VIABILITY INTERACTIONS, *IN VIVO* ACTIVITY AND THE G6PD POLYMORPHISM IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Several biochemical studies have suggested that in *Drosophila melanogaster* the two common allozymes of G6PD differ in their *in vitro* activities and thermal stabilities. Yet, it remains to be shown that these characterizations reflect actual *in vivo* differences and are not artifacts of the biochemical approach. In this study it is shown that *in vivo* activity differences must exist between these two variants. This conclusion arises from the observation that the viability of flies bearing a low activity allele of 6PGD is strongly dependent on the genotype at the G6PD (*Zw*) locus, whereas no measurable difference in viability can be detected between *Zw* genotypes in a normal activity 6PGD background. These viability interactions are in the direction predicted by the reported *in vitro* activities of the allozymes and the proposed deleterious effects of 6-phosphogluconate accumulation.——In addition, a genetic scheme is used that uncouples and quantifies the effects of viability modifiers in the region of the *Zw* locus, while homogenizing 98% of the *X* chromosome. The viability of different *Zw* genotypes is measured by examining whole chromosome viabilities relative to the *FM6* balancer chromosome. The advantages of this particular scheme are discussed.

IN recent years a promising approach to understanding the potential adaptive nature of enzyme polymorphism has involved the biochemical characterization of naturally occurring electrophoretic variants (KOEHN 1969; MERRITT 1972; WATT 1977; PLACE and POWERS 1979; MACDONALD, ANDERSON and SANTOS 1980; ZERA, KOEHN and HALL 1984). As direct as this approach may appear, a difficulty arises from the implicit assumption that *in vitro* characterizations reflect *in vivo* function. If the number of experimental conditions which can be varied is considered (such as pH and cofactor concentrations), and given our ignorance of the cellular microenvironment, this assumption becomes problematic. Most reported kinetic differences between naturally occurring polymorphic variants are relatively subtle, without *obvious* physiological effects. It is not clear that these biochemical differences reflect real changes in pathway flux; yet, this remains the principle argument for using the biochemical approach. However, in the last couple of years, several studies have linked allozyme phenotypes with physiological consequences related to the presumed function of the study enzyme (DIMICHELE and POWERS 1982a, b; HILBISH,

DEATON and KOEHN 1982; BURTON and FELDMAN 1983). Given these observations, the present challenge is to measure, directly or indirectly, *in vivo* flux and establish whether biochemical characterizations routinely reflect flux differences.

The experimental measurement of *in vivo* differences is a difficult technical problem. Measurement of flux through pathways is possible in some prokaryote and simple eukaryotic systems, such as Neurospora (FLINT *et al.* 1981). However, measuring flux through a single enzyme step is difficult in more complex eukaryotes such as Drosophila. In a recent study, CAVENER and CLEGG (1981) compared relative flux through the oxidative pentose phosphate shunt for two dilocus genotypes of glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) in *Drosophila melanogaster*. In their study they generated double homozygous lines from mass crosses using wild chromosomes recently recovered from a natural population. One set of lines was homozygous for the so-called high activity alleles at both loci, whereas the second set was homozygous for the low activity alleles. Utilizing radiolabeled D-glucose and the method of WOOD, KATZ and LANDAU (1963), they were able to measure the ratio of glucose-derived carbon passing through glycolysis relative to that passing through the pentose shunt for both genotypes. Their results clearly indicate that the high activity, dilocus genotype showed statistically greater flux through the pentose shunt in comparison with the relative flux for the low activity genotype. They did not partition the effect into locus-specific contributions, although they suggested that the difference between dilocus genotypes could be largely attributed to the alleles at the *6Pgd* locus and not *Zw*.

In this study, I use a unique viability relationship between G6PD and 6PGD to develop a scheme whereby the *in vivo* flux through G6PD can be *indirectly* assessed in *D. melanogaster*. This experimental approach is based on the following set of observations. It has been shown by a number of investigators that low, or null, activity variants of 6PGD substantially reduce the viability of flies carrying them (BEWLEY and LUCCHESI 1975; GVOZDEV *et al.* 1976). At first appearance this is surprising because most enzymes of central metabolism do not exhibit gross effects on viability or fertility as null alleles (VOELKER *et al.* 1980; LANGLEY *et al.* 1981). This observation would have suggested that the pentose shunt was uniquely vital to basic survival, if it were not for the subsequent observation that flies simultaneously null at both 6PGD *and* G6PD are fully viable and fertile (HUGHES and LUCCHESI 1977; GVOZDEV *et al.* 1977). Consequently, blockage of the pentose shunt prior to 6PGD "rescues" *6Pgd* lethals. Because the G6PD step is effectively irreversible (HUGHES and LUCCHESI 1978), the cause of this lethality is apparently the buildup of 6-phosphogluconate, which in turn inhibits glycolysis, probably at the initial step of phosphoglucose isomerase. This enzyme is strongly inhibited by 6-phosphogluconate in both vertebrates and Drosophila (see review by NOLTMANN 1972; SMIRNOVA, KOGAN and GVOZDEV 1982). It appeared that this relationship might be useful in assessing the presence or absence of *in vivo* flux differences for alleles in the G6PD electrophoretic polymorphism. This is because the

relative viability of flies carrying a "leaky" *6Pgd* allele should to some degree be sensitive to the activity of *Zw* alleles preceding it in the pentose shunt, but of course only if those alleles possess activity differences sufficient to accumulate different concentrations of 6-phosphogluconate. The relative viability of flies bearing the leaky *6Pgd* allele should vary in an inverse fashion with the measured activities of different *Zw* alleles.

The natural polymorphism for G6PD (EC 1.1.1.49) has received increasing attention in recent years as a model polymorphism in natural populations. This sex-linked polymorphism, at the *Zw* (Zwishewferment) locus (YOUNG, PORTER and CHILDS 1964) consists of dimeric and tetrameric variants, hence, is a polymorphism for quaternary structure, at least as is determined *in vitro* (STEELE, YOUNG and CHILDS 1968; LEE, LANGLEY and BURKHART 1978; HORI and TANDA 1980). We have shown that the dimeric or fast electrophoretic allele (*Zw-A*) varies in frequency from nearly 100% in some European populations to only rare frequencies in southern and equatorial populations (OAKESHOTT *et al.* 1983). Sequential electrophoresis indicates that most probably only these two molecular variants are present in any appreciable frequencies on a global basis, and that little hidden variation at the protein level exists (EANES 1983). Biochemical characterization of both variants has shown repeatable differences in specific activities, thermal stabilities and K_m's for glucose-6-phosphate (STEELE, YOUNG and CHILDS 1968; BIJLSMA and VAN DER MEULEN-BRUIJNS 1979; HORI and TANDA 1980; W. F. EANES, unpublished observations). The fast allele is generally described as having lower activity than the slow or tetrameric form of the enzyme, being less stable, and possessing lower apparent K_m's for glucose-6-phosphate over the temperature range 20 to 32°.

In this investigation, I examine the viability differences between the two *Zw* variants in association with both a normal and a low activity *6Pgd* allele, possessing less than 10% wild-type activity. A genetic scheme is described and utilized that homogenizes most of the linked-*X* chromosomal background (the autosomes randomize) for both *Zw* alleles. Viabilities are then estimated as whole chromosome measurements relative to the *FM6 X* chromosome balancer, much as is routinely used in autosomal studies using *Cy/Pm* and other balancers. The results indicate that viabilities of *Zw* genotypes are equal against a "normal" *6Pgd* background, whereas large viability differences can be shown for both male hemizygous and female genotypes in the leaky *6Pgd* background. This clearly indicates that *in vitro* biochemical characterizations mirror *in vivo* flux through G6PD.

MATERIALS AND METHODS

Test chromosomes: In measuring viability differences between *Zw* genotypes, I have attempted to control for allelic viability variation linked to and cosegregating with the *Zw* alleles on the *X* chromosomes. The experimental approach used here exploits crossing over between a pair of visible marker loci flanking the enzyme locus to generate a set of recombinant *X* chromosomes which are homozygous for the entire length of the chromosome outside the flanking loci yet segregating for the *Zw* alleles and small adjoining regions of known average size. The specific genetic scheme and parental chromosomes are illustrated in Figure 1. Male *car* progeny were collected from females heterozygous for the marked *X* chromosomes *In(1)dl-49, y v f Zw-B* and *car*

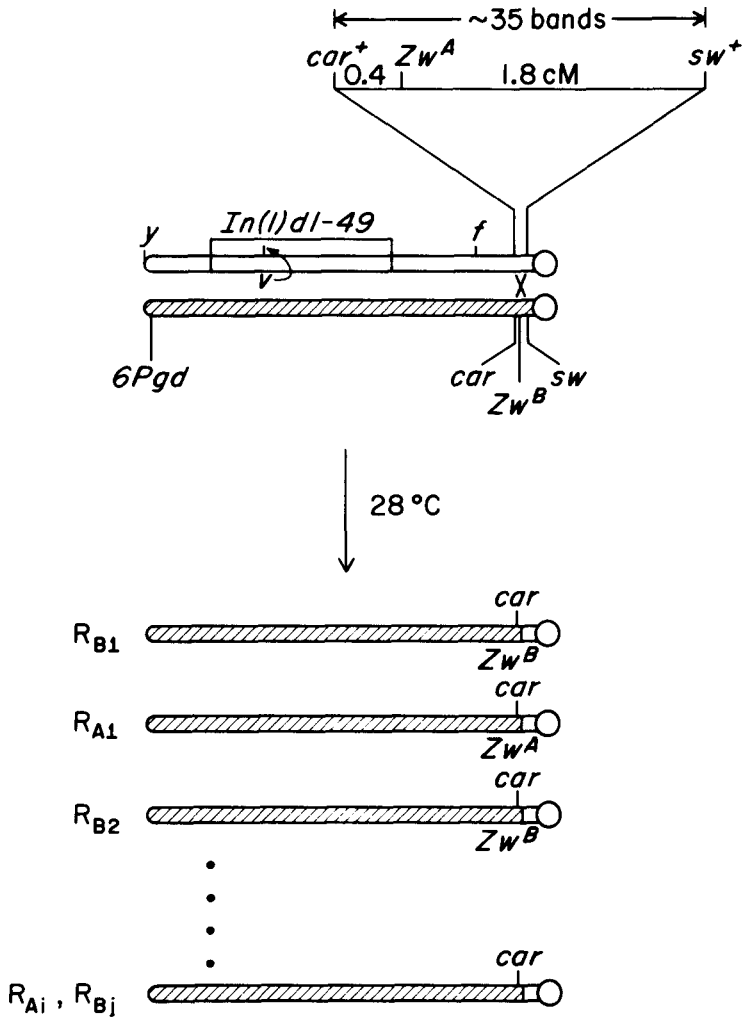

FIGURE 1.—The genetic scheme used to create a set of recombinant test chromosomes which are identical for the distal portion of chromosome *car sw* and the proximal section of *In(1)dl-49, y v f*. The position of *Zw* relative to its flanking markers is shown.

Zw-A sw. The visible markers *car* and *sw* (short wing is lethal at 28°, EKER 1935) flank the *Zw* locus 0.35 and 1.8 cM to the left and right, respectively (EANES 1983). Male *car* progeny recovered at 28° represent surviving recombinants between *car* and *sw* and carry either the *Zw-A* or *B* allele depending upon whether the recombination event occurred to the right between *Zw* and *sw* or to the left between *Zw* and *car*. The presence of the marked inversion *In(1)dl-49* prevents crossing over in the distal half of the chromosome (STURTEVANT and BEADLE 1936), and the visible markers permit the detection of rare double crossover events. The close proximity of *sw* to the centromere greatly lowers the probability of double crossovers to the right of the *sw* locus.

The *car* and *sw* markers span a distance of 2.2 of the total 66 cM on the *X* chromosome.

Consequently, recovered chromosomes are identical by descent for a minimum of 97% of their *X* chromosomes. Moreover, *Zw-B*-bearing chromosomes are on the average identical by descent for 99.7% of their *X* chromosomes, and all replicates of *Zw-A*-carrying chromosomes are homozygous for an average of 98.6% of that chromosome. Finally, in comparisons of *A*-bearing chromosomes to *B* chromosomes, such chromosomes are identical for an average of 98.4% of their *X*-linked loci. Recovered males bear a common autosomal background, derived from the parental stem chromosome stocks, and this background is assumed to be randomized relative to the *X* chromosome and the *Zw* alleles. This is important because autosomal "modifiers" of activity for both of these enzymes have been reported (see LAURIE-AHLBERG *et al.* 1980). All recombinant male progeny were mated with *C(1)DX* compound-*X* females and their F_1 male progeny electrophoresed (YOUNG, PORTER and CHILDS 1964) to determine their *Zw* genotypes. Recombinant lines are designated R_{Ai} and R_{Bj} for the *i*th and *j*th recombinant chromosomes bearing the *A* and *B* alleles, respectively. Recombinant chromosomes were crossed and maintained with the *FM6* balancer chromosome. Because the *6Pgd* locus lies at the distal end of the *X* chromosome at 0.64 cM, different *6Pgd* alleles can be substituted into the genetic background by incorporation onto the distal tip of the original parental *car Zw sw* stem chromosome.

In this study, two separate sets of independent recombinant chromosomes were generated; one set of 93 chromosomes (for experiments 1 and 2) bearing the common *6Pgd*-slow electrophoretic allele, and a second set of 31 chromosomes (for experiment 3) carrying the leaky allele, *6Pgd-lo1*, obtained from J. C. LUCCHESI. This allele possesses less than 10% "normal" activity (BEWLEY and LUCCHESI 1975). Since it was not known how this leaky allele might suppress viability and distort the recovery of chromosomes bearing specific *Zw* alleles, all parental females were mated with males bearing a w^+Y translocated chromosome. This translocation spans the region of the *6Pgd* locus, thereby masking any effects of the *6Pgd* leaky allele (HUGHES and LUCCHESI 1977; GVEZDOV *et al.* 1977). Finally, the regions flanking *Zw* are unequal in size; therefore, the large majority of recombinant chromosomes carry the *B* allele. In each experiment, approximately the same number of chromosomes bearing each *Zw* allele were used; therefore, a limited subset of each of the sets of chromosomes were actually incorporated (22 in experiment 1, 25 in experiment 2, and ten in experiment 3).

Relative viability measurements: Since the two different alleles of *Zw* are carried in essentially identical and replicated chromosomes, viability differences between alleles can be estimated by measuring *whole X* chromosomes viabilities using multiply marked balancer chromosomes such as *FM6*. The relative viability of each specific chromosome is measured relative to the *FM6* chromosome as illustrated in the scheme shown in Figure 2.

The relative viability of each genotype is computed as the number of emerging flies of the tested genotype (male or female), divided by the sum of the number of that genotype and the number of *FM6* males, as in the work of MUKAI (1964). If Mendelian segregation, and equal sex ratio, is assumed, the mean viability should be about 50.0%, except that the lowered viability of *FM6* males results in a higher average of about 60%.

The design of all viability experiments involves a hierarchical arrangement of levels in which viability differences are examined not only between *Zw* genotypes, but also variation is examined between crosses involving different recombinant chromosomes but the same *Zw* genotype. For example, in experiment 1, 22 *types* of interchromosomal crosses were set up to generate the three female and two male *Zw* genotypes. Each individual type of cross represents combining a pair of different independent recombinant chromosomes bearing the appropriate *Zw* alleles. Furthermore, each type of cross is replicated seven times (a single replicate involves the mass crossing of eight virgin $FM6/R_{Ai}$ or $FM6/R_{Bj}$ females with four to eight males of the appropriate chromosomal type to generate the necessary *Zw* genotype in females). Each replicate was placed in an 8-dram vial and allowed to oviposit for 4 days, with transfers to fresh food vials on days 4, 8 and 12. All replicates were discarded on day 16. Emerging flies were counted by genotype for each vial on days 11, 14 and 20 and the counts pooled across vials for each replicate. This generates a single viability observation.

In the viability tests using the *6Pgd-lo1* allele, it was discovered that *FM6* males bearing the w^+Y chromosome suffered greatly reduced viability with an inflated experimental variance, thereby questioning its utility as a standard genotype. Moreover, the estimation of male viabilities required the removal of the w^+Y chromosome and replacement with a normal *Y*. As a result, hemizygous

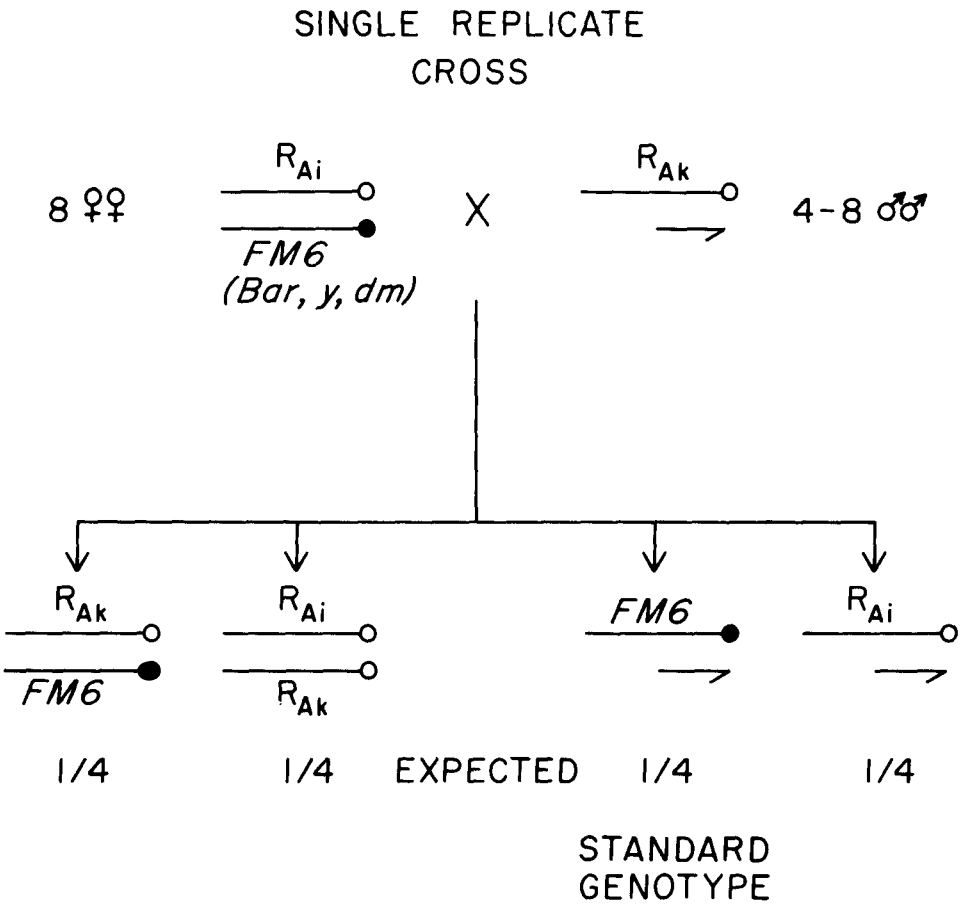

FIGURE 2.—The genetic cross used to measure relative viability for a single replicate in experiments 1 and 2. An example for the *Zw-A* and *Zw-AA* genotypes is shown. In experiment 3 female viabilities were estimated from crosses in which the male carried the w^+Y chromosome and male viabilities from crosses in which the male parent was *FM6/Y*.

male viabilities were obtained in the last step by crossing normal *FM6/Y* males with the respective females and comparing as described before. However, it was necessary to compute female viabilities by comparing counts relative to the $FM6/R_{Ai}$ and $FM6/R_{Bj}$ females as a standard. Therefore, unlike the determination of viabilities with the normal *6Pgd* background, the estimation of viabilities with the *6Pgd-lo1* allele required separate crosses to measure viabilities in males and females.

All flies were reared at 25° on standard cornmeal food as in the work of MUKAI (1964), except that Karo syrup was substituted for molasses.

RESULTS

In total, more than 110,000 flies were counted in the three viability experiments involving both a normal *6Pgd* and leaky *6Pgd-lo1* backgrounds. The results of experiment 1, using 22 independently recovered recombinant chromosomes possessing the *Zw-A* and *B* alleles in combination with the normal *6Pgd*-slow allele are presented in Table 1. This experiment, represents not only a control set, but also provides an estimate of inherent viability differences

TABLE 1

Relative viabilities of Zw *genotypes with the* 6Pgd-*slow allele*

Source of viability variance	d.f.	MS	*F*	P
Males				
Zw genotype	1	0.009	0.002	NS
Chromosome crosses within *Zw* genotypes	20	37.3	1.95	<0.014
Replicate crosses	130	19.1		
Females				
Zw genotype	2	134.1	5.53	<0.013
Chromosome crosses within *Zw* genotype	19	24.2	1.14	NS
Replicate crosses	130	21.3		

MS = mean squares.

between the common polymorphic *Zw* genotypes under standardized conditions. This is necessary because substantial viability differences between these genotypes have been reported in laboratory experiments (BIJLSMA and VAN DELDEN 1977; BIJLSMA 1978; BIJLSMA and KERVER 1983). The large size of experiment 1 was predicated on the assumption that inherent viability differences between G6PD genotypes would be subtle.

There are three levels into which the total viability variance of replicate crosses can be partitioned in a nested analysis of variance (ANOVA). The lowest level represents the contribution of replicate crosses involving the same pair of recombinant chromosomes. This level includes the error variance due to binomial sampling, environmental differences between vials, autosomal segregation of loci that interact epistatically with *X*-linked loci, the contribution of *de novo* polygenic mutations affecting viability (MUKAI 1964) and rare recombinational events with the *FM6* chromosome. Preliminary investigations showed that sampling error and environmental differences contribute the majority of the variance at this level. The second level includes all sources assumed in the former level, whereas significant variance at this level represents the added effect of genetic viability variation segregating within the regions flanking the *Zw* locus (between *car* and *sw*), and again the effects of *de novo* mutations; particularly those mutations that have arisen prior to the crosses. Variance at this level is among chromosomes bearing the same *Zw* allele but recovered as independent recombination events in the small flanking regions. The third and final level in the nested analysis represents the contribution to the total variance of the different *Zw* male and female genotypes.

In experiment 1, no significant viability variation can be attributed to different *Zw* alleles in hemizygous males. The mean hemizygous viabilities of the two genotypes were 58.35 and 58.40% for the *Zw-A* and *Zw-B* males, respectively. In contrast, the genetic variance among replicate chromosomes bearing the same *Zw* allele (level two) was statistically significant ($F = 1.95$, $P < 0.014$) and contributed about 12% to the total hemizygous viability variance. In females, statistically significant viability variation was detected among *Zw* genotypes ($F = 5.53$, $P < 0.013$), explaining about 7% of the total female viability

variance between crosses. The means for the three genotypes were *Zw-AA* = 61.53, *Zw-AB* = 58.48 and *Zw-BB* = 61.03%. Therefore, the significant difference is due to heterozygous females being underdominant for viability by about 1.5–2%. In contrast to the male viabilities, no significant genetic variance was observed at the second level. If *X*-linked viability mutations have similar effects in males and females, then the difference in genetic variance between the sexes at this level effectively reflects a difference between inbred and outbred crosses with replicate chromosomes (males are homozygotes). To examine the nature of the genetic variance at this level, a second viability experiment was carried out in which male viabilities were determined as in experiment 1, whereas female viabilities were determined for homozygous replicate chromosomes. The results are given in Table 2.

In experiment 2, again, no statistically significant differences were observed between male genotypes, and the means were 58.05 and 59.05% for the *A* and *B* genotypes, respectively. The between-replicate chromosome genetic variance was near statistical significance ($P < 0.06$). Once again, the female homozygous *Zw* genotypes were not significantly different. However, the inbred genetic variance between chromosomes measured at the second level was highly significant ($F = 2.2$, $P < 0.001$) and explained about 20% of the overall variance. Consequently, forcing chromosomal homozygosity in females reveals a level of genetic variance in viability comparable to that observed in males.

In summary, it is clear that viability differences emerge between replicate chromosomes (level two); however, little viability variation can be attributed to the *Zw* genotypes alone. Any genotype-related differences must be on the order of 2% or less and incorporate underdominance in females.

The results of experiment 3, determining the viability relationships of *Zw* genotypes when associated with the *6Pgd-lo1* allele are given in Table 3 and are in striking contrast to the outcomes of experiments 1 and 2. The mean hemizygous viability of *Zw-B* males was only 12.21% compared with 43.31% for *Zw-A* males, and despite the smaller sample sizes in this experiment were highly significant ($F = 65.56$, $P \ll 0.001$). Likewise, in females the mean viabilities of the *Zw* genotypes were *AA* = 25.32, *AB* = 19.06 and *BB* = 10.50% and statistically significant ($F = 6.5$, $P < 0.025$). In neither case were the within-genotype components (level two) statistically significant, although this is probably due to the smaller scale of this experiment and the increased variance at the lowest level. Although these viability data do not strictly conform to the assumptions of ANOVA (equal variances and normality), this is not a serious deficiency in experiments 1 and 2. However, because the viabilities are so markedly different in experiment 3, this could be a problem (the variances will depend on the means). Therefore, a two-sample, nonparametric test (Mann-Whitney *U*-test, SOKAL and ROHLF 1969) was also applied to that outcome. The viabilities of both male *Zw* genotypes and both female homozygous genotypes were contrasted and found to be statistically significant (males, $P < 0.01$; females, $P < 0.05$).

In experiment 3, female viabilities were measured relative to the *FM6/R* genotypic class. Consequently, it is necessary to determine whether these

TABLE 2

Relative viabilities of Zw *genotypes with* 6Pgd-*slow allele (homozygous chromosomes, see text)*

Source of viability variance	d.f.	MS	*F*	P
Males				
Zw genotype	1	31.0	0.73	NS
Chromosome crosses within *Zw* genotypes	23	42.5	1.61	NS
Replicate crosses	100	26.5		
Females				
Zw genotype	2	2.3	0.40	NS
Chromosome crosses within *Zw* genotypes	23	52.3	2.20	$\ll$0.001
Replicate crosses	100	23.8		

MS = mean squares.

TABLE 3

Relative viabilities of Zw *genotypes with* 6Pgd-lo1 *allele*

Source of viability variance	d.f.	MS	*F*	P
Males				
Zw genotype	2	9679.6	65.58	$\ll$0.001
Chromosome crosses within *Zw* genotypes	8	147.6	1.07	NS
Replicate crosses	30	138.1		
Females				
Zw genotype	2	826.2	6.5	<0.025
Chromosome crosses within *Zw* genotypes	12	127.0	1.67	NS
Replicate crosses	45	75.9		

"standard" genotypes are equivalent in viability. In experiment 3, the separate measurement of male hemizygous viabilities also permits an independent measurement of viabilities for the *FM6/R* genotypes relative to the *FM6/Y* males. It is important that the viabilities for *FM6/Zw-A* and *FM6/Zw-B* females be estimated and, if not equal, the viabilities of genotypes for which these genotypes were used as a standard be adjusted accordingly. In experiment 3, the viabilities of the two standard females with respect to the *FM6/Zw-A* = 74.18 and *FM6/Zw-B* = 68.00. These two mean viabilities are near statistical significance ($P < 0.10$), and the direction of the difference is as predicted since the *FM6* chromosome carries the *Zw-A* and *6Pgd*-fast alleles. If these genotypes are equal, then using them as standards for female viabilities in experiment 3 presents no problem. However, if they are different, adjusting those standards for viability differences actually results in increasing the viability differences shown for the *Zw* genotypes in females. If this is carried out, the viabilities of the *Zw* genotypes in the presence of the *6Pgd-lo1* allele are *Zw-AA* = 50.0, *Zw-AB* = 33.1 and *Zw-BB* = 20.0%. As the "normal" female viability relative to *FM6* is 60.0, this results in respective reductions in viability relative to normal of 19.0, 43.4 and 67.2% for the three female genotypes. The corresponding

male reductions in viability relative to normal males are 28.3 and 80.0% for the *A* and *B* genotypes, respectively.

DISCUSSION

In their study, CAVENER and CLEGG (1981) suggested that the principal contributor to the dilocus *in vivo* differences in pentose shunt flux was the *6Pgd* locus. They point out that specific activity differences associated with the *Zw* genotypes depend upon stability in the assay buffer, thereby rightly questioning the presence of *in vivo* differences for those allozymes. This study demonstrates that *in vivo* flux differences must also exist between *Zw* genotypes, because of the dramatic viability interactions with the leaky *6Pgd* allele. The viability interaction is in the direction predicted by the *in vitro* differences reported for the two common *Zw* variants.

CAVENER and CLEGG proposed from preliminary observations that about a 5% difference in flux existed between *Zw* genotypes, whereas the difference between *6Pgd* genotypes was on the order of 20%. My study is not inconsistent with that observation. This is because the relative magnitude of activity and flux differences cannot be ascertained from this viability information alone. If relative viability responds linearly as an additive function of G6PD activity against the leaky 6PGD background (complete nulls show normal viability), then a direct association could be assumed. However, it can only be assumed that viability is some negative function of the buildup of 6-phosphogluconate, and a knowledge of the shape of the viability relationship is necessary to make statements about flux differences from viability data. Therefore, a doubling of relative viability does not necessarily indicate a comparable reduction in activity or flux of 50%. Nevertheless, it is clear from this study that real flux differences must exist for these G6PD variants.

The outcome of the control viability experiment comparing *Zw* genotypes against a "normal" (*6Pgd*-slow) 6PGD background shows that relative viability differences between *Zw* genotypes under unstressed conditions are very small, if they exist at all. The observation of underdominance and equal viabilities in homozygous females is difficult to reconcile with any simple biochemical or physiological mechanism at this time. The possibility also arises that viability differences might exist between *Zw* alleles in a *6Pgd*-fast background, but it is doubtful that they would ever approach the large differences seen with the *6Pgd-lo1* allele. The next approach planned is to subject these particular chromosome sets to stress conditions known to induce pentose shunt activity (see BIJLSMA and VAN DELDEN 1977; BIJLSMA 1978; BIJLSMA and KERVER 1983) and see whether fitness differences are consistently present.

The genetic scheme utilized in these three experiments ensures greater control over the confounding effects of linked loci and allows their contribution to be quantified. The genetic background of most of the *X* chromosome (>98%) is forced to homozygosity in a single generation. Given the genetic size of this region, and the cytogenetic positions of *car* (at 18D1-2, LINDSLEY and GRELL 1968) and *sw* (at 19B3-C3, SCHALET and LEFEVRE 1976), this region should bound only about 35 complementation groups (of a total of approxi-

mately 1012 on the *X* chromosome), or about seven to eight loci to the left of *Zw* and 27–28 loci to the right, assuming a one-band/one-gene relationship (JUDD, SHEN and KAUFMAN 1972). The recovery of replicate sets of chromosomes representing repeated recombinant exchanges in each flanking region ensures that segregating viability variation can to a degree be uncoupled from effects due to the *Zw* locus. A putative regulatory region immediately contiguous with the structural locus for G6PD will not be readily uncoupled from the locus, unless the scheme were taken to its hypothetical limit by using markers immediately flanking the locus, which is unfeasible. However, there is also a legitimate interest in examining the potential contribution of less tightly linked loci, which under traditional approaches would contribute to spurious fitness differences between allozyme genotypes. For instance, functionally interacting temporal and tissue-specific regulatory loci such as the *adp* and *map* loci in *D. melanogaster* (ABRAHAM and DOANE 1978; DOANE 1980), which are associated with amylase regulation and closely linked to the *Amy* locus (at 5.7 and 2.1 cM, respectively), would be successfully uncoupled using this approach. It is both this type of regulatory variation and linked viability in general that this genetic scheme is designed to control.

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