SEGMENTAL ANEUPLOIDY AS A PROBE FOR STRUCTURAL GENES IN DROSOPHILA: MITOCHONDRIAL MEMBRANE ENZYMES[1]

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ABSTRACT

A method for detecting possible structural genes in *D. melanogaster* based on gene dosage dependency is presented. By making thirty crosses between Y-autosome translocations, and an attached-4 cross, it is possible to produce large duplications (approximately 150 salivary gland chromosome bands in length) for every autosomal region with the exception of 83DE. The usefulness of the technique was demonstrated by dosage dependency of three known gene-enzyme systems: *α-glycerophosphate dehydrogenase-1*, *alcohol dehydrogenase* and *malate dehydrogenase*. A screen for genes affecting two enzymes localized on the inner membrane of the mitochondrion, α-glycerophosphate oxidase (αGPO) and succinic dehydrogenase (SHD), produced a dosage-sensitive region in each case. Region 50C–52E affected αGPO activity and region 28D–29F affected SDH activity. The latter region apparently includes the *malic dehydrogenase-1* gene. The methodology and limitations of the technique are discussed.

OVER thirty genes in Drosophila have been described which specifically affect the appearance or kinetics of certain macromolecules (O'BRIEN and MACINTYRE 1971). These genes, most of which are enzyme structural genes, have been detected and mapped by one of the following standard procedures. These include the educated surmise of a biochemical defect from the nature of a morphological phenotype; e.g., the enzymic defects in pigment production detected in various eye color mutants. A second approach has been the search for nutritional mutants which specify genes for biosynthetic enzymes. A third and more generally successful technique has been the detection of iso-allelic enzyme differences (allozymes) following gel electrophoresis of flies from natural populations. The widespread polymorphism of these loci—39% in *D. pseudoobscura* (LEWONTIN and HUBBY 1966) and 54% in *D. melanogaster* (O'BRIEN and MACINTYRE 1969)—has provided a number of electrophoretic variants which have been useful in biochemical genetic localization studies.

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The use of mutagens, such as ethylmethanesulfonate (EMS), which is so useful in producing alleles for known loci, has not been so successful in the detection of new gene enzyme systems. The tremendous mutagenic efficiency of EMS generates lethal mutations at many loci, which makes the construction of homozygous mutagenized chromosomes exceedingly difficult. We describe here the use of a technique for detecting autosomal structural genes based upon gene-dosage-dependent enzyme activity in segmental aneuploids.

The methodology makes use of the collection of Y-autosome translocations throughout the autosomal genome of *D. melanogaster*. These were produced and analyzed recently by the *Seattle-La Jolla Drosophila Laboratories* (LINDSLEY *et al.* 1972). The cross of two translocations with adjacent autosomal breakpoints produces four progeny types: the two parental translocations, a fly with a deficiency of the region between the two translocation breakpoints, and a sibling with a duplication for the same region. Hence, it is possible by pair-wise crosses of selected Y-autosome translocations to generate interstitial deficiencies for at least 85% of the autosomal genome, and duplications for all of the autosomal regions except 83DE, which contains an aneuploid-lethal locus.

The procedure we have employed involves the comparative enzyme analysis of flies containing a duplication for a given region *vs.* their diploid siblings. We would expect that the duplicated region containing the structural gene for a studied enzyme would possess enzyme levels elevated 50% over the normal diploid (GRELL 1962). Hence, a duplication to diploid ratio of 1.5± would indicate that the region in question contained the structural gene. Once a trisomic region was implicated in enzyme elevation, it was further dissected genetically with included translocations to more precisely localize the responsible locus.

The success of a probe for gene-dosage-sensitive structural genes of enzymes depends upon three assumptions about the gene in question: (1) the gene is nuclear, (2) the locus is autosomal, (3) gene dosage is the rate-limiting step of measurable enzyme activity. Since, *a priori*, it is difficult to assess the validity of any of these assumptions, they merely provide alternative explanations for negative results.

We selected 30 Y-autosome translocations, whose breakpoints are approximately 150 salivary gland chromosome bands apart for our analysis. As a control, aneuploids for two known dosage-sensitive gene enzyme loci, *α-glycerophosphate dehydrogenase-1* (*αGpdh-1*) (GRELL 1967; O'BRIEN and MACINTYRE 1972b) and *alcohol dehydrogenase* (Adh) (URSPRUNG and LEONE 1965), were examined. Two enzymes located on the inner membrane of the mitochondrion, α-glycerophosphate oxidase (αGPO) and succinic dehydrogenase (SDH), were screened for elevated activity. αGPO was dosage-sensitive to a region on 2R, and SDH was dosage-sensitive to a region in 2L. The latter region proved to include *malate dehydrogenase*. In addition to these data, we report a number of characteristics about the crosses themselves, with special emphasis on their use as a probe for structural genes.

MATERIALS AND METHODS

Culturing techniques: Each cross (Table 1) was made with two males and two females in vials with five replicas. The parents were transferred to a fresh vial every third day for three successive transfers. Flies were grown on a high sucrose-yeast extract medium at 25° in the absence of chloramphenicol (NASH and BELL 1968).

Production of the segmental aneuploids: The basis for these crosses are described in LINDSLEY *et al.* (1972). A cross between any two different translocation heterozygotes will produce four types of offspring: males and females which are heterozygous for one of the two parental translocations (and are euploid), and males and females which are aneuploid for the region between the two autosomal breakpoints of the translocations. One of these (either the males or the females) will be deficient for the region in question, and the other will be the reciprocal duplication.

In the initial screen, it was decided to construct and examine only duplications. There are three reasons for this decision and each bears on the observation that duplications are generally healthier than deficiences: First, one can produce viable deficiencies for 85% of the autosomal genome with the available translocations, while the entire genome (except the 83DE region) can be duplicated with these translocations. Second, because of the small size necessary for deficiency survival, it would take approximately 80 crosses to examine the 85% which are amenable to deficiency recovery. With the duplications, the entire autosomal genome can be sampled with 30 crosses. Finally, the use of deficiencies would depend upon the detection of a diminished enzyme activity in aneuploids relative to euploids. Since the general health of deficiencies is usually poor, and because *Minutes* (there are at least 34 different autosomal *Minute* loci, any one of which will show the Minute phenotype when hemizygous) are known to cause reduced levels of enzyme activity (FARNSWORTH 1965), the possibility of false putatives due to factors other than structural gene dosage would be immense. These factors would not be expected to elevate enzyme levels in duplications of the same region.

It is possible, however, that duplication aneuploidy could cause a drop in enzyme activity due to the size of the duplication and its overall effect on the fly. This generalized drop could mask an increase in the activity of a locus included within the duplicated region. Therefore, duplication sizes were chosen which would minimize the occurrence of this masking effect.

The size of the duplications produced in this study was approximately 105 salivary bands. This size was chosen as one which would permit survival to adulthood of about 50% of the terminal duplication aneuploid zygotes (Table 4 of LINDSLEY *et al.* [1972]). Additionally, the survival of each duplication was estimated (Table 1) by the *R. V.* index. *R. V.* (Relative Viability) is the number of duplications recovered divided by the number of regular euploid progeny of the same sex. Since the frequency of progeny from alternate disjunction (which are the regular euploid progeny) is approximately the same as the frequency of progeny from adjacent I disjunction (which are the duplications and deficiencies), the ratio of the duplications to regular progeny will give an estimation of the frequency of duplications that survive to adulthood. If all of the duplications survive, the *R. V.* index would be approximately 1.00. Several crosses in which the *R. V.*'s were less than 50% also had duplication progeny with reduced αGPO, αGPDH, and SDH activities. These regions were subsequently dissected genetically with crosses using other translocations with autosomal breakpoints within the regions.

Three basic types of crosses were made: (1) those which produced intersitial aneuploids where both translocations were broken in the same autosomal arm (left or right), (2) those which produced intersitial aneuploids that spanned the centromere—thus the translocations were broken in different autosomal arms, and (3) those which produced terminal aneuploids. Each of these types of crosses will be considered separately.

The majority of crosses listed in Table 1 were Type (1). For this type of cross, there are three basic rules of thumb to consider when selecting the parents:

A. Whenever possible, select parents with breakpoints in opposite-*Y*-arms. This permits a phenotypic distinction between the four common classes of offspring: two parental euploids, deficiency, and duplication. Considering just the phenotype with respect to *Y* markers ($y+$ for

TABLE 1

The biochemical and biological characteristics of thirty-one segmental aneuploids (duplications) of D. melanogaster

Region		Approx. size	Parents[1] ♀	♂	R.V.[9]	No. progeny	Phenotype of duplication	Sex of Dp	Activity Dp/Act. αGPDH[11]	αGPO[12]	+[10,15] SDH[13]
21A	25A	145	+[2]	J96	0.41	1282	Cy Sco	♂	0.87	1.03	0.95
25A	27E	115	J96	H52	0.43	287	y Cy BB	♀	1.45	0.69	0.87
27E	30F	130	H52	L52	0.58	418	Hw Cv	♀	—	1.09	1.20
30F	35BC	180	L52	R15	0.81	414	y Cy BB	♀	0.88	0.78	0.97
35BC	38C	155	R15	B110	0.49	364	Hw Cy	♀	1.2	0.88	0.97
38C	41	100	B177	B110	0.63	270	y Cy BB	♂	0.93[14]	0.44	1.01
40	43C	100	D20	R155	0.29	315	y Cy BB	♂	0.93[14]	1.11	0.99
43C	45F	115	R155	L23	0.50	166	Hw Cy	♂	0.65	0.85	0.81
45F	47E	110	B107	L23	0.35	82	y Cy BB	♀	1.0	0.68	1.11
47E	50C	170	B107	L110[3]	0.45	25	Cy B	♂	—	1.04	1.02
50C	52E	150	R14	L110[3]	0.71	82	Sco B	♀	—	1.46	1.00
52E	54F	130	H149	R14	0.89	294	Hw Cy	♀	0.73	0.92	1.09
54F	57B	135	L107	H149	1.01	525	y Cy BB	♀	0.94	0.95	1.08
57B	59B	110	P59	L107	0.68	143	Hw Cy	♀	—	0.73	0.97
59B	60F	115	+[2]	P59	0.56	1120	y Cy Sco B	♂	—	0.99	0.88
61A	64E	185	B141	+[4]	0.19	240	y Ubx Sb B	♂	—	0.90	0.86
64E	67C	165	B141	G122	0.73	357	Hw Ubx	♀	—	0.79	0.95
67C	70C	140	G122	H156	0.33	180	y Ubx BB	♀	—	0.82	0.87
70C	74A	135	H156	D228	0.64	76	Hw Ubx	♀	—	0.71	0.96
74A	76F	120	D228	J147	0.00	27	y Ubx BB	♀	—	—	—
76E	79D	95	A112	J162[5]	0.64	130	y Ubx B	♀	—	1.04	0.89
74A	79D	215	J162[5]	D228	0.06	460	y Ubx B	♂	—	0.52	1.02
79D	83CD	125	L132	J162[5]	0.15	302	Hw Ubx	♀	—	0.77	0.95
83EF	86B	175	L136[6]	R36	0.72	426	y Ubx B	♂	1.0	0.85	0.94
86B	88C	160	G48	R36	0.64	499	Hw Ubx	♀	0.65	0.77	1.01
88C	91B	165	A89	G48	0.43	144	y Ubx BB	♀	—	0.93	0.76
91B	93F	165	B93	A89	0.54	337	Hw Ubx	♀	—	0.62	1.00
93F	96A	150	G73	B93	0.83	81	y Ubx BB	♀	—	0.73	0.89
96A	97F	130	R128	G73	0.73	195	Hw Ubx	♀	—	0.94	0.99
97F	100F	150	+[7]	R128	0.27	711	y Ubx Sb B	♂	—	0.73	0.70
101A	102F	140	+[8]	$\overline{44}$	—	869	{Ubx B or Sb B}	♂	—	1.02	0.98
101A	102F	140	+[8]	$\overline{44}$	—	869	{Ubx B or Sb B}	♀	—	1.07	0.92

[1] Unless indicated otherwise, all female parents were of the genotype *C(1)RM, y/T(Y;2)/Cy* or *C(1)RM, y² bb/T(Y;3)/In(3LR)TM6, Ubx^67b e* and the male parents were *Y^S X·Y^L, In(1)EN, y/T(Y;2)/Cy* or *Y^S X·Y^L, y/T(Y;3)/In(3LR)TM6, Ubx^67b e*, where *Cy* is either *In(2L+2R)Cy, Cy cn²* or *In(2LR)SM1, al² Cy cn² sp²*.

[2] *Y^S X·Y^L, In(1)EN,y/Y^S X·Y^L, In(1)EN,y; In(2LR)SM1/Sco.*

[3] *Y^S X·Y^L, In(1)EN, y/T(Y;2)L110/Sco* because Y breaks in L110, B107, and R14 are all in Y^L. See text for explanation.

[4] *Y^S X·Y^L, In(1)EN, y; In(3LR)TM6, Ubx^67b e/Sb.*

[5] *T(Y;2)J162* has lost *B^S*.

[6] *T(Y;2)L136* has lost *B^S*.

[7] *Y^S X·Y^L, In(1)EN, y/Y^S X·Y^L, In(1)EN, y; In(3LR)TM6/Sb.*

[8] The cross was *Y^S X·Y^L, In(1)EN, y/Y^S X·Y^L, In(1)EN, y; In(3LR)TM6/Sb* ♀ ♀ crosses to *Y^S X·Y^L, In(1)EN, v f B/0; C(4), ci ey^R/0* ♂ ♂

[9] *R.V.* = Relative viability, expressed as number of duplications/number of regular progeny of the same sex. See text for further explanation.

[10] Ratio of activity in duplication divided by activity detected in euploid sibling of the same sex. Ratios of αGPDH and αGPO represent αGP-dependent activity only, i.e. total activity minus a

Y^S and B^S for Y^L), in the cross where the Y breaks are in opposite arms, the regular euploid progeny will be $y+B^S$, and are the products of alternate disjunction in both parents. Since the sex chromosomes are marked with *yellow*, the aneuploids (products of adjacent-I disjunction in both parents), will be yB^SB^S and $y+y+B+$ (phenotypically yellow double-Bar, and Hairy wing Bar+, respectively). The duplication will show one phenotype and the deficiency will show the other.

In some regions, translocations with opposite Y arm-breaks were not available (e.g., region 47E–50C; 50C–52E in Table 1). Therefore, crosses between translocations with the same Y arm-breaks were made, and these crosses produce the four classes of progeny with the same visible phenotype, $y+B^S$. In order to recognize the aneuploid progeny, we substituted the marker *Sco* for *Cy* in the male translocation parent (see the "Report of the Seattle-La Jolla Drosophila Laboratories", supplement to *Drosophila Inform. Serv.* 47: 1971 and LINDSLEY *et al.* 1972). From the segregation of the *Sco* and *Cy* markers from the translocation and sex chromosomes, it is possible to determine the phenotype of the aneuploid duplication.

There is one potential problem which manifests itself in the crosses involving same-Y-arm breaks. Approximately 5% of all the offspring recovered from Y-autosome translocation crosses are the products of adjacent II disjunction in both parents (R. C. GETHMANN, unpublished). These offspring will be aneuploid for the region in question; however, the duplication from adjacent I disjunction will be phenotypically indistinguishable from the deficiency from adjacent II disjunction. Likewise, the deficiency from adjacent I disjunction is phenotypically the same as the duplication of adjacent II. If the *R. V.* of the deficiency is low, selection of specific aneuploid types would be equivocal. In the crosses reported here, this was not a problem as all the deficiencies were lethal.

B. The duplication from adjacent I disjunction will be recovered in the same sex as the parent with the more distal autosomal breakpoint. In these crosses, the duplications were recovered in females whenever possible, since more females were recovered in any one class than males.

C. For opposite-Y-arm crosses, the phenotype of the duplication will be a double dose of the gene marker present in the broken Y arm of the translocation with the more distal autosomal breakpoint. For example, in the cross of J96 females to H52 males (line 2, Table 1), J96 is the

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no-substrate control of the same sex. The rate of NADH formation or tetrazolium reduction (Δ1.0 O.D.) was rapid, 4 and 12 minutes, respectively, for a single fly. The amount of non-specific reduction amounted to less than 0.05 O.D. units in the incubation period. Thus in any region background fluctuation would be negligible. SDH ratios are ratios of total ΔO.D. rather than subtracting controls. This is because SDH activities are considerably less than the αGP dehydrogenases, i.e. extracts from two flies were necessary to give ΔO.D. of 0.25 over a 20-minute period In this period the ΔO.D. of any non-substrate control was approximately 0.25. Hence the total O.D. in any measurement of the diploid was approximately 0.5, the sum of the background and substrate dependent activity. Since background and SHD activity fluctuated from region to region a single control measurement could not be validly subtracted from each genotype. To prevent doubling of efforts by controlling each region, we alternatively used ΔO.D. ratios with more strict putative criteria. All regions which gave duplication:euploid ratios ≥ 1.1 were retested with individual controls. Each activity is the average of at least 4 assays per genotype. Putative regions were retested with 10–20 more flies before preparing multiple fly homogenates for confirmation.

[11] αGPDH—average activity is ♂ 8.7 nmoles/min/fly (range: 7.2–10.1)
♀ 11.4 nmoles/min/fly (range: 7.5–16.4)

[12] αGPO—average activity: ♂ 0.046 nmoles/12min/fly (range: 0.024–0.069)
♀ 0.057 nmoles/12min/fly (range: 0.025–0.072)

[13] SDH—average activity: ♂ 0.012 nmoles/20min/2 flies (range: 0.009–.014)
♀ 0.016 nmoles/20min/2 flies (range: 0.011–.021)

[14] These values are for a duplication generated from B110 × R155 covering this region but not listed above.

[15] Activity could conceivably be lowered as a result of position effect variegation of a gene proximal to the breakpoints in the presence of the Y heterochromatin (BAKER 1968; BAHN 1971). This would not affect the screen however, since the euploid sibling should also variegate and show similar lowering of enzyme activity. The problem of implicating the wrong region could be detected by subsequent genetic dissection, e.g. Table 2 and 3.

more distally broken translocation and is broken in Y^L. Since Y^L contains Bar, the duplication was recovered as a *y Cy BB* female.

The second type of cross to consider is that which produces a duplication that spans the centromere. In these crosses each translocation is broken in a different autosomal arm, and to produce duplications and deficiencies with a unique phenotype, both translocations must be broken in the same *Y* arm. If they are broken in opposite *Y* arms, the same problems encountered with the like-*Y*-arm breaks in the first type of a cross will be found. In these crosses spanning the centromere, the duplication will be recovered in a male, and the deficiency will be in a female, regardless of which way the cross is made. The phenotype of the flies carrying the duplication will be a double dose of the marker in the broken *Y* arm.

The final crosses made were those which produced terminal aneuploids. There were five of these crosses, including one which yielded triplo-4 flies. For the four major autosome arms, crosses were made between the appropriate translocation and a non-translocated stock (see footnotes, Table 1). With these crosses, the duplications were recovered as males, and were marked by the *Y*- marker carried on the non-broken *Y* arm. The triplo-4 flies were produced by crosses between a non-translocated stock and a stock carrying an attached-fourth chromosome.

A few final comments should be made about these stocks and crosses. First, although all crosses were made under identical conditions and with the same number of parents, the number of offspring from the crosses varied greatly. Additionally, it has been found that many of these translocations are unstable, in that they tend to lose the *Y* markers, and to a lesser extent, the *Cy* marker. The B^S marker is the one most frequently lost, and it appears that the marker is simply lost, rather than undergoing any spontaneous "healing" of the translocation or exchange with Y^S to produce a translocation that now carries $y+$ on both pieces of the translocation. Because of this instability, it has been necessary to select each stock on a regular basis.

Finally, it was necessary to subdivide some of the original regions by further crosses. Both centromeric regions appear to be quite sensitive to duplication aneuploidy, as was the region between 74A and 79D. The second chromosome centric region and the 74A–79D regions were dissected into two smaller regions. To accomplish this with one translocation would require the use of the *Sco* marker to recognize the duplication phenotype in one of the crosses, as both *Y* breaks would have to be in the same *Y* arm in one of the crosses. To avoid a same *Y*-arm-cross, a region was dissected with *two* included translocations and the crosses were made such that the two resulting duplications were overlapping and had a unique phenotype. (See regions 38C–43C and 74A–79D, Table 1). In both cases, the duplication inviability seemed to be associated with one part of the larger region.

ENZYME ASSAYS

All enzymes were assayed spectrophotometrically on a Gilford model 2000 recording spectrophotometer at 30°. Flies were collected within 12 hours after eclosion and aged for 3–5 days in fresh vials. Developmental differences of each of the enzymes during this period was minimal. In every case the ages of aneuploids and euploids was within 12 hours of each other.

α-Glycerophosphate dehydrogenase: αGPDH was assayed by following the production of NADH at 340 nm in response to exogenous α-glycerophosphate as described by O'BRIEN and MACINTYRE (1972a).

Alcohol dehydrogenase: ADH was assayed by following NADH production at 340 nm according to the method of SOFER and URSPUNG (1968). The reaction mixture contained: 1.0 ml 0.05M Tris-HCl pH 8.5, 0.2 ml 0.01 M NAD (Sigma), 0.05 ml 20% ethanol, 0.2 ml enzyme. The enzyme extract was the supernatant following homogenizing two adults (age 2–5 days) in 0.2 ml reaction buffer and centrifugation on a Beckman microfuge.

α-Glycerophosphate oxidase: Mitochondrial αGPO was assayed in single fly homogenates in a modification of a procedure previously presented (O'BRIEN and MACINTYRE 1972a). Single adults were homogenized at 4° in a motor-driven glass micro-homogenizer (0.5 ml capacity) in 0.2 ml of isolation medium: 100 ml 0.05 M potassium phosphate pH 7.1, 37.2 mg Na_2 EDTA, 500 mg bovine serum albumin, 13 g sucrose, 1 ml Triton X-100 (Sigma). This extract was incubated at

30° for 5 minutes after which 1 ml of the assay mixture was added. The assay mix contained 100 ml 0.05 M potassium phosphate pH 7.1, 100 mg bovine serum albumin, 203 mg $MgCl_2$, 238 mg HEPES (N-2 Hydroxyethylpiperazine-N¹-2-ethanesulfonic acid), 40 mg gelatin, 15 mg phenazine methosulfate (PMS) (Sigma) and 40 mg p-Iodonitrotetrazoluim violet (INT) (Dajac). The mix was incubated with enzyme for 5 minutes after which 0.1 ml $Na_2\alpha$-glycerophosphate (0.23 g/ml) (Sigma) was added to start the reaction. The reaction is light-sensitive and is carried out in the dark. INT formazan production is linear for 20 minutes and proportional to enzyme concentration. In exactly 12 minutes the reaction was terminated by the addition of 0.05 ml 1 N HCl, and the mixture was read at 490 nm. No-substrate controls were treated identically except 0.2 ml distilled water was added in the place of α-glycerophosphate.

For multiple-fly assays, αGPO was extracted as follows: 100 mg of adults were homogenized with a mortar and pestle in the above isolation medium, minus the Triton X-100. Preparation of mitochondria is described elsewhere (O'BRIEN 1973). αGPO was extracted by resuspending the final mitochondrial pellet in 1% Triton X-100, 0.05 M potassium phosphate pH 7.1. Assay of αGPO was in the same assay mix described above and was followed for 4 minutes at 490 nm.

Succinic dehydrogenase: Two adults were homogenized as with αGPO in 0.2 ml isolation medium: 100 ml 0.01 M potassium phosphate pH 7.1, 37.2 mg Na_2EDTA, 500 mg bovine serum albumin, 13 g sucrose. One ml of assay mix (100 ml 0.01 M potassium phosphate pH 7.1, 900 mg KCl, 238 mg HEPES, 100 mg bovine serum albumin, 40 mg gelatin, 40 mg INT, and 15 mg PMS) was added to the extract. After 5 minutes at 30°, 0.05 ml Na succinate was added to start the reaction. After 20 minutes at 30° in the dark, the reaction was terminated by the addition of 0.05 M 1NHCl and was read at 490 nm. INT formazan production was linear with enzyme concentration and with time for 60 minutes. SDH was measured in batch preparations in intact mitochondria isolated as αGPO except with the SDH isolation medium. SDH was measured by reduction of the INT tetrazolium at 490 nm in the same manner as αGPO but in the SDH assay mix.

Malate dehydrogenase: There are two distinct malate dehydrogenases in Drosophila, a mitochondrial and a cytoplasmic form (MCREYNOLDS and KITTO 1970). Mitochondria are prepared as described above for αGPO and mitochondrial MDH is released by gentle sonication (O'BRIEN 1973). The cytoplasmic form remains in the supernatant from the start. Following separation of the two components, each is assayed by following (2 mM) oxaloacetate-dependent NADH oxidation in 5 mM pyrophosphate pH 8.3 at 340 nm (O'BRIEN 1973).

RESULTS

Table 1 lists the 31 crosses constructed to produce duplications for every region of the autosomal genome except 83DE. The length of the regions varies from 100 to 215 chromomeres with a mode near 150. The relative viability (*R.V.*) ranges from 0.15 to 1.01, with the exception of two regions—83DE, which is a triplo-lethal and 74A-76F—which could only be obtained in a larger duplication (74A to 79D) with an *R.V.* of 0.06. There was no obvious correlation between *R.V.* and duplication length (Table 1).

Controls with known gene-enzyme loci: In order to test the efficacy of the segmental aneuploid approach in detecting structural genes of enzymes, we selected two known gene-enzyme loci and examined flies aneuploid for their chromosomal regions for dosage dependency. The first system was *αGpdh-1*, the structural gene for NAD-α-glycerophosphate dehydrogenase, which has been mapped genetically to II-20.5 and cytologically between 25E1 and 26C1 (O'BRIEN and MACINTYRE 1972b, GRELL 1967). The duplication that included this region (25A–27F, Table 1), exhibited approximately 50% more αGPDH activity than its euploid siblings, as predicted.

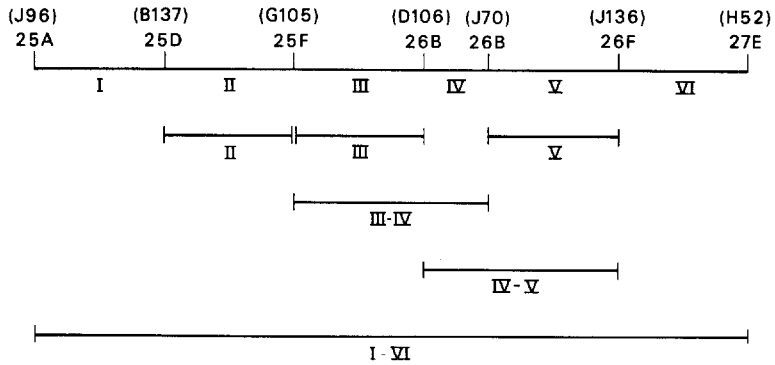

FIGURE 1.—Genetic dissection of 25A–27E with segmental aneuploids of 2L for localization of *αGpdh-1*. The translocation parents (in parentheses) and their breakpoints are listed. The six regions between the translocations are indicated as I–VI. The shorter horizontal lines represent the limits of the aneuploid region of the crosses which generated them. αGPDH activity of these offspring are presented in Table 2.

In order to localize the locus more specifically, the region was dissected by the six crosses listed in Figure 1. The enzyme activities of flies containing various doses of the six regions are presented in Table 2. From this table it can be seen that the dosage-dependent region falls between 25F and 26B, in agreement with GRELL's (1967) localization. From Table 2 it is also evident that none of the neighboring sub-regions exhibits any control over αGPDH activity. Furthermore, eleven other large regions were tested (Table 1) for dosage effects on αGPDH and no significant elevations in duplications were observed.

Adh, the gene for alcohol dehydrogenase, has been mapped genetically to II-50.1 and cytologically between 34E3 and 35D1 (GRELL, JACOBSON and MURPHY 1965; LINDSLEY and GRELL 1968). The duplication 30F–35BC listed in Table 1 was tested for dosage control of alcohol dehydrogenase. The duplication had 5.8 units of activity (expressed as nmoles/min/fly), as compared to 4.0 units of activity for the euploid sibs. The duplication/euploid ratio was 1.45. Hence, with two known gene-enzyme systems, the segmental aneuploid analysis was successful in detecting structural genes.

TABLE 2

*αGPDH activity in segmental aneuploids in the 25A–27E region of 2L**

Region†	Parents ♀	Parents ♂	Region doses 1	Region doses 2	Region doses 3
II	B137	G105	.027	—	—
IV–V	J136	D106	.027	—	.022
V	J136	J70	.029	—	.027
III	G105	D106	.014	.021	.033
III–IV	G105	J70	.010	—	.022
I–VI	J96	H52	—	.027	.038

* The αGPDH activity is expressed in μmoles/min/fly. Each measurement represents the average of four adults.
† Regions presented in Figure 1.

Succinic dehydrogenase: The search for chromosomal regions affecting SDH activity was complicated by very low levels of detectable SDH activity in Drosophila. The background levels of succinate-independent "nothing" dehydrogenase were as great as, if not greater than, the succinate-dependent activity. Moreover, activity fluctuations from genotype to genotype were significant with both background and SDH activity. Hence, a single no-substrate control for all the genotypes would not suffice even for the initial screen. This necessitated the retesting of any significant elevation (10%+) of the duplication over the euploid sibling.

Table 1 lists the ratios of activity of duplication/activity of sibling in the 31 crosses. Only one region was dosage-sensitive, 27E–30F on 2L. This region was dissected by further crosses between translocations with included breakpoints. The dissection (Table 3) localized the region responsible for enzyme elevation to be between 28D and 29F.

Since SDH has been localized to the inner membrane of mitochondria (SCHNAITMAN and GREENAWALT 1968; REED and SACKTOR 1971; and S. J. O'BRIEN, unpublished), isolated mitochondrial membranes were assayed for SDH. Two observations suggested that the elevated oxidation reaction observed in the duplication was not SDH but a background "nothing" dehydrogenase: (1) The elevation did not localize to mitochondria, rather to crude supernatants. (2) Elevated dehydrogenase levels, albeit much lower than succinate-dependent, were observed in duplications in the absence of succinate. Genetically, malate dehydrogenase has been localized to this region (map locus=40), and the structural similarity between malic acid and succinic acid suggests that the increase in activity could be due to MDH rather than SDH. Hence, both cytoplasmic and mitochondrial MDH were extracted from the aneuploids and euploids and examined for dosage dependency. Dosage dependency has been observed in the segmental aneuploids between 28D–29F with cytoplasmic preparations for malate oxidation as well as for succinate and for no-substrate controls (Table 3

TABLE 3

*Succinic-malate dehydrogenase activity in segmental aneuploids in 2L**

Region		Parents ♀	Parents ♂	Sex Dp	Region doses 3	Region doses 2	Ratio DP/+
27E	28B	R50	H52	♂	8.0	7.0	1.1
27E	28B	H52	R50	♀	11.9	9.8	1.2
28B	28C	B66	R50	♂	12.0	8.8	1.3
28B	28C	R50	B66	♀	11.9	12.6	0.94
28C	28D	A111	B66	♂	8.8	9.5	0.93
28C	28D	B66	A111	♀	13.3	18.3	0.73
28D	29F	A145	A111	♂	11.2	5.3	2.1
28D	29F	A111	A145	♀	13.0	8.3	1.6
29F	30F	L52	A145	♂	9.1	10.6	0.86
29F	30F	A145	L52	♀	12.5	10.5	1.2
27E	30F	H52	L52	♀	15.3	6.7	2.3

* Units are nmoles/20min/fly. Activity proved to represent malate dehydrogenase which used succinate.

and O'BRIEN 1973). Thus it is suggested that *Mdh-1* is cytologically localized between 28D and 29F with the reservations stated below.

DR. E. H. GRELL (personal communication) has recently induced a deficiency for *Jammed* and *Mdh-2* (GRELL 1969) which is cytologically localized between 30D and 32A on 2L. These breakpoints overlap our large duplication (27E–30F), but do not include the smaller region (28D–29F) which exhibits dosage dependency (Table 3). Furthermore, the overlapping region (29F–30F) does not exhibit dosage dependency in our hands. The possibility that the published breakpoint of A145 (29F) was incorrect was graciously reexamined by A. ATTALLAH and W. NASH. They confirmed the breakpoint of A145 to 29F which is to the left of GRELL's deficiency. It is not clear from the available data if *Mdh-1* and *Mdh-2* are the same locus and, if so, why there is the apparent discrepancy in the independently determined cytological localization. The 28D–29F locus is probably not the mitochondrial *Mdh* in light of lack of dosage dependency for that enzyme in these same aneuploid individuals (O'BRIEN 1973). Further investigation of this region is certainly necessary to unequivocally resolve this dilemma.

αGPO: The screen for αGPO (Table 1) detected one region which possessed elevated αGPO in duplications, region 50C–52E in 2R. Mitochondria were prepared from duplications and euploids of this region and αGPO was extracted from the inner membrane. The elevation does reside in the inner membrane, and the activity is α-glycerophosphate-dependent. Unfortunately, this region could not be further dissected genetically because there are no other translocations with breakpoints within this region. Thus we were unable to construct and test a deficiency for this region at this writing. Nevertheless, this is the first case, to our knowledge, of a nuclear gene's affecting the activity of a mitochondrial inner membrane enzyme in a dose-response manner.

The duplications, general characteristics: As previously stated, there was no obvious correlation between the *R.V.* and the length of the duplications. We also compared the duplication: euploid enzyme activity ratio of αGPO *vs.* duplication size and found no correlation. Likewise, no apparent relationship existed between *R.V.* and αGPO ratio or between *R.V.* and SDH ratio. There was, however, a striking increase in the variance of all the enzyme ratios in crosses in which the *R.V.* was less than 50%. For the most part, we rejected those translocation crosses which resulted in low αGPO or SDH ratios or a low *R.V.*

DISCUSSION

The results outlined in these crosses demonstrate the usefulness of segmental aneuploidy as a technique for localizing dosage-dependent gene loci, as proposed in LINDSLEY *et al.* (1972). The procedure followed in this paper was a two-step approach involving first, the screening of duplication aneuploid offspring of 31 crosses for increased levels of enzyme activity, and second, the subsequent dissection of putative regions by translocations with breaks within the cytological limits of the larger duplication. This dissection can generate both viable deficiencies and

duplications, and can be used to more specifically localize the dosage-sensitive locus. Although strict dosage dependency (1:2:3 in monosomics, euploids, and trisomics, respectively) is a characteristic only of structural genes, it is not conclusive evidence for the structural gene identity. Further credence can be obtained, as it has in other systems, by analysis of electrophoretic variation, by the demonstration of interallelic complementation of recessive "null" alleles (O'BRIEN and MACINTYRE 1972b; BELL, MACINTYRE and OLIVIERI 1972), and by the presence of immunologically crossreacting material in "null" alleles (GLASSMAN 1965; BELL and MACINTYRE 1973). Hence, the segmental aneuploid approach can only indicate probable structural gene loci; it cannot confirm the nature of the locus. It is important, however, to realize that the analysis of aneuploids gives two kinds of evidence pertinent to the identity of a structural gene: enzyme elevation in duplications and enzyme diminution in deficiencies. There are few, if any, other types of genes which are capable of producing both effects on the product of a second gene. It is unfortunate that in the case of αGPO, only one of these results (an elevation in duplications) could be examined.

The data obtained with the three known gene-enzyme loci emphasizes the feasibility of the aneuploid technique within the limits of the three assumptions discussed in the introduction: The enzymes are (1) nuclear in their inheritance, (2) located on the autosomes, and (3) dependent upon gene dosage for their level of activity. Any of these assumptions could be wrong with respect to the mitochondrial enzymes.

In Neurospora and in rat liver, there is strong evidence for mitochondrial-specific ribosomal synthesis of mitochondrial inner membrane components. These data show chloramphenicol-sensitive incorporation of radioactive amino acids into the inner membrane and into subunits of cytochrome oxidase (WEISS, SEBOLD and BUCHER 1971; NEUPERT, BRDICZKU and BUCHER 1967; COOTE and WORK 1971). Thus, it is possible that the mitochondrial enzymes examined in this study might be coded by mitochondrial DNA.

It is also possible that these genes might be sex-linked. Since the *X* chromosome comprises 20% of the Drosophila genome, and since genes appear to be randomly distributed with respect to chromosome location, it would be expected that a number of enzyme loci would be sex-linked. Such genes would not be detected in these experiments, although they could be recognized through the construction of *X* chromosome aneuploids in a manner similar to the construction of the autosomal aneuploids.

Finally, for mitochondrial enzymes, the rate-limiting step in enzyme activity might not be gene dosage, but rather, the number of mitochondrial sites or seats of enzyme action on the inner membrane. If this is true, one would expect to find no response to duplication aneuploidy, although it is possible that such enzymes might be sensitive to deficiency aneuploidy. In these experiments, only duplication aneuploids were examined in the initial screen, thus the structural genes would not have been detected.

In spite of these restrictions, a dosage region for one of the mitochondrial enzymes (αGPO) was demonstrated. The failure to find an SDH-sensitive region

could have been for any one of the above three reasons. However, since most, if not all, nuclear enzyme loci do show dosage sensitivity, the use of these *Y*-autosome translocations to produce segmental aneuploids should prove to be a useful and relatively simple technique for localizing new genes, as well as being useful in studies of regulation of these genes.

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