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Studies on Lethal Factors in *Drosophila*
I. The Time of Action of Some Sex-Linked Recessive Lethals Induced by Ultrasonics.*

By

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A Mendelian unit which causes the deah of the individual before the reproductive age is reached may be designated as a lethal. Although it is well known that lethals are most common among mutations, there is little knowledge of their time and manner of actions. LI (1927), POULSON (1940, 1945), and HADORN (1948, 1951) investigated the details of the action of certain recessive lethals and chromosome deficiencies in *Drosophila melanogaster*. MEDVEDEV (1939), OSTER (1952), RIZKI (1952), and SETO (1954) reported the time of action of lethals of spontaneous occurrence and of X-ray and ultra-violet induced origins.

This paper deals with the results of studies on the time of action of 20 ultrasonic induced X-chromosome recessive lethals in *Drosophila melanogaster*. (SHIOMI, 1955).

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Materials and Methods

Sex-linked recessive lethal stocks used for materials in the present study were obtained from KATO who exposed wild *Tokyo* males of *Drosophila melanogaster* to ultrasonics in 1953 (KATO, 1955). The lethal containing X-chromosomes were extracted by means of the Muller-5 method and were balanced with *Muller-5* (*Basc*) balancer. These stocks are called *lethal* (1)-*ultrasonic* induced strains (abbr. *l-us*).

In order to determine the time of action of the lethal factors the following

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method was employed. Three males and a single female, 3 days after emergence and well nourished, were put in each glass tube of 3 cm in diameter and 7 cm in length which stood on Petri dish containing culture medium and was stopped with a cotton plug at the top. After 12 to 24 hours, when an adequate number of eggs was laid, the tubes were removed leaving the eggs for observation. Under the condition of this work (at 25°C), the majority of eggs hatched within 24 hours after oviposition. As there was some delay in hatching, hatched and unhatched eggs were counted twice, firstly 24 hours and secondly 48 hours after removing the flies from the Petri dish. Not only lethal eggs but those whose death is not due to lethal factors were included among unhatched eggs. The unhatched eggs were dechorionated with 3% solution of sodium hypochlorite (or in commercial Antiformine) and brought under the microscope to examine at what stage the eggs had ceased to develop. As had been pointed out by HADORN (1951) lethal eggs that die at a very early stage of development cannot be distinguished from the unfertilized ones without cytological examination. Therefore, both the direct count and microscopical examination of the unhatched eggs do not yield the mortality of lethal eggs, it only provides the total mortality of eggs laid. This fact is not restricted to the egg stage but to all stages of development. Thus the statistical comparison of total mortality in each stage of the progenies of the lethal strains and those of the control crosses was necessitated to get the true mortality of lethal embryos in addition to morphological observation. For the control cross in the present work, *Oregon-RS* wild, *Muller-5*, and *Tokyo/Muller-5* × *Muller-5* were used to get the hatchability and the stage mortalities of non-lethal ones.

It is rather difficult to know the number of larvae of the first instar due to their small size, and therefore the number of hatched eggs was taken for the number of the larvae. The larvae of the second or third instar were transferred to small culture bottles to allow further development to imagos. The difference between the number of hatched eggs and that of pupae provided a measure of the total larval mortality, and the total pupal mortality was estimated by subtracting the number of empty cases from the total pupal number. By examining eyes of dead pupae it was possible to eliminate some of occasional death not due to lethal factors from the total pupal mortality. Because, as the X-chromosome of *Muller-5* carries *Bar* and *apricot* genes it is clear that dead pupae whose eye characters are of *B wa* or *B* (in female) and *B wa* (in male) ought not be killed by the action of the recessive lethal factor while death of pupae of wild eye character was supposed to be due to the action of lethal factors in hemizygous condition. Number of imaginal death caused by the lethal factors was obtained by counting dead male imagos of wild eye character.

For determination of the loci of lethal factors on X-chromosomes, females of each strain were crossed to *white miniature* and *yellow white forked* males and F_1 females carrying lethal factors were backcrossed to *w m* and *y w f* males re-

spectively. From the recombination value of F_2 segregation the loci were calculated.

Results

The results obtained are listed in Table 1. In the majority of the strains the rate of tolal unhatched eggs does not or slightly exceeds that of the controls, but in three strains (i.e. *l–us* 20, 33, and 48) it is distinctly higher. Except the three strains the microscopical examination of unhatched eggs of the strains reveals that they are very much alike in appearance to unfertilized eggs, the condition is quite similar in the controls. These eggs underwent autolysis at the time of the second count. Contrary to these, most of the unhatched eggs in three strains are in late embryonic stage, so it can reasonably be surmised that the action of lethal factors concerns the cessation of the embryonic development in these cases.

During larval development, death caused by the action of the lethal factors occurs more frequently than in the egg stage in most of the strains except two (*l–us* 20, and 48), in which the mortality of larvae is far less than that of the eggs and about the larval mortality of *Muller–5*. It is remarkable that the larval death of *l–us* 15 mainly happens both late in the third instar and early in the pupal stage. This fact would mean that the lethal factor becomes effective at the larval-pupal boundary.

Usually the pupal mortality is low both in mutants and in controls, although in *l–us* 15 it is highest in this stage. During pupal stage imaginal eyes gradually become developed to such an extent that one can distinguish the wild character from those of *Bar* or *Bar apricot*. As stated before the individual whose eye character is of the wild type carries the recessive lethal factor in hemizygous condition, and such pupae are included among dead pupae of seven strains (*l–us* 9, 16, 17, 22, 33, 46, and 47).

Imaginal death in the present work means the death of imagos just at or shortly after the emergence from pupal cases. There are some which partially emerge and these cases are also included in imaginal death. Occurrence of imaginal death is recognized in six strains, in three of which (*l–us* 33, 46, and 47) dead imagos are mostly males of the wild eye type. Wings of these lethal flies died after emergence are alike in appearance to those of *rudimentary* mutant.

The total mortality in the whole period from the egg to the early imaginal stage of the lethal strains lies between 27.9% and 41.0%. The mortality exceeds more or less the expected mortality caused by the recessive lethal factor located on the X-chromosome.

Based on the results mentioned above, the lethals may be sorted in the following four groups according to the stage at which death is brought about. They are 1) two egg lethals of late embryonic (E), 2) ten larval (L), 3) one larval-pupal boundary (L/P), and 4) seven lethals in which time of death is not

Table 1. Mortality and time of action of lethal factors.

Strains	Eggs laid	Eggs unhatched	%	Larvae died	%/E	Pupae unemerged	%/E	Imagos died	%/E	*M-5* ♂	*l/M-5* ♀	*M-5/M-5* ♀	Imagos total	Total Mortality %/E	Lethal stage
l-us 2	600	13	2.2	165	*27.5*	33	5.5	—	—	123	147	119	389	35.2	L
l-us 5	515	42	8.2	122	*23.7*	4	0.8	6	1.2	99	120	122	341	33.9	L
l-us 6	459	26	5.7	109	*23.7*	11	2.4	—	—	85	116	112	313	31.8	L
l-us 7	978	35	3.6	267	*27.3*	27	2.8	—	—	175	253	221	649	33.7	L
l-us 9	700	45	6.4	150	*21.4*	48	*6.9*	—	—	124	193	140	457	34.7	L-P
l-us 11	1508	108	7.2	437	*29.0*	73	4.8	—	—	269	347	274	890	41.0	L
l-us 12	568	30	5.5	154	*27.1*	11	1.9	—	—	123	136	114	373	34.5	L
l-us 13	768	40	5.2	222	*28.9*	31	4.0	—	—	139	156	180	475	38.1	L
l-us 14	484	29	6.0	132	*27.3*	12	2.5	—	—	98	101	112	311	35.8	L
l-us 15	632	39	6.2	76	*12.0*	98	*15.5*	2	0.3	151	114	152	417	34.0	L/P
l-us 16	545	26	4.8	117	*21.5*	54	*9.9*	—	—	91	134	123	348	36.2	L-P
l-us 17	486	37	7.6	97	*20.0*	33	*6.8*	—	—	111	104	104	319	34.4	L-P
l-us 20	626	195	*31.2*	37	5.9	14	2.2	—	—	111	142	127	380	39.3	E
l-us 22	518	26	5.0	82	*15.8*	37	*7.1*	—	—	120	128	125	373	27.9	L-P
l-us 25	505	18	3.6	147	*29.1*	12	2.4	—	—	116	97	115	328	35.1	L
l-us 26	540	31	5.7	128	*23.7*	20	3.7	—	—	117	130	114	361	33.1	L
l-us 33	1334	165	*12.4*	290	*21.7*	38	*2.8*	35	*2.6*	278	295	233	806	39.5	E-I
l-us 46	540	24	4.4	133	*24.6*	22	*4.1*	26	*4.8*	74	138	122	360	37.9	I-I
l-us 47	730	66	9.0	100	*13.7*	73	*10.0*	27	3.7	113	178	173	464	36.4	L-I
l-us 48	703	217	*30.9*	55	7.8	14	2.0	2	0.3	111	169	135	415	41.0	E
Oregon -RS	581	18	3.7	21	3.6	4	0.7	—	255 (wild ♂)		283 (wild ♀)		538	8.0	
Muller -5	500	36	7.2	37	7.4	6	1.2	—	—	203	—	218	421	15.8	
Tokyo/ Muller-5	496	17	3.4	18	3.6	2	0.4	—	85 (wild ♂)		101 (+/*M-5*)	92	359	7.4	

* *M-5*: *Muller-5*

Table 2. The loci of lethal factors on X-chromosome.

Lethal strains \ Mutant crossed	*w m*	*y w f*	Dosages of Ultrasonics **	Lethal stage
l-us 2	48.5	56.1		L
l-us 5	50.8	56.1		L
l-us 6	48.5	56.1		L
l-us 7	51.6	55.7		L
l-us 9	56.1	54.0		L-P
l-us 11	51.7	56.5		L
l-us 12	52.9	52.4	560 K C	L
l-us 13	48.2	55.9	1,500 V	L
l-us 14	56.5	55.9	250 mA	L
l-us 15	51.6	56.0	5 min.	L/P
l-us 16	52.6	56.5		L-P
l-us 17	51.1	54.8		L-P
l-us 20	50.5	56.5		E
l-us 22*	—	—		L-P
l-us 25	47.6	53.3		L
l-us 26	47.4	56.5		L
l-us 33	26.3	27.3	560KC, 1,500 V 250 mA, 30 min.	E-I
l-us 46	28.1	31.0	450 KC 1,500 V 200 mA 5 min.	L-I
l-us 47	26.3	28.4		L-I
l-us 48	33.9	56.0		E

* Back to wild and discarded. ** From KATO (1955)

restricted to one stage but extends over two or more stages of development. Among the latter seven lethals, death occurs both in larval and pupal stages (L–P) in four strains, from larval to imaginal stage (L–I) in two strains and in the remaining one the time extends over all successive stages from egg to imaginal (E–I).

The loci of the lethal factors are roughly estimated from recombination values obtained by crossing with *w m* and with *y w f* (Table 2). In some lethals, two recombination values fairly approximate each other while in some others *l–us* 48 for example, the values are so widely apart that one may imagine that the crossing over is suppressed by some kind of chromosomal aberrations. Moreover, it would not exclude the possibility that the lethal factor itself is the chromosomal aberration.

There is a strong tendency that loci of factors produced by same dosage of ultrasonic vibration are concentrating in a rather small portion of the X-chromosome. For example, 15 lethal factors produced by ultrasonic treatments of the same dosage (560 KC, 1,500 V, 250 mA for 5 min.) seem to be located near *forked* or between *garnet* and *forked*. In the present work it is not possible to

decide whether those factors are same mutations, alleles or different mutations. Usually, cross experiments are employed to test the identity of mutations but in the present cases they are not practicable, since these lethals are all hemizygous in males and die in the course of development. However, from differences in the time of action of the lethal factor it is clear that some of the factors are not identical mutation with each other.

Among the lethals employed, it is noted that heterozygous females (*l/Muller-5*) are more numerous than those expected from the theoretical point of view and they develop faster than their normal mates. Whether the superviability is a consequence of faster development under favorable laboratory conditions or that females heterozygous for the lethal factor have a real heterotic superiority in rigorous competition is not easy to be solved.

Discussion

Of lethal *Drosophila* mutants HADORN (1951, 1955) classified the great majority into eight categories according to the stage at which their development comes to a halt and death occurs. He also emphasized that the mutants have their definite lethal stage, in other words, there are "phase specificities". It is true that there are many reports which seem to corroborate his concept of the phase specificity (MEDVEDEV, 1939; RIZKI, 1952; SETO, 1954), although some workers would not agree to his opinion because some lethal factors investigated by them seem to exert their effects not at any definite stage of development (BREHME, 1937; KALISS, 1939; BRODY, 1940). BRODY states that such breakdown of the developmental process into specific sensitive periods is only arbitrary since the development itself is a continuous process in nature.

On the viewpoint of the phase specificity the results obtained in the present work seem to be conflicting. Both in *l-us* 20 and 48 the egg stage is fatal, 31.2% and 30.9% of eggs laid die in this stage respectively. In 10 strains it is the larval stage in which about 25% of the total progenies die. Thus the phase specificity may be recognized at least for these cases. Contrary to these, however, the time of death extends over two or more stages in the remaining cases, and such phenomena have been reported by LI (1927) for *tetra-IV* and by BREHME (1937) for *ClB* lethal in which lethal effect appears chiefly in the egg stage but partly in the larval stage.

In discussing the problem of phase specificity of lethal factors it should be considered that the manifestation of the factor can be influenced by the "genotypic milieu" and some other factors such as environment. The viability, in the same time, may be suffered by lethal factors not in some definite stage or stages but through whole process of development. Such lowering of viability is not an unusual phenomenon among visible mutants, and in the controls of the present work viability of *Muller-5* is lower than those of the other two in every

stage. On the other hand, the result obtained shows that development may go beyond the critical stage. Presumably, various numbers of individuals survive the critical period and are able to develop further. Such "break-throughs" (Hadorn, 1948) may die later during the second or third critical points. Thus there may be a possibility of concealing locus-specific pattern of lethal factors by general weakness and by "break-throughs" even if there is phase specificity. Under the present results, therefore, it is uncertain that whether the seven strains (i. e., *l-us* 9, 16, 17, 33, 46, and 47) have the phase-specific lethal stage and whether locus-specific pattern of damage exist in them.

Hereafter the investigation would be concentrated on the biochemical and physiological analysis of the developmental history of the pattern of damages as well as morphological observations.

Summary

1. The time of action of 20 sex-linked ultrasonic induced recessive lethals of *Drosophila melanogaster* was determined.
2. The lethals were distributed as follows: two egg lethals (E), ten larval lethals (L), and one larval-pupal boundary case (L/P). Seven lethals were affected at several stages.
3. Of the seven which were affected at several stages, four were affected from larval to pupal (L-P), two from larval to imaginal (L-I), and one at all stages (E-I).
4. The loci of lethal factors on X-chromosome were roughly estimated.
5. A brief consideration on lethals which seem to have no phase specificity is given.

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