A CYTOGENETIC ANALYSIS OF THE CHROMOSOMAL REGION SURROUNDING THE α-GLYCEROPHOSPHATE DEHYDROGENASE LOCUS OF *DROSOPHILA MELANOGASTER*

MICHAEL A. KOTARSKI, SALLY PICKERT AND ROSS J. MACINTYRE

Section of Genetics and Development, Cornell University, Ithaca, New York 14853

Manuscript received April 12, 1982
Revised copy accepted June 9, 1983

ABSTRACT

The chromosomal region surrounding the structural gene for α-glycerophosphate dehydrogenase (*αGpdh*, 2-20.5) of *Drosophila melanogaster* has been studied in detail. Forty-three EMS-induced recessive lethal mutations and five previously identified visible mutations have been localized within the 25A-27D region of chromosome 2 by deficiency mapping and in some cases by a recombination analysis. The 43 lethal mutations specify 17 lethal loci. *αGpdh* has been localized to a single polytene chromosome band, 25F5, and there apparently are no lethals that map to the *αGpdh* locus.

RECENTLY much effort has been directed in Drosophila to the genetic analysis of elements that are linked to structural genes and influence their expression (CHOVNICK *et al.* 1976; CHOVNICK, GELBART and MCCARRON 1977; GELBART, MCCARRON and CHOVNICK 1976; THOMPSON, ASHBURNER and WOODRUFF 1977). To date, however, these efforts have been directed toward relatively few loci. To determine whether the findings from these few loci are of general significance, we have begun a genetic fine structure analysis of the gene encoding the enzyme, α-glycerophosphate dehydrogenase in *Drosophila melanogaster* (αGPDH: EC 1.1.1.8). Our overall goal is to dissect the *αGpdh* locus in order to elucidate both its structural organization and its mechanism of control. Nonstructural genetic elements that contribute to the control of αGPDH expression will be revealed through a combination of biochemical methods and a genetic fine structure analysis.

A considerable amount is known about this Drosophila protein. αGPDH is abundant in the fly comprising 2% of the protein synthesized in newly eclosed adults (D. T. SULLIVAN, personal communication; M. KOTARSKI, unpublished observation), and it has been purified to homogeneity (COLLIER, SULLIVAN and MACINTYRE 1976). The active enzyme is a homodimer with a subunit molecular weight of 32,000 daltons, and its primary structure is presently being elucidated (G. CHAMBERS, personal communication). Of particular significance is the role of αGPDH in the energy metabolism of the insect flight muscle. Mutants of *αGpdh* with less than a threshold amount of enzymatic activity are unable to fly (O'BRIEN and MACINTYRE 1972).

We have examined in detail a 20-band region of the left arm of chromosome 2 (25E-26A) that contains the structural gene for αGPDH. In this study we have used the available visible mutations, numerous ethyl methanesulfonate (EMS)-induced lethal mutations and several stable deficiencies constructed from Y-autosome translocations (LINDSLEY *et al.* 1972). The lethal mutations were produced as part of a search for loci closely linked to *αGpdh* which will be used to select for recombinants within the locus. In this paper we present our findings on the genetic structure of the region immediately surrounding the structural gene for αGPDH.

MATERIALS AND METHODS

Culture conditions: All stocks were maintained at 25° on a standard medium of cornmeal, molasses, yeast and agar in which tegosept was added as a mold inhibitor. Some crosses were performed at 27° to enhance the expression of Curly.

Stocks: The following is a description of the chromosomes used in this study. Descriptions of all visible mutations can be found in the work of LINDSLEY and GRELL (1968).

(1) Balancer chromosomes: Second chromosomes were balanced using *In(2LR)O, Cy* dp^{lvl} *pr* cn^2 or *In(2LR)SM1,* al^2 *Cy* dp^{lvl} *pr Bl* cn^2 L^4 or *In(2LR)SM5,* al^2 *Cy* lt^v cn^2 sp^2 or *In(2L)Cy, Cy* dp^{lv2} *b pr.* The *In(2LR)O* balancer carries the $\alpha Gpdh^A$ (fast) electrophoretic allele and will be abbreviated as "*CyO*" throughout.

(2) Interstitial deficiencies: *Df(2L)GdhA* is an X-ray-induced deficiency for clot and *αGpdh* (GRELL 1967). This chromosome also carries a dp^{ov} allele.

Df(2L)cl-1, cn bw; *Df(2L)cl-2, cn bw* and *Df(2L)cl-7, b pr cn bw* are X-ray-induced deficiencies of clot (VELISSARIOU and ASHBURNER 1980).

Df(2L)50075a and *Df(2L)50078a* were kindly provided by ROBERT VOELKER. They are γ ray-induced deficiencies and were recovered as *αGPDH* null activity mutants (RACINE, LANGLEY and VOELKER 1980).

Df(2L)2802 was recovered in this study as a lethal following EMS mutagenesis of a *cl* $Gpdh^B$ *pr* chromosome.

(3) Other chromosomes: Chromosomes that carry the $\alpha Gpdh^B$ (slow) electrophoretic allele include: *cl* $\alpha Gpdh^B$ *pr*, $\alpha Gpdh^B$ spd^{lfg} *pr*, $\alpha Gpdh^B$ spd^{lfg} and *cl* $\alpha Gpdh^B$ spd^{lfg}.

Chromosomes synthesized carrying the $\alpha Gpdh^A$ (fast) allele include: *cl* $\alpha Gpdh^A$, $\alpha Gpdh^A$ spd^{lfg} and *cl* $\alpha Gpdh^A$ spd^{lfg}.

(4) *Y;2* translocations: The *Y;2* translocations with autosomal break points in the region 25A-27D that were used in this study include: *T(Y;2)J96*; *T(Y;2)D6*; *T(Y;2)P51*; *T(Y;2)B137*; *T(Y;2)B236*; *T(Y;2)H164*; *T(Y;2)G105*; *T(Y;2)H151*; *T(Y;2)D222*; *T(Y;2)H69*; *T(Y;2)D211*; *T(Y;2)J70*; *T(Y;2)J136* and *T(Y;2)A171* (LINDSLEY *et al.* 1972).

(5) Synthetic deficiencies: In addition to the interstitial deficiencies mentioned, we constructed stable translocation deficiency stocks, abbreviated here as *Tdf* translocations. These were made by combining different *Y;2* translocation elements as described by PYE, KNIPPLE and MACINTYRE (1980). The *TDf(Y;2)* stocks used include: *TDf(Y;2)B137-B236; TDf(Y;2)B236-B222*; *TDf(Y;2)H164-H151*; *TDf(Y;2)G105-D222*; *TDf(Y;2)H151-D222*; *TDf(Y;2)D222-H69* and *TDf(Y;2)H69-D211*. Additional regions were made deficient by crossing *T(Y;2)* stocks differing in their second chromosome break points.

Production of recessive lethal mutations: Lethals within the chromosomal region defined by *Df(2L)GdhA* were produced as follows: Males homozygous for the *cl* $\alpha Gpdh^B$ *pr* or the $\alpha Gpdh^B$ spd^{lfg} *pr* chromosome were starved on agar for 24 hr, then fed 0.03 M EMS according to the procedure of LEWIS and BACHER (1968). The males were mated *en masse* to *CyO, pr/Df(2L)GdhA* dp^{ov} females, and individual F_1 Curly, purple males were backcrossed to *CyO, pr/Df(2L)GdhA* dp^{ov} females. Matings that produced only Curly offspring were taken as putative lethals within the deficiency. Stocks were initiated using the *CyO, pr/cl* $\alpha Gpdh^B$ *pr* or the *CyO, pr/*$\alpha Gpdh^B$ spd^{lfg} *pr* sibs. Once in stock, lethality was verified by crossing *CyO, pr/cl* $\alpha Gpdh^B$ *pr* or *CyO, pr/*$\alpha Gpdh^B$ spd^{lfg} *pr* flies again

to the *CyO, pr/Df(2L)GdhA* stock. Only the stocks that produced no Curly$^+$ F_1 progeny in such a cross were kept.

Lethals in the region delineated by *Df(2L)cl-7* were produced in a similar manner. Males of the genotype *CyO, pr/αGpdh^B l(2)gdhA-2 spd^{fg} pr* were fed EMS and mated *en masse* to *CyO, pr/Df(2L)cl-7, b pr cn bw* females (see following data for a description of *l(2)gdh-2*). Single F_1 Curly, purple males were backcrossed to *CyO, pr/Df(2L)cl-7, b pr cn bw* females. Stocks containing a recessive lethal balanced by *CyO* were recovered from vials that produced only Curly offspring. Flies from the putative lethal stocks were crossed once again to *CyO, pr/Df(2L)cl-7* flies to verify the lethality.

Once the recessive lethals had been assigned to complementation groups, the lethal loci represented by these groups were numbered consecutively. The lethal loci identified in this study are labeled as *l(2)gdh-1* through *l(2)gdh-17*. Only lethal mutations of independent origin, *i.e.*, those that came from different sets of mutagenized males, were used in this study.

Criteria for noncomplementation: Complementation tests consisted simply of crossing *CyO/l* or *CyO/Df* flies from different stocks. In all complementation crosses, chromosomes were judged complementary (a "+" in the tables) if the number of viable heterozygous progeny exceeded one-half the expected value of 33.3%. Less than complete complementation is indicated in the tables by the fraction of the progeny that were heterozygotes for different lethal alleles. Only some of the data from all of the complementation tests were reported here. The complete data set can be found in the work of KOTARSKI (1982).

Cytology: Crosses that produced third instar larvae for cytological observation were made at 16–18° on media consisting of 10% glucose (or fructose), 10% dried Brewer's yeast and 1% agar. Salivary gland material was prepared for observation according to the method of STRICKBERGER (1959), with the following modification: Immediately prior to squashing, a drop of 2% orcein-0.25% carmine stain in 50% propionic acid was added to the dissected gland. Canton-S was used as the standard wild type in all crosses.

αGPDH electrophoresis: αGPDH was visualized after electrophoresis on cellulose acetate membranes according to the procedure of COLLIER and MACINTYRE (1972)

RESULTS

Cytology

Figure 1 diagrams the chromosomal aberrations used in this study, and Table 1 lists the break points that we, or others, have determined. Most of the break points of the chromosome aberrations in this study are located in or near the 25D-26B region of the second chromosome. We note parenthetically that, since this area is at the base of a large puff (25A-25E), cytological observation can be difficult at times. This is especially true of very small deficiencies in the 25E-F area that cause little or no asynapsis of the homologs. On the other hand, we have identified all of the bands reported by BRIDGES (1942) in the 25D-26B region.

Deficiency complementation

Table 2 presents the results of crosses between the interstitial deficiencies and the *TDf(Y;2)* stocks. The translocation deficiency, J70-J136, is not included in Table 2, since *TDf(Y;2)J70-J136* males are sterile despite the fact that they carry the $Y^SX{\cdot}Y^L$, *In(1)EN, y* chromosome. Crosses involving strains with break points in this region are also complicated by the sterility of $Y^SX{\cdot}Y^L$, *In(1)EN, y/T(Y;2)J70/Df(2L)GdhA* males and $Y^SX{\cdot}Y^L$, *In(1)EN. y/T(Y;2)J70/Df(2L)cl-7* or *Df(2L)cl-1* or *Df(2L)cl-2* males. It was also difficult to obtain data for the J136-A171 region due to the sterility of *X/T(Y;2)J136/Df(2L)GdhA* and *X/T(Y;2)J136/Df(2L)cl-7* males. We have not explored further the cause of the

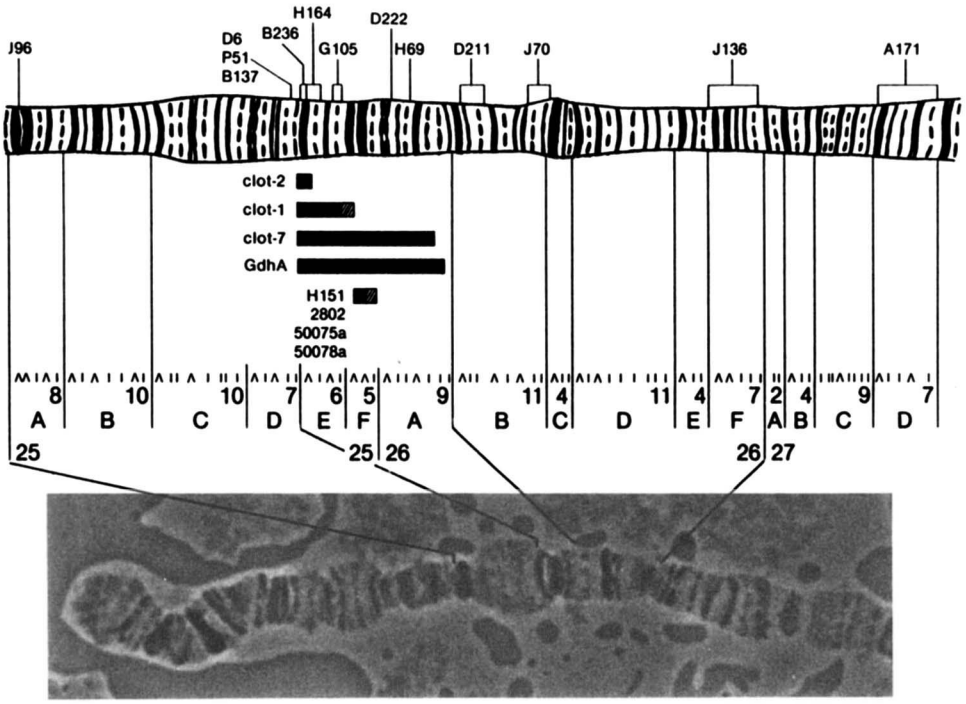

FIGURE 1.—Cytological map of chromosome region 25A-27D showing break points of aberrations. Deficiencies are shown as filled bars. Diagonally marked areas indicate break point uncertainties. Translocation break points are shown above the diagram of the chromosome. Deficiencies (*2L*) H151, *2802*, *50075a* and *50078a* all have the same cytological break points, which are indicated next to H151 on the figure.

sterility of flies with these chromosome constitutions, although LINDSLEY *et al.* (1972) have noted that the sterility of some segmental aneuploids can result from the effects of autosomal aneuploidy as well as from *Y* chromosome hyperploidy.

Crosses between all of the interstitial deficiencies were also made. The only combinations between two such deficiencies that did not result in lethality were *Cl-1* or *cl-2* over *2802*, *50075a* or *50078a*. We should point out here that the lethality of *Df(2L)50078a/Df(2L)cl-7* heterozygotes does not agree with the data of RACINE, LANGLEY and VOELKER (1980), who reported that some of these heterozygotes survive. It will be shown that the *50078a* and *cl-7* deficiencies overlap for at least three recessive lethal loci and both are deficient for *αGpdh*.

To determine whether any of the *Y;2* translocation break points might themselves be deficiencies or broken within lethal loci, the translocation stocks were crossed to each of the interstitial deficiencies. From those data, which were reported by KOTARSKI (1982), it was clear that only the *T(Y;2)B137* and *T(Y;2)H151* autosomal break points are lethal over deficiencies. Specifically, *T(Y;2)B137/cl-1* or *cl-2* heterozygotes are lethal, as are *T(Y;2)H151/Gdh-A*, *cl-7*, *2802*, *50075a* or *50078a* heterozygotes. The lethality of the *T(Y;2)B137/cl-1* or *cl-2* heterozygotes indicate that the left hand break points of the *cl-1* and

TABLE 1

A description of the second chromosome deficiencies and Y;2 *translocations*

Aberration	Autosomal break point(s)
Df(2L)GdhA	25D7–E1; 26A8–9
Df(2L)cl-7	25D7–E1; 26A7–8[a]
Df(2L)cl-2	25D7–E1; 25E2–3[b]
Df(2L)cl-1	25D7–E1; 25E6–F3[b]
Df(2L)2802	25F2–3; 25F4–26A1
Df(2L)50078a	25F2–3; 25F4–26A1
Df(2L)50075a	25F2–3; 25F4–26A1
T(Y;2)J96[c]	25A2–3[b]
T(Y;2)D6	25D6–7 and In(2L)24C4–6; 25D2–3 and Df(2L)24C3; 25E1–2[b]
T(Y;2)P51	25D6–7[b]
T(Y;2)B137	25D6–7[b]
T(Y;2)B236	25D7–E2
T(Y;2)H164	25E1–4
T(Y;2)G105	25E5–F1
T(Y;2)H151	25F2–3; 25F4–26A1
T(Y;2)D222	26A2–3
T(Y;2)H69	26A4–5
T(Y;2)D211	26B2–5
T(Y;2)J70	26B9–C1
T(Y;2)J136	26E4–27A1[d]
T(Y;2)A171	27C9–27E1[d]

[a] Personal communication, V. VELISSARIOU.
[b] VELISSARIOU and ASHBURNER (1980).
[c] *T(Y;2)J96* is a *T(Y;2;4)*.
[d] Data of LINDSLEY *et al.* (1972).

cl-2 deficiencies are more distal than are the lefthand break points of *Df(2L)cl-7* and *Df(2L)GDHA*, even though the four break points cannot be distinguished by cytological analysis alone. Once again this is in slight disagreement with the data of RACINE, LANGLEY and VOELKER (1980). Those authors report that *T(Y;2)H151/Df(2L)cl-7* heterozygotes are viable and *T(Y;2)H151/Df(2L)cl-2* heterozygotes are lethal. As will be seen, *T(Y;2)H151* is deficient for at least four recessive lethal loci that are also uncovered by the *cl-7* deficiency. Further, we have found that *Df(2L)cl-2* does not overlap *T(Y;2)H151* genetically or cytologically.

Mapping recessive lethal mutations

The screen for recessive lethal mutations in the *αGpdh* region of chromosome *2* produced 32 independently derived chromosomes that are lethal in combination with *Df(2L)GdhA* (n = 3177). Of these 32 mutagenized chromosomes, 26 were characterized in detail: 24 chromosomes bear single recessive lethal mutations within *Df(2L)GdhA*; one chromosome contains two recessive lethals, only one of which, 401a, is within *Df(2L)GdhA* (see following data), and one chromosome carries the interstitial deficiency, *Df(2L)2802*. The muta-

TABLE 2

Results of crossing stocks with interstitial deficiencies to TDf(Y;2) *stocks with autosomal break points in region 25A–27D*

TDf	GdhA	*cl-7*	*cl-2*	*cl-1*	*2802*	*50075a*	*50078a*
J96-B137	+	+	0/288	0/224	NT	NT	NT
J96-P51	NT	+	+	+	NT	NT	NT
D6-P51	+	+	+	+	NT	NT	NT
P51-D6	+	+	+	+	NT	NT	NT
D6-B137	+	+	0/253	0/119	NT	NT	NT
P51-B137	+	+	0/101	0/185	NT	NT	NT
B137-B236	0/92	0/399	0/225	0/122	+	+	+
B232-D222	0/300	0/153	0/153	0/298	0/202	0/177	0/190
H164-H151	0/170	0/200	0/100	0/141	0/200	0/200	0/200
G105-D222	0/130	0/200	+	+	0/232	0/195	0/347
H151-D222	0/310	0/379	+	+	0/136	0/200	0/200
D222-H69	0/200	0/100	+	+	+	+	+
H69-D211	0/200	+	+	+	+	+	+
D211-J70	+[a]	NT	+	+	+	NT	NT

NT = not tested.

[a] D. KNIPPLE, personal communication.

genesis using *Df(2L)cl-7* in the lethal screen produced 35 lethal chromosomes (n = 3765). Eighteen were studied in depth, and all proved to carry a single recessive lethal mutation within the limits of *Df(2L)cl-7*.

The recessive lethal mutations that are clearly in the 25D-26B region were more finely localized by crosses to the series of second chromosome translocation deficiency stocks and to the individual translocation stocks. The results of these crosses are summarized in Table 3 and in Figure 2. Of the 43 recessive lethal mutations listed, three are lethal when heterozygous with *TDf(Y;2)B137-B236*, 14 can be positioned between the autosomal break points of *T(Y;2)B236* and *T(Y;2)H164* and four lethals can be localized to the H164-G105 region. A single lethal (*3301*) has been positioned between *T(Y;2)H151* and *T(Y;2)D222* exclusive of the *T(Y;2)H151* deficiency, whereas ten recessive lethals are lethal in combination with *T(Y;2)H151* alone. The *TDf(Y;2)H69-D211* region includes 11 lethals. The chromosome carrying lethal *401* is lethal when heterozygous with *TDf(Y;2)H69-D211* and when heterozygous with a deficiency for the J70-J136 region. Thus, this chromosome must carry at least two lethal genes, designated here as *401a* and *401b*.

The lethals in each small region were then crossed *inter se* to determine their complementation groups. When viability is used as the only phenotype, the distinction between mutations showing interallelic complementation and adjacent, but nonallelic, mutations may not always be clear. This is especially true of mutations that are not separated by deficiency or translocation break points. With the criterion used here for complementation (50% or greater of the expected number of Curly$^+$ offspring), no single recessive lethal fell into more than one adjacent complementation group. It is presumed then that each complementation group represents a separate genetic locus. For reference, the

TABLE 3

Lethal complementation groups and their alleles

Locus	Allele	Chromosome	Deficiency used in the mutagenesis screen	TDf region
l(2)gdh-1	*1902*	*cl pr*	*Df(2L)GdhA*	
	11	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	H164-G105
	14	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
	31	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
l(2)gdh-2	*801*	*spd^{fg} pr*	*Df(2L)GdhA*	
	2002	*cl pr*	*Df(2L)GdhA*	H69-D211
	1301	*spd^{fg} pr*	*Df(2L)GdhA*	
l(2)gdh-3	*3301*	*cl pr*	*Df(2l)GdhA*	H151-D222
l(2)gdh-4	*401a*	*cl pr*	*Df(2L)GdhA*	
	3101	*cl pr*	*Df(2L)GdhA*	H69-D211
	1601	*spd^{fg} pr*	*Df(2L)GdhA*	
l(2)gdh-5	*2201*	*cl pr*	*Df(2L)GdhA*	H69-D211
l(2)gdh-6	*1401*	*cl pr*	*Df(2L)GdhA*	
	3501	*cl pr*	*Df(2l)GdhA*	
	502	*cl pr*	*Df(2L)GdhA*	H69-D211
	3001	*cl pr*	*Df(2L)GdhA*	
l(2)gdh-7	*901*	*spd^{fg} pr*	*Df(2L)GdhA*	
	25A	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	B236-H164
l(2)gdh-8	*1501*	*cl pr*	*Df(2L)GdhA*	
	001	*cl pr*	*Df(2L)GdhA*	
	26	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	B236-H164
	29	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
	38	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
l(2)gdh-9	*701*	*cl pr*	*Df(2L)GdhA*	
	2901	*cl pr*	*Df(2L)GdhA*	
	01	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	B236-H164
	16	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
l(2)gdh-10	*201*	*cl pr*	*Df(2L)GdhA*	
	41	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	D236-H164
	03	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
l(2)gdh-11	*07*	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	B137-B236
l(2)gdh-12	*3701*	*cl pr*	*Df(2L)GdhA*	
	40	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	B137-B236
l(2)gdh-13	*401b*	*cl pr*	*Df(2L)GdhA*	J70-J137

TABLE 3—Continued

Locus	Allele	Chromosome	Deficiency used in the mutagenesis screen	TDf region
l(2)gdh-14	*3102*	*cl pr*	*Df(2L)GdhA*	
	2001	*cl pr*	*Df(2L)GdhA*	
	301	*cl pr*	*Df(2L)GdhA*	H151
	13	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
	15	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
	802	*spd^{fg} pr*	*Df(2L)GdhA*	
	33B	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	
l(2)gdh-15	*3002*	*cl pr*	*Df(2L)GdhA*	H151
l(2)gdh-16	*25B*	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	H151
l(2)gdh-17	*10*	*l(2)gdh-2 spd^{fg} pr*	*Df(2L)cl-7*	H151

recessive lethal mutations are arranged according to their complementation groups in Table 3. Complete complementation data matrices were compiled by KOTARSKI (1982).

The crosses between the lethals and the deficiencies allow us to resolve differences between several break points that cannot be differentiated by cytological analysis alone. Thus, all of the H151 region lethals are lethal in combination with deficiency *2802*, but only the loci *l(2)gdh-15* and *l(2)gdh-16* are within the *50075a* and *50078a* deficiencies. Since *Df(2L)50078a* uncovers *l(2)gdh-3* but not *l(2)gdh-1*, these deficiencies must lie in the righthand part of the H151 region. This also suggests that the lethal loci, *l(2)gdh-14* and *l(2)gdh-17*, are to the left of *l(2)gdh-15* and *l(2)gdh-16*. Furthermore, *l(2)gdh-3* is uncovered by *50075a* and *50078a* but not by *2802* nor by the deficiency associated with the break point of *T(Y;2)H151*. Thus, the latter two deficiencies do not extend as far to the right as *50075a* and *50078a*.

We failed to recover any EMS-induced lethal or visible mutation in the D222-H69 interval (26A2-3; 26A4-5). However, there must be at least one recessive lethal locus in this interval since *TDf(Y;2)D222-H69/Df(2L)GdhA* heterozygotes fail to survive, whereas *T(Y;2)H69* or *T(Y;2)D222/Df(2L)GdhA* heterozygotes are viable. This lethality is termed simply, l^x with the reservation that more than one lethal locus may be present in this region. The deficiencies *50075a* and *50078a* are l^{x+} but not *Df(2L)cl-7* (see Figure 2).

Finally, the recessive lethal, *l(2)gdh-13*, represented by a single allele (*401b*), maps to the J70-J136 interval (26B9; 26E4-27A1). Since this interval is outside the deficiencies used to screen for the recessive lethal mutations, the discovery of the *l(2)gdh-13* locus was fortuitous. When we screened the mutagenized chromosomes for new recessive visible loci between the autosomal break points of *T(Y;2)B137* and *T(Y;2)A171*, we found that the 401/*TDf(Y;2)J70-J136* heterozygotes were lethal, even though no new recessive visible loci were recovered in this search. The *401a* and *401b* lethals are the only two lethals

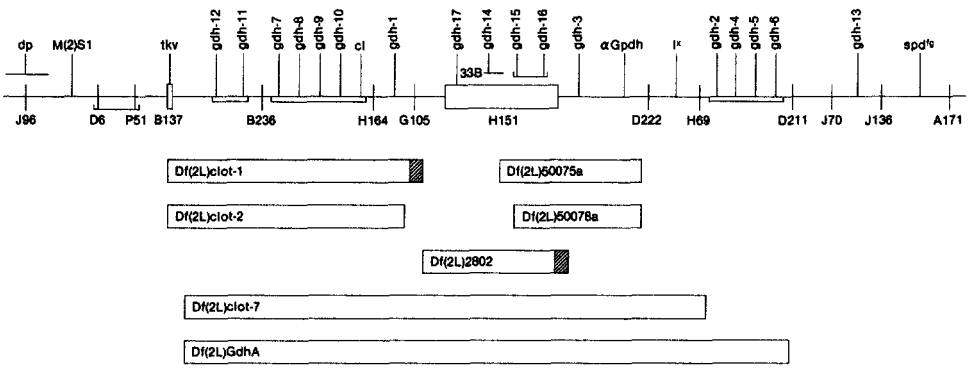

FIGURE 2.—Cytogenetic map of the *αGpdh* region of chromosome 2. Adjacent loci grouped by brackets have not been separated by a rearrangement, and their left-right order is unknown. Stippled areas at the ends of several deletions represent break point uncertainties.

induced simultaneously on the same chromosome within the 25A-27D region in this study.

Cytogenetic localization of visible and enzyme loci

M9(2)S1: Heterozygotes of the genotype, *TDf(Y;2)J96-B137/+*, display a Minute phenotype, whereas *TDf(Y;2)D6-B137/+* flies do not. This Minute is extreme and presumed to be *M(2)S1*. This locus has previously been positioned between *T(Y;2)J96* and *T(Y;2)B137* (LINDSLEY *et al.* 1972).

dp: Female flies of the genotype, C(1)RM, y/*TDf(Y;2)J96-B137/dp^{ov}*, are dumpy oblique and dumpy vortex in phenotype (as well as Minute). Additionally, *T(Y;2)J96/dp^{ov}* heterozygotes also express the dumpy oblique and vortex phenotypes. This suggests that the *T(Y;2)J96* break point may be at or near the dumpy locus. The cytological localization of the *T(Y;2)J96* break point at 25A2–3 (Table 1) and the reported position of *dp* as 25A1–4 (VELISSARIOU and ASHBURNER 1980) support this contention. To distinguish between the possibility that the J96 break point is at the dumpy locus and, thus, effectively a deficiency of *dp*, and the possibility that the break point is altering dumpy expression via a position effect, we made crosses to determine whether the dumpy phenotype of *T(Y;2)J96/dp* heterozygotes is suppressed by extra *Y* chromosomes. The crosses were of two types: Cross A was between $Y^{S}X \cdot Y^{L}$, *In(l)EN, y/Y; CyO dp^{lvl}/dp^{ov}* males and *C(l)RM, y/T(Y;2)J96/Cy* females, and cross B was between $Y^{S}X \cdot Y^{L}$, *In(l)EN, y/T(Y;2)J96/Cy* males and *X/X; CyO dp^{lvl}/dp^{ov}* females. Male offspring from the two crosses differ in the presence (cross A) or absence (cross B) of a *Y* chromosome. The *CyO dp^{lvl}/T(Y;2)J96* and the *dp^{ov}/T(Y;2)J96* male progeny from each cross were scored for the degree of dumpy vortex and dumpy oblique expression, using the phenotypic index devised by GRACE (1980). In this classification, dumpy expression ranges from 0 (wild type) to 5 (extreme dumpy). The data (see KOTARSKI 1982) indicate that the dumpy phenotype of *T(Y;2)J96/dp* heterozygotes is suppressed by the presence of an extra *Y* chromosome and, thus, is probably due to a position effect. Cross

A (extra *Y* chromosome) produces progeny that are much less dumpy (index = 0–1) than the progeny of cross B (index = 4–5). The progeny of both crosses show a range of expression of dumpy spanning at least two classes of the phenotypic index. In addition, there was a relatively small number of *CyO* dp^{lvl}*/T(Y;2)J96* progeny recovered from cross B, suggesting that the position effect affects the expression of the dp^{l} sublocus as well.

tkv: The recessive visible mutant, thick vein (*tkv*), is positioned within the P51-B137 interval (Figure 2), and since *T(Y;2)B137/tkv* heterozygotes themselves have a thick vein phenotype, it is concluded that *tkv* is also at or within the B137 break point. The thick vein mutation is also within the *cl-1* and *cl-2* deficiencies. Based on the evidence presented earlier, it should not be concluded that the B137 translocation break point is also an autosomal deficiency. There may be only a single lethal locus associated with *T(Y;2)B137*, and it may be allelic to the *tkv* mutation.

cl: The recessive visible mutation, clot was positioned by observing pseudodominance of a clot allele with the translocation deficiency B236-D222. The pseudodominance was not observed when clot was heterozygous with the translocation deficiencies H164-H151 or H151-D222. This confirms that the cytological position of clot is $25E_{1-2}$ as reported by VELISSARIOU and ASHBURNER (1980).

αGPDH: αGPDH was positioned using alleles specifying electrophoretic variants of the enzyme. All deficiency and translocation stocks are homozygous for the $\alpha Gpdh^{A}$ (fast) allele. Flies from a stock homozygous for $\alpha Gpdh^{B}$ were crossed to each of the deficiency stocks. The heterozygous progeny were subjected to electrophoresis, and the cellulose acetate membranes were stained for αGPDH activity. A heterozygous A/B pattern indicated that the *αGpdh* locus was not within the limits of the deficiency. From the data (see KOTARSKI 1982), the *αGpdh* locus can be localized cytologically to 25F5 (or in an interband region adjacent to 25F5). This is based on the inclusion of the *αGpdh* locus in the deficiencies *50075a* and *50078a* but not in *2802* or H151. Note that all of these deficiencies lack bands 25F3 and F4 but not 25A1 (see Table 1 and Figure 1). These data are consistent with the cytological localization of *αGpdh* by O'BRIEN and GETHMANN (1973) who placed it between *T(Y;2)G105* (25F) and *T(Y;2)D106* (26B).

β-galactosidase: The H69-D211 interval (26A4-5; 26B2-3), in addition to containing the four lethal loci, is also the dosage-sensitive region for a Drosophila β-galactosidase activity (EC 3.2.1.23) (R. J. MACINTYRE, unpublished data). All of the lethal heterozygotes have wild-type levels of β-galactosidase, and all of the lethals tested fully complement a null allele for β-galactosidase (D. KNIPPLE and R. J. MACINTYRE, unpublished results). In fact, the dosage-sensitive locus for β-galactosidase and the four lethal loci are uncovered by *Df(2L)GdhA* but not *Df(2L)cl-7*. There are, then, at least five loci in a one-band interval, 26A8.

spd^{fg}: *TDf(Y;2)J136-A171/*spd^{fg} heterozygotes display a spade phenotype. The position of $spade^{flag}$ proximal to *αGpdh* is in agreement with its reported genetic position on chromosome *2* (DOANE 1961) and consistent with the cytological position of the spade allele within $27C_1$-$28C_1$ as determined by LIN-

DSLEY and GRELL (1968). The cytological position of the spade locus can now be narrowed down to the region between $27C_1$ and $27E_1$.

Recombination mapping

The two recessive visible mutations close to *αGpdh*, clot and spade^{flag}, were mapped genetically in a standard three-point cross. The data are summarized in Table 4 and are also presented in Figure 3. The data place clot 1.67 cM to the left of *αGpdh* and spade^{flag} 4.65 cM to the right. The position of *αGpdh* as 2-20.5 is taken from O'BRIEN and MACINTYRE (1972).

To confirm the left-right order of some of the lethal loci and to obtain better estimates of the genetic map distances within the *αGpdh* region of chromosome 2, selected lethal loci were mapped relative to *αGpdh*, clot and spade^{flag}. The recessive lethals, *l(2)gdh-1* and *l(2)gdh-3* were mapped by constructing $\frac{cl^+\ l^+\ \alpha Gpdh^A\ spd^{fg}}{cl\ l\ \alpha Gpdh^B\ spd^{fg+}}$ heterozygous females and mating them to *cl* l^+ $\alpha Gpdh^A$ spd^{fg} homozygous males. The cl^+ spd^{fg+} male recombinants were individually mated to *CyO/Df(2L)GdhA* females to test for the presence of lethals on the recombinant chromosome. The fraction of the recombinant progeny that carried the cl^+ *l* $\alpha Gpdh^B$ spd^{fg+} and cl^+ l^+ $\alpha Gpdh^B$ spd^{fg+} chromosomes, together with the *cl-αGpdh* distance of 1.67 cM, position *l(2)gdh-1* 1.59 cM to the left of *αGpdh* and *l(2)gdh-3* 1.04 cM in the same direction (Figure 3). The absence of a cl^+ *l* $\alpha Gpdh^A$ spd^{fg+} recombinant class unequivocally places *l(2)gdh-1* and *l(2)gdh-3* between clot and *αGpdh*.

The *l(2)gdh-2* locus was mapped in a similar manner. In this case $\frac{cl^+\ \alpha Gpdh^B\ l\ spd^{fg}}{cl\ \alpha Gpdh^A\ l^+\ spd^{fg+}}$ females were mated to *cl* $\alpha Gpdh^A$ l^+ spd^{fg} homozygous males and the resulting cl^+ spd^{fg+} recombinant F_1 males tested for the presence of the lethal as described earlier. The absence of a cl^+ $\alpha Gpdh^A$ *l* spd^{fg+} recombinant class positions *l(2)gdh-2* between *αGpdh* and spd^{fg}. The fraction of the recombinant progeny that carried the cl^+ $\alpha Gpdh^B$ l^+ spd^{fg+} and cl^+ $\alpha Gpdh^B$ *l* spd^{fg+} chromosomes together with the *αGpdh-spd*^{fg} distance of 4.65 cM places *l(2)gdh-2* 0.048 cM to the right of *αGpdh* (Figure 3).

DISCUSSION

Summaries of the data produced by this study are shown in Figures 2 and 3. Figure 2 presents the cytogenetic map of the *αGpdh* region, whereas Figure 3 presents the recombination map of the same region. We have identified at least 17 recessive lethal loci and have mapped five previously identified visible mutations within the 25A-27D region of chromosome 2. Selected lethal and visible loci were genetically mapped relative to the *αGpdh* locus. No new visible mutations were found in this region.

A total of 4455 EMS mutagenized chromosomes were tested to recover a total of 43 recessive lethal mutations that mapped within *Df(2L)GdhA*. With a Poisson distribution (which assumes that all lethal loci in the *GdhA* region are equally mutable) we can estimate the probable number of lethal loci that are

TABLE 4

Results of the 3-point cross: clot-αGpdh-spade^{flag}

cl αGpdh^{A} spd^{fg+}/cl^{+} αGpdh^{B} spd^{fg} ♀♀ × *cl αGpdh^{B} spd^{fg}/cl αGpdh^{B} spd^{fg}* ♂♂	
Phenotype of progeny	Frequency
cl αGpdh$^{A/B}$ spd^{fg+}	577
cl^{+} αGpdh$^{B/B}$ spd^{fg}	492
cl αGpdh$^{B/B}$ spd^{fg}	3
cl^{+} αGpdh$^{A/B}$ spd^{fg+}	18
cl^{+} αGpdh$^{B/B}$ spd^{fg+}	32
cl αGpdh$^{A/B}$ spd^{fg}	20
cl αGpdh$^{B/B}$ spd^{fg+}	0
cl^{+} αGpdh$^{A/B}$ spd^{fg}	1
	1143

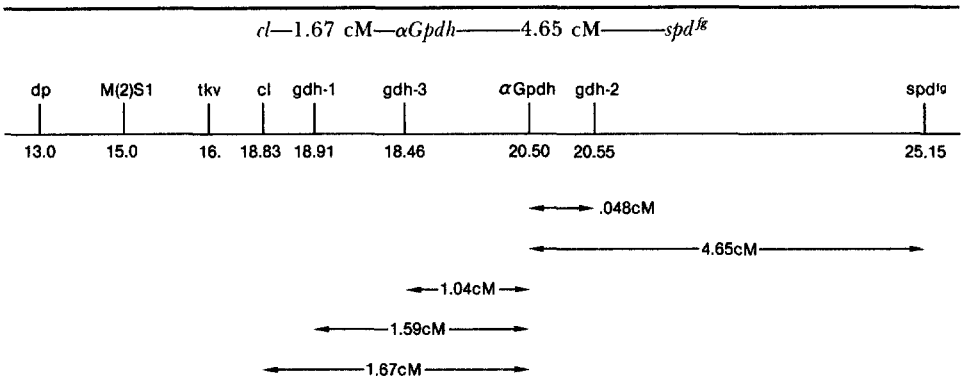

FIGURE 3.—Genetic map of the *αGpdh* region of chromosome 2. Map positions of *dp*, *M(2)S1* and *tkv* from LINDSLEY and GRELL (1968).

unidentified. The estimated number of undiscovered loci is one to two. The 16 lethal loci within *Df(2L)GdhA* that we have identified and used in this calculation do not include l^{x}. Thus, at least one of the remaining loci has already, in a sense, been found. Our intent, however, was not to saturate the *αGpdh* region of the chromosome as much as to reach a point at which further mutagenesis would be less productive.

The mapping of 16 lethal loci, plus the clot locus, l^{x}, αGPDH and β-galactosidase, means that 20 loci have been accounted for within the limits of the *GdhA* deficiency. This corresponds well with the number of bands (19) in this area. An overall correspondence between the number of bands and the number of loci in a particular region of the Drosophila genome has been noted by many other workers, *viz*, JUDD, SHEN and KAUFMAN (1972) for the zeste-white region, HILLIKER, CLARK and CHOVNICK (1980) for the rosy region, WOODRUFF and ASHBURNER (1979a,b) for the *Adh* region and HOCHMAN (1971; 1972) for the fourth chromosome. Although the overall correspondence between loci and band number is good in the 25D7-E1; 26A8–9 region, the loci are very unevenly distributed among the 19 bands (Figure 4). The region of overlap of *Df(2L)cl-1* and *Df(2L)cl-2* includes the bands 25E1–2. This large doublet is

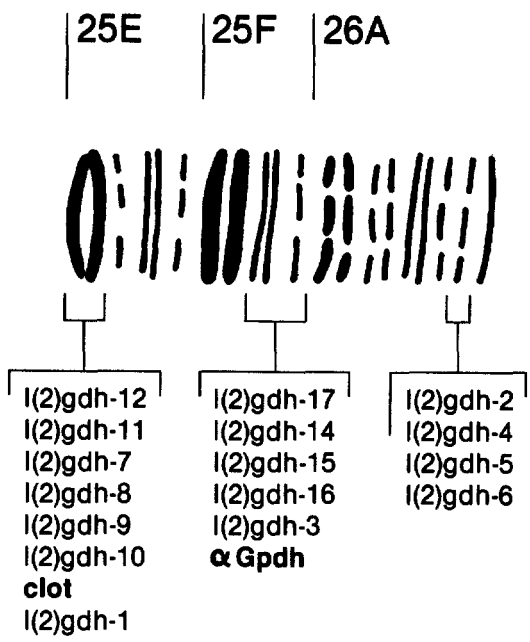

FIGURE 4.—The distribution of loci identified in this study within the 25E-26A region of chromosome *2*.

the site of seven lethal loci and the clot locus. In contrast, the six-band region between the righthand break point of *Df(2L)cl-2* (25E2–3) and the lefthand break points of *Df(2L)2802* and *T(Y;2)H151* (25F2–3) does not include any loci that we have identified in our search. The cytological region delineated by *Df(2L)2802*, *T(Y;2)H151*, *Df(2L)50075a* and *Df(2L)50078a* is, at most, three bands (25F2–3; 25F4-26A1), but it contains five lethal loci and the *αGpdh* locus. Again, the area between the proximal break points of the *GdhA* and *cl-7* deficiencies consists of a single band (26A8), yet it contains four lethal loci and the structural gene for β-galactosidase. A lack of correspondence between identifiable loci and cytologically observable bands has been described for the lB and *N-dm* regions of the *X* chromosome by LEFEVER (1974) and for the *Ddc* region by WRIGHT *et al.* (1981). It should be stressed, however, that only loci that are separated by cytological break points can definitely be said to be separate genetic units.

With regard to the positions of the *Y;2* translocation break points and deficiency break points within the *αGpdh* region, we observe a clustering of the break points at the cytological level but very little clustering at the genetic level. The cytological regions with the highest incidence of break points are the 25D6–7 area, where the autosomal break points of *T(Y;2)D6*, *T(Y;2)P51* and *T(Y;2)B137* are located, the 25D7-25E1 area where *T(Y;2)B236* and the distal break points of the *GdhA*, *cl-7*, *cl-2* and *cl-1* deficiencies have been localized and the 25F2-26A1 area which includes the deficiencies *2802*, *T(Y;2)H151*, *50075a* and *50078a*. At the genetic level, however, we have been

able to separate the autosomal break point of *T(Y;2)B137* and those of the *Y;2* translocations, *D6* and *P51*, and have presented evidence suggesting that the distal break points of the deficiencies *cl-1* and *cl-2* are to the left of the distal break points of the deficiencies *GdhA* and *cl-7*. In the 25F2-26A1 area, where four deficiencies appear to be cytologically coincident, we have shown that, in fact *Df(2L)50075a* and *Df(2L)50078a* are distinct from *T(Y;2)H151* and *Df(2L)2802* in their right- and lefthand break points (Figure 2). There appears, therefore, to be no real "hot spot" of chromosomal breakage in the 25A-27D region that can be identified from the small number of aberrations analyzed here.

One goal of this work was to identify the lethal and visible loci that are very close to the *αGpdh* locus. Thus far, the closest lethal loci are *l(2)gdh-3*, 1.04 cM to the left, and *l(2)gdh-2*, 0.048 cM to the right, of *αGpdh*. The closest visible mutations are clot (1.67 cM left of *αGpdh*) and spade^{flag} (4.65 cM to the right of *αGpdh*). These visible mutations, together with the closely linked lethal mutations, make it possible to construct an effective cross scheme for the selection of rare recombinational events within the *αGpdh* locus. The fine structure recombinational analysis of the *αGpdh* gene is presently underway in our laboratory.

We would also like to emphasize two other points concerning the *αGpdh* locus that are revealed by the data presented in this paper. First, *αGpdh* has been localized to a single polytene band, 25F5. The localization of *αGpdh* to such a small region will make the identification of chromosomal aberrations involving the *αGpdh* locus easier and will also be useful in *in situ* hybridization analysis of putative *αGpdh*-bearing recombinant DNA clones. Finally, we wish to point out that of the 43 lethal-bearing chromosomes studied here, none carried an altered *αGpdh*. Indeed, all of the recessive lethal loci, with the exception of *l(2)gdh-3*, were separable from the *αGpdh* locus by deficiency break points. The chromosome on which *l(2)gdh-3* was induced still carries a normal *αGpdh^{B}* allele, however. In view of the central role of αGPDH in the intermediary metabolism of the fly (see SACKTOR 1965 and HANSFORD and SACKTOR 1971 for reviews), it is perhaps surprising that no lethals at the *αGpdh* locus were recovered. However, the failure to identify a lethal component of the *αGpdh* locus is consistent with the observation that flies completely lacking αGPDH activity are viable (O'BRIEN and MACINTYRE 1972) as are flies that have no detectable amounts of αGPDH cross-reacting material (KOTARSKI *et al.* 1983).

We wish to thank DOUGLAS CAVENER for many helpful suggestions concerning the manuscript. Thanks are due also to MICHAEL ASHBURNER and ROBERT VOELKER for supplying Drosophila stocks used in this study. A special acknowledgement goes to VOULA VELISSARIOU for assistance in determining cytological break points and allowing us to quote unpublished work. This work was supported by the National Institutes of Health grant AG01811 to R. J. MACINTYRE and by the Genetics Training grant GM07617 to M. A. KOTARSKI.

LITERATURE CITED

BRIDGES, P. N., 1942 A new map of the salivary gland 2L-chromosome of *Drosophila melanogaster*. J. Hered. **33:** 403–408.

CHOVNICK, A., W. GELBART and M. MCCARRON, 1977 Organization of the rosy locus in *Drosophila melanogaster*. Cell **11:** 1–10.

CHOVNICK, A., W. GELBART, M. MCCARRON, B. OSMOND, E. P. M. CANDIDO and D. L. BAILLE, 1976 Organization of the rosy locus in *Drosophila melanogaster*: evidence for a control element adjacent to the xanthine dehydrogenase structural element. Genetics **84:** 233–255.

COLLIER, G. E. and R. J. MACINTYRE, 1972 New mutants. Drosophila Inform. Serv. **49:** 41.

COLLIER, G. E., D. T. SULLIVAN and R. J. MACINTYRE, 1976 Purification of α-glycerophosphate dehydrogenase from *Drosophila melanogaster*. Biochim. Biophys. Acta **429:** 316–323.

DOANE, W. W., 1961 New mutants. Drosophila Inform. Serv. **35:** 45–46.

GELBART, W., M. MCCARRON and A. CHOVNICK, 1976 Extension of the limits of the XDH structural element in *Drosophila melanogaster*. Genetics **84:** 211–232.

GRACE, D., 1980 Genetic analysis of the dumpy complex locus in *Drosophila melanogaster*: complementation, fine structure and function. Genetics **94:** 647–662.

GRELL, E. H., 1967 Electrophoretic variants of α-glycerophosphate dehydrogenase in *Drosophila melanogaster*. Science **158:** 1319–1320.

HANSFORD, R. G. and B. SACKTOR, 1971 Oxidative metabolism of Insecta. Chem. Zool. **6:** 213–248.

HILLIKER, A. J., S. H. CLARK and A. CHOVNICK, 1980 Cytogenetic analysis of the chromosomal region immediately adjacent to the rosy locus in *Drosophila melanogaster*. Genetics **95:** 95–110.

HOCHMAN, B., 1971 Analysis of chromosome 4 in *Drosophila melanogaster*. II. Ethylmethanesulfonate induced lethals. Genetics **67:** 235–252.

HOCHMAN, B., 1972 The detection of 4 more vital loci on chromosome 4 in *Drosophila melanogaster*. Genetics **71**(Suppl): S71.

JUDD, B. H., M. W. SHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the X chromosome of *Drosophila melanogaster*. Genetics **71:** 139–156.

KOTARSKI, M., 1982 A genetic and biochemical analysis of the α-glycerophosphate locus of *Drosophila melanogaster*. Ph.D. Thesis, Cornell University, Ithaca, New York.

KOTARSKI, M. A., S. PICKERT, D. A. LEONARD, G. T. LAROSA and R. J. MACINTYRE, 1983 The characterization of α-glycerophosphate dehydrogenase mutants in *Drosophila melanogaster*. Genetics **105:** 387–407.

LEFEVRE, G., JR., 1974 Exceptions to the one gene-one band hypothesis. Genetics **77**(Suppl): s39.

LEWIS, E. and F. BACHER, 1968 Method of feeding ethyl methanesulfonate (EMS) to *Drosophila* males. Drosophila Inform. Serv. **43:** 193.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627

LINDSLEY, D. L., L. SANDLER, B. S. BAKER, A. T. C. CARPENTER, R. E. DENELL, J. C. HALL, P. A. JACOBS, G. L. G. MIKLOS, B. K. DAVIE, R. C. GETHMANN, R. W. HARDY, A. HESSLER, S. M. MILLER, H. NOZAWA, D. M. PARRY and M. GOULD-SOMERO, 1972 Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71:** 157–184.

O'BRIEN, S. J. and R. C. GETHMANN, 1973 Segmental aneuploidy as a probe for structural genes in Drosophila: Mitochondrial membrane enzymes. Genetics **75:** 155–167.

O'BRIEN, S. J. and R. J. MACINTYRE, 1972 The α-glycerophosphate cycle in *Drosophila melanogaster*. II. Genetic aspects. Genetics **71:** 127–138.

PYE, Q., D. KNIPPLE and R. J. MACINTYRE, 1980 Construction of segmental deficiency stocks from Y-autosome translocation stocks. Drosophila Inform. Serv. **55:** 170.

RACINE, R. R., C. H. LANGLEY and R. A. VOELKER, 1980 Enzyme mutants induced by low-dose-rate-irradiation in *Drosophila*: Frequency and characterization. Environ. Mutagen. **2:** 167–177.

SACKTOR, B., 1965 Energetics and respiratory metabolism of muscle contraction. pp. 483–580. In: *Physiology of Insecta*, Vol. 2, Edited by M. ROCKSTEIN. Academic Press, New York.

STRICKBERGER, M. W., 1959 An improved salivary technique. Drosophila Inform. Serv. **33:** 183.

THOMPSON, J. N., JR., M. ASHBURNER and R. C. WOODRUFF, 1977 Presumptive control mutation for alcohol dehydrogenase in *Drosophila melanogaster*. Nature **270:** 363.

VELISSARIOU, V. and M. ASHBURNER, 1980 The secretory proteins of the larval salivary gland of *Drosophila melanogaster*: cytogenetic correlation of a protein and a puff. Chromosoma (Berl.) **77:** 13–27.

WOODRUFF, R. C. and M. ASHBURNER, 1979a 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.

WOODRUFF, R. C. and M. ASHBURNER, 1979b The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. II. Lethal mutations in the region. Genetics **92:** 133–149.

WRIGHT, T. R. F., W. BEERMANN, J. L. MARSH, C. P. BISHOP, R. STEWARD, B. C. BLACK, A. D. TOMSETT and E. Y. WRIGHT, 1981 The genetics of dopa decarboxylase in *Drosophila melanogaster*. IV. The genetics and cytology of the 37B10-37D1 region. Chromosoma (Berl.) **83:** 45–58.

Corresponding editor: T. C. KAUFMAN