QUANTITATIVE ANALYSIS OF *X* CHROMOSOME EFFECTS ON THE ACTIVITIES OF THE GLUCOSE 6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

By combining 20 *X* chromosomes with five autosomal backgrounds, the relative importance of these factors with respect to the activity variations of G6PD and 6PGD in *Drosophila melanogaster* were investigated. Analysis of variance revealed that there exist significant *X* chromosome, autosomal background and genetic interaction effects. The effect of the *X* chromosome was due mainly to the two allozymic forms of each enzyme, but some within-allozyme effects were also detected. From the estimated variance components, it was concluded that the variation attributed to the autosomal background is much larger than the variation attributed to the *X* chromosome, even when the effect of the allozymes is included. The segregation of the allozymes seems to account for about 10% of the total activity variation of each enzyme. The variation due to the interaction between the *X* chromosome and the autosomal background is much smaller than variations attributed either to the *X* chromosome or to the autosomal background. The interaction effect is indicated by the change of the ranking of the *X* chromosomes for different autosomal backgrounds. Highly significant and positive correlation between G6PD and 6PGD activities was detected. Again, the contribution of the autosomal background to the correlation was much larger than that attributed to the *X* chromosome.

IN *Drosophila melanogaster*, glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) of the oxidative pentose phosphate shunt have been studied genetically and physiologically by many investigators (see reviews by LUCCHESI, HUGHES and GEER 1979; GEER *et al.* 1981; LAURIE-AHLBERG 1985). The structural loci for both enzymes have been localized on the *X* chromosome, that for G6PD to the proximal end (*Zw* at 1-63 and 17B-18F, YOUNG, PORTER and CHILDS 1964; STEWART and MERRIAM 1974) and that for 6PGD to the distal tip (*Pgd* at

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1-0.6 and 2D3-5, YOUNG 1966; GERASIMOVA and ANANIEV 1972). Each locus is polymorphic in natural populations for two common electrophoretic variants (O'BRIEN and MACINTYRE 1969). The 6PGD enzyme is a dimer, and thus female heterozygotes express an intermediate hybrid band of the fast and slow monomers (YOUNG, PORTER and CHILDS 1964; HORI and TANDA 1980); however, female heterozygotes of G6PD allozymes do not have an intermediate hybrid band. It has been shown for G6PD that the slow allozyme is a tetramer and the fast variant is a dimer (STEELE, YOUNG and CHILDS 1968; HORI and TANDA 1980).

An activity level difference between allozymes has been reported for both G6PD and 6PGD. The slow form of 6PGD shows a greater specific activity in crude homogenates than does the fast form (BIJLSMA and VAN DER MEULEN-BRUIJNS 1979; CAVENER and CLEGG 1981; HORI and TANDA 1981). However, the two 6PGD allozymes do not appear to differ in kinetic parameters (KOGAN, ROZOVSKII and GVOZDEV 1977; BIJLSMA and VAN DER MEULEN-BRUIJNS 1979; HORI and TANDA 1981), in thermal stability (BIJLSMA and VAN DER MEULEN-BRUIJNS 1979) or in specific activity when purified (HORI and TANDA 1981). Thus, the activity difference between 6PGD allozymes may be due to concentration of enzyme molecules. The tetrameric slow form of G6PD also appears to have a greater specific activity in crude homogenates than the dimeric fast form (STEELE, YOUNG and CHILDS 1969; BIJLSMA and VAN DER MEULEN-BRUIJNS 1979; HORI and TANDA 1981), but CAVENER and CLEGG (1981) report that this difference is highly dependent on the extraction buffer. In all cases of a reported difference, a Tris-HCl extraction buffer was used. CAVENER and CLEGG (1981) found that the fast G6PD is highly unstable in the Tris-HCl buffer and that no specific activity difference is observed when the enzymes are extracted in a phosphate buffer. Here, we report a difference in activity level in crude homogenates (in a phosphate buffer) between the G6PD allozymes that is not due to differential instability. The fast G6PD dimer is consistently reported to be more thermal labile than is the slow tetramer, but reports about kinetic parameter differences are not consistent (KOMMA 1968; STEELE, YOUNG and CHILDS 1968; BIJLSMA and VAN DER MEULEN-BRUIJNS 1979; HORI and TANDA 1980, 1981; CAVENER and CLEGG 1981). HORI and TANDA (1981) found no significant difference in specific activity between purified preparations of the two allozymes, so it appears that there may be a differential accumulation of the allozymic forms *in vivo*.

In addition to the allozymic differences, the activities of these two enzymes are also modified by genetic factors on the second and third chromosomes (LAURIE-AHLBERG *et al.*1980, 1981; HORI and TANDA 1981). Furthermore, there exists a significant interaction effect between the second and third chromosomes, such that the second chromosome activity modifiers seem to be influenced by the third chromosome background (TANDA and HORI 1983; MIYASHITA and LAURIE-AHLBERG 1984). Thus, it has been well established that the allozymic forms of the structural genes and the autosomal background are both very important factors responsible for variation in the activities of G6PD and 6PGD. However, the relative contributions of these factors have

not yet been reported. Here, we investigate this problem by combining wild-derived *X* chromosomes, which have the structural genes of the two enzymes, with the autosomal backgrounds that we previously studied (MIYASHITA and LAURIE-AHLBERG 1984).

MATERIALS AND METHODS

Line construction: First, 29 *X* chromosomes ($+_1$) sampled from natural populations (Texas, North Carolina and Fukuoka, Japan) and maintained in isofemale lines were extracted into an isogenic background consisting of the second and third chromosomes (i_2,i_3) from a highly inbred line, Ho-R (Figure 1). Thirteen of the *X* chromosomes have the fast form of the G6PD allozyme, and 24 have the fast form of the 6PGD allozyme. These lines were used to screen for the *X* chromosome effect on the activity variations of G6PD and 6PGD (experiment 1).

From these 29 substitution lines, 20 *X* chromosomes were chosen at random and were combined with four autosomal backgrounds randomly chosen from the 93 autosomal combination lines studied previously (MIYASHITA and LAURIE-AHLBERG 1984). Ten of these *X* chromosomes have the fast form of the G6PD allozyme, and 15 have the fast form of the 6PGD allozyme. The procedure for constructing a line with a particular combination of *X* chromosome and autosomal background is shown in Figure 2. Only 71 of the possible 80 combinations were established. The original 20 *X* chromosome substitution lines with the Ho-R background and the newly established 71 lines were used in experiment 2. Together these lines represent 20 *X* chromosomes combined with five different autosomal backgrounds. Before using any of the lines for screening activity variation, electrophoresis for eight common polymorphic enzymes was conducted to check for contamination or errors in the construction scheme. No problem was 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) were described by LAURIE-AHLBERG and WEIR (1979).

Activity 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 dithiothreitol and 0.5% (v/v) Triton X-100 and was centrifuged for 10 min at 10,000 × *g*. The supernatant was used for activity measurements and the determination 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, 0.18 mM $NADP^+$ and 1.8 mM glucose-6-phosphate for G6PD activity, and contains 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 1:9 ratio with distilled water, then the amount of protein was determined by the method of LOWRY *et al.* (1951).

Since CAVENER and CLEGG (1981) have reported that the stability difference between G6PD slow and fast allozymic forms is caused by the homogenization buffer, a preliminary test to check the effect of the homogenization buffer used in this experiment on the allozymes of G6PD was performed for seven randomly selected lines. Four lines have the slow form and three lines have the fast form of G6PD. After the homogenate sat for 4 hr on ice, a slight decrease of G6PD activity was observed for all lines, but there was no differential decrease between allozymes or among lines. For all samples in this report, enzyme activities were measured within 3 hr after homogenization of samples. Therefore, it can be assumed that the effect of homogenization buffer on the difference between allozymes is negligible in this study.

Experimental design and statistical analysis

Experiment 1: For the screen of the *X* chromosome effects on G6PD and 6PGD activity, 29 isogenic *X* chromosome lines established by the procedure in Figure 1 were used. For each line, 50 pairs of flies were placed in a half-pint milk bottle and were

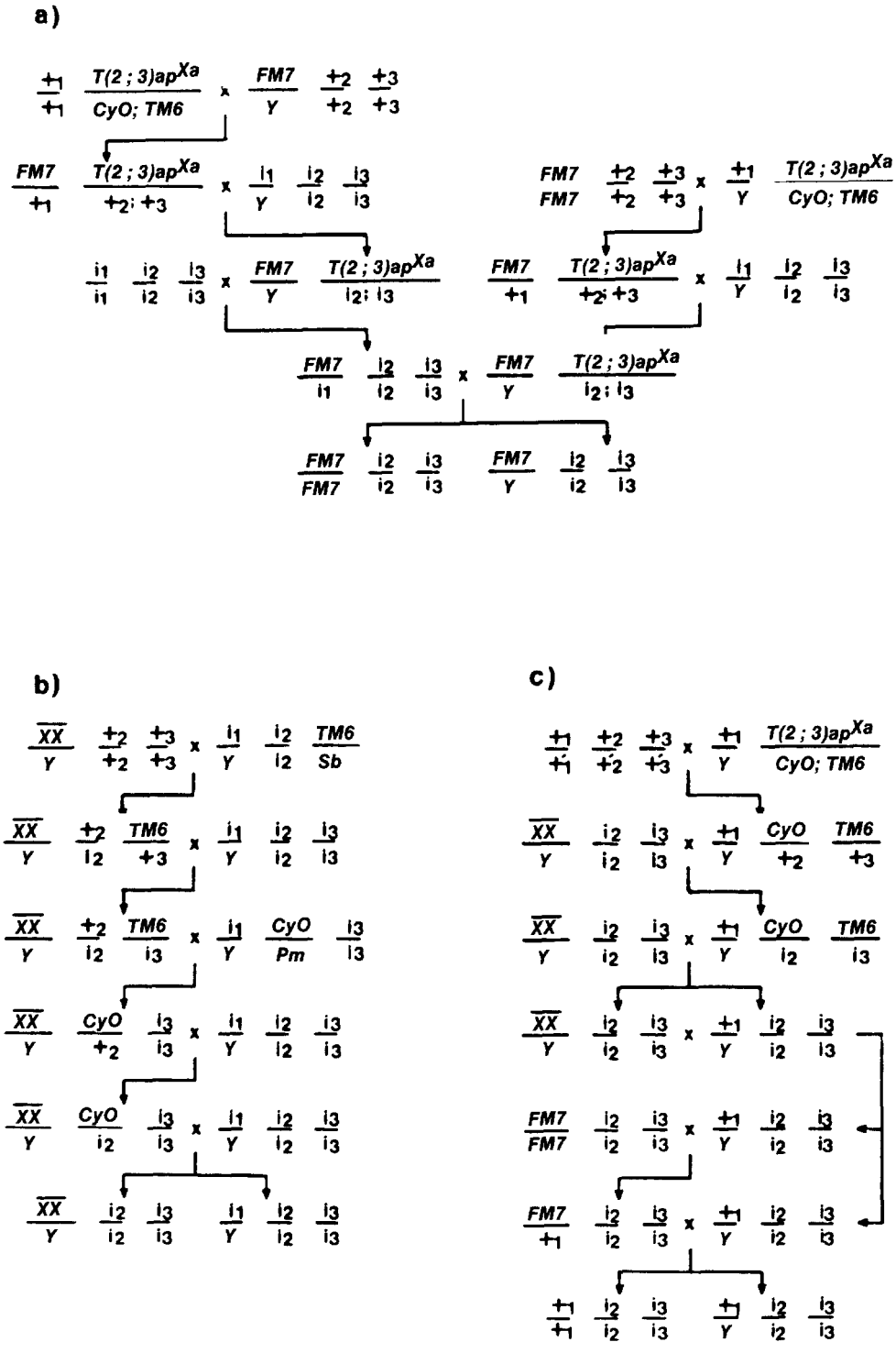

FIGURE 1.—Crossing scheme for construction of isogenic *X* chromosome substitution lines. *FM7* = *In(1)FM7*, y^{31d} sc^{8} w^{a} v^{of} *B*; *CyO* = *In(2LR)O*, *Cy* dp^{lwl} *pr* cn^{2}; *Pm* = *In(2LR)*bw^{v1}, bw^{v1}; *Ubx* = *In(3LR)TM6*, Hn^{P} ss^{P88} bx^{34e} Ubx^{P15} *e*; $\overline{XX}$ = *C(1)DX*, *y f.* *i* refers to a chromosome from the highly inbred line Ho-R. Construction of the *Sb/Ubx* and *CyO/Pm* isogenic stocks used in (b) is described by LAURIE-AHLBERG *et al.* (1980). Markers are described by LINDSLEY and GRELL (1968).

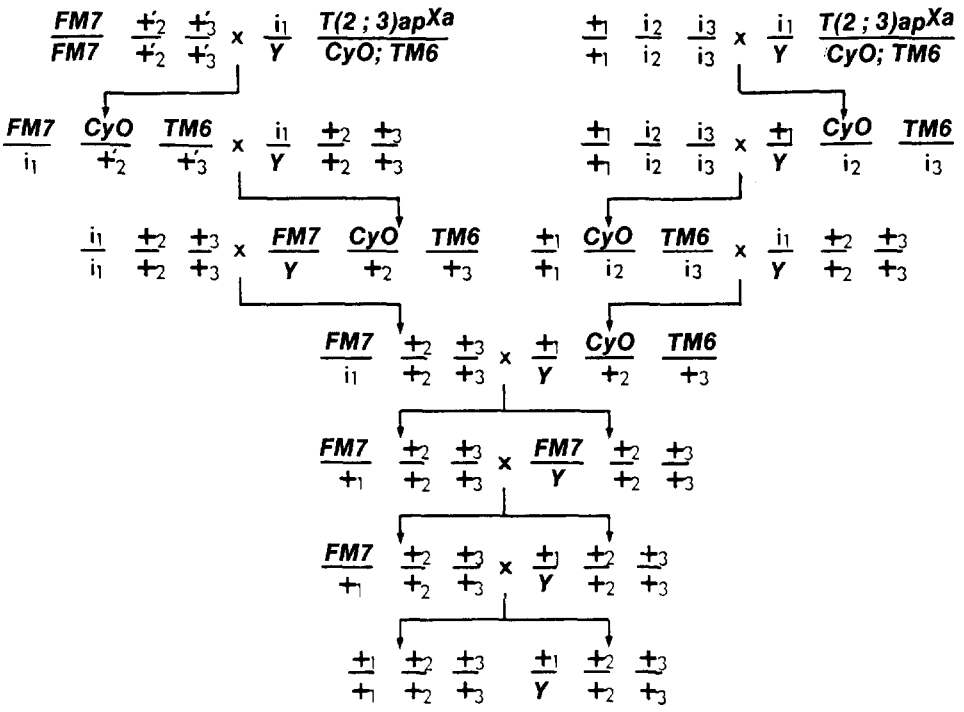

FIGURE 2.—Procedure for construction of a line homozygous for an *X* chromosome and an autosomal background. See legend of Figure 1 for the markers.

allowed to lay eggs for 4 days at 25°. From each bottle, two samples of 10 4-day-old males were collected. They were weighed and kept frozen at −70° until the activity assay. The setup of the bottles and collection of samples were repeated twice (two blocks). Samples from each of the blocks were assayed in one day. This design yields a total of 116 samples.

The model for analysis of variance (ANOVA) of each enzyme activity for experiment 1 follows:

$$Y_{il(jk)m} = \mu + B_i + G_j + P_k + (G^*P)_{jk} + X_{l(jk)} + (B^*G)_{ij} + (B^*P)_{ik} + (B^*G^*P)_{ijk} + (B^*X)_{il(jk)} + e_{il(jk)m},$$

where μ is the overall mean, B_i is the effect of the ith block (i = 1, 2), G_j is the jth allozyme effect of G6PD (j = *S*, *F*), P_k is the kth allozyme effect of 6PGD (k = *S*, *F*), $X_{l(j,k)}$ is the lth *X* chromosome effect of the haplotype of the jth G6PD and the kth 6PGD allozymes, and the terms in parentheses represent the interaction effects. The effects of the allozymes of G6PD and 6PGD are assumed to be fixed, and the other effects are assumed to be random.

Experiment 2: In order to investigate the effect of the autosomal background of the *X* chromosome action on G6PD and 6PGD activities, 91 combination lines between five autosomal backgrounds and 20 *X* chromosomes were used. For each line, 15 pairs of flies were placed in a 10-dram shell vial and were allowed to lay eggs for 4 days at 25°. Two samples of ten 4-day-old males per vial were weighed and kept frozen at −70° until the activity assay. The setup of vials and collection of samples were repeated three times (3 blocks). A replication of samples from each of the blocks was assayed in one day (two assay days within each block). This design yields a total of 546 samples.

The model for ANOVA for experiment 2 is the following for each of the variables

(G6PD, 6PGD, weight and protein):

$$Y_{ij(i)kl} = \mu + B_i + R_{j(i)} + X_k + (B*X)_{ik} + (R*X)_{j(i)k} + BG_l + (B*BG)_{il} + (R*BG)_{j(i)l} + (X*BG)_{kl} + (B*X*BG)_{ikl} + e_{ij(i)kl},$$

where μ is the overall mean, B_i is the effect of the ith block ($i = 1, 2, 3$), $R_{j(i)}$ is the jth assay day effect within the ith block ($j = 1, 2$), X_k is the effect of the kth X chromosome ($k = 1, 2, \cdots, 20$), BG_l is the lth background effect ($l = 1, 2, \cdots, 5$) and $e_{ij(i)kl}$ is the error term. The effects in parentheses represent the interactions.

For calculation of the sums of squares for the unbalanced data of both experiments 1 and 2, a "GLM procedure" of the "SAS" statistical analysis system was used (SPEED, HOCKING and HACKNEY 1978; HELWIG and COUNCIL 1979). The F-tests in the ANOVAs were constructed as described by NETER and WASSERMAN (1974, p. 664).

Adjustment of raw activity

Experiment 1: The activities of both enzymes were adjusted by live weight. The line means of the raw activities of G6PD and 6PGD ($\overline{Y}_{il\cdot}$) were regressed on the line mean of weight ($\overline{WT}_{il\cdot}$) for each block. The sums of squares and products were then pooled to obtain a single slope (b) for each of the enzyme activities. The adjusted variables ($\hat{Y}$) were then obtained as follows for each enzyme:

$$\hat{Y}_{ilm} = Y_{ilm} - b*(WT_{ilm} - \overline{WT}\cdot\cdot\cdot).$$

Experiment 2: For the adjustment of raw activities (Y) of G6PD and 6PGD, the line means within each block were regressed on the line mean of the amount of protein ($\overline{PRO}$). The sums of squares and products were then pooled over blocks in order to obtain a single slope for G6PD. Since the residual variances of 6PGD activity on the amount of protein were heterogeneous over blocks, the adjustment was performed with the slope (b_i) for each block. The heterogeneity of residual variances was not detected for G6PD activity. The adjustment of G6PD activity was done as follows:

$$\hat{Y}_{ij(i)kl} = Y_{ij(i)kl} - b*(PRO_{ij(i)kl} - \overline{PRO}_{\cdot\cdot(\cdot)\cdot\cdot}),$$

and for 6PGD activity,

$$\hat{Y}_{ij(i)kl} = Y_{ij(i)kl} - b_i*(PRO_{ij(i)kl} - \overline{PRO}_{i\cdot(\cdot)\cdot\cdot}).$$

The decision to adjust the enzyme activities by the amount of protein was based on the results of partial regression analysis of activity on both weight and protein. The partial regression coefficient of each enzyme activity on protein after weight ($b_{pro\cdot wt}$) was highly significant, whereas the partial regression coefficient on weight after protein was not significant. This result indicates that, after the adjustment by the amount of protein, the activity variations are not significantly correlated with the variation of weight and that the adjustment by protein is sufficient.

RESULTS

Experiment 1: *X* chromosome effects on G6PD and 6PGD: The effects of the 29 X chromosomes included in experiment 1 are summarized by the ANOVAs in Table 1. The following points are of interest:

1. A significant effect of X chromosomes within allozymic haplotypes (*i.e.*, the Line(*Zw*Pgd*) source) was detected for each enzyme both before and after weight ajdustment. Separate analysis for each allozyme of each enzyme (not shown) reveal significant within-allozyme X chromosome effects for G6PD-S lines and for 6PGD-F lines, but not for G6PD-F or 6PGD-S lines. The latter is not surprising, however, since only five of the 29 lines are 6PGD-S.

TABLE 1

Analyses of variance of G6PD and 6PGD activities for experiment 1

Source	d.f.	G6PD		6PGD	
		Raw	Adjusted	Raw	Adjusted
Block	1	NS	***	NS	***
Zw	1	***	***	NS	NS
Pgd	1	***	**	***	***
*Zw*Pgd*	1	NS	NS	NS	NS
Line(*Zw*Pgd*)	25	*	***	***	***
Block**Zw*	1	NS	NS	NS	NS
Block**Pgd*	1	NS	NS	NS	NS
Block**Zw*Pgd*	1	NS	***	NS	NS
Block*Line(*Zw*Pgd*)	25	***	NS	*	NS
Residual	58				

Zw and *Pgd* allozyme effects are assumed to be fixed.
NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

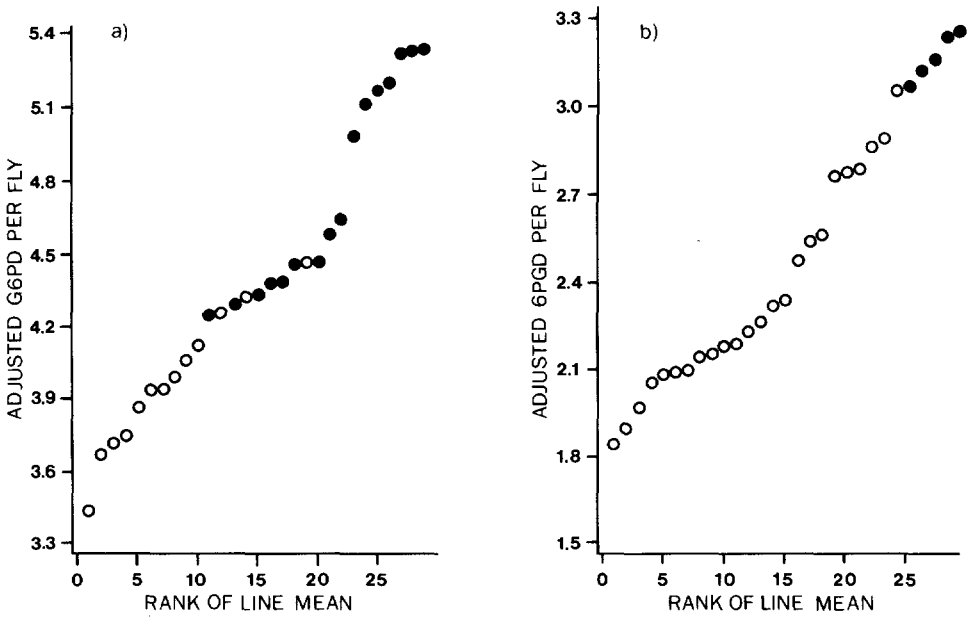

FIGURE 3.—Plot of weight-adjusted activity per fly against rank of line mean for experiment 1. a) G6PD, b) 6PGD. O = Fast allozyme; ● = Slow allozyme.

2. The *Zw* allozymes have a highly significant effect on G6PD activity, as do the *Pgd* allozymes on 6PGD activity. As previously reported in the literature, the slow form of each enzyme has higher activity than does the fast form. The degree of difference between allozymes relative to the within-allozyme variation is illustrated by the line mean distribution in Figure 3.

3. Table 1 also provides a test for the effects of one allozyme on the activity of the other enzyme. These enzymes catalyze consecutive steps in the pentose monophosphate shunt and they share a cofactor, NADP, so it is possible that

TABLE 2

Summary of analyses of variance for experiment 2

Source	d.f.	G6PD		6PGD		PROTEIN	WEIGHT
		Raw	Adjusted	Raw	Adjusted		
Block	2	NS	NS	NS	NS	NS	NS
AD(Block)	3	*	**	NS	NS	*	*
X	19	***	***	***	***	NS	NS
Block*X	38	NS	NS	**	NS	NS	NS
AD(Block)*X	57	NS	NS	NS	NS	NS	NS
BG	4	***	***	***	***	***	***
Block*BG	8	NS	*	***	*	NS	NS
AD(Block)*BG	12	*	*	NS	*	NS	**
X*BG	67	***	***	***	***	NS	***
Block*X*BG	134	**	***	NS	***	*	***
Residual	201						

AD = assay day, X = *X* chromosome, BG = autosomal background.
NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 3

Estimates of the variance components for the *X* chromosome, the autosomal background and their interaction

Variable		*X* chromosome	Autosomal background	Interaction
G6PD Overall	Raw	1.14 (0.17)	3.76 (0.57)	0.29 (0.04)
	Adj	1.33 (0.22)	2.53 (0.42)	0.35 (0.06)
Zw = F	Raw	−0.01 (0)[a]	2.89 (0.64)	0.36 (0.08)
	Adj	−0.02 (0)[a]	1.95 (0.49)	0.45 (0.11)
Zw = S	Raw	0.49 (0.07)	4.66 (0.67)	0.21 (0.03)
	Adj	0.54 (0.09)	3.12 (0.53)	0.26 (0.04)
6PGD Overall	Raw	0.98 (0.20)	3.01 (0.62)	0.15 (0.03)
	Adj	1.18 (0.28)	1.85 (0.43)	0.19 (0.04)
Pgd = F	Raw	0.29 (0.07)	2.97 (0.73)	0.18 (0.04)
	Adj	0.32 (0.10)	1.77 (0.53)	0.22 (0.07)
Pgd = S	Raw	−0.02 (0)[a]	3.12 (0.76)	0.09 (0.02)
	Adj	−0.03 (0)[a]	2.05 (0.61)	0.10 (0.03)
Weight		−0.02 (0)[a]	0.87 (0.67)	0.13 (0.10)
Protein		0.09 (0.01)	5.98 (0.44)	0.27 (0.02)

The proportion of each component to the total variance is shown in parentheses.
[a] Negative estimate was assumed to be zero.

one enzyme affects the other's stability or regulation. In fact, there is a highly significant effect of the 6PGD allozyme on G6PD activity, but not the reverse.

Experiment 2: *X* chromosome, autosome and epistatic interaction effects: The ANOVAs for experiment 2, which deals with the lines representing combinations of 20 *X* chromosomes with five autosomal backgrounds, are summarized in Tables 2 and 3. The following points are of interest:

1. For both G6PD and 6PGD activities, the *X* chromosome and autosomal background are each highly significant sources of variation (both before and after protein adjustment). Although the autosomal background also has highly significant effects on body weight and protein content, no significant *X* chromosome effect was detected.

2. As detected in experiment 1, both enzymes show an association of allozymic and activity variation. For each enzyme, *X* chromosomes that have the slow allozymes have significantly higher activity than do those having the fast allozyme. This result was obtained by the analysis of the marginal *X* chromosome means over autosomal backgrounds ($t = 6.16$, d.f. = 18, $P = 0.0001$ for raw G6PD; $t = -6.55$, d.f. = 18, $P = 0.0001$ for adjusted G6PD; and $t = -6.19$, d.f. = 18, $P = 0.0001$ for raw 6PGD; $t = -6.63$, d.f. = 18, $P = 0.0001$ for adjusted 6PGD) and by separate analysis of the *X* chromosome means within each of the five autosomal backgrounds.

3. The *X* chromosomes were categorized according to the G6PD and 6PGD allozymes in order to eliminate the effects of allozyme segregation on the activity variation of each enzyme. As detected in the analysis of experiment 1 (Table 1), significant *X* chromosome effects were detected for the *X* chromosomes with the slow form of G6PD and the *X* chromosomes with the fast form of 6PGD for G6PD and 6PGD activities, respectively. The significance of the interaction between the *X* chromosome and the autosomal background effects was confirmed for each allozymic class except for the *X* chromosomes with the slow form of 6PGD for 6PGD activity.

4. The effect of the allozyme of one enzyme on the activity of the other enzyme was tested as in experiment 1. A significant 6PGD allozyme effect on the activity of G6PD was detected for two of the five autosomal backgrounds at the 1% level. This result suggests that the effect is influenced by the background.

5. Table 2 also shows a highly significant epistatic interaction between the *X* chromosome and autosomal background for both enzymes. In order to investigate the possibility of a multiplicative action between *X* chromosome and autosomal background, a logarithmic transformation of the raw G6PD and 6PGD activities was performed. ANOVAs of the transformed variables gave essentially the same results as those for the raw variables. In particular, the genetic interaction remained highly significant, which does not support a multiplicative epistasis model.

6. In order to characterize the detected interaction effect between the *X* chromosome and the autosomal background further, the heterogeneity of the *X* chromosome variance components over the autosomal backgrounds and the change of rankings of the *X* chromosomes over the autosomal backgrounds were investigated. The variance components of the *X* chromosome for both enzyme activities were fairly constant over autosomal backgrounds, and no significant heterogeneity was detected either before or after categorizing the *X* chromosomes by the allozyme and the bilocus haplotype, except for the adjusted G6PD activity of the *X* chromosomes that have the fast forms of both enzymes (significant at the 5% level). For both enzyme activities, when the

effects of allozyme segregation are included, the mean correlations over *X* chromosomes and between autosomal backgrounds are fairly high (0.73 and 0.72 for raw and adjusted G6PD activity, respectively, 0.88 and 0.86 for raw and adjusted 6PGD activity, respectively). In other words, the rankings of the *X* chromosomes do not change substantially over different autosomal backgrounds. However, after classifying the *X* chromosomes by the allozymes, a marked reduction in the correlation of the *X* chromosomes was observed for G6PD activity (0.30 and 0.18 for the *X* chromosomes with the slow form of G6PD, and 0.59 and 0.57 for the *X* chromosomes with the fast form of G6PD, for raw and adjusted G6PD activity, respectively) and for the *X* chromosomes that have the slow form of 6PGD for 6PGD activity (0.08 and 0.13 for raw and adjusted 6PGD activity, respectively). Although the mean correlation of the *X* chromosomes that have the fast form of 6PGD is still high (0.88 and 0.86 for raw and adjusted 6PGD activity, respectively), the correlations of the *X* chromosomes between some pairs of the autosomal backgrounds are very low. This variation in the magnitudes of the correlations among backgrounds was manifested as significant heterogeneity. This result suggests that the association of activity difference with the allozyme of each enzyme is so strong that the ranking of the *X* chromosomes is not strongly influenced by the change of autosomal background. However, the ranking of the *X* chromosomes within the allozyme group seems to be sensitive to the change of autosomal background. These observations suggest that the detected interaction effect between the *X* chromosome and the autosomal background is mainly due to the change of the rankings of the *X* chromosomes, particularly within an allozymic class.

7. In order to quantify the importance of the *X* chromosome, the autosomal background and the interaction effects, the variance components of these effects were estimated for G6PD and 6PGD activities by equating mean squares to expected values and then solving the equations (Table 3). Despite large differences due to the allozymes of both enzymes on the activities, the *X* chromosome variance component accounts for only 20% of the total G6PD activity variation and 25% of the total 6PGD activity variation, whereas 50–60% of the total variation of each enzyme is attributed to the autosomal background. The variance components attributed to the interaction between the *X* chromosome and the autosomal background are very small and account for $<10\%$ of the total variation of G6PD and 6PGD activities. After the adjustment of the enzyme activities by protein, the variance components due to the autosomal background were reduced. This result suggests that the effects of the autosomal background on the activity variations are associated with the variation of the amount of protein to some extent. However, the effects of the autosomal background are very large even after the adjustment.

8. Table 3 also gives the estimates of the variance components of the *X* chromosome, the autosomal background and the interaction for each of the allozymes of both enzymes. Because of the strong difference in the enzyme activities due to allozymes, a drastic reduction of the *X* chromosome variance components for both enzyme activities was observed. The magnitudes of the

TABLE 4

Correlation between G6PD and 6PGD activities over line means

Haplotype	*N*	Correlation	
Experiment 1: *X* chromosome line means			
Overall	29	0.58***	(0.49**)
Zw = F and *Pgd* = F	12	−0.03 NS	(0.55 NS)
Zw = S and *Pgd* = F	12	0.17 NS	(−0.02 NS)
Zw = S and *Pgd* = S	4	0.98*	(0.77 NS)
Average of the three correlations above		0.19	(0.34)
Experiment 2: Marginal *X* chromosome means			
Overall	20	0.70***	(0.74***)
Zw = F and *Pgd* = F	9	0.22 NS	(0.33 NS)
Zw = S and *Pgd* = F	6	0.42 NS	(0.35 NS)
Zw = S and *Pgd* = S	4	0.94 NS	(0.97*)
Average of the three correlations above		0.42	(0.48)

Adjusted data are shown in parentheses. Average correlations were obtained by Fisher's z-transformation. Heterogeneity of the three correlations was not detected.

NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. N = the number of lines.

reduction of the *X* chromosome variance components suggest that the segregation of the allozymes accounts for about 10% of the total variation of G6PD activity and about 15% of the total 6PGD activity variation. These values are much smaller than those of the autosomal background.

Correlation between G6PD and 6PGD activities: Table 4 summarizes the results concerning the correlation over *X* chromosomes between G6PD and 6PGD activities. In experiment 1, a highly significant correlation of 0.58 over the 29 *X* chromosomes was observed. This value is similar to the value of 0.70 for the correlation over the marginal *X* chromosome means from experiment 2, but somewhat less than the correlation estimates previously reported for variation due to autosomal modifiers, which are generally >0.8 (LAURIE-AHLBERG *et al.* 1981; WILTON *et al.* 1982). Further analysis of experiment 2 confirms the lower degree of correlation due to *X* chromosome than to autosomal factors. The average of the 20 correlations over the five autosomal backgrounds for each *X* chromosome is 0.98 (before and after protein adjustment), whereas the average of the five correlations over the 20 *X* chromosome for each autosomal background is 0.79 for raw data and 0.75 for adjusted data.

It is desirable to quantify the relative contributions to the correlation between enzymes of the *X* chromosome *vs.* the autosomal background. A cross-product analysis is appropriate, but experiment 2 is unbalanced, so an exact analysis could not be performed. However, estimation of missing values allowed an approximate analysis. The activities of the nine missing combination lines

TABLE 5

Estimates of the components of covariance between G6PD and 6PGD activities

Haplotype	*X* chromosome	Autosomal background	Interaction
Overall	1.15 (1.30)	3.65 (2.28)	0.13 (0.17)
Zw = F and *Pgd* = F	0.38 (0.26)	3.15 (1.90)	0.17 (0.25)
Zw = S and *Pgd* = F	0.56 (0.56)	4.23 (2.70)	0.12 (0.14)
Zw = S and *Pgd* = S	0.12 (0.05)	3.33 (2.23)	0.06 (0.13)

Adjusted data are shown in parentheses.

were estimated as the sum of the marginal *X* chromosome effect and the marginal autosomal background effect for each of the replicates within each block. Estimates of the covariance components are given in Table 5. As expected, the contribution of the autosomal background to the covariance between enzymes is much larger than the *X* chromosome contribution.

The *X* chromosome contribution to the correlation between G6PD and 6PGD levels appears to be due mainly to allozymic effects. Table 4 shows that the correlations within allozymic haplotype are substantially lower than the correlations over *X* chromosomes ignoring allozymes. Similarly, Table 5 shows that the covariance component for the *X* chromosome is much reduced when the analysis is performed separately for each allozymic haplotype. These results strongly suggest that the within-allozyme *X* chromosome factors do not contribute substantially to the correlation between G6PD and 6PGD activities, in strong contrast to the autosomal factors.

DISCUSSION

The results presented here demonstrate highly significant allozyme effects on the activities of both G6PD and 6PGD. Consistent with previous reports (see Introduction), we find for both enzymes that lines with the slow allozyme have greater activity than those with the fast allozyme. This result is not confounded by a differential effect of the homogenization buffer on stability of the G6PD allozymes, as suggested by CAVENER and CLEGG (1981) for some other published reports. Furthermore, the allozymic difference is not affected substantially by variation in the autosomal background. Although the *X* chromosome effects are dominated by the allozymic differences, significant within-allozyme effects due to *X* chromosome factors were also observed for both enzymes. It is possible that the genetic variation within allozymes is due to hidden structural variation that cannot be detected by our electrophoretic procedure. However, there is evidence that hidden structural variation for G6PD is very rare (EANES 1983). Of course, some of the within-allozyme *X* chromosome variation may be due to modifier genes similar to those on the autosomes.

Both the *X* chromosome and autosomal background are very important contributors to the activity variation of G6PD and 6PGD. However, despite the large magnitude of the allozymic effects, the variance component estimates

suggest that the autosomal background has a considerably larger contribution than does the *X* chromosome. Of the total variance in activity, roughly 50% is due to autosomes, 20% to the *X* chromosome and 5% to the *X*-autosome interaction. At least half of the *X* chromosome contribution is due to the allozyme effect. It should be noted that the autosomal backgrounds used in this experiment are artificial combinations between second and third chromosomes and that these backgrounds do not exactly constitute a random sample from natural populations. Although our previous work indicates it is possible to obtain much larger autosomal effects than in the present experiment, more work is needed to draw definite conclusions about the relative contributions of the *X* chromosome and autosomes to G6PD and 6PGD variation.

The results also clearly demonstrate an epistatic interaction between the *X* chromosome and autosomal background. However, the interaction effect is considerably smaller in magnitude than either main effect and is very complicated in nature. It appears to be due mainly to change in ranking of *X* chromosomes within allozymes among the different autosomal backgrounds. These results are very similar to our analysis of the interaction between second and third chromosome modifier effects (MIYASHITA and LAURIE-AHLBERG 1984).

This study also demonstrates significant positive correlation between G6PD and 6PGD activities due to *X* chromosome factors, as previously reported for autosomal modifiers (LAURIE-AHLBERG *et al.* 1981; HORI and TANDA 1981). This correlation is more likely due to pleiotropy than to linkage disequilibrium, since a positive association is found for all three major chromosomes. However, the autosomal factors have considerably stronger effects on the correlation between enzymes than do the chromosomes, suggesting perhaps a different mechanism. The *X* chromosome variation may be due largely to structural gene variation, whereas the autosomal variation is obviously nonstructural.

Several studies deal with the possibility of differential selection of the allozymic forms of G6PD and 6PGD (reviewed by ZERA, KOEHN and HALL 1983). In one study, CAVENER and CLEGG (1981) demonstrated differential flux through the pentose shunt in Zw^F Pgd^F vs. Zw^S Pgd^S flies, where the latter genotype, which shows higher enzyme activity levels, also shows higher flux levels. In another study, EANES (1984) showed that, in the presence of a null *Pgd* allele, the genotypes with the fast G6PD allozyme have higher viability than those with the slow allozyme. This result is also consistent with the direction of the activity level difference, since *Pgd* null alleles have a lethal effect that can be rescued by loss of G6PD activity (HUGHES and LUCCHESI 1977). These studies clearly demonstrate the potential for selection, but estimation of the relation between fitness-related traits and activity over naturally occurring ranges of activity variation is still needed. The results presented here clearly point to the need to consider and control genetic background effects in studies of this type. In particular, it is important to sample many representatives of each allozyme because of substantial within-allozyme effects on activity, some of which may be closely associated with the structural gene.

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