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Genetic Variation for Superoxide Dismutase Level in *Drosophila melanogaster*

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We have studied genetic variation for levels of activity of the enzyme superoxide dismutase (SOD) in Drosophila melanogaster. *We have constructed 34 lines homozygous for a given second and a given third chromosome derived from eight original lines; all lines were homozygous for the "fast" (F) allele of* Sod. *The variation in the relative levels of SOD CRM ranges from 1 to 1.6. The second chromosomes modify the SOD level, even though the structural* Sod *locus is in the third chromosome, and the specific effect of a given second chromosome depends on the particular third chromosome with which it is combined. This indicates that the variation in SOD content is controlled by polygenic modifiers present in the second (and in the third) chromosome. In addition to these trans-acting modifiers, we have isolated a cis-acting element* (Sod^{CAl}) *that reduces SOD CRM levels to 3.5% of a typical F/F homozygote.* Sod^{CAl} *is either a mutation in a regulatory site closely linked to the structural locus or a change in the coding sequence affecting the rate of degradation of the enzyme.*

INTRODUCTION

The superoxide dismutases (SOD; EC 1.15.1.1) have the property of converting the very active superoxide radicals to hydrogen peroxide and oxygen.

Three distinct classes of superoxide dismutases have been described, and all aerobic cells and organisms seem to contain at least one type of SOD (Fridovich, 1975). It has been postulated that SOD is part of the cell's defense mechanisms against the toxicity of oxygen metabolites (Fridovich, 1975, 1978).

The cytoplasmic superoxide dismutase (Cu–Zn SOD) from *Drosophila melanogaster* has been purified in our laboratory and shown to be a homodimeric enzyme of 32 kdaltons (Lee *et al.,* 1981a). Natural populations are polymorphic, with two common electromorphs designated "fast" (F) and "slow" (S). Lee *et al.* (1981b) have, however, shown that the "fast" electromorph conceals two electrophoretically cryptic allozymes. In addition, they have shown that SOD-slow differs from SOD-fast in thermal stability and specific activity. Clearly, functional differences of this kind suggest that *Drosophila* SOD might be a good model for studying the adaptive value of enzyme polymorphism. One must keep in mind, however, that the adaptive response at the biochemical level might involve quantitative changes in gene expression (McDonald *et al.,* 1977) as well as structural differences.

Laurie-Ahlberg *et al.* (1982) have shown that in *D. melanogaster* the activity levels of many enzymes are subject to genetically controlled quantitative variation. Superoxide dismutase is not included in their investigation, and we have no information on the amplitude of quantitative differences in SOD between different individuals or strains. In the present article we describe a genetic component of variation for SOD levels in *D. melanogaster* and report the genetic mapping of a cis-acting element which determines low levels of SOD activity.

MATERIALS AND METHODS

Strains

We use "isofemale" lines originated from single females collected in Furnace Creek (Death Valley, California) in April 1980. From each of these lines one second chromosome and one third chromosome were extracted and made homozygous following the procedure described by McDonald and Ayala (1978), by means of the balancer chromosomes *CyO* and *TM6*. The stock used (*CyO;TM6;T(2;3)ap^{Xa},Xa*) had been previously made homozygous for an X chromosome through many generations of inbreeding. Hence, the resulting isogenic lines may be considered homozygous for the first, second, and third chromosomes, or probably more than 95% of the genome. Substitution lines (i.e., lines homozygous for a second and a third wild chromosome derived from two different strains) were constructed according to the scheme described by McDonald and Ayala (1978).

Additional isofemale lines were isolated in October 1981 from a natural population near Davis, California. These were used for an experiment on divergent selection (high or low SOD activity), from which a strain with very low SOD activity was isolated after only a few generations of selection for low activity.

The third-chromosome marker stocks *se h st in ri p^p* and *ru h st p^p ss e^s* used for mapping this low-activity variant were obtained from Dr. M. M. Green.

All strains were maintained on standard cornmeal–yeast–sugar medium at 25°C.

Developmental Study

Two isogenic strains carrying the *Sod^F* allele were used to establish the developmental profile of the enzyme. Eggs were collected following the procedure of Elgin and Miller (1978), over a 12-hr period. Samples of 20 mg each were kept at -70°C for eventual assay. Aliquots of about 200 eggs were placed in fresh bottles, from which first-, second-, and third-instar larvae were later collected and frozen. After 4 days, the larvae remaining in these cultures were resynchronized: third-instar larvae crawling along the sides of the bottles (and thus ready to pupate) were transferred to disposable petri dishes over 2-hr periods. From these, samples of pupae were collected at 24-hr intervals and frozen, and emerging adults were collected over 4-hr periods and then aged in fresh bottles. All cultures were maintained at 25°C.

Sampling of Isogenic Lines

We placed 50 pairs of adult flies from each isogenic line in a half-pint bottle and allowed them to lay eggs for 2 days. The offspring was reared at 25°C and collected within 2 days of emergence. These flies were aged in groups of 100 to 200 flies per bottle for 6 days. We then sampled 24 males from each bottle and kept them at -70°C until the assays were performed.

Enzyme Assays

Samples of 24 adult males (6 to 8 days posteclosion) were homogenized in 0.9 ml of 10 mM potassium phosphate, 1mM EDTA (pH 7.4) with a Teflon homogenizer on ice. The extract was centrifuged at 10,000g for 5 min, and the supernatant was collected.

SOD activity was measured using a modification of the epinephrine assay (Misra and Fridovich, 1972). First, the rate of autooxidation of epinephrine in a solution containing 0.6 mM epinephrine, 0.1 mM EDTA, and 0.05 M sodium carbonate at pH 10.2 and 30°C was determined using a Gilford

spectrophotometer at 480 nm. Then different quantities of crude extract were added (to 1 ml of total reaction mixture) until three assays produced inhibition values comprised between 40 and 60%. The quantity inhibiting the standard rate by 50% was graphically interpolated. We arbitrarily define a unit of SOD activity as the quantity producing a 50% inhibition of the epinephrine autooxidation under our assay conditions.

Electrophoresis

SOD phenotypes were determined by electrophoresis either in 12% polyacrylamide (Davis, 1964) or in starch gel (gel buffer—9 mM Tris, 2.9 mM citric acid, 1mM EDTA, pH 7.1; electrode buffer—135 mM Tris, 40 mM citric acid, 1 mM EDTA). Polyacrylamide gels were stained according to Beauchamp and Fridovich (1971). For starch gels, the technique described by Ayala *et al.* (1972) was used.

Preparation of Antibodies

Cu–Zn SOD ("fast" allozyme from *D. melanogaster*) was purified by the method of Lee *et al.* (1981a). The pure SOD (800 μg) was then electrophoresed in a polyacrylamide–sodium dodecyl sulfate (SDS) slab gel according to Laemmli (1970), modified so that the cathodal electrode tray contained 0.13% SDS. The protein was visualized in the gel using a solution of 2 M sodium acetate, pH 7.0. The band containing SOD was cut from the gel and divided into two parts, which were kept at $-70°C$. One part was homogenized with an equal volume of Freund's complete adjuvant and injected intradermally in the back of two female New Zealand White rabbits. The other part was injected 14 days later following the same protocol, except that Freund's incomplete adjuvant was used. Finally, a booster of 70 μg of the pure antigen (without acrylamide) was injected in the popliteal lymph nodes of each rabbit, on the 28th day from the first injection. Seven days thereafter, blood was collected from ear veins and allowed to clot overnight, followed by centrifugation at 3000g for 10 min. The antibody titer was tested and the serum was kept in 1-ml aliquots at $-70°C$. Subsequent collections of blood were done every 5 days (20 ml per rabbit). When the titer dropped, a new booster of pure antigen was given.

The antiserum produced a single precipitin line when tested against extracts of homozygous flies, using immunoelectrophoresis on agarose gels.

CRM Determinations

The amount of CRM in crude extracts was determined using the immunodiffusion technique of Mancini *et al.* (1965). Immunodiffusion gels contained 1%

agarose, 0.5% SOD antiserum in Tris–barbital buffer, *p*H 8.6. Ten microliters of sample was placed in 4-mm wells and allowed to diffuse for 4 days at 4°C. The gels were then soaked overnight in 0.15 M NaCl, washed in distilled water for 1 hr, dried, and stained with Coomassie blue. Serial dilutions of the pure antigen were used to test the linear relationship between ring area and enzyme concentration.

The samples were prepared as follows: 24 individuals (adult flies, pupae, or third-instar larvae) or 20 mg of eggs or young larvae was homogenized in 300 μl of Tris–barbital, *p*H 8.6, using a Teflon homogenizer. The extract was centrifuged at 10,000*g* for 5 min, and 10 μl of supernatant was applied to the wells.

All assays were performed in the presence of a standard: aliquots of a solution of pure SOD (kept at −70°C) were applied to two wells in each immunodiffusion gel.

Protein Determinations

Total protein content was measured in each sample according to Lowry *et al.* (1951) with bovine serum albumin (BSA) as a standard.

Concentration Procedure for SOD

In some cases, it was necessary to increase the SOD concentration of crude extracts before measuring the activity or CRM level. For this purpose, we have applied one step of the purification procedure described by Lee *et al.* (1981a), namely, the ethanol–chloroform precipitation followed by centrifugation and freeze-drying of the supernatant. The lyophilized sample was then dissolved in distilled water to a final volume of 0.3 ml/g of flies (fresh weight). According to Lee *et al.* (1981a) and to our experience, the yield in Cu–Zn SOD is higher than 90%.

RESULTS

Developmental Profile

Figure 1 shows the changes in SOD content during the development of two isogenic lines, based on CRM determinations. These lines were selected on the basis of their divergence in the quantitative level of SOD expression. For the preadult stages, three samples per strain were measured each day; for the adults, three samples per sex per strain were measured.

SOD content is high in eggs and embryos. It decreases during the larval stages and stays at a relatively low level during the third instar and in the pupae. It rises steadily from emergence until the sixth day of adult life, when it reaches a plateau.

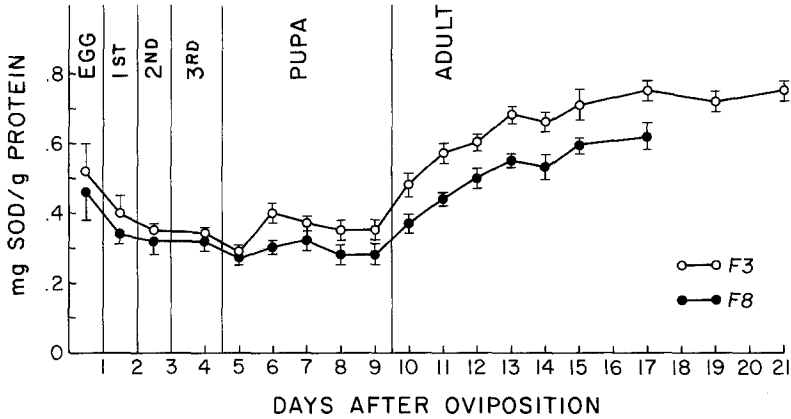Our results show the same general pattern as the developmental profile of

Fig. 1. Developmental profile of SOD content in two strains of *Drosophila melanogaster*, F3 and F8. Each point is the mean of three independent measures. Adult levels are the means of male and female levels. Vertical lines indicate standard errors. The two strains are homozygous for the "fast" electromorph of SOD.

D. melanogaster SOD activity presented by Nickla *et al.* (1983). However, some differences need to be pointed out. First, these authors found the peak of preadult activity in first-instar larvae, whereas we found it in eggs. Second, their results show activity variations ranging from 0 (late pupae) to 5 U/mg protein (adults), while our means range from 0.25 to 0.75 mg SOD/g protein. This discrepancy can be explained, at least partially, by a methodological difference. According to our experience, many of the published activity assays for SOD are not very suitable for determining the enzyme level in crude extracts, certainly due to the interference of other molecules with the test reaction. For example, the epinephrine assay did not reveal any measurable activity in crude extracts of certain pupal stages, whereas electrophoresis (PAGE) of the same extracts showed fairly intense bands of SOD activity (data not shown). For this reason, we think that the immunoassay is more reliable for the determination of developmental changes in the quantitative level of SOD expression.

Adults of the isogenic line F3 show a higher SOD content than those of strain F8. This difference is constant throughout development, but it becomes significant only during the pupal and adult stages.

Quantitative Differences Between Strains

We first attempted to analyze quantitative variation by measuring SOD activity in crude extracts from adult flies. However, we found that the assays based on activity were not very reliable when used with crude extracts instead of purified SOD. Therefore, we changed to an immunoassay, which produced reproducible and more precise results.

Forty isogenic lines, all carrying the Sod^F allele, were screened for variation of SOD activity, Eight lines were selected on the basis of their high (lines 1–4) or low (lines 5–8) level of SOD activity. From these eight strains, 34 substitution lines were constructed by combining second and third chromosomes of different origins. SOD content was determined in the eight original strains and in 26 of the 34 substitution lines using the technique of radial immunodiffusion (eight substitution lines were discarded for reasons of sterility or very low viability). The 34 lines assayed were sampled at each of three times (corresponding to different generations), which were assayed on ' each of 3 days.

Raw data include the amount of SOD CRM (Y) and the total protein content (X) detected in line i on day j, with $i = 1, 2, \ldots, 34$ and $j = 1, 2, 3$. Regression of SOD (Y_{ij}) on total protein (X_{ij}) was performed for each day separately. Because the two variables, Y and X, were found to be significantly correlated with each other ($P < 0.01$), adjustment of raw SOD CRM levels by the total protein content of the samples was necessary. Adjusted variables (Y'_{ij}) were obtained using the formula [modified from Laurie–Ahlberg *et al.* (1981)]

$$Y'_{ij} = Y_{ij} - b_j(X_{ij} - X_j),$$

where b_j is the regression coefficient, and X_j the total protein content, averaged over the 34 lines, for the experiment performed on day j.

The results are summarized in Table I, which shows the mean SOD CRM levels, calculated from adjusted data, for each of the 34 lines studied.

In order to discriminate between the effect of day and the effect of line on the total variation, a two-way analysis of variance was made using the adjusted CRM levels. The results are given in Table II. The effect of line and the effect of day are both highly significant.

The data in Table I allow one to gain insight into the effect of individual chromosomes on the level of SOD CRM. Chromosomes are numbered from 1 to 8, according to the original strain from which they had been extracted. For simplicity, substitution lines and original strains are designated by the ordered numbers of their second and third chromosomes (e.g., 4–8 for the homozygous line carrying the second chromosome from strain 4 and the third chromosome from strain 8). Each column in Table I illustrates the effects of different second chromosomes when associated with a given third chromosome. Similarly, each row shows the effects of different third chromosomes when associated with a given second chromosome. In order to test the significance of differences between individual lines, the Student–Newman–Keul's multiple-range test (Steel and Torrie, 1960) was applied to the mean CRM levels calculated for each of the 34 lines (Table III). Four main results were observed:

Table I. Mean SOD CRM Levels (±SE) of 34 Isogenic Lines of *Drosophila melanogaster*[a]

Second chromosome	Third chromosome							
	1	2	3	4	5	6	7	8
1	76.10 ± 0.25	—	86.63 ± 0.71	70.53 ± 3.06	—	—	69.93 ± 1.12	66.50 ± 0.57
2	85.83 ± 2.68	69.97 ± 1.77	—	85.83 ± 2.18	59.43 ± 1.53	58.67 ± 5.53	—	57.10 ± 1.96
3	80.43 ± 3.27	66.03 ± 3.33	76.67 ± 0.70	67.40 ± 1.39	64.47 ± 0.48	59.63 ± 0.38	—	—
4	68.47 ± 3.57	70.47 ± 1.53	78.93 ± 4.74	76.57 ± 2.07	—	—	—	58.33 ± 0.71
5	—	56.90 ± 2.65	62.80 ± 0.15	—	62.80 ± 2.55	54.03 ± 2.57	—	—
6	—	58.50 ± 1.78	60.53 ± 3.38	—	61.53 ± 1.86	62.77 ± 4.91	—	—
7	72.67 ± 2.80	—	—	—	—	—	64.20 ± 2.31	—
8	79.23 ± 3.52	—	—	—	—	—	—	60.67 ± 2.13

[a]The lines are various combinations of second and third chromosomes from eight original strains. The results are given as nanograms of SOD per fly, after adjustment for the total protein content.

Table II. Analysis of Variance of SOD CRM Levels in 34 Isogenic Lines
of *Drosophila melanogaster*

Source of variation	df	Sum of squares	Mean square	F	P
Line	33	8271.66	250.66	14.53	<0.001
Day	2	198.88	99.44	5.77	<0.01
Error	66	1138.20	17.25	—	—

(1) Adjusted CRM levels vary from 54.03 (line 5–6) to 86.63 (line 1–3), which means a 60% increase between the line with the lowest and the line with the highest level of SOD CRM.

(2) Although the structural gene for SOD is located in the third chromosome, the adjusted SOD content can be significantly modified by genes in the second chromosome.

(3) On the average, substitution lines containing both a second and third chromosome from any of the original strains numbered 1, 2, 3, and 4 exhibit higher levels of SOD CRM (mean, 76.05) than lines containing one or two chromosome pairs from any of the original strains with a low SOD content, namely, 5, 6, 7, and 8 (mean, 62.53).

(4) The relative effects of specific second chromosomes (e.g., chromosomes 2 and 4) are dependent upon the third chromosome with which they are combined (Table IIIa). Similarly, the effects of specific third chromsomes depend on the accompanying second chromosome (Table IIIb). This result suggests that the observed variation of SOD content is controlled, in part at least, by polygenic modifiers with epistatic effects.

Finally, it must be noted that sampling days are a significant source of variation for SOD CRM levels. The day effect includes environmental factors (i.e., differences in rearing conditions) as well as experimental error. Laurie-Ahlberg *et al.* (1980, 1982) also found that variation of activity levels among sampling days was significant for most enzymes.

Characterization of a Low-Activity Allele of *Sod*

For the purpose of obtaining quantitative variants for SOD, we submitted 20 isofemale lines carrying the Sod^F allele to divergent selection for increased and decreased levels of SOD CRM. In the third generation of selection, one of the lines (line F36) selected for reduced SOD did not show any detectable SOD CRM. Analyses of individual flies from this line by means of starch gel electrophoresis did not reveal any SOD activity either. These results strongly suggested that line F36 was homozygous for a null allele of *Sod*.

In order to determine whether this variant was a complete "null" allele or

Table III. Analysis of the Effects of Individual Chromosomes on SOD CRM Levels Using the Student–Newman–Keul's Multiple-Comparison Test: (a) Comparison of Different Second Chromosomes Combined with a Given Third Chromosome; (b) Comparison of Different Third Chromosomes Combined with a Given Second Chromosome

(a)	
Third chromosome	Effects of second chromosomes*
1	$2 \gg 4 = 7$ ($1 = 3 = 8$)
2	$2 = 4 > 5$ ($3 = 6$)
3	$1 = 3 = 4 \gg 5 = 6$
4	$2 \gg 1 = 3$
5	$2 = 3 = 5 = 6$
6	$2 = 3 = 5 = 6$
7	$1 = 7$
8	$1 = 2 = 4 = 8$

(b)	
Second chromosome	Effects of third chromosomes*
1	$3 \gg 4 = 7 = 8$
2	$1 = 4 \gg 2 = 5 = 6 = 8$
3	$1 = 3 \gg 5 = 6$ ($2 = 4$)
4	$3 = 4 \gg 8$ ($1 = 2$)
5	$2 = 3 = 5 = 6$
6	$2 = 3 = 5 = 6$
7	$1 = 7$
8	$1 \gg 8$

*($>$) Greater than, with $P < 0.05$; ($\gg$) greater than, with $P < 0.01$; ($=$) not significantly different.

a quantitative variant with a low CRM level and low activity, we concentrated the SOD possibly present in flies from line F36 by treating crude homogenates of adult flies with the technique described in Materials and Methods. The enriched extracts were then tested for SOD activity (electrophoresis and epinephrine assay) and CRM level.

The results are presented in Table IV. They show that the variant from line F36 is not a null, but a low-activity variant of *Sod*, the product of which comigrates with the SOD-S allozyme. For simplicity, this variant is designated Sod^{CA1} (California-1). The homozygous Sod^{CA1} strain (i.e., line F36)

expresses only 3.5% of the SOD CRM and 8.6% of the SOD activity found in a normal strain. The difference between these two results could be explained by the fact that, according to our experience, the SOD activity assay is not strictly specific for Cu–Zn SOD. Heterozygotes with various Sod^F strains have lower CRM levels compared to Sod^F homozygotes (Table V), the difference being significant in three of four combinations. It appears that the SOD CRM in heterozygotes is not correlated to the CRM in the corresponding Sod^F homozygotes.

Mapping of Sod^{CA1}

We decided to map the Sod^{CA1} variant in order to learn about the nature of this low-activity gene. Crosses using the *CyO* and *TM6* chromosomes indicated that this element is located in the third chromosome. Cytological analysis of salivary gland chromosomes from larvae homozygous for Sod^{CA1} and from heterozygotes with Canton-S revealed the presence of the rare inversion *Inv(3L)69F;75CD* in the third chromosomes carrying the Sod^{CA1} mutation. It was therefore necessary to determine first whether the low-activity gene was located within the inverted segment or out of it. Line F36 was crossed with a strain carrying the visible markers *se h st in ri p^p*, which mark a segment extending between the cytological limits 66D and 85A (Lindsley and Grell, 1968). Heterozygous females were then backcrossed to males from the marker strain, and their progeny were screened for recombinants. Eighteen recombinant third chromosomes were made homozygous and tested for SOD activity; two of them (M6 and M9) were found to carry the Sod^{CA1} mutation together with the standard chromosome sequence and the visible markers *st in ri p^p*. One of these lines (i.e., M9) was subsequently used for the genetic mapping of Sod^{CA1}.

Line M9 was crossed with a strain bearing the third-chromosome markers *ru h e^s* together with Sod^F. Female offspring were mated to males

Table IV. SOD Activity, CRM Level, and Electrophoretic Phenotype of Lines F36 (Presumed "Null") and F5 ("Fast" Allozyme) of *Drosophila melanogaster*[a]

Line	SOD CRM[b]	$\bar{X}$ (%)	SOD activity[c]	$\bar{X}$ (%)	Electrophoretic phenotype[d]
F36	2.35	3.5	0.07	8.6	Slow
F5	67.43	100	0.81	100	Fast

[a]Two samples have been treated for each line, using the same concentration procedure; the mean values are given.
[b]Expressed as nanograms of SOD per milligram of tissue.
[c]Expressed as units (defined in Materials and Methods) per milligram of tissue.
[d]Determined in starch gels at pH 7.1 and in polyacrylamide at pH 8.6.

Table V. SOD CRM Level of Sod^{CAl}/Sod^F Heterozygotes Compared to That of Homozygotes for the Corresponding F Allele

Line	CRM in homozygotes ($\bar{X}^a \pm$ SE)	CRM in heterozygotes with Sod^{CAl} ($\bar{Y}^a \pm$ SE)	Student's t test[b]	
			$\bar{X}-\bar{Y}$	$\bar{Y}$-additivity
F1	76.10 ± 0.25	45.18 ± 1.10	***	**
F3	76.67 ± 0.70	45.20 ± 3.75	***	ns
F5	62.80 ± 2.55	46.33 ± 2.75	*	*
F6	62.77 ± 4.91	49.60 ± 0.35	ns	***

[a] Mean of three independent assays, corrected for total protein content. Units: nanograms of SOD per fly.
[b] Significance of the difference when heterozygotes are compared to Sod^F homozygotes ($\bar{X}-\bar{Y}$) or to the expected heterozygote levels assuming additivity of the effects of the two alleles ($\bar{Y}$-additivity).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

bearing ru h st p^p ss e^s as well as Sod^S. Males issued from this cross were screened for recombination events in region I, II, III, or IV (Table VI). They were subsequently crossed with one of the lines homozygous for Sod^{CAl} and adequate visible markers, so that the recombined chromosomes could be tested in the presence of the variant Sod^{CAl}. The SOD phenotypes were determined from individual flies, with the knowledge that Sod^{CAl} homozygotes do not produce any SOD band in starch gels, whereas heterozygotes with either Sod^F or Sod^S produce clear and distinct zones of SOD activity.

The results of the mapping experiment are presented in Table VI. The Sod^{CAl} mutation maps to 3–33.4, based on the distribution of SOD phenotypes among the recombinants in region II. Previous studies [references given by Doane and Treat-Clemons (1982)] have attributed the Sod structural gene to either 3–32.5 or 3–34.6, the second location resulting from the use of more adequate markers (V. Finnerty, personal communication). The map position of Sod^{CAl} suggests that this mutation is very closely linked to the Sod locus, especially in view of the fact that the mapping experiment gave no evidence of recombination between the electrophoretic variants of Sod and the Sod^{CAl} "modifier."

DISCUSSION

Studies by McDonald and Ayala (1978) and Laurie-Ahlberg et al. (1980, 1982), have shown that genetic variation for enzyme activity or CRM level is

Table VI. Mapping of the Sod^{CAI} Mutation in the Third Chromosome
of *Drosophila melanogaster*

Region:		I		II		III		IV	
Position:		0.0		26.5		44.0		48.0		70.7
A (Sod^F):		ru		h		+		+		e^s
B (Sod^{CAI}):		+		+		st		p^p		+

Sequence[a]	A-B	B-A	A-B	B-A	A-B	B-A	A-B	B-A
SOD$^+$ (F)	0	4	26	18	3	0	4	0
SOD$^-$	6	0	16	26	0	5	0	4

[a]The column below A-B (respectively, B-A) indicates recombinants with the alleles from chromosome A (respectively, B) to the left of the corresponding region.

a general phenomenon in *Drosophila melanogaster*. This quantitative variation is explained largely by polymorphism for modifier genes, which can be either linked or unlinked to the structural gene that they influence.

The results presented here extend these observations to Cu–Zn SOD. We have assayed 34 isogenic lines bearing the Sod^F electrophoretic allele and have observed a range of variation from 1 to 1.6 in the relative levels of SOD CRM (adjusted for protein content). Given that the *Sod* structural gene is located in the third chromosome, we have demonstrated that linked as well as unlinked loci significantly affect the SOD content of flies. However, because of the relatively low amplitude of the differences between lines and the large variance within lines, we have not attempted to map these modifiers.

In a detailed study of *Adh* expression in *D. melanogaster,* Maroni and Laurie-Ahlberg (1983) have shown that ADH activity can be affected by two distinct types of modifiers. In the sample that they studied, they found that ADH levels in lines homozygous for the "fast" allele were affected primarily by distant polygenic modifiers with trans-acting effects, whereas ADH levels in the "slow" lines were controlled by cis-acting modifiers that mapped to or very close to the *Adh* structural gene. The variability due to the distant trans-acting modifiers was not very large, with relative ADH levels ranging from 1 to 1.7; the variation caused by cis-acting modifiers was, however, substantially larger, ranging from 1 to 4.

The present study describes a related situation for *Sod* expression. In most cases, differences in SOD levels between lines seem to be due to polygenic modifiers with epistatic effects, as it appears from the data in Tables I and III. One line, however, showed a drastic reduction of the SOD CRM level: to 3.5% of the level found in a normal strain. We have designated the mutation responsible for this difference Sod^{CAI}. It appears to be tightly linked to the *Sod* structural gene ("slow" allele): no recombinants were

obtained between this putative modifier and the electrophoretic site. CRM levels are lower in Sod^{CA1}/Sod^F heterozygotes than in Sod^F homozygotes (Table V). However, the heterozygote levels are higher (significantly so in all but one case) than the levels expected when assuming that the effects of the two "alleles" are additive. Moreover, electrophoresis of single flies heterozygous for Sod^{CA1} and Sod^F reveals only two bands of SOD activity: one corresponded to the "fast" allozyme, while the other, much weaker, is intermediate between the position of SOD-fast and the position of SOD-slow (data not shown). We assume that this intermediate band represents the heterodimer. These results strongly suggests that Sod^{CA1} is either a cis-acting modifier or an allele of Sod.

Comparable situations have been described for other enzymes from $D.$ $melanogaster$ (Bentley et $al.,$ 1983; Shaffer and Bewley, 1983). In each case, there was a considerable reduction of enzyme activity and CRM level caused by a cis-acting element tightly linked to the structural gene. Several lines of evidence suggest that this element is, in each case, a quantitative site controlling the rate of synthesis of the enzyme. The low-activity "allele" described in the present study displays similar characteristics as the ones analyzed in articles just cited. Our data, however, do not allow us to make an unequivocal statement about the nature of the Sod^{CA1} allele. It could be either a mutation in a regulatory site or a change in the coding sequence which would affect the rate of degradation of the enzyme.

It is worth noticing that the Sod^{CA1} allele was discovered in an isofemale line in which it was tightly linked to the unique inversion Inv(3L)69F;75CD. Burkhart et $al.$ (1984) have described a close association between unique inversions and 4 of 58 null or low-activity mutations recovered from natural populations of $D.$ $melanogaster$. They took this association as an indication that some of these mutations might be the result of hybrid dysgenesis. In the case of Sod^{CA1}, the presence of an inversion with a breakpoint near the Sod structural locus suggests that a dysgenic event might be involved. But we must keep in mind that the isofemale line segregating for Sod^{CA1} had not been previously crossed with any laboratory stock. This means that the presumptive dysgenic event must have occurred in the natural population or within the line itself if it happened in the laboratory.

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