Oster, Irwin, I. Accentuation of distinctions in larval Malpighian coloration by the feeding of riboflavin.

Kikkawa (DIS-22) reported that riboflavin is the substance which gives the Malpighian tubes their coloration. Following this, a statement, of undetermined origin, has received circulation, that the feeding of riboflavin to larvae results in a deepening of the color of the Malpighian tubes. In order to study this phenomenon, I tried feeding larva with a concentration of 0.3 g riboflavin per liter of food. It was found that the larvae of flies of genotypes giving adults with phenotypically normal eye color were thereby caused to acquire a deep yellow-orange color in their Malpighian tubes. Eight wild-typ stocks derived originally from very diverse sources (including Florida, Japan, and Argentina) were all found to give this deep color, as also were stocks showing the phenotypes of 21 different mutant genes not affecting eye color. Stocks with mutant eye colors gave the following results for the color of the Malpighian tubes (second column) as compared with the colors of larvae not fed extra riboflavin (first column), as previously reported by Brehme and others (see Bridges and Brehme, 1941) or as determined by us.

Eye-Color Phenotypes of Adults	Color of Malpighian Tubes of Larvae	
	Without extra riboflavin feeding	With extra riboflavin feeding
p^p, st, ca, w^a, w^e	colorless	colorless
cm	very pale yellow	very pale yellow
bw, cn, cn bw, st, v	pale yellow	pale yellow
bur, cn, car	pale yellow	deep orange
glass-like (DIS-25), pr, pu, ras^2, se	bright yellow	deep orange

From the above it is evident that the feeding of riboflavin accentuates the difference between the colorless and the bright yellow (= normal) tubes, and also between the colorless and certain of the pale yellows, as well as between the other pale yellows and those ordinarily bright yellow. Hence, this method should be useful for the better discrimination of larvae of different genotypes, whose classification would otherwise be more difficult and uncertain. At the same time, interesting biochemical problems are raised concerning the basis of these differences in the treatment of riboflavin by Malpighian cells of different genotypes, and the relation of this to the processes occurring in the development of eye pigments.

Oster, Irwin I. An analysis of ultraviolet-induced lethals due to gene mutation.

Medvedev (1938), in a developmental analysis of 30 cases of sex-linked lethals that had arisen spontaneously and that showed no apparent changes in their salivary-gland chromosomes, found that 15 exerted their lethal effect during the pupal stage. (It is not stated to what extent the possibility of allelism among these lethals had been ruled out.) In 1939 he reported that of 12 of these same spontaneous sex-linked lethals, which killed in some prepupal stage, 2 caused death during the embryonic period, 9 during the 1st larval instar, and 1 during the 2nd larval instar. Five others among the total of 30 were observed to die at about the time of pupation. He interpreted this apparent grouping as showing three "sensitive periods"--in the embryo, the first instar, and the time of pupation. Poulson (1937 et seq.) found that deficiencies usually cause embryonic death, as might be expected in view of their involving multi-locus losses, since the earliest-acting loss would be the one whose effects were seen. Hadorn (1948) tabulated the stages of death for 38 lethals reported on in previous work of various authors.

Death was found to occur at various stages, and Hadorn interpreted the results as showing a clustering of deaths at four periods: the early embryo, the period just before or after hatching from the egg case, just before or after pupa formation, and just before or after eclosion from the pupa case. He regards "phases of high developmental activity" as especially subject to the action of lethals. However, since many of the lethals were known to be allelic, and many were deficiencies, the collected data which he presented are such as to provide little evidence of what the relative frequency of death at different stages would be for gene mutations.

We had at our disposal a group of sex-linked lethals which had been obtained by ultraviolet irradiation of D. melanogaster spermatozoa in experiments by J. T. McQuate. These had been localized by McQuate in linkage tests and had then been determined by Dr. J. I. Valencia by cytological examination of the appropriate portions of the salivary-gland chromosomes to entail no visible structural changes of the X chromosome(see Valencia and McQuate, Rec. Gen. Soc. Amer., 1951). Thus these cases represent point mutations and are suited for a study of the action of individual genes on the physiologival processes involved in development. The lethals were contained in balanced stocks, with the lethal X chromosome distinguished from its homologue by the markers yellow body (y) and white eye (w). These allow the male larvae with the lethal chromosome to be recognized by their light mouth parts (due to y) and their colorless Malpighian tubes (due to w). Death in embryonic stages was shown by the presence of brwonish eggs and the absence of y w larvae. When death occurred in larval stages, the instar to which viability extended could be determined by the morphology of the spiracles. The moribund larvae were observed and dissected in insect Ringer's solution.

Forty-three of the ultraviolet-induced gene-mutational lethals were subjected to the above type of study. Of these only 4 were found to cause the death of the embryo (in all cases this occurred during the late embryonic stage), 21 caused death during the first larval instar, none during the second larval instar (or at least this was not the limit beyond which they could not pass), 7 during the third larval instar, 8 during the early pupal period, none during the middle pupal period (with the same qualification as above), and 3 during the late pupal period. Of these 43 stocks, 36 showed no morphological abnormalities as determined by gross inspection and dissection of the dead or dying stage, while 7 exhibited some derangement which might be related to the cause of death. The locus in chromosome 1, and the characteristic effects, of these 7 lethals are as follows:

1jl: lethal jawless. 14. Dies during the first larval instar, mouthparts poorly formed and sometimes absent.

1ml: melanoma-like. 1. Dies during the third larval instar; larvae at time of death have internal black and brown melanotic masses (usually one or two, sometimes as many as ten), may represent a malignancy.

1rr: lethal, ring gland rudimentary. 0.3. Dies during the third larval instar, reduction of the ring gland with associated failure to undergo the third molt; otherwise normal, may live 15-30 days.

1te: lethal, tracheae enlarged. 0.3. Dies during the third larval instar; main tracheal tubes greatly enlarged, may lack functional posterior spiracles.

1tl: lethal, tracheae lacking. 59. Dies during the first larval instar; no evidence of main tracheal tubes, although small side branches are present.

1tr: lethal, tracheae ramified. 56. Dies during the first larval instar; main tracheal tubes are thick and have very many side branches.

1ts: lethal, tracheae stretched. 8. Dies during the first larval instar; very large larvae; all the tracheal tubes are very thin, suggesting

that they do not grow at the same rate as the larvae and thus become stretched.

A consideration of our data indicates that there is a clustering of mortality at the end of embryonic life, at the beginning and end of larval life, and at the beginning and end of pupal life. These are all stages where many newly developed parts and processes become functionally active for the first time. The lethal gene may have influenced development earlier, but the effect of this did not become exerted on the survival until the given part or process was called on to participate in some vital physiological function. This is well illustrated in stock lrr. These larvae remain small, and live about 15 days as third-instar larvae but are unable to pupate because of a reduced ring gland. However, the ring-gland deficiency already was determined during the first and second larval instars by a failure of the gland's cells to enlarge. Although the greatest developmental changes of all take place in the embryo, relatively few lethals were found to act there, doubtless because functioning of the new parts is not yet active and they are not so important for life. On the other hand, a great preponderance of lethals express themselves during the first larval instar, when a manifold physiological (nervous, secretory, muscular, circulatory, etc.) processes characteristic of larval life are required. Similarly, during the end of larval and the beginning of pupal life, new and different physiological processes must take place and thus many lethals were found which express themselves then. Furthermore, the fact that no lethals were found which act mainly during the second instar was to be expected, since that period is not characterized by radically new physiological functions but only by slight morphological changes.

In addition to the above cases of gene mutation, one case of ultraviolet-induced deficiency (bands 15A2-3 to 15C4-5, according to J. I. Valencia) and one of translocation were studied similarly. The former proved to be lethal to the embryo, like most other deficiencies, although death occurred at a late embryonic stage. The latter caused death during the first larval instar.

Prevosti, A. The vti and vli characters in a wild population of D. subobscura. Collins.

The frequency of the characters vti (venae transv. incompl.) and vli (venae long. incompl.) has been studied in a population of subobscura of Barcelona, using the method of inbreeding by F_1 pair matings. The experimental cultures were reared at 25° C, a temperature almost at the upper limit for the subsistence of the species, and greater than those employed by Buzzati-Traverso and Gordon, Spurway, and Street in their studies on natural populations of the same species. The results obtained by these authors, and my own results, can be summarized as follows:

	Gordon, Spurway, and Street			Buzzati-Traverso	Prevosti
	Slough	New Forest	Studland	Bellumo	Barcelona
% of wild ♀♀ with vti in their descendants	38.3±7.1	26.2±6.8	25.5±5.7	15.5±3.9	61.5±9.5
with vli	23.4±6.2	11.9±5.0	20.0±5.4	8.3±3.0	34.6±9.3

The greater frequency of these characters in the population of Barcelona may be atttibuted, at least partically, to the higher temperature of the experiment, for other experiments show that their penetrance increases greatly with the temperature. When eggs laid by the same female were reared at 20° C and 27° C, the average increase of penetrance with the higher temperature was from 45.9±2.6 to 87.2±2.4 per cent for vti, and from 28.4±2.2 to 89.5±5.0 per cent