Title
DEVELOPMENTAL EFFECTS OF X RAYS ON EMBRYOS OF DROSOPHILA MELANOGASTER

Author
Hildreth, Philip E.

Publication Date
1967-08-08

UCRL-17720
c2

University of California

Ernest O. Lawrence Radiation Laboratory

DEVELOPMENTAL EFFECTS OF X RAYS ON EMBRYOS OF DROSOPHILA MELANOGASTER

Philip E. Hildreth

August 8, 1967

Berkeley, California

DISCLAIMER

Submitted to the Proceedings of the National Academy of Science (U.S.)

UCRL-17720
Preprint

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

DEVELOPMENTAL EFFECTS OF X RAYS ON EMBRYOS OF DROSOPHILA MELANOGASTER

Philip E. Hildreth

August 8, 1967

DEVELOPMENTAL EFFECTS OF X RAYS ON EMBRYOS OF DROSOPHILA MELANOGASTER*

Philip E. Hildreth**

Lawrence Radiation Laboratory and Department of Zoology
University of California
Berkeley, California

August 8, 1967

In Drosophila melanogaster the gene erupt (er) causes malformation of the eye. Another gene, suppressor of erupt (Su-er), effectively blocks the action of er so that Su-er/Su-er; er/er individuals develop with normal eyes. It was reported (Glass and Plaine[1], and Glass[2]) that X irradiation of embryos homozygous for these two genes causes inactivation of the suppressor so that the gene er manifests its effect. Inactivation could be produced even if the egg were irradiated immediately after fertilization and before pronuclear fusion, thus indicating a very early time of gene action of Su-er. Experiments in our laboratory (Hildreth[3]) failed to completely corroborate these findings possibly due to genetic differences in the stocks used; inactivation resulted when 18-hour old embryos were irradiated, but did not when embryos one half hour old and younger were subjected to the same treatment. It was decided to continue the investigations in order to determine more precisely at which developmental stage the suppressor effect can first be inactivated by X rays. The results of this investigation are reported below.

MATERIALS AND METHODS. — The flies used were homozygous for Su-er bw (brown eyes); st (scarlet eyes) er and were from the same stock B 91; 83 used in our previous experiments. The techniques for culturing and mating the flies, collecting and treating the eggs, and classifying the eye phenotype are basically the same as those given in the earlier report.[3] Eggs to be irradiated were collected over either 10 or 20-minute periods and control samples were always obtained from the same females on the same day as for the treated series. The times at which the flies were mass mated, and the eggs collected and irradiated, were recorded. In some experiments, in order to determine if the embryos were homogeneous in age, we examined eggs at hourly intervals and the numbers of eggs hatched was recorded. X rays were administered by the same Andrex industrial machine and under the same conditions as reported previously, the total dose being 1000r delivered at about 105 r/minute.

RESULTS. — Table 1 shows that X rays administered to embryos six hours old, or older, have a strong effect in blocking the action of Su-er. Whether the gene effect is inactivated when younger embryos are irradiated is questionable. Among embryos five hours old and younger the frequency of the extreme-erupt phenotype is less in the irradiated than in the control group ($\chi^2 = 0.0109$, D. F. = 1, P = 0.90 to 0.95). Even in the 3 and $3\frac{1}{2}$-hour series in which the frequencies among the treated individuals are higher (0.46 and 2.70% respectively) than for their controls (0.15 and 1.91% respectively), the deviations are non-significant, the P values being 0.3 - 0.5 for the former and 0.7 - 0.8 for the latter age group. Apparently X rays do not

inactivate the suppressor effect in the young embryos to the extent that the extreme-erupt phenotype is often produced.

An analysis of the total frequencies of erupt-phenotype individuals fails to disclose conclusive evidence that X rays even inactivate the suppressor effect to the extent that the weak-erupt phenotype is produced more frequently in the treated than in the control groups. Among the seven age groups from one to five hours old, four have a higher frequency of erupt individuals among the controls, two have a higher frequency among the treated individuals, and in the seventh group no adults eclosed in the treated series. A homogeneity test of the seven control groups yields a χ^2 value of more than 110 with six degrees of freedom (P value much less than 0.0001). Obviously the control data are extremely heterogeneous. The total frequency of erupt individuals is significantly higher in the group irradiated when three hours old than in the controls ($\chi^2 = 19.67$, D. F. = 1, $P < 0.0001$) and also in the group irradiated when $3\frac{1}{2}$ hours old ($\chi^2 = 6.3972$, D. F. = 1, P = 0.01 - 0.02). The total frequency of erupt individuals from embryos irradiated when five hours old or less is also significantly higher than for the controls ($\chi^2 = 14.30$, D. F. = 1, P = 0.0001 - 0.0002). Because of the heterogeneity of the data, one should be aware that the deviation observed may have arisen from causes other than the X-ray treatment. When embryos six hours or more old were irradiated, the frequency of the extreme-erupt phenotype was increased so greatly over that among the controls that a statistical analysis of these data would be superfluous. The fact that 50% of the adults arising from eggs treated six hours after oviposition show the extreme-erupt phenotype may be misleading

unless one realizes that only two individuals from the 3574 eggs treated survived to adulthood. However, there can be little doubt of the effectiveness of X rays in causing inactivation of the gene effect when administered to embryos older than six hours.

In Table 2 are presented the average numbers of eggs laid per hour per female, and the maximum age possible for an embryo when irradiated, if an egg was fertilized immediately after the males were placed with the females and was retained until the last collection for that day. Where two egg-laying rates are given, experiments were conducted on two or more days and the high and low rates are indicated.

If the developmental ages of eggs collected over a ten-minute period do not vary by more than ten minutes, then it would be expected that all eggs would hatch within a few minutes of each other. The assumption is that the genetic backgrounds and external environmental conditions are identical for all eggs tested. The stocks were not made isogenic, so undoubtedly the genetic background varied. In two experiments eggs were collected over ten-minute periods and observed at hourly intervals from 19 to 27 hours after oviposition and then again the next day. No eggs hatched before 19 hours and a few hatched more than 27 hours after oviposition. The results are presented graphically in Fig. 1. The wide range in time over which the eggs hatched could reflect the different developmental rates among eggs that were homogeneous in age when collected or could have resulted from eggs being in different developmental stages when oviposited.

Eggs were collected from flies that were mass-mated, without assurance that every female had copulated or was inseminated; therefore there were unfertilized eggs among the samples. In Table 3 are

recorded the numbers of eggs and the numbers that survived to adulthood. The adjusted % survival is equated to the % survival in the controls for each developmental age group.

$$\left(\frac{(\text{No. of adults eclosed from treated eggs})(100)}{\left(\frac{\text{No. of control adults eclosed}}{\text{No. of control eggs}}\right)(\text{No. of treated eggs})} \right)$$

Thus the adjusted % is higher than the actual %. With the exception of that for the 15-hour embryos the eclosion frequency was higher for the control than for the treated group. The killing effect of X rays is extreme during certain developmental stages but slight during other stages. Other interesting aspects of sensitivity to X rays are observed when one plots hatching of the eggs and survival from the time of hatching to eclosion of the adults. The results are presented in Fig. 2.

The information on mortality during embryonic and later stages, given in Fig. 2, is presented as an adjunct to the main purpose of the report. What is shown is first the probability that an egg will hatch after being irradiated at a specific developmental stage, and second the probability that the emerged larva will survive to adulthood. Irradiation causes the greatest depression in hatching frequency when administered to one-hour old embryos. An interesting aspect is that the larvae that survived then had a very high chance of becoming adults. A similar situation has been described for individuals that have genes causing lethality, yet occasionally even though they have the genetically lethal constitution, some individuals survive to adulthood. These individuals that have overcome the crisis in development have been called "escapers"

by Hadorn[4]. When embryos seven and eight hours old are irradiated there is a high probability that the eggs will hatch, yet a low probability that the larvae will become adults. When embryos older than nine hours are irradiated, the probability for both hatching and eclosion are high. Those individuals from 0 to 2 hours old and from 4 to 8 hours old are very sensitive to X radiation, yet those individuals from $2\frac{1}{2}$ to $3\frac{1}{2}$ hours old are much less sensitive. It was not intended to investigate aspects of mortality, and therefore critical analyses of precise stages and causes of death were not made. It is intended to continue research on the mortality effects of X rays administered during the various stages of development, but with vigorous stocks that are better suited for the purpose.

DISCUSSION. — The inactivating influence of X rays on the Su-er system becomes clearly apparent only when embryos six hours old, and older, are irradiated. In fact, one cannot be certain that the inactivating effect occurs in six-hour embryos. We know that the eggs were laid six hours prior to being irradiated but we can not be sure what the true developmental age was when treated. The way to be certain of the true age is to do a histological study of the embryo but then, of course, there would be no adults to classify. In the earlier cytological analysis,[3] slightly more than 92% of the eggs, collected over ten-minute periods and immediately fixed, were in stages developmentally restricted to about 30 minutes (anaphase I of meiosis to second cleavage). Among the 10 remaining eggs, six were in 3rd, two in 4th, and two in 7th or 8th cleavage stages; the latter two would be, developmentally, approximately 78 to 93 minutes old. If the eggs from which

the data for Fig. 1 were gathered were as homogeneous in age, then as development proceeds asynchrony must result. An alternative is that the eggs sampled here were not as homogeneous in age as those studied cytologically. The egg-laying rates from one experiment to another varied from about 1 to 3 eggs per female per hour. That this is not a rapid rate is evidenced by the fact that under the same conditions, wildtype stocks generally lay at least 6 to 8 and frequently 10 to 12 eggs per female per hour. It would not be prudent to equate a slow rate of egg laying with retention of eggs after fertilization; it is possible that eggs are produced slowly but then are laid soon after fertilization. The cytological evidence tends to support this latter view. At any rate, we must be aware that despite all the precautions taken to ensure homogeneity in the ages of the embryos, there will probably be a degree of heterogeneity that must be considered in the final analysis. Thus, it is possible, and perhaps, even likely as evidenced from the cytological and time-of-hatching data that the 0.06% and 0.33% survivors classified in the 6 and 7 hour groups respectively (see Tables 1 and 3) are developmentally older than is indicated. If true, then the effective period for inactivation of the Su-er gene effect would be when the embryo is 8, 9, or perhaps 10 or more hours old. The cells from which the eyes develop originate in the frontal sacs, which are ectodermal evaginations from the dorsal wall of the pharyngeal cavity. According to Rabinowitz[5] and Poulson,[6] stomodaeal invagination begins at $5\frac{1}{2}$ hours after oviposition and evagination of the frontal sacs occurs at about $10\frac{1}{2}$ hours. From the evidence presented herein, it would appear that X irradiation causes inactivation of the suppressor effect only if

administered after stomodaeal invagination begins and possibly only if administered during or after the time of frontal-sac formation.

SUMMARY. — In Drosophila melanogaster the effect of the gene er (erupt eyes) is suppressed by the gene Su-er (suppressor of erupt); thus, Su-er/Su-er; er/er individuals have normal eyes. X irradiation causes inactivation of the suppressor effect. Embryos from one to fifteen hours old were irradiated with 1000r or X rays to determine the earliest stage during which inactivation would result. Embryos six hours old are the youngest in which inactivation by X rays resulted in a significant increase in the expression of the extreme-erupt phenotype. Whether X rays cause inactivation in embryos younger than six hours is questionable. It is suggested that inactivation results, probably, if the embryos are in or beyond the stage of stomodaeal invagination when irradiated. Data on the frequencies of hatching and eclosion, after irradiation of the above embryonic stages, are presented.

The author is grateful to Dr. Curt Stern for his comments and suggestions and Mrs. Cole Ulrichs for her help in conducting the experiments.

*This work was done under the auspices of the U. S. Atomic Energy Commission.

**Present address: Department of Biology, University of North Carolina at Charlotte, Charlotte, N. C. 28205.

[1]Glass, B., and H. L. Plaine, these Proceedings, 36, 627 (1950).

[2]Glass, B., Science, 126, 683 (1957).

[3]Hildreth, P.E., these Proceedings, 54, 736 (1965).

[4]Hadorn, E., Developmental Genetics & Lethal Factors (Methuen & Co. LTD., London, 1961).

[5]Rabinowitz, M. J., J. Morphol., 69, 1 (1941).

[6]Poulson, D. F., in Biology of Drosophila, ed. M. Demerec (New York, John Wiley, 1950), Chap. 3.

FIGURE LEGENDS

Fig. 1. Hours from egg laying to hatching. In one experiment (-----), a total of 142 eggs hatched from females laying at the rate of 1.3/♀/hr. In the second (————), 110 eggs hatched from females laying at the rate of 1.4/♀/hr.

Fig. 2. The effects of irradiation on the survival rate of eggs of different ages.

Table 1. Influence of X Rays in Blocking the Action of Su-er When Eggs Were Irradiated at Specified Hours after Oviposition.*

*The minimum number of adults scored was 133 (2.5-hr control) and the maximum was 655 (3-hr control).

†Eggs collected over ten-minute periods; therefore age is 1 hr ± 5 min. All other collections over 20-minute periods and ages are ± 10 min.

Eggs	Adults							
Age when X-rayed (hr)	Number	Eclosed	Erupt Phenotype Total	%	Control	Extreme	%	Control
1†	3014	15	0	0		0	0	0.97
2	4669	149	2	1.34	2.06	0	0	0.40
2½	1564	203	27	13.30	15.79	2	0.99	1.50
3	6110	1940	97	5.00	1.07	9	0.46	0.15
3½	2099	185	35	18.92	10.83	5	2.70	1.91
4	2156	2	0	0	0.36	0	0	0
5	2398	0	-	-	2.80	-	-	0.80
Total	22010	2494	161	6.46		16	0.64	
Control	4133	2253	90	3.99		15	0.67	
6	3574	2	1	50.00	14.14	1	50.00	1.01
7	3021	10	10	100	6.77	9	90.00	1.99
8	2608	117	104	88.89	10.75	87	74.36	1.08
9	1135	377	359	95.23	12.00	262	69.50	1.43
10	430	146	121	82.88	1.93	87	59.59	0.55
15	519	314	299	95.22	5.89	265	84.39	1.31
Total	11287	966	894	92.55		711	73.60	
Control	2811	1235	115	9.31		17	1.38	

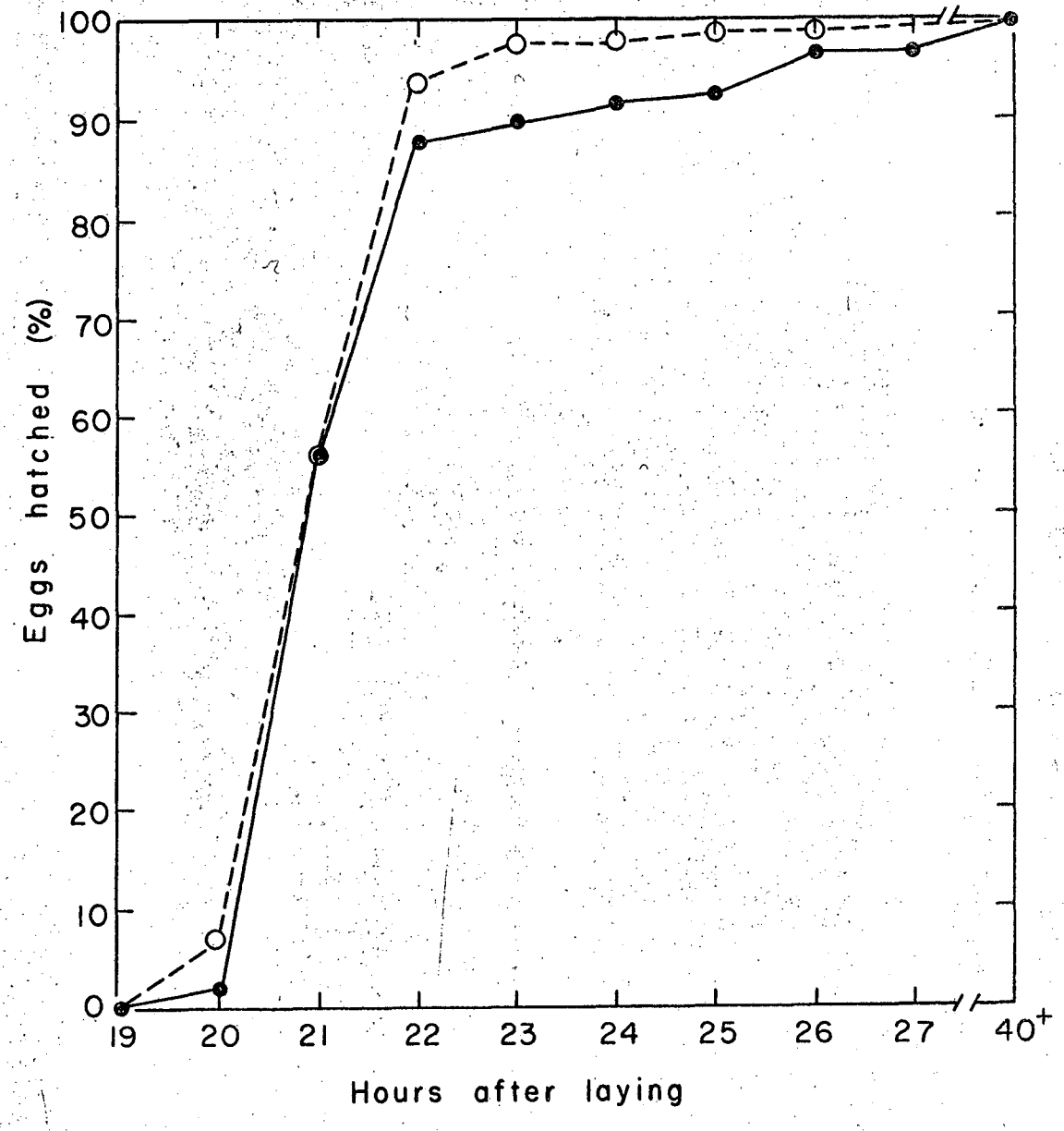
Table 2. Egg-Laying Rates and Maximum Ages of Eggs Possible If Fertilized Immediately after Males and Females Were Placed Together and the Eggs Were Retained until Time of Last Collection.

Age of eggs at irradiation		Eggs/♀/hr	Maximum age if retained	
Hours	Minutes		Hours	Minutes
1 ± 5		1.1 - 1.5	8	23
2 ± 10		1.1 - 2.9	9	32
2½ ± 10		1.5 - 1.8	9	46
3 ± 10		0.9 - 2.6	11	15
3½ ± 10		1.5 - 1.9	9	46
4 ± 10		2.2 - 2.4	9	45
5 ± 10		1.1 - 1.9	14	15
6 ± 10		1.3 - 2.2	12	40
7 ± 10		2.0 - 2.8	14	45
8 ± 10		1.5 - 2.8	14	30
9 ± 10		1.5 - 2.8	14	20
10 ± 10		1.2	16	16
15 ± 10		1.4	21	23

Table 3. Survival Frequencies from Egg to Adult.

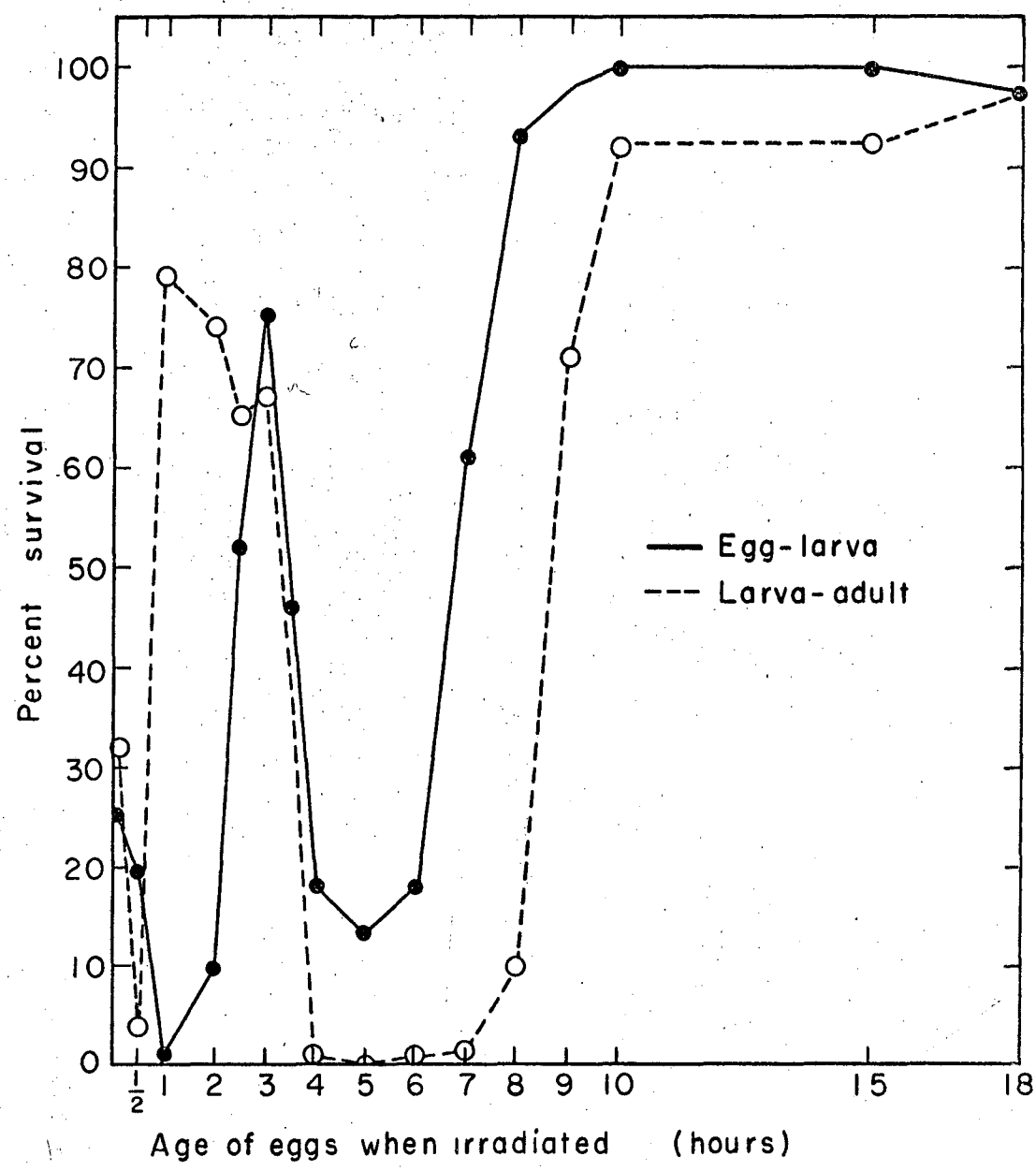
Eggs	Adults Eclosed				
Age when irradiated	Total	Total	%	% in Control	Adjusted %
$5\frac{1}{2}$ min*	3134	133	4.24	55.70	7.62
30 min*	1334	6	0.45	55.70	0.81
1 hr	3014	15	0.50	65.64	0.76
2 hr	4669	149	3.19	43.09	7.40
$2\frac{1}{2}$ hr	1564	203	12.98	38.33	33.89
3 hr	6110	1940	31.75	51.62	61.51
$3\frac{1}{2}$ hr	2099	185	8.81	46.59	18.92
4 hr	2156	2	0.09	68.29	0.14
5 hr	2398	0	0	52.85	0
6 hr	3574	2	0.06	40.74	0.14
7 hr	3021	10	0.33	39.84	0.83
8 hr	2608	117	4.49	47.35	9.47
9 hr	1135	377	33.22	47.23	70.34
10 hr	430	146	33.95	42.49	79.78
15 hr	519	314	60.50	51.17	118.04
18 hr*	1163	641	55.12	55.70	98.92

*Data from Hildreth, 1965. Eggs collected over 10-11 minute periods. See Table 1 for explanation of other collection periods.



XBL678 - 3835

Fig. 1



XBL678-3836

Fig. 2