DIFFERENTIAL RESPONSE TO GENE DOSAGE EXPERIMENTS INVOLVING THE TWO LOCI WHICH CONTROL XANTHINE DEHYDROGENASE OF DROSOPHILA MELANOGASTER*

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With 1 Figure in the Text
(Received September 19, 1962)

Introduction

The elegant manner in which JACOB and MONOD (1961) have applied gene dosage analysis to the mutants involved in the production of β-galactosidase of Escherichia coli has prompted a similar study of mutants involved in the production of xanthine dehydrogenase of Drosophila melanogaster. The two mutant loci involved in this study are maroon-like (ma-1) and rosy (ry) eye color, and are located on the first and the third chromosome, respectively (BRIDGES and BREHME 1944). The mutants show many similarities. Both have a reddish-brown eye color due to a partial lack of red eye pigment, and a deficiency of the enzyme, xanthine dehydrogenase (FORREST, GLASSMAN and MITCHELL 1956). As a result of this deficiency, ma-l and ry mutants accumulate the enzyme substrates (hypoxanthine and 2-amino-4-hydroxypteridine), and show no trace of the products (uric acid and isoxanthopterin) formed from these compounds (HADORN and SCHWINK 1956, MITCHELL, GLASSMAN and HADORN 1959). No xanthine dehydrogenase activity could be detected in partially purified extracts of the mutants (GLASSMAN and MITCHELL 1959a).

HUBBY and FORREST (1960) have reported that ry/+ and ma-l/+ heterozygotes have 53% and 67% of the xanthine dehydrogenase activity of Canton-S wild-type, respectively. The present study confirms that the enzyme activity in ry/+ is probably half of the usual wild-type, but indicates, on the other hand, that ma-l/+ has the same activity as +/+ . In addition, through the use of a small chromosomal fragment containing ma-l+, we have been able to show that +/+ has the same amount of enzyme activity as +/ma-l/ma-l and +/+ma-l.

While this manuscript was in preparation, we learned of the elegant experiments of GHELL, who extends our results by showing that flies with three doses of the ry+ gene have three times the amount of enzyme as one dose of the gene (E. GHELL, personal communication).

Mutants used. The following mutant of Drosophila melanogaster were used: Bx3 = Beadex wing; f = forked bristle; ma-l = maroon-like eye color; ma-l3z = bronzy allele of ma-l;

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* This work was supported by a grant (RG-8202) from the National Institutes of Health.
** Senior Research Fellow of the National Institutes of Health (GSF-14, 911).
* Predoctoral Trainee supported by a Genetics Training Grant (2 G-685) of the National Institutes of Health.
** Postdoctoral Trainee supported by a Genetics Training Grant (2 G-685) of the National Institutes of Health.
ma-I = the wild-type allele of ma-I; ry = rosy eye color; ry² = rosy² allele of ry; ryA = rosyA allele of ry; ryT = the wild-type allele of ry; y = yellow body color. The wild-type strain utilized was an Oregon-R stock derived from the strain at The Johns Hopkins University. \( \text{In}(3 \text{RC}:3 \text{LP})\text{Sb} \) e is a third chromosome inversion containing a ry allele (Hubby and Forrest 1960). \( \text{Dp}(1;3)\text{B}\text{za} \) is a duplication of the X-chromosome containing ma-I which is inserted into the 3rd chromosome. It can easily be followed since flies carrying it have Bar eyes.

**Fly media.** Flies were grown on a medium similar to that developed by Dr. E. B. Lewis (see D.I.S. 34: 117, 1960) and contained 81.0% water, 1.2% agar, 5.0% dextrose, 2.5% sucrose, 8.3% corn meal, 2.3% dried yeast, 0.06% phosphoric acid and 0.4% propionic acid.

**Enzyme assay.** Xanthine dehydrogenase activity was measured by the rate of the conversion of 2-amino-4-hydroxypteridine to isoxanthopterin. The amount of xanthine dehydrogenase activity in a single adult *Drosophila melanogaster* was determined by a modification of the fluorometric technique used by Glassman and Mitchell (1959a) and Glassman and Pinkerton (1960) and described in detail elsewhere (Glassman 1962). A unit of enzyme activity is defined as that amount of enzyme which will convert 1 \( \mu \) mole of 2-amino-4-hydroxypteridine in one minute under the conditions of the assay.

**Results**

All data on xanthine dehydrogenase activity are expressed either as the ratio between the activity for the fly in question and those obtained from Oregon-R wild-type flies which were always assayed at the same time, or as the enzyme units per fly. Sexes were treated separately, a procedure which has the misleading effect of making the activity ratio of males and females equal. However it should be pointed out that males had consistently lower absolute activity than females. Indeed, in some experiments, the females had up to 80% higher xanthine dehydrogenase activity than the males. This value seems to be too high to be accounted for by differences in size alone.

Assays of xanthine dehydrogenase in flies heterozygous for an ry mutant indicate that these heterozygotes have a combined mean ratio of 0.73 ± 0.016 (S.D. \( \overline{X} \)) of the activity of Oregon-R (Fig. 1, A—D). This effect can be observed for wild-type heterozygotes of \( \text{ry}^1, \text{ry}^2, \text{ry}^A \), and the ry allele contained in the \( \text{In}(3 \text{RC}:3 \text{LP})\text{Sb} \) e chromosome (Hubby and Forrest 1960). Thus, the ry mutants appear to be similar to other genes in which the heterozygote has a