A THIRD LOCUS (*lxd*) AFFECTING XANTHINE DEHYDROGENASE IN DROSOPHILA MELANOGASTER[1]

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TWO genes which affect xanthine dehydrogenase in *Drosophila melanogaster* are already known. These are maroon-like (*ma-l*) on the X chromosome, and rosy (*ry*) on the third chromosome. The phenotype of both of these mutants is a dark red-brown eye color. When either of these genes is homozygous, the mutant flies have no detectable amounts of xanthine dehydrogenase (FORREST, GLASSMAN, and MITCHELL 1956; GLASSMAN and MITCHELL 1959). In such flies, the substrates of the enzyme accumulate and the products are absent (HADORN and SCHWINK 1956; MITCHELL, GLASSMAN, and HADORN 1958). In vitro complementation between extracts of *ma-l* and *ry* has been reported (GLASSMAN 1962a). Recently, we have found a strain of flies having only about 25 percent of the xanthine dehydrogenase activity found in standard wild-type strains. These flies with low enzyme activity have wild-type eye color and wild-type amounts of the substrates and products of xanthine dehydrogenase. This paper is concerned with the genetic analysis of this strain (designated Low).

MATERIALS AND METHODS

The activity of xanthine dehydrogenase in individual adult flies was determined in randomly coded extracts using the following modification of the fluorometric assay described by GLASSMAN (1962b). All preparative procedures were carried out at temperatures of 5°C or less. A single fly was homogenized in 4 ml of 0.1 M Tris buffer, pH 7.5, containing 5 mg crystalline bovine albumin (Armour) per ml. Approximately 40 mg of Norite-A (Fisher) was added and the mixture was allowed to stand (with occasional stirring) for 1 hour. The solution was then centrifuged 30 min at 30,000*g*. The supernatant was removed and filtered through a coarse sintered glass filter to remove the last remnants of the charcoal. One ml of the supernatant was placed into a fluorometer cuvette and 0.02 ml of 1×10^{-3} M methylene blue was added. The solution was allowed to stand for 4 min in a Thermolyne Dri-Bath adjusted to 30°C, after which 0.01 ml of 6.67×10^{-4} M 2-amino-4-hydroxypteridine was added. The solution was then mixed and the readings were taken at 2 min intervals for 10 min in a No. 540 Photovolt fluorometer equipped with a 347 mμ primary filter (Photovolt) and a 405 mμ secondary filter (Turner, No. 110–812). The fluorometer was initially adjusted each day so that a standard solution of 1.6×10^{-6} M quinine in 0.1 M sulphuric acid read 100. The high blank of the enzyme reaction mixture was suppressed by appropriate adjustment of the photometer. During the assay, the cuvettes were returned to the Dri-Bath between readings. One unit of enzyme activity is defined as that

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amount of enzyme which converts one $\mu\mu$mole of 2-amino-4-hydroxypteridine to isoxanthopterin per minute. Under the conditions of the assay the rate of the reaction was linear for at least 30 min and was found to be proportional to enzyme concentration. All flies were grown at 25 ± 1°C using medium described by GLASSMAN, KARAM, and KELLER (1962) and were less than 12 hours old when the assays were done.

The mutants, strains, and abbreviations used in this study were as follows. *by:* blistery, 3-48.7 (Chromosome 3, locus 48.7), a recessive wing mutant. *ci^D:* cubitus interruptus, 4, a dominant wing mutant. *jv:* javelin, 3-19.4, a recessive bristle mutant. Low: strain with low xanthine dehydrogenase activity, from The Pennsylvania State University [A31X^{89}(18)]. *Ly:* Lyra, 3-40.5, a dominant wing mutant. *lxd:* low xanthine dehydrogenase, 3-33±, a new recessive mutant producing a low level of xanthine dehydrogenase activity. *ma-l:* maroon-like, 1-64±, a recessive eye color mutant (lacks xanthine dehydrogenase). *Mio: Microphthalmia*, 3-100.5, a dominant eye mutant. Or-R: Oregon-R wild-type strain (derived from the stock at The Johns Hopkins University). Or-S: Oregon-S wild type (from Oak Ridge National Laboratory). PAC: Pacific wild-type strain (from The Institute of Animal Genetics, Edinburgh, Scotland). *Pm:* Plum, a dominant eye mutant marking an inversion of the second chromosome. *R:* Roughened, 3-1.4, a dominant eye mutant. *ru:* roughoid, 3-0, a recessive eye mutant. *ry:* rosy, 3-52±, a recessive eye color mutant (lacks xanthine dehydrogenase). *se:* sepia, 3-26, a recessive dark brown eye color mutant. *Sb:* Stubble, 3-58.2, a dominant bristle mutant, which, in some crosses is used to mark an inversion of the third chromosome. See BRIDGES and BREHME (1944) for descriptions of most of these mutants.

MATINGS AND RESULTS

Figure 1 summarizes the results of preliminary attempts to ascertain the genetic control of the reduced xanthine dehydrogenase activity of the Low strain. The low enzyme level of the Low strain is due to an autosomal recessive trait because reciprocal crosses of this strain (Low, Figure 1A) to wild type (Or-R, Figure 1A) resulted in F_1 progeny (Figure 1B) with a mean enzyme activity that exceeded the mean activity of the Or-R parental strain) see also GLASSMAN,

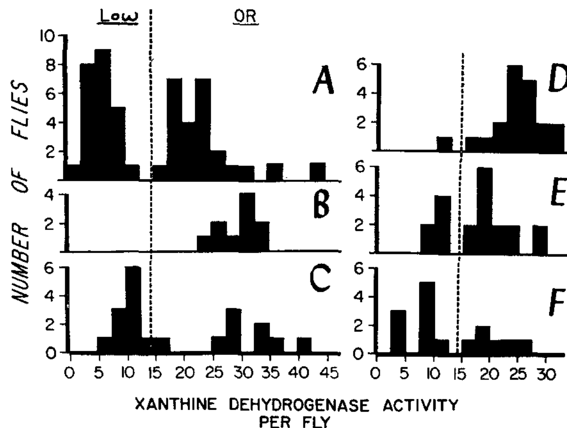

FIGURE 1.—Genetic analysis of the Low strain. A. Distribution of enzyme activity of the Low and Or-R strains. B. Combined F_1 data: Low × Or-R and Or-R × Low. C. Backcross data: Low females × F_1 males from B (Low females × Or-R males). D. Combined F_1 data: Low × *ma-l* and *ma-l* × Low. E. Combined F_1 data: Low × *ry* and *ry* × Low. F. Backcross data: Low females × F_1 males from E (Low females × *ry* males).

KARAM, and KELLER 1962 for this heterozygotic effect). The mean enzyme activity for the Low strain was 5.8 enzyme units per fly ($S_{\bar{x}} = 0.48$), for Oregon-R it was 23.4 enzyme units per fly ($S_{\bar{x}} = 1.17$) and for the F_1 it was 29.5 enzyme units per fly ($S_{\bar{x}} = 0.96$). A 1:1 segregation of high to low enzyme activities was observed when the F_1 males derived from the cross, Low females × Or-R males, were mated to Low parental-type females (Figure 1C). Although not shown here, backcross segregation ratios of 1:1 were also obtained using PAC or Or-S instead of Or-R.

When the Low strain is crossed to *ma-l* or *ry* (Figure 1D, 1E), the progeny contain approximately wild-type levels of enzyme activity; therefore the Low factor is not identical to either *ma-l* or *ry* (both of which lack detectable amounts of xanthine dehydrogenase). The Low factor resembles *ma-l* in dosage, in that the mutant effect on enzyme activity is completely recessive to wild type. This differs from dosage at the *ry* locus, since flies which are ry^+/ry have about half of the activity of the wild type (GLASSMAN, KARAM, and KELLER 1962; GRELL 1962). As expected, a 1:1 backcross ratio was also observed when the F_1 males derived from the cross, Low females × *ry* males were mated to Low females (Figure 1F). The dosage effect of the *ry* heterozygotes is apparent in the shift toward lower mean enzyme activities shown in Figures 1E and 1F as compared to Figures 1B and 1C.

The chromosome on which the Low factor is located was determined by crossing Low females × $Pm;Sb;ci^D$ males and backcrossing the $Pm;Sb;ci^D$ F_1 males to Low females. The F_2 progeny were assayed individually for xanthine dehydrogenase activity. In Figure 2, the dark symbols indicate F_2 flies which are heterozygous for the third chromosome from the Low stock while the open symbols indicate F_2 flies with homozygous third chromosomes from the Low stock. With few exceptions, flies which were homozygous for the third chromosome from the

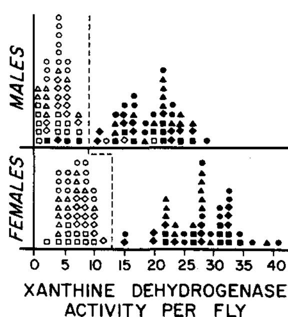

FIGURE 2.—The localization of the Low factor on the third chromosome. Low females were crossed to $Pm;Sb;ci^D$ males, and the resulting $Pm;Sb;ci^D$ F_1 males were crossed to parental-type Low females. The progeny were individually assayed for xanthine dehydrogenase activity. Each point on the graph represents the activity of a single fly. □ = $Pm;+;ci^D$. ○ = +; +; +. △ = $Pm;+;+$. ◇ = $+;+;ci^D$. ■ = $Pm;Sb;ci^D$. ● = $+;Sb;+$. ▲ = $Pm;Sb;+$. ◆ = $+;Sb;ci^D$. Open symbols indicate a fly containing homozygous third chromosomes from the Low strain. Closed symbols indicate that the fly carried a 3rd chromosome from the Low strain heterozygous with a *Sb* chromosome.

Low strain have low xanthine dehydrogenase activity while those with a heterozygous third chromosome from the Low stock had high activity. There was no relationship of enzyme activity to chromosomes 2 or 4. This indicated that the Low factor, named "low xanthine dehydrogenase" (*lxd*), was on the third chromosome.

Preliminary results using the dominant genes, *Mio*, *R*, and *Sb*, indicated that *lxd* was on the left arm of chromosome 3 between loci 27 and 35. To locate *lxd* more accurately, we crossed + + + *lxd* +/*ru jv se* + *by* females to *ru jv se* + *by* males. Selected male progeny were crossed to + + + *lxd* + females and the F_1's were inbred to establish a homozygous stock. In all cases, the presence of *lxd* was determined by assaying individual flies for xanthine dehydrogenase activity. In eight stocks, where se^+ and by^+ were recovered together, all but one stock was of the parental $se^+ lxd\ by^+$ type. In 12 stocks where *se* and *by* were recovered together, all were of the parental $se\ lxd^+\ by$ type. Hence, *lxd* is located between *se* and *by*. In 15 stocks where a crossover was observed between *se* and *by* (i.e., $se\ by^+$ and $se^+\ by$) eight stocks had *lxd* and seven did not. Therefore, *lxd* is about midway between *se* and *by* (about locus 37).

To obtain more extensive crossover data we crossed *ru jv se lxd* +/+ + + + *Ly* females to *ru jv se lxd* + males. Flies exhibiting crossovers between *se* and *Ly* were assayed to ascertain whether *lxd* was present. Other crossover types were also assayed (data not shown) and no inconsistencies were observed with the data discussed here. From flies showing crossover chromosomes between *se* and *Ly*, 26 of 65 *ru jv se Ly* chromosomes contained *lxd*, and 52 of 103 + + + + chromosomes contained *lxd*. Thus, *lxd* is about 0.45 of the distance between *se* and *Ly*, closer to *Ly*. The reported crossover distance between *se* and *Ly* is 14.5 (BRIDGES and BREHME 1944). However, in our experiment there was 14.1 percent crossing over between *se* and *Ly* (N = 624). *lxd* is therefore placed at 33±.

The mechanism whereby *lxd* affects xanthine dehydrogenase is obscure. Preliminary tests indicate that no inhibitor for wild-type xanthine dehydrogenase is present in extracts of *lxd* flies. Furthermore, there do not appear to be any qualitative differences between the enzyme isolated from *lxd* and wild-type strains with respect to electrophoretic mobility on paper (KELLER, SAVERANCE, and GLASSMAN 1963), acrylamide gels (GLASSMAN and SAVERANCE 1963), heat stability, or Km (GLASSMAN and MCLEAN, unpublished). Perhaps the *lxd* locus normally exercises a regulating function on xanthine dehydrogenase, and it is this function which is deranged in *lxd*. About 150 wild-type strains from all over the world have been examined for the presence of *lxd*, and it has been found in only six of 17 inbred strains obtained from a single sampling of one wild population near The Pennsylvania State University (KELLER 1961).

Other mutant genes for low tyrosinase activity (LEWIS and LEWIS 1962) and low ali-esterase activity (OGITA 1961) have been reported. These are not related to *lxd* since low ali-esterase strains (kindly provided by DR. T. WRIGHT) have normal xanthine dehydrogenase activity and the *lxd* strain itself has normal tyrosinase activity (MCLEAN and GLASSMAN, unpublished).

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Note added in proof:

It has not been shown that *lxd* flies have a substance (or substances) which inhibits the activity of the *mal*+-complementation factor described by GLASSMAN (1962a). This is shown in the complementation reaction in vitro by using extracts from *ma-l;lxd* and *lxd ry* with *ma-l* and *ry*, and by the fact that *lxd* lacks detectable amounts of the pyridoxal oxidase activity described by FORREST, *et al.* It is of interest that *lxd* has the same amount of CRM units per mg protein as wild type.

SUMMARY

Strains of *Drosophila melanogaster* with low xanthine dehydrogenase activity have been analyzed genetically. It was found that this condition is due to a recessive gene (low xanthine dehydrogenase, *lxd*) on the left arm of the third chromosome near locus 33. This gene is not allelic to *ma-l* or *ry*. When homozygous, *lxd* reduces the xanthine dehydrogenase activity to about 25 percent of wild type. The low activity does not appear to be associated with a substance that inhibits the wild-type xanthine dehydrogenase.

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