromosome II segment, forward and reverse α-GPDH activity were markedly reduced (by approximately 30 %) while chromosome II-linked ADH was not affected (Tables 2 and 3).

Flies hyperploid for two subdivisions of the region in question, 21A–21D and 22D–25CD, were significantly decreased in α-GPDH activity (Fig. 4). The magnitude of the effects exerted by these two subdivisions collectively approximate the α-GPDH decrease in 21A–25CD hyperploids. Although the latter failed to exhibit altered G6PD of IDH levels, flies hyperploid for the 21A–21D subdivision displayed reduced levels of both enzymes.

(ii) *35A–40*

Flies trisomic for this region had clearly depressed α-GPDH levels (by approximately 27 %) when either the reverse (Table 2) or forward (Table 3) reaction was observed. ADH levels were normal.

Table 3. *Additional enzyme activities in males hyperploid for chromosome II segments*

Aneuploid segment	Genotype	Forward α-GPDH	ADH
21A–25CD	Hyperploid	2·71 ± 0·08 (4)*	0·99 ± 0·08 (4)
	Control	4·31 ± 0·10 (4)	0·87 ± 0·07 (4)
27D–21E	Hyperploid	2·86 × 0·12 (3)*	0·97 ± 0·04 (3)
	Control	3·92 ± 0·12 (3)	0·99 ± 0·06 (3)
35A–40	Hyperploid	2·91 ± 0·09 (3)*	0·99 ± 0·04 (3)
	Control	4·06 ± 0·05 (3)	1·03 ± 0·01 (3)
41–45F	Hyperploid	2·82 ± 0·14 (7)*	0·70 ± 0·04 (6)
	Control	3·72 ± 0·24 (7)	0·80 ± 0·04 (6)
57B–60F	Hyperploid	2·77 ± 0·05 (3)*	0·69 ± 0·01 (3)
	Control	3·65 ± 0·12 (3)	0·76 ± 0·06 (3)

Enzyme activities are expressed as mean units per mg protein ± S.E.
The number of determinations is in parentheses.
* Hyperploid values differing from their control values at the 1 % level of significance.

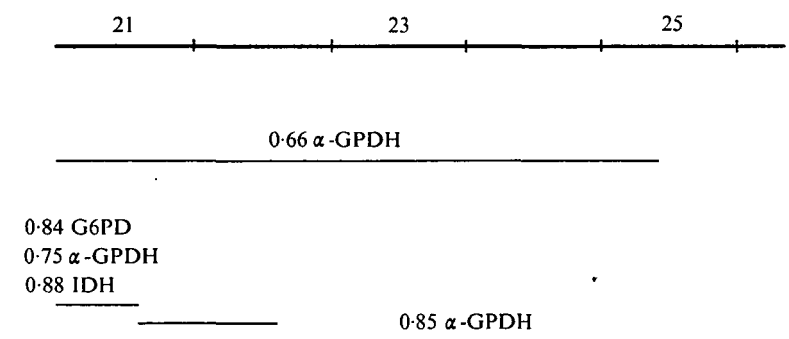


Fig. 4. Enzyme activity changes in flies trisomic for subdivisions of the 21A–25CD region. G6PD, α-GPDH, and IDH activities were monitored for each aneuploid segment and only significantly deviant enzyme changes ($P \leqslant 0{\cdot}05$) are listed as the ratio of aneuploid fly activity to control fly activity.

Dissection analysis of this region failed to implicate a single site in the α-GPDH response since the α-GPDH levels of flies hyperploid for regions 34B–37D, 36C–39C, and 37D–40 did not display sufficient deviations from controls to account for the large decrease seen in 35–40 hyperploids. It should be pointed out that these results do not eliminate the possibility that an effective site lies in the centric heterochromatin of chromosome II since the 35A–40 duplication may contain more of this heterochromatin than does the particular 37D–40 segment tested.

35A–40 hyperploids were increased in IDH activity but this increase, although statistically significant was very small and is disregarded.

(iii) *57B–60F*

Flies trisomic for this region displayed reduced α-GPDH levels (by approximately 25 and 30% for the forward and reverse reaction, respectively) but unaltered G6PD, IDH, and ADH activities (Tables 2 and 3).

(iv) *41–45F and 41–43A*

Hyperploids for the 41–45F region displayed reduced α-GPDH (by approximately 24 and 20% for the forward and reverse reaction, respectively) but G6PD, IDH, and ADH levels were no different from control values (Tables 2 and 3). Flies trisomic for a subdivision of this region (i.e. 41–43A) also displayed reduced α-GPDH levels but G6PD and IDH levels were simultaneously elevated in these flies (Fig. 1). The appearance of novel enzymatic changes in the 41–43A subdivision may indicate the presence of dosage sensitive sites in the centric heterochromatin of chromosome II since the relative positions of the proximal limits of the 41–43A and 41–45F duplications are unknown.

(v) *27D–31E*

Flies trisomic for this segment displayed a 25% decrease in forward and reverse α-GPDH levels while ADH activities were normal (Tables 2 and 3). IDH activity was clearly increased in these flies (by approximately 27%) while G6PD levels were normal.

(vi) *45F–50C*

Although α-GPDH and IDH levels in flies trisomic for this region were normal, a sizeable decrease (by approximately 20%) in G6PD activity was evident. The viability of this genotype was only 0·13 but the size and body weight of trisomics was not different from that of controls; therefore, changes in tissue distributions are not suggested to account for the observed effect.

4. DISCUSSION

The usefulness of segmental aneuploidy as a probe for genes involved in enzyme regulation is limited by the resolution with which the enzymatic changes can be described. The current study has shown that, in *Drosophila*, aneuploidy for specific chromosome segments evokes characteristic modifications in the whole-fly activities of particular enzymes. Flies trisomic for five chromosome segments – 21A–25CD, 27D–31E, 35A–40, 57B–60F, 70CD–71B – exhibited changes in enzyme levels equal to or greater than 25% and are of primary interest. The following discussion is aimed toward distinguishing indirect effects on apparent enzyme activity from direct regulatory effects and suggesting possible mechanisms mediating aneuploidy-induced changes.

That the results encountered in aneuploids of chromosomes II and III in this study are not due to imbalance of chromosome IV and Y material has been previously argued. Further support for this contention and the demonstration that autosomal aneuploidy *per se* does not give rise to enzymatic changes are provided by the observed failures of some large chromosome III regions to have any effects on the enzymes studied. In addition, for a given enzyme, the majority of the aneuploid segments tested had no apparent effects.

IDH is unevenly distributed among various adult organs in *D. melanogaster* and the bulk of its activity resides in the abdomen (Fox, Conscience-Egli & Abücherli, 1972), whereas adult α-GPDH activity is most concentrated in the thorax (Rechsteiner, 1970). Because of the uneven distribution of these enzymes in normal flies, tissue derangements in aneuploid flies could give rise to some of the significant enzyme activity changes evident in Table 2. It seems unlikely, however, that large (greater than 20 %) changes in enzyme levels can be explained in this manner since there is no evidence for massive changes in the amounts of various tissues in aneuploids.

It appears that the inviability caused by trisomy for a large chromosome region is usually due to the additive effects of the duplication of many genes which are each contributing slight deleterious effects (Lindsley *et al.* 1972). If the enzyme responses of flies aneuploid for relatively large autosome regions were also due to such additive effects, these responses should disappear when small subdivisions of the regions are tested. This seems to be the case for the 35A–40 region's influence on α-GPDH levels. On the other hand, changes in the level of the same enzyme in 21A–25CD hyperploids (Fig. 4) and of IDH in 67D–73AB hyperploids (Fig. 3) more likely result from changes in the dosage of a few or a single site within those segments.

Some changes in enzyme quantity may not reflect enzyme regulation, since the sensitivity of an enzyme activity to dosage of an autosomal segment may indicate the presence within that segment of a structural gene for the enzyme. Such responses have, in fact, been used for the localization of structural gene loci (Carlson, 1972; O'Brien & Gethmann, 1973). Although the duplication and deletion of known structural gene loci was avoided in the present study (except in specific instances of structural gene dosage studies), some aneuploid enzyme responses could represent dosage changes of previously unknown structural genes. A structural gene dosage response should involve an increase in the enzyme levels of duplication-bearing flies. Since, with the exception of aneuploids for the known α-GPDH structural gene locus, all hyperploids with altered α-GPDH activity exhibited a decrease in activity, none of these autosome segments appear to contain an α-GPDH structural gene. Several hyperploid flies, however, displayed elevated IDH levels and could be expressing structural gene dosage responses. The failure of flies aneuploid for 27D–31E, 41–43A and 70CD–71B to display levels of IDH directly proportional to dose of the aneuploid segment (i.e. increased by approximately 50 %) is not inconsistent with the presence of structural genes therein since 50 % changes in activity are expected only for trisomy of loci pro-

ducing an element that is rate-limiting in the assembly of the enzyme. The 53% IDH increase of 66B–67C hyperploids implies that the rate-limiting element in IDH activity is the product of the *Idh–NADP*$^+$ locus. It is unlikely that all of the chromosome segments that affected IDH levels contain structural genes for that enzyme. We therefore feel it is reasonable to proceed on the assumption that most enzyme responses produced in segmental aneuploids are of a regulatory nature and do not represent structural gene dosage responses.

Since regulation may occur by increasing or decreasing enzyme levels, the effect of a regulatory product which normally exists at sub-saturating concentrations on a structural gene's activity could be either directly or inversely proportional to the dosage of the regulatory gene which produces it. Such a relationship predicts maximum responses consisting of a 33% decrease or a 50% increase in enzyme levels in flies trisomic for the regulatory locus. No hyperploid flies studied displayed enzyme alterations outside of these limits and enzyme changes intermediate to these limits might result from lack of strict proportionality between enzyme expression and regulatory product concentration.

An interesting relationship is brought out by comparing the location of known structural genes for the enzymes studied with the locations of effective aneuploid segments. It is apparent that while IDH activity was altered in flies aneuploid for regions of both autosomes, a linkage relationship exists between the α-GPDH structural gene, *Gdh*$^+$, and the autosome segments that were found to influence α-GPDH activity levels (Figs. 1 and 2). While most autosome segments studied are on chromosome II, enough of chromosome III was studied to suggest that a real disparity exists between the number of sites on these two autosomes that affect α-GPDH and that most dosage-sensitive loci regulating α-GPDH activity are in the same linkage group as the *Gdh*$^+$ locus. That the effective chromosome II sites do not regulate the activities of all enzymes linked to that chromosome was shown by the failure of ADH to respond in aneuploids displaying altered α-GPDH levels.

It is also noteworthy that the response to hyperploidy of effective segments always entailed a decrease in α-GPDH levels while the IDH response was always an increase in activity. This may reflect the positive or negative nature of the regulatory systems controlling the activities of these particular enzymes.

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