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The α-Glycerophosphate Cycle in Drosophila melanogaster. III. Relative Viability of "Null" Mutants at the α-Glycerophosphate Dehydrogenase-1 Locus

Stephen J. O'Brien
Cornell University, sobrien1@nova.edu

Bruce Wallace
Cornell University

Ross J. MacIntyre
Cornell University

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LETTER TO THE EDITORS

THE α-GLYCEROPHOSPHATE CYCLE IN *DROSOPHILA MELANOGASTER*. III. RELATIVE VIABILITY OF "NULL" MUTANTS AT THE α-*GLYCEROPHOSPHATE DEHYDROGENASE-1* LOCUS*

The description of genetic variation in natural populations has involved three different general approaches: (1) the detection, by means of special genetic techniques, of genes in *Drosophila* which affect viability, fertility, or developmental rates (e.g., Dobzhansky and Spassky 1953); (2) the measurement of chromosomal inversion polymorphism in *Drosophila* (Dobzhansky 1970, chap. 5); and (3) the electrophoretic monitoring of gene-enzyme (allozyme) variation in a number of species from many different animal and plant phyla (e.g., Lewontin and Hubby 1966; O'Brien and MacIntyre 1969; Allard 1971; Prakash, Lewontin, and Hubby 1969; Selander and Yang 1969). The first approach uncovers cryptic variation with respect to various components of fitness; such variation is revealed by techniques that render chromosomes homozygous. The other two approaches reveal genetic variation virtually by direct observation. The relation of this variation to fitness is not at all immediately obvious; indeed, there are cogent arguments that much allozyme variation is selectively neutral (King and Jukes 1969; Kimura and Ohta 1971).

The first method of measuring relative viability of wild chromosomes has provided a wealth of information to the field of population genetics mainly through the efforts of Dobzhansky and his colleagues (for references, see Dobzhansky 1970, p. 118; Wallace 1968, p. 36). Relative viability is determined through the use of invested balancer chromosomes which contain a dominant marker mutation and a recessive lethal. In these studies, male and female flies, heterozygous for the balancer chromosome and the same sampled chromosome, are mated to each other and their progeny are scored. The ratio of homozygous wild-type offspring to balancer heterozygous offspring has been determined for large numbers of wild chromosomes from natural populations of several *Drosophila* species. This ratio can be related to the ratio obtained by comparing the relative viability of heterozygotes for different wild chromosomes with that of the balancer heterozygotes in control cultures. It is striking that in the majority of analyses reported, regardless of which chromosome, population, or species is sampled, the frequency distribution of chromosome viability followed a uniquely distinct

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pattern. The frequency of lethals and near-lethals is high; they represent anywhere from 10%–15% to 40%–50% of all chromosomes tested depending upon the species and geographic location of the sampled population. Relatively few chromosomes are in the semilethal range, that is, with relative viabilities between 10% and 50% of expected. This class rarely contains more than 10%–15% of the chromosomes tested (Dobzhansky 1970, chap. 4; Wallace 1968, chap. 3). The remaining chromosomes are quasi-normal with a mean frequency about equal to that expected. Control cultures of heterozygous wild chromosomes yield only a few allelic lethals. The vast majority of heterozygote combinations are quasi-normal with mean viability slightly but consistently greater than the mode of the homozygous wild chromosomes. Semilethal combinations are virtually absent in the heterozygote control cultures. When isogenic chromosomes are subjected to X radiation or chemical mutagenesis with ethyl methanesulfonate (EMS) and tested in the same manner, virtually identical distributions of viabilities are obtained in both the homozygous test and heterozygote controls (Timofeef-Ressovsky 1935; Kerkis 1938; Mukai 1970).

In spite of considerable data which exist describing the extent and distribution of relative viabilities, the biological basis of some aspects of the bimodal distribution remains obscure. We have made some observations, which are relevant to this problem, concerning the viability of certain combinations of mutants affecting the enzymes of the α-glycerophosphate cycle in *D. melanogaster* (O'Brien and MacIntyre 1972*a*). Four "null" or "zero" alleles of the α-*glycerophosphate dehydrogenase*-1 (α*Gpdh*-1) gene were induced with EMS as described elsewhere (O'Brien and MacIntyre 1972*b*). Flies with any one of these alleles designated as α*Gpdh*-1^{B0} on one chromosome and the deletion of the α*Gpdh*-1 *locus* (Grell 1967) on the other have drastically reduced levels of the soluble NAD-linked α-glycerophosphate dehydrogenase (from 0% to 10% wild type). Because lethals are induced at loci other than α*Gpdh*-1 by the mutagenic treatment, the mutant chromosomes must be balanced over SM-1; *al Cy sp* (Lindsley and Grell 1968), a chromosome II balancer. All possible crosses (at least five replicates) were made between the four mutants. Each test culture produced over 200 flies. The ratio of Cy^+ to *Cy* flies was determined; the expected frequency was 33.3% Cy^+. The Cy^+ flies which were heterozygous for the various mutant alleles were then ground up and the specific activity of αGPDH of the Cy^+ flies for each cross was measured (O'Brien and MacIntyre 1972*b*). Levels of αGPDH ranged from 0% to 25%. Some α*Gpdh*-1^{B0} allele combinations showed interallelic complementation; that is, the heterozygotes had enzyme activities greater than the sum of the activities of the two mutants when carried over the deletion. The specific activity of each class of heterozygote was then plotted against the percentage of wild-type flies which emerge in the cultures. The results (fig. 1) show an obvious correlation between αGPDH levels and viability. Crosses producing heterozygotes with low αGPDH levels (less than 5%) yielded less than 50% of the expected number of Cy^+ offspring. This level of viability falls precisely into the rare semilethal range

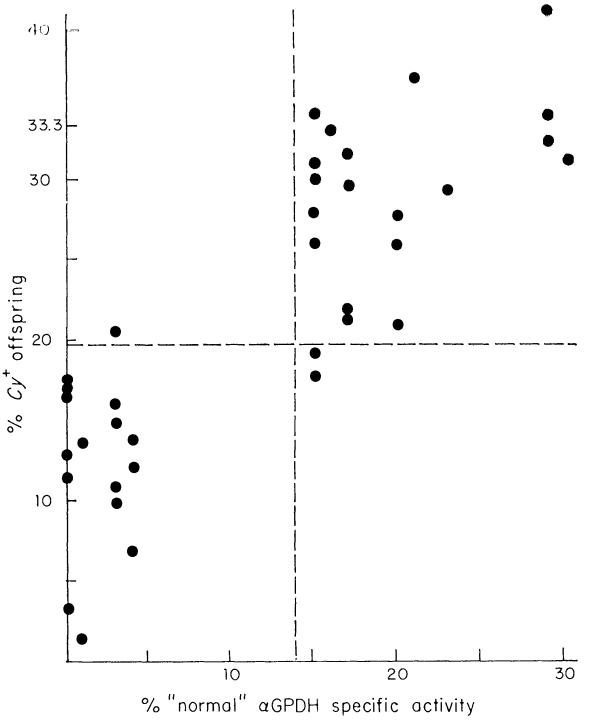

FIG. 1.—Relative viability of flies heterozygous for various "null" alleles of α*Gpdh*-1; 100% αGPDH level equals 0.37 μmoles NAD per minute per milligram protein.

alluded to above. Alternatively, the heterozygotes with greater than 15% normal αGPDH activity are more viable, and if the αGPDH activity is 25% of wild-type enzyme levels, the flies have "quasi-normal" viability.

For two reasons we believe the pattern seen in figure 1 is attributable to the effect of selection on the altered products of the mutant α*Gpdh*-1 alleles as opposed to the numerous background mutations induced during the mutagenesis. First, there is no reason to expect that the recessive lethals or semilethals induced by the EMS produce any effect on heterozygotes. Dobzhansky and Spassky (1963), in an exhaustive test of the viabilities of carriers of lethals, semilethals, quasi-normals, and supervitals, failed to demonstrate any correlation between the fitness of a homozygote and that of its heterozygous counterpart. The second reason lies in the data themselves. If viability differences were due to selection on the products of genes other than α*Gpdh*-1 on the second chromosome, there would be no correlation between enzyme activity and viability.

The observation that all flies with less than 5% of wild-type enzyme activity fall into the semilethal class may provide some insight into the reason(s) for the apparent scarcity of this class in nature. Insectan α-GPDH has been implicated in three important functions in *Drosophila* (Sacktor

1965; O'Brien and MacIntyre 1972*a*): regeneration of NADH in the cytoplasm for continued glycolysis, energy production, and phospholipid anabolism. Because the enzyme does occupy such a crucial and multifunctional role in insect metabolism, it is somewhat surprising that flies deficient for αGPDH survive at all. The explanation may be found in the flies' suspected ability to compensate biochemically for the three functions by alternative pathways, that is, malate dehydrogenase for NADH metabolism, the citric acid cycle for energy production, and the phosphorylation of glycerol for the continuation of phospholipid synthesis (Sacktor 1965; Kennedy 1957). The relative inefficiency of the genetic and/or physiological compensatory mechanisms could be the basis of the observed diminished viability. Perhaps it is only those mutant blocks that can be alternatively but inefficiently compensated for which can and do represent the semilethal class.

One prediction of such a hypothesis would be the recovery of rare "null" alleles in natural populations at a number of different loci. These "nulls" would be metabolically compensated by alternative pathways but inefficiently enough to lower relative viability. Although several examples of rare naturally occurring "null" alleles are known in *Drosophila* (Johnson, Wallis, and Denniston 1966; Glassman 1965; Dickinson 1970) few data are available concerning their viability.

If the semilethal class is a population of compensated "null" alleles, then those genes which function in essential and unique processes may well be the lethals in the populations. The so-called subvital chromosomes (50%–90% normal viability) could represent missense mutations in genes of both classes which only partially obstruct normal function (e.g., allozyme variation).

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Stephen J. O'Brien*
Bruce Wallace
Ross J. MacIntyre

Section of Genetics, Development, and Physiology
Cornell University
Ithaca, New York 14850
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* Present address: Gerontology Research Center, National Institute of Health, Baltimore City Hospitals, Baltimore, Maryland 21224.