EFFECT OF SEX TRANSFORMATION GENES ON
GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY
IN *DROSOPHILA MELANOGASTER*¹

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GLUCOSE-6-PHOSPHATE dehydrogenase (G-6-PD) is produced in *Drosophila melanogaster* by a gene carried on the X chromosome (YOUNG, PORTER and CHILDS 1964). G-6-PD is thus a favorable system for investigation of dosage compensation at the level of enzyme synthesis. Dosage compensation in *Drosophila* is apparently brought about by some form of genetic regulation rather than by X-chromosomal inactivation such as occurs in mammals (COCK 1964; KAZAZIAN, YOUNG and CHILDS 1965). MULLER (1932, 1950) has presented evidence that each gene carried on the X is subject to the effects of a set of "compensatory modifiers," which are apparently different from the genes controlling sexual development. He found furthermore that the mutant gene transformer (*tra*; STURTEVANT 1945), which produces morphologically normal males carrying two X chromosomes, has no effect on the phenotypic expression of sex-linked genes, and concluded that sexual development probably plays no role in dosage compensation. This conclusion has been strongly disputed by GOLDSCHMIDT (1954).

Different genes on the X chromosome may have different compensation mechanisms, and it is desirable in any case to investigate the phenomenon of dosage compensation at the level of gene action. This report, therefore, describes the effect of sex transformation genes on G-6-PD activity. In addition to *tra*, the mutant doublesex (*dsx*; HILDRETH 1965) was also tested. This mutant, when homozygous, causes both XX and XY embryos to develop into intersexes. Both *tra* and *dsx* are on the third chromosome, but they are not alleles.

The data presented here utilized X chromosomes obtained from stocks showing dosage compensation for G-6-PD, as judged by criteria to be described. A later report will describe the effects of *tra* and *dsx* on X chromosomes from stocks showing abnormalities of dosage compensation. Data are also included on the levels of 6-phosphogluconate dehydrogenase (6-PGD) in the stocks used. This enzyme is also produced by X-chromosome genes (KAZAZIAN, YOUNG and CHILDS 1965).

MATERIALS AND METHODS

The stocks used were ones in which both *tra* and *dsx* were maintained in balanced condition

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with the dominant third-chromosomal marker gene *Dichaete* (*D*), which is lethal when homozygous. The genetic constitutions of the stocks were such that the pseudomales produced by *tra* were visibly different from genuine males, and XX intersexes different from XY intersexes. Most of the data presented are from stocks in which the two X's of the female are attached to one another and inherited as a unit. Both attached-X's of the unit carry the mutant yellow (*y*). The free X of the males carries the wild-type allele of *y* (*y*⁺). Thus all females, pseudomales and XX intersexes in these stocks have yellow bodies, while real males and XY intersexes are wild-type in body color. All the chromosomes in these stocks carried the *ZwA* allele (fast electrophoretic form) of G-6-PD.

Attached-X females carry a Y chromosome, a possible complicating factor. Two other *tra* stocks were therefore set up for comparison. Both carry the mutant white (*w*) on all X chromosomes, but the wild-type allele, *w*⁺, is carried on a small piece of the X attached to the Y. Females and pseudomales are therefore white-eyed, while real males have the Y with *w*⁺ on it and are therefore wild-type. One of these two stocks also carries the *ZwA* allele of G-6-PD; the other carries *ZwB*, as well as miniature (*m*) and forked (*f*).

The attached-X stocks were grown at 21°, 25° and 29°C to test for reported effects of temperature on sexual development of intersexes (DOBZHANSKY 1930) and pseudomales (BROWN and KING 1961). The stocks with the abnormal Y were grown at 25°C.

The assay system used was that of GLOCK and McLEAN (1953). Samples of ten flies were homogenized in a Ten Broeck glass homogenizer with 3.5 ml of a buffer containing 0.05 M tris(hydroxymethyl)amino-methane, 0.0295 M HCl and 0.01 M EDTA, pH 7.5, to which 0.3 mg/ml of NADP had been added. The homogenates were then centrifuged for 30 minutes at 30,000 g and 0°C. One ml of the supernatant was added to 0.5 ml stock substrate solution and 0.5 ml of 0.12 M MgCl₂. The stock substrate solutions were 0.0008 M Ba 6-phosphogluconate in cuvette #1 and 0.0008 M Ba 6-phosphogluconate plus 0.0008 M Ba glucose-6-phosphate in cuvette #2. Another 1 ml of the homogenate was used to measure soluble protein (LOWRY *et al.* 1951).

For assay of 6-PGD, flies were homogenized as above, but with no NADP in the buffer. One ml of the homogenate was added to 0.5 ml of 0.6 mg/ml NADP solution and 0.5 ml of an 0.0008 M solution of the trimonocyclohexylammonium salt of 6-phosphogluconic acid dissolved in 0.12 M MgCl₂. The control cuvette had 0.12 M MgCl₂ substituted for the substrate. Activity was otherwise measured in the same way as for G-6-PD. Activity for both enzymes was expressed as μ moles NADP reduced per min per mg of soluble protein.

RESULTS

Table 1 shows the specific activities of the stocks from which the X chromosomes used were derived. These stocks were maintained in parallel with the sex-

TABLE 1

G-6-PD and 6-PGD specific activities of stocks carrying the X chromosomes used

X chromosome	G-6-PD allele	Activity*			
		G-6-PD		6-PGD†	
		Males	Females	Males	Females
<i>w m f</i>	<i>ZwB</i>	3.0 ± .1‡	3.1 ± .1	2.0 ± .15	2.1 ± .1
<i>w</i>	<i>ZwA</i>	2.0 ± .1	2.2 ± .1	2.2 ± .15	2.1 ± .15
<i>y</i> attached-X	<i>ZwA</i>		2.1 ± .1		1.2 ± .1
+§	<i>ZwA</i>	2.2 ± .1	2.1 ± .15	2.1 ± .1	2.1 ± .1

* μ moles NADP reduced per minute per milligram soluble protein.

† All stocks carry the *PgdA* allele of 6-PGD (KAZAZIAN *et al.* 1965).

‡ $s_x = s/\sqrt{N}$, the standard error of the mean of assays performed on 10-fly homogenates taken from different bottles at different times. N=5 in this table, N=3 in each of the other tables.

§ This is the free X chromosome found in males of the attached-X stocks.

TABLE 2

Effect of tra and dsx on G-6-PD activity at three different growth temperatures

Genotype	Sexual type	Growth temperature ($\pm 0.3^\circ$)		
		21°C	25°C	29°C
<i>D/tra</i> ; γ attached-X	Female	$1.2 \pm .1$	$1.3 \pm .1$	$1.6 \pm .25$
<i>tra/tra</i> ; γ attached-X	Pseudomale	$2.4 \pm .2$	$1.8 \pm .1$	$1.6 \pm .2$
<i>D/tra</i> ; γ^+	Male	$2.2 \pm .2$	$2.0 \pm .1$	$2.4 \pm .2$
<i>tra/tra</i> ; γ^+	Male	$2.2 \pm .1$	$2.2 \pm .1$	$2.4 \pm .2$
<i>D/dsx</i> ; γ attached-X	Female	$2.0 \pm .1$	$2.2 \pm .1$	$2.4 \pm .25$
<i>dsx/dsx</i> ; γ attached-X	XXY intersex	$3.4 \pm .1$	$3.0 \pm .2$	$2.8 \pm .2$
<i>D/dsx</i> ; γ^+	Male	$2.2 \pm .1$	$2.2 \pm .1$	$2.0 \pm .1$
<i>dsx/dsx</i> ; γ^+	XY intersex	$2.0 \pm .1$	$2.2 \pm .2$	$2.2 \pm .2$

transformation stocks made from them, and assays were made during the same period of time. All the stocks show normal dosage compensation for G-6-PD, assuming that activity per unit of soluble protein is the proper criterion for judging dosage compensation for this enzyme. No value is given for γ males, since the attached-X stocks do not produce yellow males. The very high values obtained for the *w m f* stock are characteristic of stocks carrying the *ZwB* allele of G-6-PD. This phenomenon is probably unrelated to dosage compensation. With regard to 6-PGD, it is noticeable that the attached-X females have rather low specific activity.

The effect of *tra* and *dsx* on G-6-PD levels is shown in Table 2. Both pseudomales and XXY intersexes show higher specific activities than their normal

FIGURE 1.—Least-squares regression lines for G-6-PD activity as a function of growth temperature in γ attached-X flies of Table 1. Using $b = 0$ as the null hypothesis, t scores and probabilities are as follows:

D/tra females: $t=2.89$, $P < 0.05$
tra/tra pseudomales: $t=4.79$, $P < 0.01$
D/dsx females: $t=2.37$, $P=0.05$
dsx/dsx intersexes: $t=4.81$, $P < 0.01$

TABLE 3

G-6-PD activities of flies heterozygous for D and tra

Cross	F, flies tested			
	<i>D</i> /+ Female	<i>tra</i> /+ Female	<i>D</i> /+ Male	<i>tra</i> /+ Male
<i>D/tra</i> ; <i>y</i> attached-X ♀ × X ♂	2.1 ± .1*	2.0 ± .2*	2.0 ± .1	2.2 ± .2
+ ♀ × <i>D/tra</i> ♂	2.0 ± .1†	2.0 ± .05†	2.2 ± .1	2.1 ± .1

* Attached-X females (XXY).

† Free-X females.

sisters, and the activity is negatively correlated with growth temperature, whereas the females show a positive correlation (Figure 1). No differences are seen between real males heterozygous and homozygous for *tra*, suggesting that the effect seen in the pseudomales is not due to a direct influence of *tra* on the G-6-PD locus or enzyme.

The females heterozygous for *tra* in Table 2 have markedly low specific activity, only about 60% of the value found for the parent stock. This suggests a partially dominant expression of *D* or *tra* in females with respect to the G-6-PD locus. As a test of this hypothesis, reciprocal crosses to the wild-type stock were made, giving *D*/+ and *tra*/+ offspring. These offspring were grown at 25°C; their G-6-PD values are shown in Table 3. No evidence of dominance of either *D* or *tra* can be seen.

Because of the peculiarities of attached-X stocks, assays were also made on the free-X stocks in which pseudomales were distinguished from normal males by eye color. The data for these stocks are given in Table 4. For the stock carrying the *w ZwA* X chromosome, the values are essentially identical to those found for the *D/tra* attached-X stock. The values for the *w m f ZwB* stock are higher, as would be expected from Table 1, but have a similar distribution. In both these stocks, the *D/tra* females show the same unexpectedly low values relative to their brothers as do the *D/tra* females in the attached-X stock. Since this effect does not seem to be due to a dominant expression of either *D* or *tra*, but appears in all three *D/tra* stocks, the most likely explanation is that of a recessive third-chromosomal gene affecting G-6-PD activity in females and that happens to be carried on both the *D* and *tra* chromosomes. Efforts to isolate and characterize this gene are underway.

TABLE 4

G-6-PD activities of tra flies carrying free X chromosomes

X chromosome	Sexual type			
	<i>D/tra</i> Female*	<i>tra/tra</i> Pseudomale*	<i>D/tra</i> Male†	<i>tra/tra</i> Male†
<i>w m f ZwB</i>	1.7 ± .2	2.7 ± .2	2.6 ± .1	2.8 ± .2
<i>w ZwA</i>	1.3 ± .1	2.0 ± .1	2.0 ± .2	1.8 ± .1

* White-eyed.

† Red-eyed because of the presence of *w*⁺ on the Y.

The apparent effect of the sex transformation genes on specific activity for G-6-PD could possibly be explained by assuming that there is a sharp decrease in the total soluble protein in the pseudomales and XX intersexes, without an accompanying decrease in G-6-PD synthesis. This hypothesis seems unlikely, since the pseudomales and XX intersexes are both much closer to females than to males with respect to size (STURTEVANT 1945; HILDRETH 1965) and total protein, and have more activity per fly as well as more specific activity. Still further evidence against this interpretation is provided by the 6-PGD values, given in Table 5. Although this enzyme is also produced by an X-linked gene, neither pseudomales nor XX intersexes show any increase in activity, and there is no correlation between activity and growth temperature, except in XY intersexes.

DISCUSSION

Three features of the data presented in this report indicate a relationship between G-6-PD activity and sexual differentiation. (1) Both pseudomales and XX intersexes show increased activity for G-6-PD as compared to their normal sisters. The *tra* and *dsx* mutants are not allelic, and there is no reason to assume that their effects on sexual differentiation are achieved in identical ways. The concordance of the results thus suggests that sexual differentiation itself is the critical factor. (2) Both pseudomales and XX intersexes show an inverse correlation between G-6-PD activity and growth temperature. This is precisely the correlation expected if sexual differentiation is involved in regulation of G-6-PD synthesis. DOBZHANSKY (1930) showed that low temperatures cause intersexes to become more male-like, while high temperatures have a feminizing effect. Lowering the growth temperature thus provides a more and more male-like "sexual environment," which is reflected in increasing G-6-PD activity. The *tra* mutant could reasonably be expected to show a similar effect, as it has been interpreted as an extreme example of an intersex gene (STURTEVANT 1945; GOLDSCHMIDT 1954). (3) No corresponding increase in 6-PGD activity is shown, suggesting that the effect on G-6-PD activity is a real effect and not merely a reflection of abnormal total protein synthesis.

TABLE 5

Effects of tra and dsx on 6-PGD activity at three different growth temperatures

Genotype	Sexual type	Growth temperature		
		21°C	25°C	29°C
<i>D/tra; γ attached-X</i>	Female	1.2 ± .1	1.6 ± .2	1.5 ± .2
<i>tra/tra; γ attached-X</i>	Pseudomale	1.4 ± .1	1.6 ± .1	1.4 ± .1
<i>D/tra; γ+</i>	Male	1.8 ± .1	2.0 ± .2	1.8 ± .2
<i>tra/tra; γ+</i>	Male	2.0 ± .1	2.0 ± .1	2.0 ± .2
<i>D/dsx; γ attached-X</i>	Female	1.4 ± .2	1.2 ± .1	1.2 ± .25
<i>dsx/dsx; γ attached-X</i>	XXY intersex	1.3 ± .2	1.2 ± .1	1.2 ± .2
<i>D/dsx; γ+</i>	Male	2.2 ± .2	2.4 ± .2	2.0 ± .25
<i>dsx/dsx; γ+</i>	XY intersex	2.4 ± .2	1.8 ± .2	1.6 ± .25

One would expect the XY intersexes to have lower enzyme activity than their normal brothers, since they have only one dose of the gene in a partly female internal environment. No such effect appears, even at the highest growth temperature used. Perhaps regulation of G-6-PD synthesis involves some sort of threshold which is not reached by these male-type intersexes.

A more serious problem presented by the data is the hypothetical third-chromosomal gene that brings about a reduction of G-6-PD activity in the *D/tra* females. It has not yet been possible to separate this gene from the *tra* gene, and its existence raises serious questions as to the true mode of action of *tra* on G-6-PD activity. An increase in rate of synthesis could be brought about either by a simple reversal of the effects of this gene, or by an influence on the normal regulatory mechanism through its effect on sexual differentiation. However, the low activity of the *D/tra* females is not temperature-dependent, and it seems a remarkable coincidence that the reversal of the effect by *tra* should be temperature-dependent in precisely the same manner as the expected effect on sexual differentiation. This result, when combined with the concordance of the *dsx* effect, thus leaves a relationship between sexual differentiation and G-6-PD regulation as the simplest explanation for the data.

Whether such a relationship is a frequent mechanism of dosage compensation in *Drosophila* remains to be determined. MULLER (1950) and STURTEVANT (1945) found no effect of *tra* on the regulation of sex-linked genes affecting such characters as eye color, bristle development or eye structure, and neither *tra* nor *dsx* seems to affect G-6-PD activity. The G-6-PD gene is therefore the only one for which such a relationship has been found. However, much evidence exists that the pseudomales produced by *tra* are only superficially similar to normal males. Besides being invariably sterile (STURTEVANT 1945), they react like females both with respect to female-lethal genes such as scute-3 (STURTEVANT 1945) and daughterless (BELL 1954) and to male-lethal factors such as the "sex ratio" spirochaetes (SAKAGUCHI and POULSON 1963). It therefore seems likely that their physiology resembles that of females more than that of males in certain critical respects. Similar arguments are certainly applicable to *dsx*, and it seems doubtful that the absence of any measurable effect of these genes on any given sex-linked gene can be used to exclude a role of sexual differentiation in the regulation of the gene. This is essentially the conclusion reached by GOLDSCHMIDT (1954) in his dissent from the MULLER model of dosage compensation.

Another complication in studying dosage compensation is the possibility that the enzyme involved may be only partly sex-linked. GLASSMAN (1965) has shown that while lack of xanthine dehydrogenase in *Drosophila* can be sex-linked, a structural subunit of the enzyme produced on chromosome 3 is the critical factor in determining the amount of activity present. In such a situation, an increase in the amount of an X-chromosomal subunit brought about by sex transformation might go undetected. The linear relationship between gene dosage and eye color excludes such a mechanism in the case of the eye color mutants studied by MULLER (1950), but it might be operative in other systems.

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SUMMARY

Partial sex transformation brought about by two different mutants markedly affected the activity of glucose-6-phosphate dehydrogenase, but not that of 6-phosphogluconate dehydrogenase. The changes in glucose-6-phosphate dehydrogenase activity were temperature-dependent, and were in accord with expectation if dosage compensation for this enzyme is dependent upon sexual differentiation.

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