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## Effects of the Triplo-X Condition on Development in *Drosophila melanogaster.*

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In a study of the giant larva, using an attached-X stock (gt $bb^{11}$/gt $w^a$), a high pupal mortality was observed, as well as a high frequency of zygotes whose pupation was retarded, as expected for giant, but which did not form large (giant) pupa cases. In order to separate the effects of the triplo-X condition from those of the giant, a study has been made of the larvae and pupae of an attached-X yellow white stock.

In this stock, male larvae and pupae can be identified by the testes, triplo-X females by the ovaries and yellow (wild type) Malpighian tubes, and XXY females by ovaries and the colorless Malpighian tubes characteristic of individuals homozygous for white. During this investigation, it was noticed that the homozygous yellow white larvae could be identified by the light color of the mouth armature, a character which could be diagnosed with certainty in the living larvae at all stages from hatching to pupation, and which, when checked against the colorless Malpighian tubes of the homozygous white larvae, proved to be an accurate means of classification. That the light mouth armature color is dependent upon the yellow gene and not the white was determined by examination of white and of yellow stocks. In the first instar larva homozygous for yellow the whole mouth armature is very light brown, almost yellow; wild type armature at this time is very dark brown. In the third instar (about 70 hours until pupation at 25°C.), the mouth hooks are as dark as those of wild type but the posterior part of the armature is light brown. Both Malpighian tube color and mouth armature color were used in classifying larvae in these experiments.

*Lethal action of the triplo-X condition.* Newly hatched larvae were cultured in 3-inch Petri dishes, 30 to a dish, on finely strained banana-agar; this colorless, translucent medium served as a good background for observation of Malpighian tube color. Of 352 larvae from matings of attached-X (y w/Y) females by wild type males, 47 died before pupation (33 in the first instar, 3 in the second, 11 in the late third), a larval mortality of $13.35 \pm 1.81\%$ of the larvae (or $10.01 \pm 0.02\%$ of the total zygotes, allowing for death

of 25% of the zygotes, Nullo-X, in the egg stage[1]). All dead third instar larvae were identified as triplo-X. It was not possible to ascertain the genotype of all the dead first and second instar larvae, as these small larvae decompose too rapidly after death to allow accurate classification of Malpighian tubes, and the discovery of the difference in mouth armature color was made too late to be used at this juncture. Of 303 pupae, 82 failed to hatch, a mortality of $23.29 \pm 2.25\%$ of the larvae, or $17.48 \pm 1.75\%$ of the total zygotes; all but 3 of the dead pupae were triplo-X. A few super-females emerged, with a frequency of $3.13 \pm 0.92\%$ of the larvae, or $2.35 \pm 0.70\%$ of the total zygotes. The dead larvae and pupae thus constitute 36.64% of the total larval population. The expected proportion of triplo-X individuals is 33.3% of the larvae. It is evident from these data that the majority of triplo-X zygotes die in the pupal stage, while a small proportion die in the third larval instar, and a few in the first and second instars.

Similar counts were then made with the giant stock, in which the high pupal mortality was originally observed; these counts corroborate the evidence from the yellow white stock. Of 298 larvae from matings of attached-X gt $bb^{11}$/Y females by gt $w^a$ males, 50 died in the larval stage (41 in the first instar, 1 in the second, 8 in the third), a larval mortality of $16.77 \pm 2.16\%$ of the larvae, or $12.58 \pm 1.66\%$ of the total zygotes; the pupal mortality was $28.18 \pm 2.65\%$ of the larvae, or $21.14 \pm 2.04\%$ of the zygotes. The total deaths, in percent of the larvae, were thus 44.95%. Here the chromosomal constitution of the dead individuals could not be ascertained.

Comparison of these data with the mortality characteristic of the Florida wild type stock (about 8% larval, almost entirely in the first instar, and 1% pupal mortality, from unpublished data of the author) lends weight to the conclusion that the lethal action of the triplo-X condition is chiefly exerted upon the pupal stage. Direct comparison of the 3 stocks cannot be made, however, since the genetic backgrounds are different in the 3 cases; the residual mortality in the Florida stock appears to be greater than in the 2 attached-X stocks. It is probable that the processes which lead to death of the triplo-X pupae are begun in the larval stage and result in death of some of the larvae, especially in the stage just before pupation, but that the majority of triplo-X zygotes are able to survive until pupation. A few triplo-X larvae appear to die in the

[1] Li, J.-C., *Genetics*, 1927, **12**, 1; Poulson, D. F., *Proc. Nat. Acad. Sci.*, 1937, **23**, 133.

first instar; this is not surprising, since the death of Mw, Ml[2] and Mfla homozygotes and ClB males in the first instar,[2] as well as of almost all of the wild type larvae which die during the larval period, indicates that the first instar is a crucial period in development.

*Delay in onset of pupation.* In the y w/+ stock, it was observed that triplo-X larvae do not begin to form puparia until about 24 hours after the mean pupation time of XY and XXY sibs at 25°C. Some *XXX* larvae did not pupate until 7 days after oviposition, and 2 were still in the larval stage at 15 and 18 days respectively. This is the longest delay in onset of pupation reported for any genotype of *D. melanogaster* and is the first delay known to be attributable to a duplication. Dobzhansky[3] has reported a delay in total time of development of superfemales, but the lengthened development has not hitherto been recognized as due to a delay in puparium formation.

*Conclusions.* The lethal effect of the triplo-X condition is chiefly exerted upon the pupal stage. A few triplo-X zygotes die in the larval period, especially at the stage just before pupation. The onset of pupation of triplo-X larvae is delayed more than 24 hours at 25°C.

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## The Estimation of Histidine.

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The estimation of arginine, histidine and lysine in small amounts of protein by the silver precipitation method[1] has been satisfactorily employed by Miller,[2] Mazur,[3] Plimmer,[4] and others. However, Abderhalden and Siebel[5] were only successful in the determination of

2 Brehme, K. S., *Genetics*, 1938, **23**, 142; *Am. Nat.*, 1937, **71**, 567.
3 Dobzhansky, Th., *Biol. Bull.*, 1930, **59**, 128.
1 Block, R. J., *J. Biol. Chem.*, 1934, **106**, 457.
2 Miller, E. J., *Biochem. J.*, 1935, **29**, 2344.
3 Mazur, A., *J. Biol. Chem.*, 1937, **118**, 631.
4 Plimmer, R. H. A., and Lowndes, J., *Biochem. J.*, 1937, **31**, 1751.
5 Abderhalden, E., and Siebel, H., *Z. physiol. Chem.*, 1935, **238**, 169.