

X-Linked Electrophoretic Variation in 6-phosphogluconate dehydrogenase

In *Drosophila melanogaster*

WILLIAM J. YOUNG

QUALITATIVE variation in enzymes, expressed as altered electrophoretic mobility in a variety of supporting media, has proved to be of value in studies of gene-enzyme relationships in a number of organisms¹⁰. Investigations in which extensive analysis of such systems in *Drosophila* have been accomplished are still limited in number, though increasing rapidly. It is hoped a number of gene-determined enzyme systems will be discovered in this diploid organism that will provide suitable assays for studies of regulation of protein synthesis, gene evolution, functional organization of chromosomes, etc. We have previously reported the X-linked control of electrophoretic variation in *Drosophila melanogaster* of glucose-6-phosphate dehydrogenase (G-6-PD)¹³. The enzyme generally considered to follow it in the hexose monophosphate pathway is 6-phosphogluconate dehydrogenase (6-PGD). The latter enzyme was chosen for study to test the possibility that its locus might be close enough to that of G-6-PD to provide a satisfactory system for the study of coordinate control of gene function. Though the locus specifying the observed electrophoretic variation in 6-PGD is X-linked, recombination is essentially random with the G-6-PD locus; no evidence on coordinate control is provided by this study. Evidence is advanced, however, that relates to the problem of dosage compensation in *Drosophila*^{2,7,11}.

Materials and Methods

The assay method for 6-PGD in *Drosophila* is similar to that described for G-6-PD¹³, and to that of Fildes and Parr⁴ with some modifications. Single flies may be conveniently assayed after homogenizing them in 0.05 ml of either distilled H₂O; a suitable

buffer; or in an aqueous solution of NADP (nicotinamide-adenine dinucleotide phosphate) (1 mg/ml). The coenzyme solution was regularly used in these studies. Vertical starch gel electrophoresis is carried out for either 15-18 hours at 2-2.5 volts/cm (4°C), or for 2-4 hours at 7-8 volts/cm (4°C). Several buffer systems have been found to be satisfactory: 0.01 M phosphate, pH 7.5, with the same solution at 0.2 M in the electrode vessels; tris (hydroxymethyl) amino methane 0.05 M, pH 8.0 and the same solution in the electrode chambers; or "EBT" (EDTA-Borate-Tris)⁹. For the first two buffers 5 ml of 0.027 M EDTA were added to 500 ml of starch-buffer solution prior to heating; in each instance, 4 mg (1 ml) of NADP was added just prior to pouring. The recombination analysis with the G-6-PD marker was run in the tris system; phosphate was employed for chromosomal localization of 6-PGD. Discrimination between the two migrating forms is adequate in all three systems, although the EBT is perhaps more convenient since the entire run can be completed in about five hours. It produces rather sharper bands since diffusion is reduced.

The sliced gels were stained, at 25°C, in a solution containing Tris (0.5 M, pH 7.5), 80 ml; 0.1 M MgCl₂, 10 ml; 6-phosphogluconate, 50 mg (4.5 ml); NADP (4 mg) 1 ml; phenazine methosulfate (8 mg), 2 ml. Nitro blue tetrazolium (20 mg), 10 ml. Tetranitro blue tetrazolium, in the same concentration, produces much more rapid staining, and a gray color this dye is now routinely used. With TNBT the bands may be resolved within 20 minutes, although staining may be carried out overnight.

G-6-PD activity was visualized in the fashion previously described¹³. *Drosophila* stocks and crosses were maintained on standard cornmeal, agar, dextrose medium, in half-pint milk bottles at 25°C. All *Drosophila* mutants employed are described in Bridges and Brehme¹.

Results

Seventeen wild-type strains were examined (Table I). Of these, 12 were found to have only a single, rapidly migrating band (A); three had only a single,

Dr. Young is Associate Professor in the Department of Anatomy, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205. Supported in part by funds from the National Institutes of Health Research Grant #HD 00486 and from the National Institutes of Health Inst. Research Grant #1 SO 1—FR-5378-03. The author wishes to thank Drs. Barton Childs and T. R. F. Wright for useful discussions.

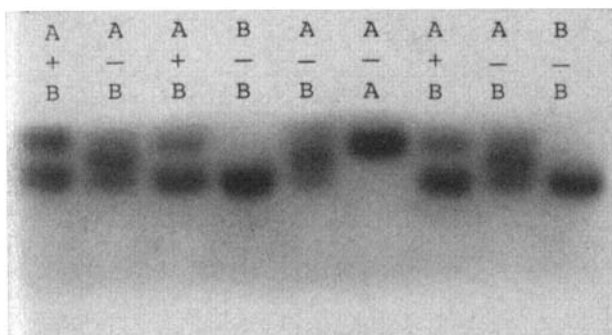


FIGURE 9—The three 6-PGD phenotypes observed in starch gels. Equal amounts of homogenates of Pgd^A/Pgd^A and Pgd^B/Pgd^B were mixed to produce A + B. The A phenotype migrates anodally.

slower migrating band (B); while individual flies in the other strains were either fast (A), slow (B), or "triple" (AB), that is, a third, "hybrid" band of intermediate mobility was present; see Figure 9. Males were either A or B; no males displaying more than one major band have been observed. In all instances, crosses of (A) females \times (B) males (or the reciprocal) have resulted in the progeny distribution expected for X-linkage, as have crosses of appropriate males with attached-X females. These observations are summarized in Table II. No exceptions to X-linkage have been observed. The locus determining the X-linked variants of 6-phosphogluconate dehydrogenase is called Pgd ; the alleles specifying the fast and slow variants are, respectively, Pgd^A and Pgd^B . It is proposed to name the previously described¹³ locus controlling the X-linked electrophoretic variation in glucose-6-phosphate dehydrogenase Zw (Zwischenferment). The allele specifying the rapidly-migrating variant is Zw^A ; the slow variant is specified by Zw^B . Preliminary studies had roughly placed the Zw locus to the right of forked (f , 56.7), based on recombination with a chromosome marked with vermilion (v , 33.0) and forked. Wright¹² has assigned the Zw locus to a position 0.5 units to the right of carnation (car , 62.5). It is thus at $63.0 \pm$.

From a cross of $\frac{Pgd^A Zw^A}{Pgd^B Zw^B} \text{ } \varnothing \varnothing \times Pgd^A Zw^A \text{ } \sigma \sigma$ 215 F_1 males were each scored for migration of both 6-PGD and G-6-PD. The data are presented in Table III. Since 41 percent of the F_1 males are recombinant, the Pgd locus is tentatively placed near the left (free) end of the X-chromosome.

Table I. 6-PGD phenotypes of *D. melanogaster* wild-type strains

Pgd Allele	
A ("Fast")	Oregon-R; Amherst-34; Florida-i; Kyoto; Varese, Crimea, Seto, Formosa, Tuscaloosa; Salta; Lausanne-S; Swedish-b
B ("Slow")	Canton-S; Stephenville; Woodbury
A and B ("Double")	Urbana-S; St. Louis-7

Appropriate crosses have localized it to the region left of eosin (w^e , 1.5), and right of scute (sc , 0.0+). Male F_1 's, offspring of females of the genotype $sc \text{ } Pgd^A w^e$ $\frac{sc^+ Pgd^B w^{e+}}$, were examined for morphological and 6-PGD phenotypes. The slightly greater (10/17) frequency of recombinants between scute and Pgd than between Pgd and eosin (7/17) places the locus closer to eosin. It is tentatively given as $0.9 \pm$.

Discussion

The X-chromosome location of Pgd places it in contrast to that of pigeons⁵, human beings^{4,8} and rats⁸, where autosomal loci have been described. In addition, in each of the instances from mammals, a "hybrid" enzyme band has been described in known or presumed heterozygotes for the gene. The present example is perhaps of special interest, therefore, because it is relevant to the problem of the mechanism of dosage compensation in *Drosophila*, in addition to providing another readily assayable biochemical marker in this animal. The hybrid phenotype is found only in the heterozygote, *in vivo*, and is not reproduced by simple *in vitro* mixing of the homogenates from Pgd^A and Pgd^B individuals (Figure 9), or by homogenizing flies of the two genotypes together. The hybrid phenotype has been reconstituted *in vitro*, however, by reassociation of subunits derived from the treatment, with a disulfide reducing agent, of partially purified homogenates of the two variants⁶. Since, *in vivo*, the hybrid phenotype would then appear to be the consequence of intracellular events, it is possible to conclude that the mechanism of X-chromosome dosage compensation, whatever it may turn out to be, does not involve "Lyonization" in *Drosophila* (see reference 3

Table II. Single-pair matings demonstrating X-linkage of the electrophoretic variant in 6-PGD

P_1	No. crosses	$F_1 \text{ } \sigma \sigma$			$F_1 \text{ } \varnothing \varnothing$		
		B	A	AB	B	A	AB
A $\sigma \times$ B \varnothing	3	19	0	0	0	0	21
A $\sigma \times$ AB \varnothing	3	14	12	0	0	13	16
B $\sigma \times$ A \varnothing (attached-X)	3	29	0	0	0	16	0

Table III. Male offspring from females heterozygous at both dehydrogenase loci. 41 percent of the F_1 are recombinant

$P_1 \text{ } \varnothing \varnothing$	$F_1 \text{ } \sigma \sigma$			
	Parental		Recombinant	
	$Pgd^A Zw^A$	$Pgd^B Zw^B$	$Pgd^A Zw^B$	$Pgd^B Zw^A$
$Pgd^A Zw^A$	64	62	40	49
$Pgd^B Zw^B$				

for review), since the participation of *both* X's in the *same* cell appears to be required, at least for the *Pgd* locus. Further discussion of this point and additional details concerned with the production of the hybrid enzyme have been presented elsewhere⁶. It may be noted that the *Pgd* locus is very close to that for eosin, a classic example of a non-compensating gene¹¹.

Summary

Electrophoretic variation, on starch gels, in 6-phosphogluconate dehydrogenase in *Drosophila melanogaster* has been observed. The responsible locus has been identified as X-linked and is at $0.9 \pm$. Two alleles, *Pgd*^A and *Pgd*^B, have been identified; the heterozygote produces a "hybrid" enzyme pattern. The significance of this observation to the problem of dosage compensation in *Drosophila* is briefly discussed.

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A List of Chromosome Numbers in Primates

D. S. BORGAONKAR*

SINCE the discovery of the correct chromosome number of man by Tjio and Levan⁵³ and Ford and Hamerton⁵⁰, and the two International Conferences in 1960 and 1963 on the methodology of karyotyping the chromosomes of man, interest in chromosomology has increased phenomenally. Whereas lists of chromosome numbers in plants are available^{27, 28, 37}, very few lists are available for chromosome numbers in animals^{40, 54}. Several reports are being published each year containing chromosome

determinations for different species. The need and importance of such information is evident; it is hoped that the present list will meet this lacuna. The total number of primate taxa for which chromosome numbers are known is 111. The diploid chromosome numbers range from 26 to 80. There were only a few good counts prior to 1951 but with the rate of progress we have seen in the last decade or so, mainly due to refinement in techniques, this list will be soon outdated. A primate chromosome bibliography prepared by this author is available in the *Mammalian Chromosome Newsletter*⁵⁵. It is hoped that this companion list of chromosome numbers will be of some aid to cytogeneticists.

* Division of Medical Genetics, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.