GENETICAL ANALYSIS OF CHROMOSOMAL INTERACTION EFFECTS ON THE ACTIVITIES OF THE GLUCOSE 6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES IN *DROSOPHILA MELANOGASTER*

NAOHIKO MIYASHITA AND C. C. LAURIE-AHLBERG

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695

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ABSTRACT

By combining ten second and ten third chromosomes, we investigated chromosomal interaction with respect to the action of the modifier factors on G6PD and 6PGD activities in *Drosophila melanogaster*. Analysis of variance revealed that highly significant chromosomal interaction exists for both enzyme activities. From the estimated variance components, it was concluded that the variation in enzyme activity attributed to the interaction is as great as the variation attributed to the second chromosome but less than attributed to the third chromosome. The interaction is not explained by the variation of body size (live weight). The interaction is generated from both the lack of correlation of second chromosomes for third chromosome backgrounds and the heterogeneous variance of second chromosomes for different third chromosome backgrounds. Large and constant correlation between G6PD and 6PGD activities were found for third chromosomes with any second chromosome background, whereas the correlations for second chromosomes were much smaller and varied considerably with the third chromosome background. This result suggests that the activity modifiers on the second chromosome are under the influence of third chromosome factors.

THE two oxidative pentose phosphate pathway enzymes of *D. melanogaster*, glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), have been the subject of a large number of genetic, biochemical and physiological studies (see reviews by LUCCHESI, HUGHES and GEER 1979; GEER *et al.* 1981). The structural genes for both enzymes have been localized to the *X* chromosome, that for 6PGD to the distal tip (*Pgd* at 1-0.6 and 2D3-5, YOUNG 1966; GERASIMOVA and ANAVIEV 1972) and that for G6PD to the proximal end (*Zw* at 1-63 and 17B-18F, YOUNG, PORTER and CHILDS 1964; STEWART and MERRIAM 1974). Each locus is polymorphic for two common electrophoretic variants in natural populations (O'BRIEN and MACINTYRE 1969), and a number of null or low activity variants of each enzyme have been induced on the *X* chromosome (see LUCCHESI, HUGHES and GEER 1979). The active form of 6PGD is a dimer (HORI and TANDA 1980; WILLIAMSON, KROCHKO and GEER 1980), whereas the nat-

ural polymorphism at *Zw* is due to instability of subunit association where one variant is a dimer and the other a tetramer (STEELE, YOUNG and CHILDS 1968; HORI and TANDA 1980).

The mechanisms that regulate or cause variation in the activity levels of these enzymes have been investigated at several levels. Environmental causes include short-term fluctuations in the concentrations of metabolites that directly modulate activity levels as well as long-term influences of the diet that affect accumulation of enzyme molecules (GEER *et al.* 1981). The genetic causes of variation include sex-specific effects such as dosage compensation (LUCCHESI 1977; BELOTE and LUCCHESI 1980) and sex-nonspecific effects such as the difference in activity level between the A and B allozymes of 6PGD (BIJLSMA and VAN DER MEULEN-BRUIJNS 1979; CAVENER and CLEGG 1981; HORI and TANDA 1981) or the activity effects due to autosomal modifiers (HORI and TANDA 1981; LAURIE-AHLBERG *et al.* 1981). Genotype-environment interaction effects have been described in terms of the response of different genotypes to the modulation of activity levels by dietary carbohydrate (COCHRANE and LUCCHESI 1980).

We have been concerned with the detection and characterization of naturally occurring genetic variants affecting the expression of G6PD and 6PGD, with particular attention on the possibility of coordinate genetic control because of the closely related functions of these two enzymes (LAURIE-AHLBERG *et al.* 1980, 1981, 1982; WILTON *et al.* 1982). These experiments have utilized two sets of chromosome substitution lines with coisogenic backgrounds (50 second and 50 third chromosomes sampled at random from four different geographic localities). We find extreme modifier genetic effects due to each autosome, which are very repeatable over time and are generally substantially larger than the effects due to uncontrolled variation in the standard laboratory culture environment. Furthermore, the genetic effects on G6PD and 6PGD are highly correlated with each other as well as with some other metabolically related enzymes (WILTON *et al.* 1982). Tissue distribution studies shows that these activity effects are not restricted to one particular body part and may even go in opposite directions in different body parts. Immunoelectrophoresis experiments show that a large part (but perhaps not all) of the modifier variation is accounted for by variation in the concentration of enzyme molecules, especially for third chromosome lines (LAURIE-AHLBERG *et al.* 1981). Here, we extend our investigation of autosomal modifier effects on G6PD and 6PGD with a study of chromosomal interaction with respect to the activity of each enzyme individually and also with respect to the correlation between their activities.

MATERIALS AND METHODS

Line construction: Ten second and ten third chromosome substitution lines with coisogenic background were selected from a total of 50 of each type so as to represent the range of G6PD activity variation (see LAURIE-AHLBERG *et al.* 1980 for construction of these original lines). The goal was to establish all of 100 possible combinations between these second and third chromosomes, which were derived from natural populations. The original 20 lines have the same *X* chromosome from a highly inbred line Ho-R. Consequently, the combination lines have the *X* chromosome, which has alleles for the fast electrophoretic forms of both G6PD and 6PGD. The procedure for con-

structing lines homozygous for particular combinations of these chromosomes is shown in Figure 1. During this procedure females are never doubly heterozygous for second and third chromosome balancers with the wild-type chromosomes. Only seven of the possible 100 combinations failed because of synthetic lethality or weak expression of the marker genes. After establishment of the 93 combination lines, a starch gel electrophoretic survey of eight commonly polymorphic enzymes was conducted to check for errors in the procedure. No problems were detected. The electrophoretic procedures for the eight enzymes (ADH, EC 1.1.1.1; GPDH, EC 1.1.1.8; ODH, EC 1.1.1.73; PGM, EC 2.7.5.5; EST-6, EC 3.1.1.1; EST-C, EC 3.1.1.2; G6PD; 6PGD) are described by LAURIE-AHLBERG and WEIR (1979).

Enzyme assay: A sample of ten males was homogenized in 0.5 ml of 0.01 M potassium phosphate buffer, pH 7.4, with 1 mM EDTA, 5 mM DTT and 0.5% (v/v) Triton X-100 and centrifuged for 10 minutes at 10,000 × *g*. The supernatant was used for activity measurements and the determi-

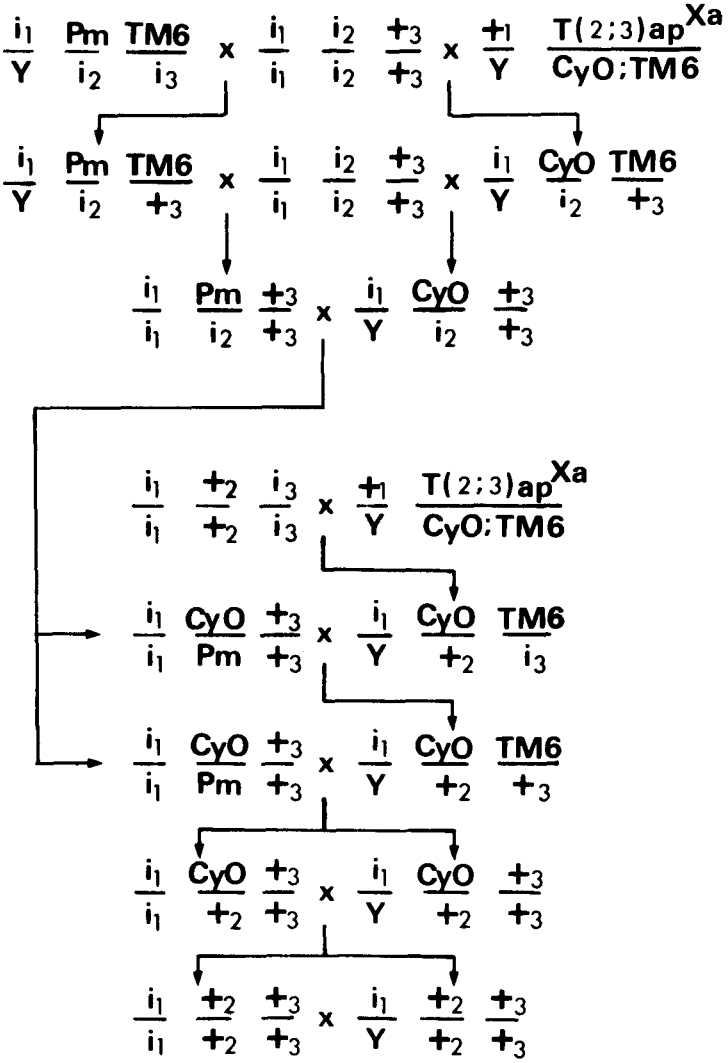

FIGURE 1.—Procedure for construction of a line homozygous for both a second and a third chromosome. *i* = chromosome from Ho-R inbred line; + = chromosome from natural population; CyO = *In(2LR)O, Cy*; Pm = *In(2LR)bw*^{V1}; TM6 = *In(3LR)Tm6, Ubx*.

nation of total amount of protein. Enzyme activities were measured by observing the reduction of NADP+ to NADPH at 340 nm. The reaction mixture contains 0.1 ml of the supernatant and 0.9 ml of 0.055 M Tris-HCl buffer, pH 7.6, with 18.5 mM $MgCl_2$, 0.18 mM NADP+ and 1.8 mM glucose-6-phosphate for G6PD activity and 0.9 ml of 0.055 M Tris-HCl buffer, pH 7.6, with 1.68 mM $MgSO_4$, 0.15 mM NADP+ and 0.34 mM 6-phosphogluconate for 6PGD activity. The crude supernatant was diluted in a 1:9 ratio with distilled water, and then the amount of protein was determined by the method of LOWRY *et al.* (1951).

Experimental design: The design of this experiment is shown in Figure 2. The combination lines were arranged in rows and columns in a random fashion according to the origins of the chromosomes. Then, the combination lines were split into four groups (H_i V_j, i,j = 1,2) in order to accommodate the number of assays that could be performed in 1 day. Two replicates of ten 4-day-old males were sampled from each line in each group. The samples were weighed and kept frozen at −70° until the assay of enzyme activities. The samples from one group were assayed in 1 day for both G6PD and 6PGD activities. The sampling and assay for each of the groups was repeated four times (four blocks). This design yields a total of eight observations per line. However, some samples were lost during the experiment due to low viability and/or fertility. Thus, a total of 728 samples was assayed. Flies were raised in the standard cornmeal-molasses medium at 25°.

The model for the analysis of variance for each of the variables, G6PD, 6PGD, weight and

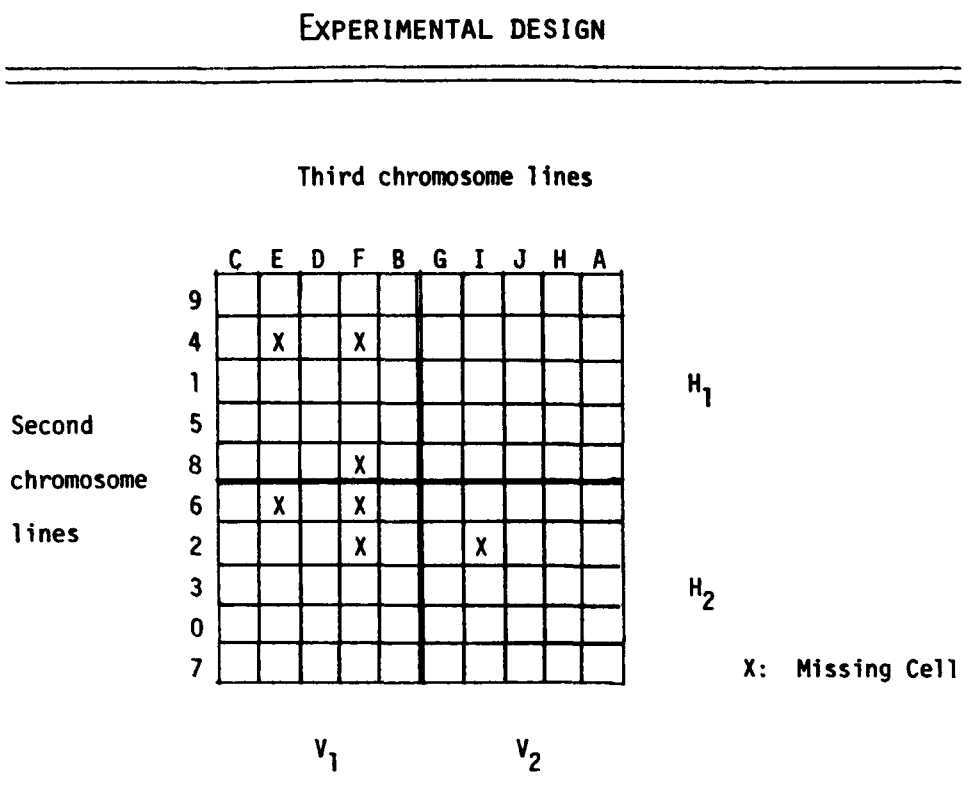

FIGURE 2.—The design of this experiment. The lines are arranged in rows and columns at random according to the origin of isogenic chromosomes. All of the combination lines are split into four groups (H_i V_j; i = 1–2, j = 1–2). Each group is assayed on 1 day within each block. Two replicates of ten males are sampled from each line. Experiment consists of four sampling times for all of the combination lines (four blocks).

protein, is the following:

$$Y_{ijk(j)lm(l)n} = U + A_i + B_j + (AB)_{ij} + C_{k(j)} + (AC)_{ik(j)} + D_l + (AD)_{il} + (BD)_{jl} + (ABD)_{ijl} + (CD)_{k(j)l} + (ACD)_{ik(j)l} + F_{m(l)} + (AF)_{im(l)} + (BF)_{jm(l)} + (ABF)_{ijm(l)} + (CF)_{k(j)m(l)} + (ACF)_{ik(j)m(l)} + e_{ijk(j)lm(l)n},$$

where U is the mean, A_i is the effect of the ith block (i = 1,2,3,4), B_j is the effect of the jth horizontal split (j = 1,2), $C_{k(j)}$ is the effect of the kth second chromosome within the jth horizontal split (k = 1,. . .,5), D_l is the effect of the lth vertical split (l = 1,2), $F_{m(l)}$ is the effect of the mth third chromosome within the lth vertical split (m = 1,. . .,5), $e_{ijk(j)lm(l)n}$ is the error term (n = 1,2). The effects in parentheses represent interactions. For the calculation of the sums of squares for these unbalanced data, the GLM procedure of "SAS" statistical analysis system was used (SPEED, HOCKING and HACKNEY 1978; HELWIG and COUNCIL 1979). The F-tests in the analysis of variance (Table 1) were constructed as described by NETER and WASSERMAN (1974, p. 664) for a completely random model.

For adjustment of raw activities (Y) of G6PD and 6PGD, which were expressed in terms of nanomoles per minute per fly, by live weight, the regression of $\overline{Y}_{ijk(j)lm(l)\cdot}$ on $\overline{WT}_{ijk(j)lm(l)\cdot}$ was performed for each of the four groups of each block; the sum of squares and products were then pooled over blocks in order to obtain a single slope (b_{jl}) for each of the horizontal and vertical combinations (groups). The adjusted variables ($\hat{Y}$) were then obtained as follows:

$$\hat{Y}_{ijk(j)lm(l)n} = Y_{ijk(j)lm(l)n} - b_{jl}(WT_{ijk(j)lm(l)n} - \overline{WT}\cdot j\cdot(j)l\cdot(l)\cdot)$$

TABLE 1

Summary of analyses of variance of combination lines

Source	d.f.	G6PD		6PGD		PRO	WT
		Raw	Adjusted	Raw	Adjusted	Raw	Raw
BLK	3	NS	NS	NS	NS	NS	NS
H	1	NS	NS	NS	NS	NS	NS
BLK*H	3	NS	*	NS	NS	NS	NS
SC(H)	8	**	*	***	***	***	***
BLK*SC(H)	24	NS	NS	NS	NS	NS	NS
V	1	NS	NS	NS	NS	NS	NS
BLK*V	3	NS	*	NS	NS	NS	NS
H*V	1	NS	NS	NS	NS	NS	NS
BLK*H*V	3	NS	NS	***	**	***	NS
SC(H)*V	8	NS	*	NS	NS	NS	NS
BLK*SC(H)*V	24	NS	NS	NS	NS	NS	NS
TC(V)	8	***	***	***	***	***	***
BLK*TC(V)	24	NS	NS	NS	NS	NS	NS
H*TC(V)	8	NS	NS	NS	NS	NS	NS
BLK*H*TC(V)	24	**	**	*	*	NS	NS
SC(H)*TC(V)	57	***	***	***	***	NS	***
BLK*SC(H)*TC(V)	167	***	**	***	***	NS	***
Error	360						
Total	727						

Adjustment of activities was done by weight. BLK = block, H = horizontal split, SC = second chromosome, V = vertical split, TC = third chromosome.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS $P > 0.05$ (significance levels of F-tests).

The decision to adjust by weight rather than protein is based on the observation of significant heterogeneity among blocks with respect to the slopes and residual variances from the regression of activity on protein, which was not found for the regression on weight.

RESULTS

Interaction between second and third chromosomes on G6PD and 6PGD activities

The ranges of line means (in nanomoles per minute per fly) are 3.94–10.18 and 2.08–5.68 for G6PD and 6PGD, respectively. The ranges after the weight adjustment are 4.52–9.72 and 2.09–5.68 for G6PD and 6PGD, respectively. Thus, weight adjustment has little effect on the ranges. The results of the analyses of variance are summarized in Table 1. As expected from our early results (LAURIE-AHLBERG *et al.* 1981), the effects of second and third chromosomes are each highly significant for both raw and weight-adjusted G6PD and 6PGD activities, as well as for weight and protein. Also, the analyses of variance show the existence of a highly significant interaction between second and third chromosomes for raw and weight-adjusted G6PD and 6PGD activities and for weight, whereas the interaction was not detected for protein.

Since the three-way interaction among block, second chromosome and third chromosome (BLK*SC*TC) is significant for G6PD, 6PGD and weight, the chromosomal interactions were tested in each the four blocks separately. Significance of the chromosomal interactions was confirmed for G6PD, 6PGD and weight in each of blocks.

The effects of weight adjustment on the significance level of the second chromosome, the third chromosome and the chromosomal interaction are small. Only the significance level of the second chromosome effect on G6PD was reduced but is still significant at the 5% level. This result suggests that the variations on either second, third chromosome or the chromosomal interaction are not explained by the variation in live weight.

To determine whether the chromosomal interaction is due to a multiplicative action between second and third chromosomes, the raw G6PD, 6PGD and weight variables were transformed onto a logarithmic scale. The analyses of variance gave essentially the same results. In particular, the chromosomal interaction remains highly significant for all three variables. Thus, the results do not support a multiplicative model.

To quantify the importance of the chromosomal interaction, the variance components of the second and third chromosome main effects and their interaction were estimated (Table 2). The variation attributed to the chromosomal interaction is approximately equivalent to the variation attributed to the second chromosome main effect for both G6PD and 6PGD activities. On the other hand, the variation due to the third chromosome main effect is more than 60% of the total variation of G6PD activity, which is five times larger than the variation due to second chromosome or to the chromosomal interaction. For 6PGD, the variation due to the third chromosome is 50% of the total variation, which is almost three times larger than the variation due to the second chromosome or the interaction. The adjustment of activity by weight did not affect these results except that the variation of G6PD due to the second

TABLE 2

Estimates of variance components

Variable	BLK	SC	TC	SC*TC	Error
G6PD (raw data)	1	0.36 (0.14)	1.63 (0.62)	0.43 (0.16)	0.20 (0.08)
	2	0.34 (0.13)	1.60 (0.62)	0.45 (0.17)	0.18 (0.08)
	3	0.27 (0.14)	1.19 (0.60)	0.30 (0.15)	0.18 (0.08)
	4	0.21 (0.10)	1.43 (0.63)	0.34 (0.15)	0.27 (0.12)
G6PD (adjusted)	1	0.17 (0.09)	1.30 (0.67)	0.27 (0.14)	0.20 (0.10)
	2	0.24 (0.14)	1.10 (0.63)	0.24 (0.14)	0.17 (0.09)
	3	0.10 (0.08)	0.83 (0.62)	0.17 (0.13)	0.24 (0.17)
	4	0.10 (0.07)	0.94 (0.60)	0.23 (0.15)	0.29 (0.18)
6PGD (raw data)	1	0.21 (0.26)	0.39 (0.48)	0.15 (0.19)	0.06 (0.07)
	2	0.11 (0.19)	0.32 (0.54)	0.08 (0.13)	0.08 (0.14)
	3	0.17 (0.25)	0.35 (0.51)	0.11 (0.16)	0.05 (0.08)
	4	0.15 (0.19)	0.48 (0.61)	0.09 (0.11)	0.07 (0.09)
6PGD (adjusted)	1	0.15 (0.23)	0.32 (0.50)	0.11 (0.17)	0.06 (0.10)
	2	0.09 (0.21)	0.21 (0.48)	0.06 (0.13)	0.07 (0.18)
	3	0.12 (0.21)	0.33 (0.58)	0.07 (0.15)	0.05 (0.16)
	4	0.12 (0.21)	0.33 (0.59)	0.06 (0.10)	0.06 (0.10)
Weight	1	0.27 (0.39)	0.19 (0.27)	0.20 (0.29)	0.04 (0.05)
	2	0.08 (0.13)	0.21 (0.32)	0.32 (0.50)	0.03 (0.05)
	3	0.18 (0.34)	0.11 (0.21)	0.21 (0.38)	0.04 (0.07)
	4	0.09 (0.16)	0.22 (0.40)	0.21 (0.38)	0.03 (0.06)

The proportion of each estimate to total variance is shown in parentheses. See Table 1 for definitions of abbreviations.

chromosome was slightly reduced. The observation of larger variance components due to the third chromosome than that to the second chromosome is consistent with our earlier results (LAURIE-AHLBERG *et al.* 1980, 1981).

The interaction in a factorial design is generated from the heterogeneous variances of levels in one factor over the levels in the other factor and/or the lack of correlation between levels in one factor over levels in the other factor. Both appear to be important causes of the chromosomal interaction reported here, as indicated by Figure 3, in which the means of the combination lines are plotted against the marginal means of the second (Figure 3a and b) or third (Figure 3c and d) chromosome background. To explore the interaction further, the second chromosome variance components were estimated for each of ten third chromosome backgrounds, and also the third chromosome variance components were estimated for each of ten second chromosome backgrounds, for raw and weight-adjusted G6PD and 6PGD activities. Then, the estimated variance components were tested for heterogeneity over backgrounds by the Bartlett's test (see SNEDECOR and COCHRAN 1967, p. 296). The heterogeneities of either the second chromosome variance components or of the third chromosome variance components were not detected for the raw G6PD and 6PGD activities. However, after removing the effect of weight by

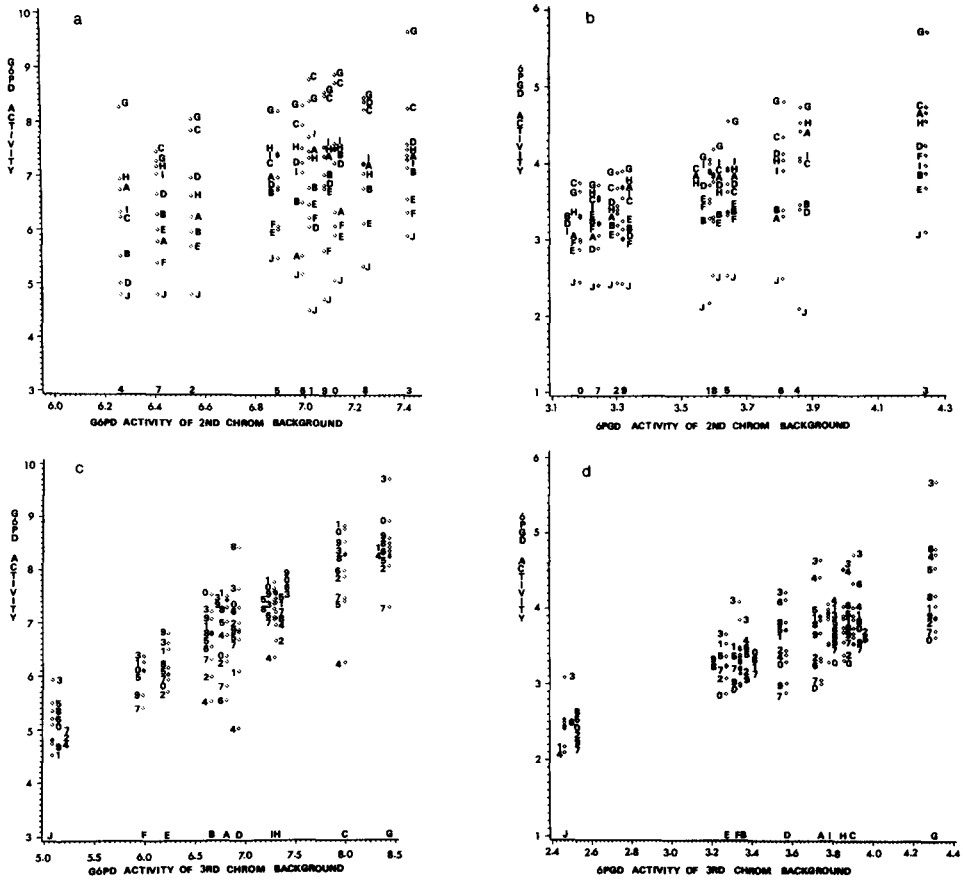

FIGURE 3.—Plots of combination line means of activity against the marginal mean of the background chromosome. a, G6PD activity against second chromosome background. b, 6PGD activity against second chromosome background. c, G6PD activity against third chromosome background. d, 6PGD activity against third chromosome background.

the adjustment, highly significant heterogeneities of the second chromosome variance components over the third chromosome backgrounds were detected for both G6PD and 6PGD activities. From Figures 3c and d, it can be seen that the magnitudes of second chromosome variance components do not have any association with the levels of the activity of the third chromosome backgrounds for either G6PD or 6PGD. In addition to the heterogeneity of the second chromosome variance components over the third chromosome backgrounds, the lack of strong correlation of one chromosome type between the other chromosome backgrounds in some cases contributes to the detected interaction for G6PD and 6PGD activities. Figure 3 gives the impression that this contribution is substantial. Although the lowest and highest of either second or third chromosomes maintain the extreme activity levels for G6PD and 6PGD even if the chromosome backgrounds change, the ranks of other chromosomes vary considerably over different backgrounds. The means of the 45 product-moment correlations of second chromosomes for pairs of the third

chromosome backgrounds are 0.48 and 0.73 for G6PD and 6PGD, respectively. The means of third chromosome correlations for pairs of second chromosome backgrounds are 0.86 and 0.87 for G6PD and 6PGD, respectively. For both enzyme activities, the correlations of third chromosomes are relatively high, whereas the correlations of second chromosomes are lower and sometimes negative. In other words, the ranks of third chromosomes are not strongly affected by the change of second chromosome backgrounds, whereas the ranks of second chromosomes are affected by the change of the third chromosome background. It is concluded that the interaction between second and third chromosomes on G6PD and 6PGD activity modification is generated from both the heterogeneous variance of second chromosomes and the weak correlations of second chromosomes for third chromosome backgrounds. The nature of the chromosomal interaction is even more complicated than indicated by the analysis because of the highly significant three-way interaction among the two chromosomes and "blocks." Variation among blocks is due to uncontrolled variation in the standard laboratory culture environment over time; so their three-way interaction represents a genotype-environment interaction. It means that the precise nature of the interaction varies over different environments. The other significant factors that involve the horizontal and/or vertical splits are also probably generated from the uncontrolled environmental variations or perhaps the unbalanced nature of the experiment (particularly the distribution of missing cells across the horizontal and vertical splits).

Effects of chromosomal background on the correlation between G6PD and 6PGD

We have previously reported high positive genetic correlation between G6PD and 6PGD activities for both second and third chromosomes on one particular genetic background (LAURIE-AHLBERG *et al.* 1981; WILTON *et al.* 1982). The combination lines used in this experiment provide information about the effect of chromosome background on this coordinated modifier action. Here, we present product-moment correlations over the line means (Table 3) rather than the usual genetic correlation (a standardized covariance component estimate), because the unbalanced nature of the experiment made the latter impossible to calculate precisely. However, we have found that the two types of correlation estimates are very similar in experiments of this kind (LAURIE-AHLBERG *et al.* 1981; WILTON *et al.* 1982). The correlations for third chromosomes are relatively constant and significantly different from zero with any second chromosome background. On the other hand, the correlations for second chromosomes are clearly affected by the change of third chromosome background. The adjustment of the activities by weight does not affect the correlation over third chromosomes appreciably, whereas the correlation over second chromosomes with some third chromosome backgrounds changed after the adjustment. However, the ten correlations for second chromosomes are not significantly heterogeneous either before or after the weight adjustment, which probably is due to the small number of second chromosomes. The ten correlations for third chromosomes, both before and after the weight adjustment, are not statistically different from each other. The pooled estimates of

TABLE 3

Correlation between adjusted G6PD and 6PGD activities

Second chromosome background	Correlation over third chromosome lines	Third chromosome background	Correlation over second chromosome lines
4	0.88** (0.89**)	J	0.87* (0.84**)
7	0.83** (0.92***)	F	0.62NS (0.64 NS)
2	0.96** (0.98***)	E	0.64NS (0.67 NS)
5	0.92** (0.95***)	B	0.15NS (0.69*)
6	0.96** (0.99***)	A	0.70* (0.69*)
1	0.86** (0.89***)	D	0.37NS (0.23 NS)
9	0.91** (0.92***)	I	−0.38NS (0.24 NS)
0	0.98** (0.96***)	H	0.13NS (0.55 NS)
8	0.90** (0.92***)	C	0.04NS (−0.08 NS)
3	0.92** (0.96***)	G	0.58NS (0.59 NS)
	Average 0.92 (0.95)		Average 0.43 (0.54)

Correlations for raw data are in parentheses. Backgrounds are given in the rank order of adjusted G6PD activity.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; NS $P > 0.05$ against H_o; $r = \phi$.

the correlation between G6PD and 6PGD activities are $r = 0.95$ and $r = 0.92$, before and after the adjustment, respectively, for third chromosomes, and $r = 0.54$ and $r = 0.43$, before and after the adjustment, respectively, for second chromosomes. Relatively higher correlation between G6PD and 6PGD activities for third chromosomes and relatively lower correlation for second chromosomes are consistent with our previous results (LAURIE-AHLBERG *et al.* 1981; WILTON *et al.* 1982). The activity level of second chromosome background does not affect the correlation for third chromosomes; on the other hand, the correlation for second chromosomes tends to decrease as the activity level of third chromosome increases. Although there is a scatter of the points, Spearman's coefficient of rank correlation (STEEL and TORRIE 1980, p. 550) for the negative relation between the second chromosome correlations and the activity of the third chromosome backgrounds was significant at 5% level for the G6PD activity of the third chromosome background ($r_s = -0.66$) but not significant for the 6PGD activity of the third chromosome background ($r_s = -0.62$).

DISCUSSION

The results clearly demonstrate the existence of epistatic interaction between second and third chromosomes with respect to G6PD and 6PGD activity modification in *D. melanogaster*. Quantitative analysis of the interaction shows the sensitivity of the second chromosome effects to the third chromosome background. Furthermore, the sensitivity is observed on the correlation between G6PD and 6PGD activities. The effect of the third chromosomes is manifested as the change of the distribution range and rank of the second chromosomes, and the slightly negative relation between the second chromosome correlations between G6PD and 6PGD activities over the third chromosome backgrounds. On the other hand, the range of distribution and rank of the third chromo-

somes do not seem to be affected by the second chromosome backgrounds, and the correlation between G6PD and 6PGD activities over third chromosomes is very high and insensitive to second chromosome background, so the genetic correlation between G6PD and 6PGD probably occurs in natural populations. Thus, the results indicate directional epistatic action of the third chromosome on the second chromosome activity modifiers. Although the incomplete correlation over third chromosomes between the second chromosome backgrounds may suggest second chromosome epistatic action on the third chromosome, the effect can be regarded small if compared with the effects of the third chromosome.

Generally, it is very difficult to determine the nature of an interaction from a quantitative analysis. Here, the detected chromosomal interaction is very complex and influenced by the environment. The activity levels of the third chromosome backgrounds are not related to the changes of the second chromosome effects, and the deviations of the line means from the sum of the second and third chromosome marginal means does not show any pattern (data not shown). The interaction may be caused by the existence of separate third chromosome epistatic factor(s) on the second chromosome activity modifiers or the epistatic action of the third chromosome activity modifiers themselves. The interaction will be very difficult to define further unless identification of the individual activity modifier loci and the characterization of the actions of these loci becomes possible. An attempt to localize the activity modifiers is in progress.

Our previous results (LAURIE-AHLBERG *et al.* 1980, 1981) have shown that the third chromosome is always a clear significant source of activity variation of these two enzymes. That result is also confirmed by this experiment. Although the third chromosome accounts for more than 50% of the total variation in both enzyme activities, the contribution from the interaction is not negligible. The interaction can explain as much variation as the second chromosome. It is not clear whether this larger contribution from the third chromosome is due to the larger effect of third chromosome modifiers than that of the second chromosome modifiers or simply to the larger number of the modifiers on the third chromosome than on the second chromosome.

The potential importance of epistasis has been well recognized in the development of evolutionary theory, even though its absence is frequently assumed. As WRIGHT (1932, 1977) pointed out, the rate of evolution is faster in the presence of epistasis, and his shifting-balance theory depends on the multiple peaks in the fitness landscape caused by epistasis. Although epistasis for quantitative traits like body size and bristle number is well documented (*e.g.*, ROBERTSON 1954; KIDWELL 1969), the experimental results with respect to epistatic effects on fitness components are inconsistent (see BARKER 1979 for a review). Although SPASSKY, DOBZHANSKY and ANDERSON (1965) and SEAGER and AYALA (1982) detected significant chromosomal interaction for viability in *D. pseudoobscura* and *D. melanogaster*, respectively, TEMIN *et al.* (1969) could not detect the interaction in spite of the large scale of their experiments in *D. melanogaster*. For the enzyme activity variations, BIRLEY, COUCH and MARSON (1981) could not detect the chromosomal interaction on ADH of *D. melano-*

gaster but detected the larger effect of the third chromosome than any other chromosomes as detected here. The interaction detected here does not involve the structural genes. It is very difficult to infer the adaptive significance of the detected interaction as long as the relation between the activity variation and the fitness is unclear. However, as JONES and YAMAZAKI (1974) recognized the importance of genetic background to the study of the allozyme polymorphisms, this result also demonstrates the importance and complexity of genetic background effects on the level of enzyme expression.

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LITERATURE CITED

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Corresponding editor: M. T. CLEGG