GENETIC VARIATION AFFECTING THE EXPRESSION OF CATALASE IN *DROSOPHILA MELANOGASTER*: CORRELATIONS WITH RATES OF ENZYME SYNTHESIS AND DEGRADATION

GLENN C. BEWLEY AND CATHY C. LAURIE-AHLBERG

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27650

Manuscript received July 20, 1983
Revised copy accepted November 15, 1983

ABSTRACT

Both second and third chromosome substitution lines isolated from natural populations of *Drosophila melanogaster* affect the expression of catalase (EC 1.11.1.6) at both the larval and adult stages of development. In each case, the level of catalase activity is strongly related to the level of catalase-specific cross-reacting material. Turnover studies employing the catalase inhibitor 3-amino-1,2,4-triazole were conducted on a selected number of lines. Although the variation in steady state levels of catalase protein was highly significant among lines, variation in intracellular degradation rate was not. These results suggest that the different steady state levels observed among lines largely reflect different rates of catalase synthesis.

THE differential expression of genomic information in both space and time is a fundamental property of eukaryotic development (DAVIDSON 1976; PAIGEN 1979; SCANDALIOS 1979). However, the relative contribution of opposing rates of synthesis and degradation that can lead to genetically controlled differences in the steady state level of a particular gene product is poorly understood. Naturally occurring genetic variants that perturb the expression of a given structural gene are, therefore, important in that they allow the identification of genome-encoded information that programs such changes and afford the opportunity to probe their function at the biochemical and molecular level.

The enzyme catalase offers a unique opportunity to examine the genetic control of enzyme turnover since simple but reliable techniques have been developed for this purpose (for review see REICHCIGL 1968). The compound 3-amino-1,2,4-triazole binds irreversibly to the active site of the protein apoenzyme, resulting in irreversible inhibition of activity (MARGOLIASH, NOVGRODSKY and SCHEJTER 1960; REID *et al.* 1981). In addition, when aminotriazole is administered to the organism under study, it destroys existing catalase molecules without interfering with *de novo* enzyme synthesis (PRICE, RECHCIGL and HARTLEY 1961; PRICE *et al.* 1962). Thus, the initial rate of reappearance of catalase activity directly reflects the rate of *de novo* synthesis, and knowledge

of steady state levels allows estimations of the rate constants for both synthesis and degradation (PRICE, RECHCIGL and HARTLEY 1961; PRICE *et al.* 1962; FRITZ and PRUITT 1977). This technique has found application for catalase turnover studies in a number of organisms, including Drosophila (RECHCIGL and HESTON 1967; GANSCHOW and SCHIMKE 1969; CRANE, HOLMES and MASTERS 1978; LUBINSKY and BEWLEY 1979; BEWLEY *et al.* 1983), and the simplicity of the experimental procedure has allowed us, for the first time, to examine turnover parameters for a specific enzyme on a population level.

The structural gene, *Cat*$^+$, for Drosophila catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) has been mapped to the cytogenetic region 75D-76A on the left arm of chromosome *3* on the basis of dosage responses to segmental aneuploidy (LUBINSKY and BEWLEY 1979). The enzyme has been purified to homogeneity, is tetrameric with a subunit molecular weight of 58,100 and catalase-specific antibodies have been raised (NAHMIAS and BEWLEY 1984). Two distinct peaks of activity are observed during development with the first peak occurring in late third instar larvae just prior to puparium formation and the second and larger of the two peaks occurring during metamorphosis (BEWLEY *et al.* 1983). Upon emergence of the adult fly, catalase activity reaches a steady state level that is maintained throughout adult life.

In the present study, we investigate several aspects of catalase activity variation using a well-defined set of lines in which a large sample of second and third chromosomes isolated from natural populations are substituted into an isogenic background. We have previously reported the occurrence of a great deal of genetic variability among these same lines for catalase and a number of other gene-enzyme systems as well (LAURIE-AHLBERG *et al.* 1980, 1982). This report presents a more detailed characterization of these lines with respect to the following questions: How repeatable are the line effects on catalase activity over time, and what is their magnitude relative to measurement error and to the effects of random laboratory culture environment? Are the line effects on activity specific for particular stages of development, or are they systemic in their mode of action? To what extent is the variation in activity due to variation in the steady state level of catalase-specific protein (*i.e.*, serologically cross-reacting material) as opposed to variation in enzyme structure? To what extent do opposing rates of enzyme synthesis and degradation contribute to the variation in steady state levels of catalase-specific protein observed among lines? The ultimate goals of this project are to identify the types of modifiers that are polymorphic in natural populations, to gain insight into the range of effects that they have on modulating synthesis and degradation of a specific gene product and to develop material suitable for investigating the molecular mechanisms whereby these modifiers exert their effects.

METHODS

Stocks: Two sets of isogenic chromosome substitution lines were used in this study. The constitution of a line of each type is $i_1/i_1;+_2/+_2;i_3/i_3$ (referred to as a second chromosome line) and i_1/i_1; $i_2/i_2;+_3/+_3$ (a third chromosome line), where *i* refers to a chromosome from a highly inbred line (*Ho* − R) and + refers to a chromosome sampled from a natural population. The + but not

the *i* chromosomes vary within a set of lines. The + chromosomes derive from four geographic localities within the United States. The construction and electrophoretic and karyotypic analyses of these lines are described by LAURIE-AHLBERG *et al.* (1980, 1982).

Assay: The preparation of extracts from both the larval and adult stages of development has been previously described (BEWLEY *et al.* 1983).

Catalase activity was determined by the spectrophotometric method of BEERS and SIZER (1952) and has been described in detail for Drosophila catalase (LUBINSKY and BEWLEY 1979; NAHMIAS and BEWLEY 1984). The disappearance of H_2O_2 was monitored at 230 nm using a molar extinction coefficient for H_2O_2 of 62.4 (NELSON and KIESOW 1972). One unit of activity is defined as 1 μmol of H_2O_2 decomposed per min at 30°.

Protein determinations were conducted by the dye-binding method of BRADFORD (1976).

Immunological procedures: The purification of Drosophila catalase to homogeneity by hydrophobic interaction chromatography and the subsequent production of monospecific antibodies have been described (NAHMIAS and BEWLEY 1984).

Radial immunodiffusion was used to estimate levels of cross-reacting material (CRM) as described previously (BEWLEY *et al.* 1983). Immunodiffusion was conducted at 4° for 8 days. Since the catalase antigen-antibody complex retains enzymatic activity, the gels were stained for activity using a ferric chloride-potassium ferricyanide stain (WOODBURY, SPENCER and STAHMAN 1971). The area of each diffusion ring was estimated using a mean radius from two determinations. The linearity of diffusion ring area with serial dilutions of a fly extract, one fly per 10 μl of buffer, is illustrated in Figure 1.

Rearing conditions and sampling: All flies were reared on standard cornmeal-molasses medium with live yeast. The general procedure for obtaining samples from a set of isogenic lines was to place 50 pairs of parents in a half-pint bottle for 48 hr, rear the offspring at 25°, collect them within 18 hr of emergence, age the male imagoes in groups of 15 per vial for 6 days, weigh the live flies and freeze the samples at −70°.

Experiment 1: a large sample of chromosomes from natural populations: The design of this experiment was described elsewhere (LAURIE-AHLBERG *et al.* 1980). Larval samples were obtained from the same bottle as the adults, at the "wandering" third instar stage. Collections were made from the second chromosome lines in April and May 1978 and from the third chromosome lines in August and September 1978.

Experiment 2: repeatability of line differences: Sixteen each of second and third chromosome substitution lines were selected from the distribution of line means of catalase activity per unit weight obtained from experiment 1, (five high, five low and six intermediate). Two replicate sets of ten males were collected from each of three sets of bottles ("blocks") during November 1978 for the second chromosome lines and during February 1979 for the third chromosome lines. The bottle sets differ by 1 or 2 days in the time that parents were added and in the time of collection of offspring. One set of ten males from each line was homogenized on a given day ("assay day"), and two replicate assays were made from each homogenate.

Experiment 3: relationship between activity and CRM level: Twelve each of second and third chromosome substitution lines were selected from the line mean distributions from experiment 2. Four replicate sets of five males were collected from each of three sets of bottles (blocks) on each of two collection days during November 1981. Two of the replicate samples were homogenized on different assay days and used for catalase activity and general protein assays. The other two samples were homogenized on the same day but placed in different immunodiffusion gels. Each gel contained one sample from each of the 24 lines (12 second chromosome and 12 third chromosome).

Adjustment of activities for body size variation: It is common practice when using the activity of an enzyme as an indicator of genetic expression to "standardize" the raw enzyme measurements (units per fly) by dividing by protein content or live-weight per fly to obtain "specific activity" or "units per milligram of live weight." This procedure is usually inappropriate for reasons discussed elsewhere (LAURIE-AHLBERG *et al.* 1980; MARONI *et al.* 1982). Instead of forming such ratios, we adjust activity by linear regression on weight or protein. The regression is taken over lines within a block (or other design factor), and the sums of squares and products are pooled over blocks (if homogeneous) to obtain a single coefficient, *b*. For example, the following equation shows adjustment of raw activity per fly (Y) from the ith sample within the jth block and kth line by the corresponding independent variable (X = weight or protein): $\hat{Y}_{ijk} = Y_{ijk} - b(X_{ijk} - \bar{X} \ldots)$.

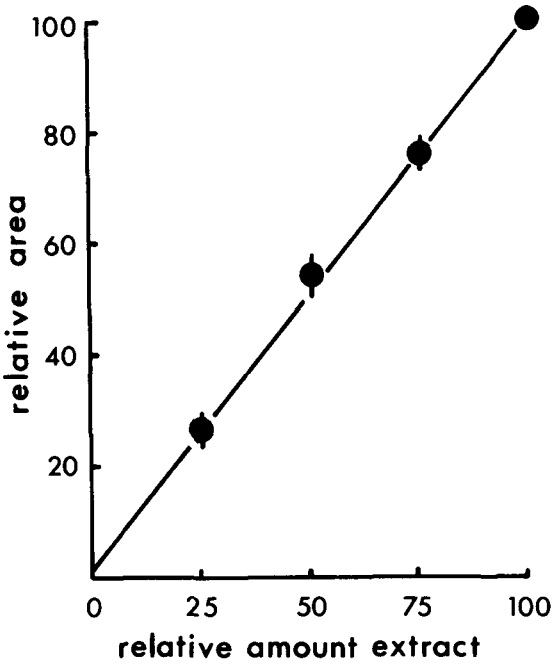

FIGURE 1.—Standard calibration curve of radial immunodiffusion illustrating the linearity of the relative area of the diffusion circle, from which the area of the well has been subtracted, as a function of the amount of extract applied. Each value plotted is the mean ± 1 SD of four determinations.

Dietary administration of 3-amino-1,2,4-triazole: Adult male flies aged for 5 days posteclosion were nutritionally deprived for 12 hr on 2.5% agar. Flies were subsequently transferred without etherization to agar bottles containing Kimwipes saturated with 3 ml of a 5 mM aminotriazole solution in 2% sucrose. Control flies were fed the sucrose solution in the absence of aminotriazole. Flies were allowed to feed for 6 hr and then placed onto chase media.

Analysis of turnover parameters: Turnover parameters were determined by the aminotriazole perturbation method of PRICE, RECHCIGL and HARTLEY (1961) and PRICE *et al.* (1962) as described for Drosophila (LUBINSKY and BEWLEY 1979; NAHMIAS and BEWLEY 1984). At specific time intervals following an aminotriazole feeding, flies were sacrificed, and the level of catalase activity was determined. The log of the difference between enzyme activity at the steady state (C_N) and activity at time intervals during the recovery phase (C_t) was plotted as a function of time. The rate constant for degradation (k_d) was calculated from the slope of this line according to the relationship $k_d = \left[\ln\left(\frac{C_N}{C_N - C_t}\right)\right] \div t$. The rate constants for synthesis (k_s) were estimated from the relationship $k_s = k_d \cdot C_N$ and half-life values for catalase were estimated from $t_{1/2} = \ln 2/k_d$ (SCHIMKE 1975).

Turnover parameter estimates for C_N and k_d were made with the following unbalanced design. From one to five lines of a chromosome type were sampled during a particular block of time. Sampling of each line consisted of setting up six bottles of adults. Flies in three of the bottles (experimentals) were fed the aminotriazole sucrose solution and flies in the other three (controls) were fed just the sucrose solution. Flies from the experimental bottles were assayed at 12, 24, 48, 72, 96, 120 and 144 hr after removal from aminotriazole; these data provide estimates of C_t for seven time points. Flies from the three control bottles were each assayed at 0 hr and at 120 or 144 hr; the average of these six values provided one estimate of C_N for calculation of one k_d value for each of the three experimental bottles. The k_d values were estimated as the slope of the linear regression of $\ln (C_N - C_t)$ on t. The average R^2 for the 128 regressions reported here was 0.90. Analyses of variance were performed on the C_N and k_d estimates.

RESULTS

Experiment 1: a large sample of chromosomes from natural populations: In order to establish the range and distribution of effects on catalase activity level caused by chromosomes sampled from natural populations, 50 second and 50 third chromosome substitution lines were assayed for catalase activity in adult males and third instar larvae. The results for the adult males have been reported (LAURIE-AHLBERG *et al.* 1980). Third chromosome lines were a significant source of variation in both raw activity (units per fly) and in activity adjusted by regression on live weight. However, second chromosome lines did not have a significant effect on catalase activity. The distributions of line means of adult male activity, from which the approximately continuous nature of the third chromosome effects is evident, are shown in Figure 2.

Here we report the results concerning variation in larval catalase activity. Again, third chromosome lines show a highly significant effect, whereas second chromosomes do not have a significant effect. The correlation over third chromosomes between larval and adult activities is highly significant ($r = 0.52$, $P < 0.001$) but weak enough in magnitude that important stage-specific genetic effects may exist (see Figure 2). To test this possibility, a weighted analysis of variance including both stages was performed, with weights equal to the inverse of the residual variances of larval and adult activities (because those residuals are significantly different). The line-by-stage interaction is highly significant and confirms the presence of stage specificity for the third chromosome effects.

Experiment 2: repeatability of line differences: Experiment 2 was designed primarily to investigate the repeatability of specific third chromosome line differences and to determine the importance of line effects relative to other sources of variation. Second chromosome lines were also included to determine whether unlinked modifier effects on catalase activity could be detected with a statistically more powerful design. The design of this experiment allows the

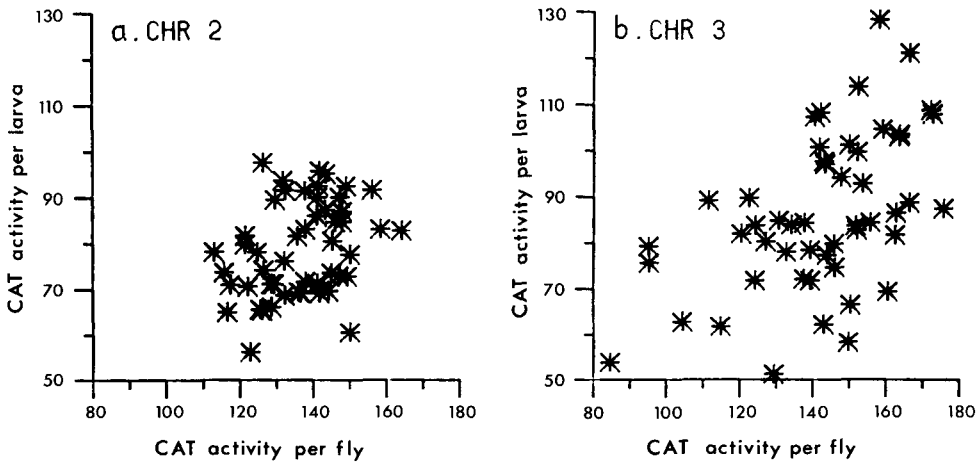

FIGURE 2.—Plots of the 50 line means of catalase activity units per adult male *vs.* the activity per third instar larva (from experiment 1). The correlations corresponding to each plot are (a) $r = 0.33$ ($P < 0.05$) and (b) $r = 0.52$ ($P < 0.001$).

variance of activity to be partitioned into lines (genotypes), blocks (random laboratory culture environments), line-by-block (a genotype-by-environment interaction), assay day nested within block (which includes the among-homogenate variation), experimental error and within-homogenate measurement error. Table 1 shows that both second and third chromosome effects are highly significant in this experiment. Table 1 also shows a variance component ratio, K, which represents the proportion of variation within a block that is attributable to differences among the lines and is thus basically a broad-sense heritability (except that the experimental unit consists of several, rather than one, individuals). The K values indicate that the third chromosome effects on catalase activity level are considerably stronger than the second chromosome effects. These results, based on raw activity per fly, are affected only slightly by weight adjustment (see K values in Table 1). This finding indicates that most of the genetic variation affecting activity is independent of body size variation. The results in Table 1 also show that the lines contribute considerably more variation than do any of the other sources.

The repeatability of specific line differences can be investigated by examination of Figure 3, which compares the line mean distributions for three experiments performed over a 3-yr span of time. The overall repeatability between experiments can be quantified as the correlation over the line means (Table 2). The third chromosome line effects are very repeatable over time (as well as showing high K values for each experiment). The second chromosome effects are much weaker within each experiment and do not appear to be repeatable over time. This lack of repeatability of second chromosome

TABLE 1

Summary of analyses of variance of raw catalase activities for experiment 2

		Catalase units per fly			
		Chromosome *2*		Chromosome *3*	
Source	d.f.	F test	σ^2	F test	σ^2
Line	15	****	1.11	****	2.32
Block	2	NS	0.40	NS	0.06
$L \times B$	30	*	0.41	NS	0.12
Assay day (B)	3	**	0.19	NS	0.03
$L \times A(B)$ = experimental error	45	****	0.68	****	0.56
Rep [$L \times A(B)$] = measurement error	96		0.43		0.10
Total	191				
$K = \sigma^2_L/(\sigma^2_L + \sigma^2_{L\times B} + \sigma^2_{EE})$			0.51		0.77
C.V. (measurement error)			5.5%		3.4%
C.V. (experimental error)			11.2%		11.9%

The significance levels of the F tests and the variance component estimates (σ^2) are given. After weight adjustment, the significance level of the F test for lines decreases to $P < 0.001$ for chromosome *2* lines but does not change for chromosome *3* lines. The K values decrease by a small amount, to 0.41 for chromosome *2* and to 0.73 for chromosome *3*. Rep refers to replicate assays from one homogenate.

NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

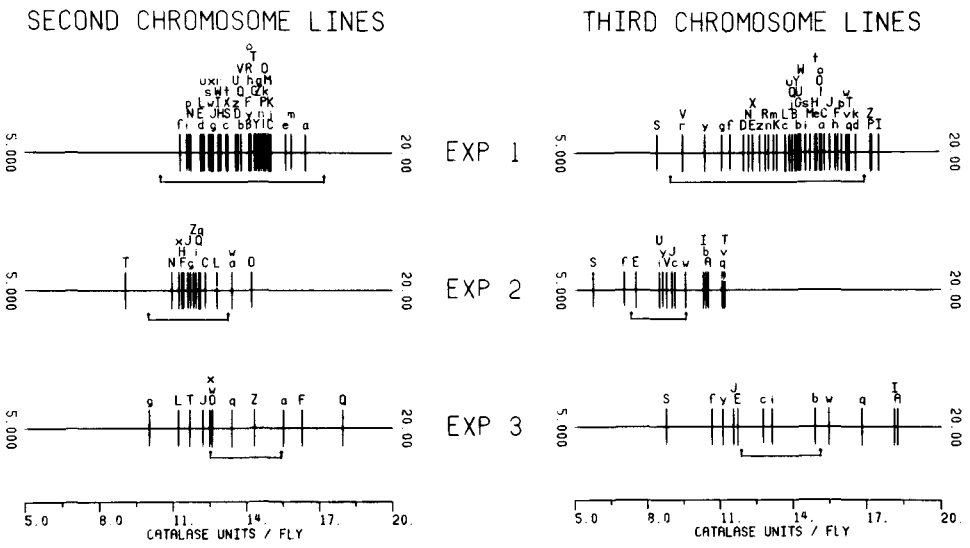

FIGURE 3.—Distributions of line means of catalase activity units per fly from three experiments. A one-letter code label for each line appears above its position in the plot. The 95% confidence interval for the difference between any two means within an experiment (Tukey's multiple comparison procedure) is indicated by the bar below each distribution. The three experiments represent independent samples collected over a 3-yr period.

TABLE 2

Correlations over line means of catalase units per fly from experiments 1–3

	Experiment		
	1	2	3
1		0.24 (n = 16)	0.51 (n = 12)
2	0.79*** (n = 15)		0.12 (n = 12)
3	0.88*** (n = 11)	0.89*** (n = 12)	

Chromosome *2* lines are above diagonal, chromosome *3* below.
*** $P < 0.001$.

effects is probably due to genotype-by-environment interaction (since the block-by-line source is significant in both experiments 2 and 3.

Experiment 3: relationship between activity and CRM level: Experiment 3 was designed to investigate the relationship between line effects on catalase activity and catalase-specific CRM level (estimated by radial immunodiffusion). Tables 3 and 4 show that both second and third chromosome lines are a highly significant source of variation for all three raw variables (activity per fly, CRM level per fly and general protein per fly). After adjustment by regression on general protein, lines remain highly significant for both catalase activity and CRM levels. This result is clearly expected for the third chromosome lines because of the lack of a significant correlation between activity and protein (Figure 4d). For the second chromosome lines, the regression of activity on

TABLE 3

Summary of analyses of variance of chromosome 2 data for experiment 3

Source	d.f.	*F* tests					
		Raw CAT/fly	General PROT/fly	CAT-CRM/fly	PROT-adj CAT/fly	CRM-adj CAT/fly	PROT-adj CAT-CRM/fly
Block	2	NS	NS	NS	NS	NS	NS
Collection day	1	NS	NS	NS	NS	NS	NS
$B \times C$	2	**	NS	NS	NS	NS	NS
Rep ($B \times C$)	6	NS	****	**	**	**	****
Line	11	***	**	****	****	NS	***
$B \times L$	22	**	NS	NS	NS	NS	NS
$C \times L$	11	*	*	NS	NS	NS	NS
$B \times C \times L$	22	*	**	***	NS	NS	NS
Error	66						
Total	143						
$K = \frac{\sigma_L^2}{(\sigma_L^2 + \sigma_{BL}^2 + \sigma_{CL}^2 + \sigma_{BCL}^2 + \sigma_E^2)}$		0.55	0.44	0.59	0.49	0.05	0.43

The significance levels of the *F* tests and a ratio of variance component estimates (σ^2), *K*, are given. For catalase (CAT) activity levels and for general protein (PROT) content, Rep refers to different assay days, whereas for immunodiffusion area (CAT-CRM/fly), it refers to different gels. The -adj suffix identifies independent variables by which CAT or CAT-CRM levels were adjusted by regression (see text).

NS, $P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

general protein is highly significant ($R^2 = 0.71$), but the variation in general protein content does not account for all of the genetic variation in catalase activity, since lines remain significant after the adjustment. In fact, the *K* value is reduced only slightly from 0.55 to 0.49. In contrast to the adjustment by general protein, adjustment of activity by CRM level causes lines to lose significance for both chromosomes. Consistent with this result is the highly significant regression of activity on CRM level for both chromosomes (Figure 4), with R^2 values of 0.93 and 0.94 for second and third chromosomes, respectively. These results clearly indicate that most, if not all, of the genetic variation affecting catalase activity level is accounted for by variation in the rate of accumulation of catalase-specific protein.

Catalase turnover studies: Variation in the rates of accumulation of catalase-specific protein must be explained by differential rates of enzyme synthesis, intracellular degradation or a combination of these two processes. Therefore, it is of interest to evaluate the relative contribution of these processes in establishing the steady state levels of catalase observed in this study. Aminotriazole perturbation of catalase activity provides an effective method for determining turnover parameters in adult flies, since steady state levels are attained within 12–24 hr of eclosion and maintained essentially unchanged throughout adult life (BEWLEY *et al.* 1983).

Results of the turnover parameter estimation experiment are summarized in

TABLE 4

Summary of analyses of variance of chromosome 3 data for experiment 3

Source	d.f.	*F* tests					
		Raw CAT/fly	General PROT/fly	CAT-CRM/fly	PROT-adj CAT/fly	CRM-adj CAT/fly	PROT-adj CAT-CRM/fly
Block	2	*	NS	NS	NS	NS	NS
Collection day	1	*	NS	NS	NS	NS	NS
$B \times C$	2	NS	NS	NS	NS	NS	NS
Rep ($B \times C$)	6	**	***	****	****	****	****
Line	11	****	****	****	****	NS	****
$B \times L$	22	NS	NS	NS	NS	NS	NS
$C \times L$	11	NS	NS	NS	**	NS	*
$B \times C \times L$	22	***	*	NS	NS	NS	NS
Error	66						
Total	143						
$K = \frac{\sigma_L^2}{(\sigma_L^2 + \sigma_{BL}^2 + \sigma_{CL}^2 + \sigma_{BCL}^2 + \sigma_E^2)}$		0.83	0.66	0.72	0.88	0.08	0.71

The significance levels of the *F* tests and a ratio of variance component estimates (σ^2), *K*, are given. For catalase (CAT) activity levels and for general protein content, Rep refers to different assay days, whereas for immunodiffusion area (CAT-CRM/fly), it refers to different gels. The -adj suffix identifies independent variables by which CAT or CAT-CRM levels were adjusted by regression (see text).

NS, $P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

Tables 5 and 6 for second and third chromosome substitution lines, respectively. The variation in steady state (C_N) among lines is highly significant for both chromosomes, whereas the variation in k_d among lines is not significant (*F* tests from analyses of variance). These results suggest that differences in steady state enzyme levels among lines are not due to variation in rates of intracellular degradation, and, therefore, imply the importance of differences in rates of enzyme synthesis. However, the power to detect differences in k_d is less than that for C_N (the coefficients of variation for C_N are 6.0 and 6.2% for second and third chromosomes, respectively, whereas the corresponding values for k_d are 40 and 28%). Comparison of the second chromosome lines NC11 and RI45 illustrates the power problem; the observed k_d difference is more than sufficient to account for the C_N difference, but the difference is not significant. If the variation in k_d's among lines accounts for the variation in C_N's, one expects a highly negative correlation between the two estimates. For second chromosomes, this correlation is −0.15, and for thirds it is −0.40, neither of which are significantly different from zero. Therefore, this experiment provides no substantial evidence for line differences in k_d, but neither can the importance of k_d variation be eliminated.

One pair each of second and third chromosome lines provides much better comparisons, since each line was sampled at four different times, and they were originally selected as high/low C_N pairs. These two pairs were analyzed separately from the other lines. Second chromosome lines KA01 and NC11

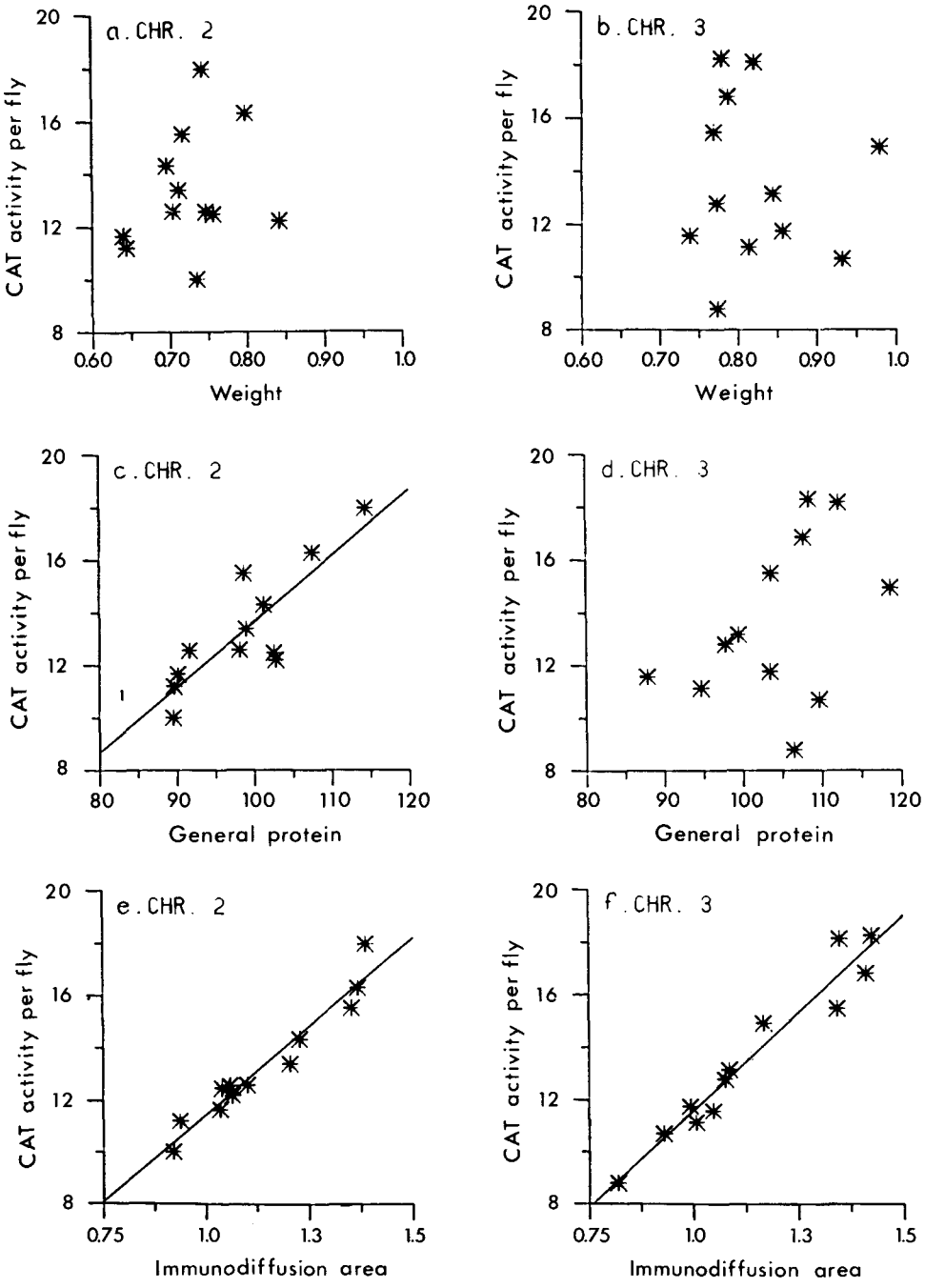

FIGURE 4.—Plots of the 12 line means of catalase activity per fly, immunodiffusion area, milligrams of live weight per fly, and micrograms of general protein per fly. The regressions of activity on weight or general protein are not significant for plots a, b and d. For plots c, e and f, the regressions are highly significant and the R^2 values are: (c) $R^2 = 0.71$; (e) $R^2 = 0.93$; and (f) $R^2 = 0.94$. For the second chromosomes, the partial correlation between catalase activity and CRM levels with general protein fixed is 0.92 ($P < 0.001$).

TABLE 5

Turnover parameter estimates for catalase among second chromosome lines

Line	Code	Sample times	C_N ± SE	k_d ± SE	k_s	Half-life (hr)
KA01	a	4	186.8 ± 3.1	0.015 ± 0.002	2.80	46.2
RI45	w	1	173.0 ± 6.2	0.008 ± 0.004	1.38	86.6
NC35	O	1	168.0 ± 7.7	0.019 ± 0.004	3.19	36.5
RI03	Q	3	166.8 ± 3.8	0.018 ± 0.002	3.00	38.5
RI15	Z	1	163.7 ± 7.7	0.019 ± 0.004	3.11	36.5
WI03	g	2	151.4 ± 6.6	0.012 ± 0.004	1.81	57.8
RI08	T	2	133.5 ± 6.6	0.016 ± 0.004	2.14	43.3
NC11	J	4	133.4 ± 3.5	0.017 ± 0.002	2.27	40.8
F test			$P < 0.0001$	ns		

The C_N and k_d values are means adjusted for empty cells due to the unbalanced design (SEARLE, SPEED and MILLIKERS 1980).

TABLE 6

Turnover parameter estimates of catalase among third chromosome lines

Line	Code	Sample times	C_N + SE	k_d ± SE	k_s	Half-life (hr)
KA24	h	1	163.2 ± 6.6	0.016 ± 0.003	2.61	43.3
WI18	I	4	159.3 ± 3.5	0.018 ± 0.002	2.87	38.5
WI08	i	1	154.8 ± 7.3	0.019 ± 0.003	2.94	36.5
WI05	H	1	151.5 ± 6.6	0.013 ± 0.003	1.97	53.3
RI31	w	3	150.4 ± 4.5	0.019 ± 0.002	2.86	36.5
RI20	q	3	149.2 ± 3.8	0.015 ± 0.002	2.23	46.2
NC23	J	2	145.0 ± 4.6	0.017 ± 0.002	2.47	40.8
KA05	c	2	134.2 ± 4.7	0.015 ± 0.002	2.01	46.2
KA26	E	1	115.1 ± 7.3	0.018 ± 0.003	2.07	38.5
KA20	f	3	107.2 ± 3.9	0.019 ± 0.002	2.04	36.5
RI33	S	4	79.9 ± 3.3	0.019 ± 0.002	1.52	36.5
F test			$P < 0.001$	NS		

The C_N and k_d values are means adjusted for empty cells due to the unbalanced design (SEARLE, SPEED and MILLIKERS 1980).

show a highly significant difference in C_N level but no significant difference in k_d. A ratio of 1.4 between the k_d's is required to account for the C_N level ratio, whereas the observed k_d ratio is just 1.1. The smallest difference in k_d between these two lines that would be significant at the 5% level is 0.004, which is sufficient to detect a 25–30% increase or decrease from the mean of the two k_d estimates. Therefore, it is very unlikely that a k_d difference accounts for the difference in steady state catalase level between KA01 and NC11. Similarly, the third chromosome lines WI18 and RI33 show a highly significant difference in C_N level (observed ratio of 2.0) but no significant difference in k_d (observed ratio of 1.1). The 5% least significant difference in this case is 0.003, which is sufficient to detect a 15–20% increase or decrease from the mean of the k_d estimates. Again, it is very unlikely that a k_d difference accounts for the C_N difference observed between these two lines.

DISCUSSION

The results reported here confirm our earlier observation of a large degree of genetic variability among chromosomes from natural populations that affect catalase activity levels (LAURIE-AHLBERG *et al.* 1980). Although environmental and measurement error effects on catalase activity are often substantial, particularly with the second chromosome lines, the between-line variation contributes more variation than any other source and is not accounted for by body size variation. A stronger and more repeatable genetic effect is evident for the third chromosome lines, which also contain the structural gene Cat^{+}. The structural gene for catalase in *D. melanogaster* appears to be relatively conserved in that no electrophoretic variants have been identified either within the lines included in this study or in another study by LUBINSKY and BEWLEY (1979). The lack of a correlation of activity level variation with specific structural gene variants could imply that most, if not all, of the activity level variations are due to modifiers that are regulatory in nature.

The results of experiment 3 demonstrate a strong relationship between catalase activity level variation and catalase-specific protein for both the second and third chromosome substitution lines. These relationships remain highly significant after adjustment for general protein (see Tables 3 and 4) and add further support to the suggestion that the genetic variation in activity is due to variation in the steady state level of catalase-specific protein rather than to catalytic efficiency of the enzyme. These results are similar to those reported for glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme (LAURIE-AHLBERG *et al.* 1981), alcohol dehydrogenase (MARONI *et al.* 1982) and glycerol-3-phosphate dehydrogenase (LAURIE-AHLBERG and BEWLEY 1983) with samples from the same set of isogenic lines. Results of this kind imply that variation in the steady state level of CRM for a specific enzyme must be due to differential rates of enzyme synthesis, intracellular degradation or a combination of these two processes. Fortunately, the relative ease of conducting turnover studies for catalase (PRICE, RECHCIGL and HARTLEY 1961; PRICE *et al.* 1962; LUBINSKY and LEWLEY 1979) has allowed us, in this case, to estimate protein turnover parameters on a population level. For the most part, our results demonstrate that, although the variation in steady state levels (C_N) of catalase is highly significant among lines, variation in intracellular degradation (k_d) is not, implying that rates of enzyme synthesis provide the most important parameter in determining catalase steady state levels. However, partly due to the unbalanced design of the turnover experiments, the power to detect differences in k_d is less than that for C_N, and, therefore, we cannot eliminate the possibility of small differences in rates of catalase degradation between lines. This point is illustrated by lines RI45 and WI03 for the second chromosome lines (Table 5) and by line WI05 for the third chromosomes (Table 6). In total, however, these data strongly suggest the presence of both unlinked, as in the case of second chromosomal lines, and linked, as in the case of third chromosomal lines, modifiers that act by modulating the rate of catalase synthesis.

The results of experiment 1 (see Figure 2) suggest the importance of stage-

specific effects with respect to catalase expression. Two distinct peaks of catalase expression have been defined during Drosophila development with the first peak occurring in late third instar larvae, just prior to puparium formation, and the second and larger of the two peaks occurring during metamorphosis (BEWLEY *et al.* 1983). Several genetic variations of this temporal program have already been isolated from the third chromosome lines represented in Figure 2b in which the larval activity peak is very much reduced in one case, *i.e.*, line RI33, and overexpressed in two others, *i.e.*, lines KA24 and WI18, relative to the second activity peak (BEWLEY 1982; G. C. BEWLEY, J. L. COOK, and J. A. NAHMIAS, unpublished data). In addition, these differences in the larval expression of catalase are most pronounced in larval fat body tissue. Further characterization of these putative temporal variants is in progress. The data in Figure 2 suggest many other possibilities for investigating stage-specific and/or tissue-specific effects of catalase.

We gratefully acknowledge the technical assistance of YOSHIKO CHEU and JUSTINA WILLIAMS. This is paper no. 9039 of the Journal series of the North Carolina Agricultural Research Service, Raleigh, North Carolina 27650. This project has been supported by Public Health Service Research grants GM-23617, AG-01739 and GM-11546.

LITERATURE CITED

BEERS, R. G., JR and I. W. SIZER, 1952 A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **195:** 133–140.

BEWLEY, G. C., 1982 Variation in the temporal expression of catalase in *Drosophila melanogaster.* Isozyme Bull. **15:** 111.

BEWLEY, G. C., J. A. NAHMIAS and J. L. COOK, 1983 Developmental and tissue specific control of catalase expression in *Drosophila melanogaster*: correlations with rates of enzyme synthesis and degradation. Dev. Genet. **4:** 49–60.

BRADFORD, M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

CRANE, D., R. S. HOLMES and C. J. MASTERS, 1978 On the relative rates of synthesis and degradation of catalase in vertebrate tissues. Int. J. Biochem. **9:** 589–596.

DAVIDSON, E. H., 1976 *Gene Activity in Early Development.* Academic Press, New York.

FRITZ, P. J. and K. M. PRUITT, 1977 Intracellular turnover of isozymes. pp. 125–157. In: *Isozymes: Current Topics in Biological and Medical Research,* Edited by M. C. RATTAZZI, J. G. SCANDALIOS and G. S. WHITT, Vol. 3. Alan R. Liss, New York.

GANSCHOW, R. E. and R. T. SCHIMKE, 1969 Independent control of the catalytic activity and the rate of degradation of catalase in mice. J. Biol. Chem. **244:** 4649–4658.

LAURIE-AHLBERG, C. C. and G. C. BEWLEY, 1983 Naturally occurring genetic variation affecting the expression of sn-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster.* Biochem. Genet. **21:** 943–961.

LAURIE-AHLBERG, C. C., G. MARONI, G. C. BEWLEY, J. C. LUCCHESI and B. S. WEIR, 1980 Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster.* Proc. Natl. Acad Sci. USA **77:** 1072–1077.

LAURIE-AHLBERG, C. C., J. H. WILLIAMSON, B. J. COCHRANE, A. WILTON and F. I. CHASALOW, 1981 Autosomal factors with correlated effects on the activities of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases in *Drosophila melanogaster.* Genetics **99:** 127–150.

LAURIE-AHLBERG, C. C., A. N. WILTON, J. W. CURTSINGER and T. H. EMIGH, 1982 Naturally occurring enzyme activity variation in *Drosophila melanogaster.* I. Sources of variation for 23 enzymes. Genetics **102:** 191–206.

LUBINSKY, S. and G. C. BEWLEY, 1979 Genetics of catalase in *Drosophila melanogaster*: rates of synthesis and degradation of the enzyme in flies aneuploid and euploid for the structural gene. Genetics **91:** 723–742.

MARGOLIASH, E., A. NOVGRODSKY and A. SCHEJTER, 1960 Irreversible reaction of 3-amino-1,2,4-triazole and related inhibitors with the proteins of catalase. Biochem. J. **73:** 339–350.

MARONI, G., C. C. LAURIE-AHLBERG, D. A. ADAMS and A. N. WILTON, 1982 Genetic variation in the expression of ADH in *Drosophila melanogaster.* Genetics **101:** 431–446.

NAHMIAS, J. A. and G. C. BEWLEY, 1984 Characterization of catalase purified from *Drosophila melanogaster* by hydrophobic interaction chromatography. Comp. Biochem. Physiol. (B). In press.

NELSON, D. P. and L. A. KIESOW, 1972 Enthalpy of decomposition of hydrogen peroxide by catalase at 25°C (with molar extinction coefficients of H_2O_2 solutions in the UV). Anal. Biochem. **49:** 474–478.

PAIGEN, K., 1979 Genetic factors in developmental regulation. pp. 1–63. In: *Physiological Genetics,* Edited by J. G. SCANDALIOS. Academic Press, New York.

PRICE, V. E., M. RECHCIGL, JR. and R. W. HARTLEY, 1961 Methods for determining the rates of catalase synthesis and destruction *in vivo.* Nature **189:** 62–63.

PRICE, V. E., W. R. STARLING, V. A. TARANTOLA, R. W. HARTLEY and M. RECHCIGL, JR., 1962 The kinetics of catalase synthesis and destruction *in vivo.* J. Biol. Chem. **237:** 3468–3475.

RECHCIGL, M., 1968 Relative role of synthesis and degradation in the regulation of catalase activity. pp. 399–415. In: *Regulatory Mechanisms for Protein Synthesis in Mammalian Cells,* Edited by A. PIETRO, S. M. R. LAMBORG and F. T. KENNEY. Academic Press, New York.

RECHCIGL, M., JR. and W. E. HESTON, 1967 Genetic regulation of enzyme activity in mammalian systems by the alteration of the rates of enzyme degradation. Biochem. Biophys. Res. Commun. **27:** 119–124.

REID, T. J., M. R. N. MURTHY, A. SICIGNANO, N. TANAKA, W. D. L. MUSICK and M. G. ROSSMANN, 1981 Structure and heme environment of beef liver catalase at 2.5A resolution. Proc. Natl. Acad. Sci. USA **78:** 4767–4771.

SCANDALIOS, J. G., 1979 Control of gene expression and enzyme differentiation. pp. 64–109. In: *Physiological Genetics,* Edited by J. G. SCANDALIOS. Academic Press, New York.

SCHIMKE, R. T., 1975 Methods for analysis of enzyme synthesis and degradation in animal tissues. Methods Enzymol. **40:** 241–266.

SEARLE, S. R., F. M. SPEED and G. A. MILLIKERS, 1980 Population marginal means in the linear model: an alternate to least squares means. Am. Stat. **34:** 216–221.

WOODBURY, W., A. K. SPENCER and M. A. STAHMAN, 1971 An improved procedure using ferricyanide for detecting catalase isozymes. Anal. Biochem. **44:** 301–305.

Corresponding editor: R. E. GANSCHOW