Coyne, J.A. The Rockefeller University, New York, New York. Quantification of xanthine dehydrogenase allozymes in mass samples of D. pseudoobscura.

The use of electrophoretic techniques to detect cryptic genetic variation in Drosophila populations has been limited by the necessity of using homogenates of individual flies; this procedure is tedious and subject to sampling errors when a small number of flies are used. Homogenization and electrophoresis of multiple-fly samples could provide a quick estimate of the degree of enzyme polymorphism in populations or, if not employed quantitatively, could at least indicate the presence or absence of such polymorphism. We have made efficient use of mass sampling in experiments with one allozyme locus in Drosophila.

Two strains of D. pseudoobscura homozygous for the allozymes .90 and 1.02 of the second-chromosome locus xanthine dehydrogenase (XDh) were obtained from stocks originally developed by Dr. Richard Lewontin at The University of Chicago. Fifty-fly samples consisting of different proportions of these genotypes were homogenized in 100 microliters of buffer. After centrifugation, 0.3 microliters of the supernatant was applied to cellulose acetate strips and subjected to electrophoresis at 100 volts for 24 minutes at room temperature. Strips were stained for XDh with a modification of the procedure of Prakash et al. (1969). After incubation, the strips were cleared and the allozyme bands quantified with a Millipore PhoroScope densitometer. Figure 1 shows the relationship between genotypic proportions of the two

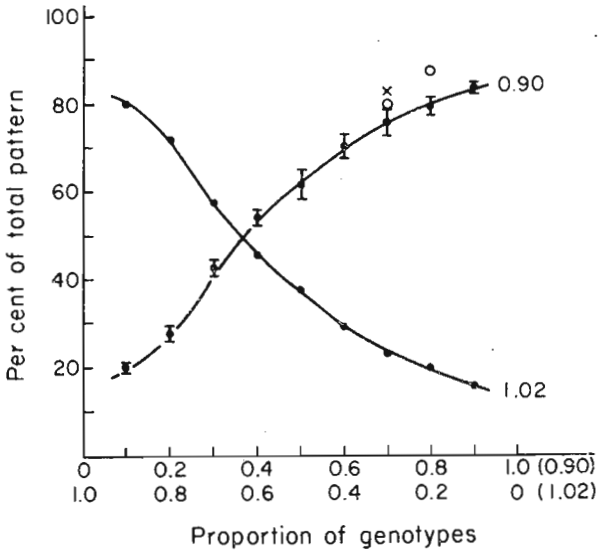

Figure 1. Standard curve of percentage of total banding pattern (quantified with densitometer) versus genotype frequency for homozygotes of the .90 and 1.02 allozymes of XDh. Open circles represent trials in which 150 microliters of homogenization buffer was used; X's represent 200-microliter trials. 100 microliters of buffer was used in all other runs.

strains and relative allozyme band intensity. The curve is roughly sigmoid, possibly indicating lack of saturation of those allozymes present in high proportions. This hypothesis is supported by the increase in relative staining of high-concentration allozymes evident when the homogenate is diluted with additional buffer.

The figure also shows the accuracy and reproducibility of the method: the maximum standard deviation in relative band intensity for any one point was only 2.7%. The experiments were expanded to include homogenates of up to 200 flies without significant change in relative intensity.

The accuracy of results obtained in construction of this standard curve thus indicates the feasibility of such quantitative analyses of mass samples of natural or laboratory populations of Drosophila. Although XDh heterozygotes - which exhibit a heterospecific band - were not employed in these experiments, the method could be useful for investigation of those allozymes which do not show hybrid bands. The development of quantitative methods for allozymes which do form heterospecific molecules should be investigated, however, for the hybrid band may enable one to calculate directly the percentage of heterozygotes in a mass population sample.

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Reference: Prakash, S., R.C. Lewontin and J.L. Hubby, 1969 Genetics 61:841.