MATERNAL EFFECT OF ma-l+ ON XANTHINE DEHYDROGENASE OF DROSOPHILA MELANOGASTER

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Received August 6, 1958

PREVIOUS work has indicated that there are at least two loci (ma-l+ and ry+) in Drosophila melanogaster capable of mutating independently to produce a deficiency of xanthine dehydrogenase (Forrest, Glassman and Mitchell 1956). Some results of this deficiency are accumulations of the substrates (hypoxanthine and 2-amino-4-hydroxypteridine) of this enzyme, and a lack of the products (uric acid and isoxanthopterin) formed from these compounds (Mitchell, Glassman and Hadorn 1959). In addition, the red eye pigment of these flies is diminished so that the color of the eye is dark red-brown; however, the direct relation between the enzyme deficiency and the eye color is obscure at present. The enzyme from wild type adults has been purified with charcoal, ammonium sulphate, and calcium phosphate gel, but treatment of the mutant extracts with this procedure has failed to elicit enzymatic activity indicating that the deficiency in the mutants is not due to simple inhibitors (Glassman and Mitchell 1958). The present report concerns a maternal effect associated with ma-l+, which is not dependent on the presence of ry+. A preliminary report of this investigation has appeared (Glassman, Hubby and Mitchell 1958).

MATERIALS AND METHODS

Unless stated otherwise, all procedures followed those reported previously (Glassman and Mitchell 1959). The enzyme was assayed in various strains as follows: 100 adult flies, one day old, were homogenized in 1.0 ml of 0.1 M Tris buffer, pH 8. Two to five mg of Norit-A were added, and after ten minutes the mixture was centrifuged to remove the charcoal. The colorless supernate (0.5 ml) was added to an assay mixture containing 0.5 ml buffer; 0.01 ml 10-3 M 2-amino-4-hydroxypteridine; and 0.01 ml 10-3 M methylene blue, and the reaction was followed photofluorometrically as described previously (Glassman and Mitchell 1959).

1 This work was supported in part by a contract from the Atomic Energy Commission (Contract No. AT(04-3)-41).

* The following abbreviations for various mutants are used (Bridges and Brehme 1944): Bez = Beadex wing; cu = curved wing; f = forked bristle; m = miniature wing; ma-l = maroon-like eye color; ry = rosy eye color; ss = spineless bristle; st = scarlet eye color; TM1 = third chromosome balancer (Lewis, pers. comm.); th = thread antennae; y = yellow body color; y f: = is an attached-X chromosome containing y and f.
1959), except that the quinine standard was set at 100 instead of 30 to increase sensitivity.

Chromatographic procedures were carried out as previously described (HADORN and MITCHELL 1951; GLASSMAN and MITCHELL 1959). Analysis of single pupae and flies was accomplished by squashing directly on the chromatography paper, but extracts were also used as follows: 50 flies were placed in a small homogenizer with 0.2 ml of distilled water or 1 percent NH$_4$OH, and subjected to a boiling water bath for a few minutes. They were then homogenized and again heated in a water bath. The mixture was then centrifuged, and the resulting supernate was applied as a one cm streak at the starting line of the paper chromatogram.

The combination of $st$ with $ma-l$ or $ry$ produces orange eyes. This greatly facilitates the separation of $ma-l^+$ from $ma-l$, and $ry^+$ from $ry$, and for this reason the stocks used in this investigation contained $st$.

RESULTS

Progeny analysis of reciprocal crosses between $ma-l$ and wild type revealed that $ma-l$ is a typical sex-linked recessive as reported by OLIVER (see BRIDGES and BREHME). However, when the F$_1$ $ma-l/ma-l^+$ females are crossed to $ma-l$ males, the expected $ma-l$ phenotype does not appear even though half the flies are genetically $ma-l$ as revealed by genetic markers and progeny tests. The effect is also obtained when attached-X females ($\gamma f; =; st$) are crossed to $ma-l; st$ males, and most of the analysis was accomplished with this type of cross.

Since $ma-l$ is probably nonautonomous (GLASSMAN 1958), one explanation might be that the wild type progeny excrete products which are capable of affecting their $ma-l$ sibs during larval development. However, this is disproved by the cross, $ma-l/ma-l \times$ wild type, which produces normal $ma-l$ males, and also by further experiments in which attached-X females ($\gamma f; =; st$, mated to $ma-l^+$ males) and $ma-l$ females ($\gamma ec ma-l; st$ females mated to males of their own genotype) were allowed simultaneously to lay eggs in the absence of the males in the same bottle, simulating the conditions found in the cross between attached-X females and $ma-l$ males. All $\gamma ec ma-l; st$ offspring had $ma-l$ eyes indicating that no cross-feeding occurs between wild type flies and $ma-l$.

The progeny from maternally-affected males and females have the normal $ma-l$ phenotype, and, therefore, the maternal effect is due to a “predetermination” of the egg and is not due to self-reproducing cytoplasmic particles. Additional tests indicated that the presence of other genes ($al, B, Br^x, ec, f, fu, m, m^b, pol, ptg^x, sn^x, st, wy, \gamma$ (see BRIDGES and BREHME)) did not alter this effect. The usual interpretation for this type of phenomenon is that the $ma-l^+$ females are passing a substance to their progeny through the egg. For convenience, we shall designate this material as “$x$.”

Biochemistry

Paper chromatography revealed significant differences between the normal $ma-l$ males and the maternally-affected ones. First, the maternally-affected males
have increased amounts of the red pigment, and their eye color resembles the wild type exactly. Second, traces of uric acid and isoxanthopterin (the enzyme reaction products) are present indicating that xanthine dehydrogenase occurs in these flies, and, indeed, small amounts of enzymatic activity were found when the maternally-affected flies were assayed (Figure 1). This small change in

![Figure 1](image_url)

**Figure 1.**—Enzyme activity in maternally-affected flies. The enzyme was prepared and assayed as described under Methods.

fluorescence in extracts of maternally-affected *ma-l* was shown to be due to the formation of isoxanthopterin as follows: one gram of maternally-affected *ma-l* males, less than one-day old, was homogenized in 2 ml 0.1 M Tris buffer, pH 8.0, and the solution was centrifuged at 30,000 × g for 15 minutes. Charcoal (Norite-A) was added to the supernatant solution in 100 mg portions until all traces of eye pigment were removed. To 0.3 ml of the colorless extract were added 0.01 ml
of $10^{-3}$ M 2-amino-4-hydroxypteridine and 0.01 ml of $10^{-3}$ M methylene blue. The mixture (0.1 ml) was applied as a one cm streak for paper chromatography before and after a three hour incubation at 25°C. The chromatogram was developed with propanol: one percent ammonia (2:1) for two hours. The use of an ultraviolet lamp emitting at 3600 Å revealed traces of isoxanthopterin after incubation, but none before. Control extracts of normal ma-l did not produce any isoxanthopterin.

Visual inspection of the chromatograms of maternally-affected flies reveals that the amounts of the enzyme substrates (hypoxanthine and 2-amino-4-hydroxypteridine) present are variable but lie between the amounts found in the usual ma-l and the wild type male. However, when the spot containing the 2-amino-4-hydroxypteridine is eluted from the paper and the amount of fluorescence of the eluate determined, it appears that more fluorescence is present in the maternally-affected flies than in ma-l or wild type (HUBBY and FORREST unpublished). These results are being pursued further, and may indicate the existence of another compound involved in this system.

**Effect of environment**

Only ma-l flies which emerge in the first six to eight days after the first flies appear are maternally-affected; after this time a short period occurs (one to two days) during which the flies emerging have intermediate eye colors between ma-l and wild type, but later none is maternally-affected and all have the typical ma-l phenotype. This reversal could be due to a depletion of "x" in the female parent, or to a change in the condition of the culture. To test these alternatives, $y f: = ; st$ females were mated to $m$ ma-l ; st males (Table 1), and transferred periodically to new bottles. Each successive bottle was then examined daily to determine the first day that males which were not maternally affected appeared. The results show that the time of disappearance of the maternal effect is the same regardless of the age of the female. Thus, it seems that the ma-l+ females are capable of producing "x" throughout their lives, and that the decline in the maternal effect is

**TABLE 1**

*The effect of age vs. culture conditions on the maternal effect of ma-l*

<table>
<thead>
<tr>
<th>Transfer</th>
<th>Number of days from previous transfer</th>
<th>Number of days between transfer and the disappearance of the maternal effect</th>
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Ten pairs of the mating $y f: = ; st$ females × $m$ ma-l; st males were successively transferred to fresh bottles at five to seven day intervals. The bottles were examined daily in order to determine the first day that male progeny which are not maternally affected appeared.
due to a change in the culture environment. This interpretation received further support when ten pairs of the mating, attached-X females × f Bx m males,* were added to food bottles in which attached-X females (mated to m ma-l males*) had been allowed to lay eggs for seven days. The bottles were then examined daily in order to determine the time at which the maternal effect diminished for the m ma-l and f Bx m ma-I males, respectively. The m ma-l males showed the usual pattern, i.e., these males were maternally affected until five to six days after the first flies emerged; however, during the time the f Bx m ma-I males developed the critical change had occurred in the culture, and none of these flies was maternally affected.

The nature of the change is not known. It may be due to the depletion of a dietary factor necessary for the maternal effect, or it may be due to the accumulation of metabolic substances which can inhibit the small amounts of enzyme present in the maternally-affected flies. Experiments designed to test these alternatives are in progress.

**Effect of ry**

The mutant ry is located on the 3rd chromosome at 51±. When females heterozygous for ry (th st cu ry ss / TM1) are crossed to ry males (th st cu ry ss or th st cu ry ss / TM1), the non-TM1 progeny are phenotypically normal ry, indicating that ry is not maternally affected by ry+. A similar result was obtained using ry*. Since both ry and ma-I are deficient in xanthine dehydrogenase, one might expect that ry / ry might negate the maternal effect of ma-l+. Accordingly, attached-X females containing st ry or st ry (i.e., ry / st ry) were mated to m ma-l ; st males. The result was unexpected since the maternal effect of ma-l+ was still manifested in the m ma-l ; st ry / st+ ry+ male progeny. Thus, the maternal effect of ma-l+ does not depend upon the simultaneous presence of ry+. It should be noted that mating the above ry / st ry females to males containing both ma-l and ry (ma-l ; st ry) produced ma-l ; st ry males which do not exhibit a maternal effect, since they are homozygous for ry.

**DISCUSSION**

Maternal effects of the type associated with ma-l+ occur in many organisms. These are characterized by a "predetermination" of the egg cytoplasm by the physiological condition of the female parent so that the progeny reflect her genetic constitution, even though they may have a different genotype. This effect does not persist beyond one generation, since maternally-affected organisms have progeny which are typically mutant. This phenomenon is interpreted as being due to a substance which the female parent passes to her progeny; the lack of persistence beyond one generation is taken to mean that this compound is not synthesized in the maternally-affected mutant, and is diluted out or used up during protoplasmic growth. In the case of ma-l+, this contribution from the female parent is designated as "x."

* The crosses were ry / ; st × f Bx ma-l ; st and ry / ; st × m ma-l ; st.
An example of this type of maternal influence is associated with sinistral and
dextral coiling in snails (Boycott et al. 1930), some egg color mutants of Bom-
byx (Kikkawa 1957), the a mutant of Ephesia (Caspari 1933), the light eye
color mutant (Beadle 1937) and the vermilion eye color mutant (Graf 1957)
of D. melanogaster, and many others. These maternal effects are associated with
processes initiating in the egg or in early development, whereas the effect seems
to be much later with ma-l+; indeed, “x” apparently persists to the adult stage
where we can observe a wild type eye color and traces of xanthine dehydrogenase
and its reaction products. This would tend to eliminate the idea that “x” is a
simple substance such as an enzyme activator or cofactor, since such compounds
which are not being resynthesized would probably be depleted prior to the adult
stage.

On the other hand, “x” may represent the enzyme, particularly since there is
approximately only 1/1000 the enzyme activity in maternally-affected ma-l
males than there is in their female parent. Comparison of the weight of the adult
female (approximately 0.8 mg) and the egg (about 0.008 mg) indicates that
1/100 of the enzyme present in the adult female can be passed to the egg, if we
assume that the enzyme is distributed equally throughout the adult and the egg.
This may not be true since the egg contains considerable yolk, which may not
carry the enzyme. In any case, the small amount of enzyme present in the adult
would be consistent with the idea that it is “x.” However, since females which
are homozygous for ry (y f: = ; st ry+) and which are therefore deficient in
xanthine dehydrogenase, can still exert a maternal effect on ma-l progeny, it is
difficult to see how this enzyme could be “x.” Another alternative is that “x” is a
component of the enzyme-forming system, which might be stable, and which
could overcome possible cytoplasmic dilution by producing many enzyme mole-

\[ \text{Figure 2.—Possible alternatives to explain the effect of ma-l and ry on x. This compound is not passed into the egg, whereas x is.} \]
cules. Data on this point are lacking, however, and it is difficult to rule out any possibility.

The question arises as to why ry flies cannot respond to "x" in the egg. Since ma-l+; ry females can exert a maternal effect on ma-l, it is obvious that ry flies can synthesize "x." Thus, it would appear that ry flies cannot respond to maternal influences because they are blocked in the utilization of "x." On the other hand, the maternal effect on ma-l most likely occurs because these flies can utilize "x" if it is present in the egg. These data suggest that a sequential relationship may exist whereby ma-l is blocked in the synthesis of "x," and ry is blocked in its utilization; a simple scheme (Figure 2a) can account for these data, but other equally likely pathways can also be applied (Figure 2b). It is of interest that the only other difference between ma-l and ry which has been found concerns the presence of a substance which can cross-react with the antibody to xanthine dehydrogenase. This substance is present in ma-l and deficient in ry (Glassman and Mitchell 1958). Its significance is not yet known.

**SUMMARY**

Previous work has shown that two eye color mutants of Drosophila melanogaster (ry and ma-l) are deficient in xanthine dehydrogenase. The present study concerns a maternal effect which ma-l+; ma-l females have upon their ma-l progeny. It is of interest that ry+; ry females do not exert a similar effect on ry. Biochemical studies have shown that in addition to increased amounts of red eye pigment, the maternally-affected ma-l flies have traces of isoxanthopterin and uric acid (the enzyme products), as well as traces of the enzyme itself. This case is unusual, since most maternal effects of this type usually affect processes initiating in the egg or in early development, while the effect here seems to be much later in development.

However, only flies which emerge in the first six to ten days of hatching show this effect; following this time the adults which emerge in the bottle have the typical ma-l eye color. This is due to a change in the culture and not to the age of the female parent, since old females transferred periodically to new bottles will also have maternally-affected progeny in each new bottle until six to ten days after the first flies emerge. The nature of this change is not known.

The data suggest that the ma-l+; ma-l females are passing a substance to their progeny in the egg cytoplasm. This substance is not made in ma-l flies, but can be utilized by this mutant; hence, the maternal effect. On the other hand, since ma-l+; ma-l; ry females can exert a maternal effect on ma-l, it is evident that ry can synthesize this compound and pass it to the egg; its utilization is probably blocked in this mutant. This suggests that a sequential relationship may exist between the reactions blocked by these mutants.

**LITERATURE CITED**


