

The results with the numbers of progeny scored and the observed frequencies of primary nondisjunction are shown in the table. With all crosses more male than female progeny were obtained. The frequencies of primary nondisjunction show some variation. In particular the HH stock displayed an unexplained high number of exceptional males. But all the nondisjunction frequencies are within the range found with wild type stocks. This demonstrates that no meiotic mutants are present in the chromosome substitution stocks studied.

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Reference: Racine, Beck and Würgler 1979, Mutation Res. 63:87-100.

Batterham, P. and G.K. Chambers\*. Monash University, Clayton, Victoria, Australia; \*Australian National University, Canberra, A.C.T., Australia. The molecular weight of a novel phenol oxidase in *D. melanogaster*.

PHOX, a newly discovered form of phenol oxidase (O-diphenol: O<sub>2</sub> oxidoreductase E.C. 1.10.3.1.) encoded by the Phox locus (II 80.6) in *D. melanogaster* has been described by Batterham and McKechnie (1980). To firmly establish the novelty of this enzyme it was important to devise a test to distinguish it from A component phenol oxidases (see Seybold et al. 1974). We report here

determination of the molecular weight of this new enzyme by Sephadex G-150 gel filtration. *D. melanogaster* pupae (48 hours old) from the Silvan (Victoria) population were homogenized (6 g pupae/4 ml buffer) in ice cold 50mM Tris/HCl buffer pH 8.3 containing 10% (w/v) sucrose and 2M urea. The homogenate was centrifuged at 10,000g for 30 min at 4°C. The supernatant (6.0 ml)

was applied to a Sephadex G-150 column (5.0 x 75 cm) equilibrated with homogenization buffer lacking sucrose and urea. Fractions (15 ml) were collected at a flow rate of 40-45 ml/hr. Effluent was monitored for absorbance at 280nm, MDH activity (malate dehydrogenase: internal standard) after McReynolds and Kitto (1970) and polyacrylamide gel electrophoresis to detect phenol oxidase (after Batterham and McKechnie, 1980). The column was calibrated with chymotrypsinogen ( $\alpha$ -CT: 25,000), ovalbumin (OA:45,000) and bovine serum albumin (BSA monomer: 68,000; BSA dimer 136,000).

The molecular weight of MDH was taken as 71,500 (G.K. Chambers unpublished). The elution position of the PHOX enzyme was judged to be  $44.5 \pm 0.5$  fractions, from which we calculated a molecular weight of  $108,000 \pm 4,000$  for the PHOX oligomer---see Fig. 1. Electrophoretic evidence (Batterham 1980) suggests that the Phox gene product is a dimer and hence we deduce the subunit molecular weight to be 54,000. From such evidence we cannot discount the possibility of higher order aggregates (e.g., a tetramer that hybridizes as pairs of dimers in heterozygotes). However, it is certain that PHOX is non-identical to the A1 phenoloxidase components described by Seybold et al. (1974) as a monomer of subunit molecular weight 77,000.

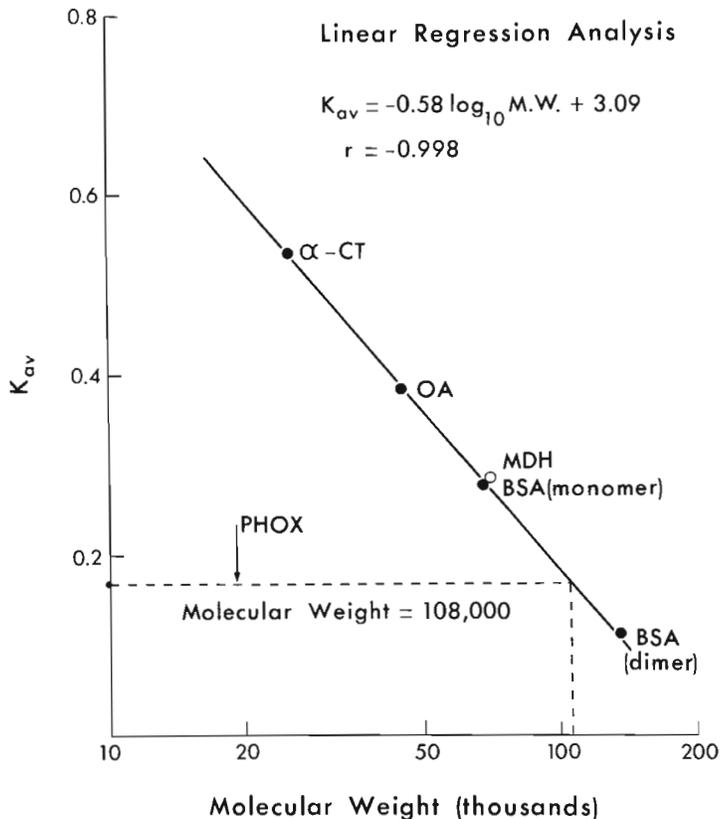


Fig. 1. Calibration of Sephadex G-150 column and calculation of the molecular weight of PHOX.

We would recommend this experimental approach to other workers involved in allozyme surveys of natural populations in view of recent suggestions that enzyme variability is correlated with subunit size (e.g., Nei et al. 1978).

We are indebted to Dr. J.B. Gibson in whose laboratory this work was carried out.

References: Batterham, P. and S.W. McKechnie 1980, submitted to *Genetica*; McReynolds, M.S. and G.B. Kitto 1970, *Biochim. Biophys. Acta.* 198:165-175; Seybold, W.D., D.S. Meltzer and H.K. Mitchell 1975, *Biochem. Genet.* 13:85-108; Nei, M., P.A. Fuerst and R. Chakraborty 1978, *Proc. Nat. Acad. Sci.* 75:3359-3362.

Bewley, G.C. and S. Lubinsky. North Carolina State University, Raleigh, North Carolina. Thermal stability of catalase during development in *Drosophila*.

An analysis of the thermal stability of the enzyme catalase ( $H_2O_2:H_2O_2$  oxidoreductase, E. C. 1.11.1.6) during *Drosophila* development was conducted on crude extracts of an Oregon-R-6 strain and the results are illustrated in Figs. 1 and 2.

The optimum temperature for this study was considered to be 56°C since about half the activity decayed in 5 min (Fig. 1). In extracts from each developmental stage, there is a break in the semilog plot, with a half-life of 6.5 min in adult and pupal extracts and 14 min in larval extracts (Fig. 2). Similar results have been obtained in screening 20 different wild type laboratory stocks. Such a bimodal curve indicates the possibility that more than one molecular form of the enzyme exists, although isozymic patterns are not yet evident on electrophoretic gels. Multiple forms could arise by one or more of the following mechanisms, although none of these have been rigorously ruled out in our current studies: (1) isozymes coded for by different structural genes, although only a single enzyme dosage-sensitive region has been identified by segmental aneuploidy (Lubinsky and Bewley 1979); (2) post-translational modification of a primary gene product leading to conformational alterations; (3) the partitioning into compartmentalized and soluble fractions of the enzyme; and (4) dissociation of the enzyme into enzymatically active subunits.

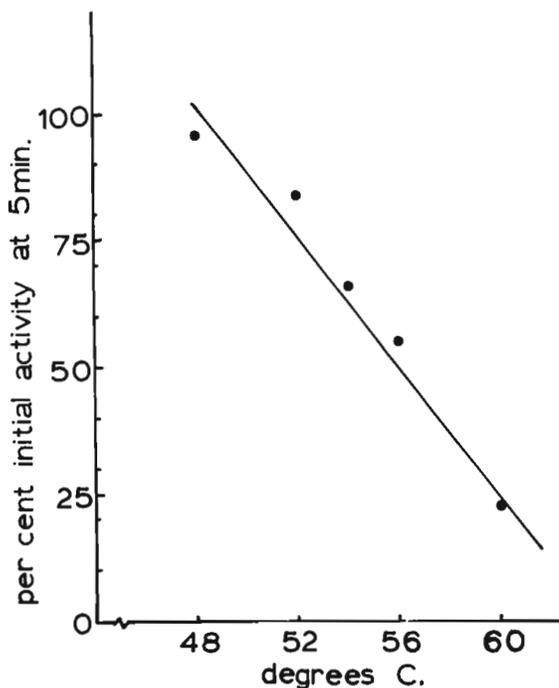


Fig. 1. The effect of increasing temperature on the thermal stability of catalase in adult crude extracts incubated for a period of 5 min.

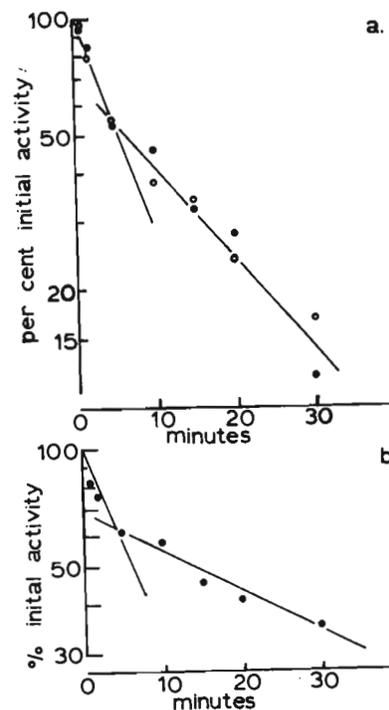


Fig. 2. Thermal denaturation at 56°C of catalase activity in crude extracts. a. Crude adult (○) and crude pupal (●) extracts. b. Crude larval extracts.