GENETIC EFFECTS OF GAMMA-IRRADIATION ON EGG PRODUCTION AND ADULT EMERGENCE OF *DROSOPHILA MELANOGASTER*¹

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THE importance of assessing the over-all genetic effects of radiation was recently emphasized by EHLING and RANDOLPH (1962). Although many human characteristics and most of the productive traits of plants and animals are biometrical in nature, little evidence is available concerning the genetic effects of radiation on biometrical traits. The study of the genetic effects of radiation on biometrical traits need not be precluded because these traits are influenced by environmental factors any more than this fact prevents the study of their heritable basis.

That genetic differences at many loci, collectively, can cause differences in radioresistance has been established (DOOLITTLE 1961). Significant differences among inbred strains of mice regarding body weight response following whole-body X-irradiation have been reported by GRAHN (1954). In a study concerning lifetime fertility of mice, HAVERLAND and GOWEN (1960) found that inbreds were more severely affected by exposure to X rays than hybrids; a comprehensive review of studies relating to strain differences in acute X ray lethality and in reproductive organ resistance was also presented by these authors.

A few investigators have found evidence of polygenic damage among the offspring of irradiated parents. RUSSELL (1957) and RUSSELL and RUSSELL (1958) reported that radiation shortened the life of offspring of irradiated mice. Inconsistent, but significant, effects of paternal irradiation on growth rate in swine were reported by WILLHAM and COX (1962). COX and WILLHAM (1962) also found a higher early mortality rate in litters of pigs from irradiated sires as compared with progeny of control males. EHLING and RANDOLPH (1962) found a distinct increase in skeletal abnormalities in offspring of irradiated male mice. CARTER and LYON (1961) reported that granddaughters of irradiated male mice had fewer corpora lutea per pregnancy than those of control males, but found no difference in litter size at birth.

WALLACE (1957, 1959) has reported that polygenic mutations induced in *Drosophila melanogaster* second chromosomes by 500r of X-irradiation have a favorable heterotic effect on viability when they are in the heterozygous condition. *Drosophila* homozygous for the unirradiated second chromosomes had a 1.5 per cent lower average fitness than those in which one member of the pair of second

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chromosomes had been previously irradiated. BURDICK and MUKAI (1958) investigated the genetic effect of lower doses of radiation on the viability of *D. melanogaster* and reported comparatively larger increases in the variances than in the means of the treated groups, indicating that mutations seemed as likely to be beneficial as detrimental to viability. Results contrary to those of WALLACE (1957, 1959) and BURDICK and MUKAI (1958), however, were obtained by FALK (1961). That the genetic background may influence the frequency and direction of radiation-induced polygenic mutations in yeast has recently been shown by MÜLLER and JAMES (1961).

That radiation reduces egg production of treated insects and decreases the viability of irradiated gametes is well established; studies concerning the effects of radiation on gamete production and hatchability in insects were recently reviewed by GROSCH (1962). The extent of genetic damage regarding these traits as expressed in subsequent generations, however, is not known, although a reduced fertility among male mice conceived after irradiation of the parents has been reported (SNELL 1935; RUSSELL 1950; RUSSELL and WICKHAM 1959). Reciprocal translocations were believed to account for part, but not all, of the reduced fertility found in the latter studies. STONE and WILSON (1958) reported that populations of *D. ananassae* were severely damaged by direct radiation and fallout in the Pacific Proving Ground Area in 1954, but that after many generations these populations had recovered or were only slightly inferior to other non-irradiated Pacific Island populations.

The primary objective of the present study was to assess the genetic effects of several levels of γ -irradiation on the egg production and percent adult emergence of *Drosophila melanogaster*, summaries of which have been presented elsewhere (DEFRIES and TOUCHBERRY 1962, 1963).

METHODS AND MATERIALS

Both newly emerged male and female flies from two populations were placed in plastic capsules and subjected to one of four levels of γ -irradiation (0, 500, 1000, or 1500r) in the University of Illinois nuclear reactor. Both populations, one inbred and one randombred, were derived from Luce's wild-type strain, the coefficient of inbreeding of the inbred population being approximately 70 percent as compared to the randombreds. Within an hour following exposure, the flies within each population were mated in all possible combinations and the resulting single-pair matings were placed in egg collection bottles. Daily egg counts over a ten-day period were then obtained, as well as the number of adults to emerge on or before 15 days after oviposition from the eggs deposited by each pair on each of the ten days. The sex ratio and the adult weights of males and females were recorded for the flies emerging from the eggs deposited by each pair on each of the ten days. A study of these latter data will be published later.

Egg production was obtained on the treated flies, on the progeny of the treated flies (Generation 1) and on the grand-progeny of the treated flies (Generation 2). Percent adult emergence, sex-ratios, and weights were obtained on Generations 1, 2 and 3; Generation 3 flies were the progeny of Generation 2. Members of both the first and second generations were full-sib mated so that the flies which arose from eggs oviposited by the second generation were the product of two generations of full-sib mating.

Two replicate experiments, differing only with respect to dose rate, were conducted during the course of the study; the dose rates were 50r and 15r per min in Experiments 1 and 2, respectively. Three mating pairs were included in each of the 16 subclasses resulting from the four

× four factorial arrangement of treatments within each population and experiment. Therefore, with two populations and two experiments, a total of 192 mating pairs were required per generation. With regard to egg production, somatic and genetic effects of radiation could be expressed by the treated flies, whereas only genetic effects (dominant, incomplete dominant and sex-linked recessive point mutations and any effects due to structural rearrangements of the chromosomes) would be expressed by Generations 1 and 2. With regard to percent adult emergence, both somatic and genetic effects could be expressed in Generation 1. Percent adult emergence in Generation 1 refers to the fraction of eggs oviposited by treated flies resulting in viable adults; thus, a decrease in percent adult emergence in Generation 1 could be due to a reduction in gamete production of treated males, the induction of lethal mutations in both male and female gametes or both. Neither somatic effects nor the effects of completely dominant lethals would be observed in the second and third generations since only the parents of the adults to emerge in Generation 1 were treated. By considering percent adult emergence as a characteristic of the emerging offspring, the adults to emerge in Generation 3 are the product of two generations of full-sib mating; thus, the probability of an induced autosomal recessive lethal being homozygous in Generation 3 is 1/16.

The parents of Generation 1 came from inbred and randombred stocks in our laboratory and were exposed to γ -irradiation. The parents of Generation 2 were chosen from Generation 1 from flies emerging from eggs oviposited by the treated flies during the interval from three to seven days after treatment. The parents of Generation 3 were chosen from Generation 2 from flies emerging from eggs oviposited by Generation 1 during the interval from three to seven days after the commencement of oviposition. Three pairs were chosen to represent each subclass for each population and experiment. Thus, the pooled 0 male × 0 female class in Generation 2, Table 1, was produced from a total of 12 pairs chosen from Generation 1.

For statistical analyses, the daily egg production data were converted to square roots ($\sqrt{X + \frac{1}{2}}$), and the daily percent adult emergence data were converted to an arc sine transformation expressed in radian measure. The ten transformed observations for each variable for each pair of flies were then fitted to a second degree polynomial from which the linear and quadratic regression coefficients of the variable in question on day of oviposition were obtained. To derive the means and the regression coefficients, the ten daily observations on egg production were weighted equally while those for percent adult emergence were weighted according to the number of eggs laid each of the respective days. Having done this, the ten observations per variable for each pair of flies were then expressed as a mean to define the magnitude of the characteristic and the two regression coefficients to describe the trend of the characteristic throughout the ten-day period. Error sums of squares were obtained from the pooled sums of squares not accounted for by the linear and quadratic regressions; error variances for the various subclasses were homogeneous. The error sums of squares regarding the percent adult emergence data were weighted according to the daily egg production. Sums of squares for the linear and quadratic main effects of level of radiation on the male, the female and the various interactions of levels were obtained by orthogonal comparisons of means pooled across populations and experiments and were tabulated according to characteristic and generation.

RESULTS AND DISCUSSION

Egg production: Table 1 presents the transformed mean daily egg production over a ten-day period of the treated flies, their offspring (Generation 1) and the progeny (Generation 2) resulting from a full-sib mating of members of the first generation. Each subclass mean per generation is based on 120 observations, or the daily egg production for each of 12 pairs of flies for each of ten days. Because analyses indicated no differences in the response to radiation of inbred and randombred populations or between experiments, the data were pooled across populations and experiments. An analysis of variance of these pooled data is pre-

TABLE 1
*Mean daily egg production**

Generation	γ -irradiation on female	γ -Irradiation on male				Mean
		0r	500r	1000r	1500r	
Treated	0r	5.7	5.1	6.2	5.6	5.6
	500r	5.9	5.7	5.6	5.6	5.7
	1000r	6.4	5.0	5.7	5.7	5.7
	1500r	6.3	5.2	5.3	6.2	5.8
	Mean	6.1	5.3	5.7	5.8	5.7
1	0r	5.8	5.2	6.3	5.1	5.6
	500r	6.1	6.0	5.4	6.4	6.0
	1000r	6.2	5.3	5.7	6.0	5.8
	1500r	5.8	6.0	5.3	6.5	5.9
	Mean	6.0	5.6	5.7	6.0	5.8
2	0r	5.3	5.5	5.5	5.8	5.5
	500r	4.7	5.6	5.6	5.7	5.4
	1000r	5.6	5.8	5.4	5.5	5.6
	1500r	5.4	5.4	5.7	4.4	5.2
	Mean	5.3	5.6	5.5	5.3	5.4

* Square root transformation ($\sqrt{X + \frac{1}{2}}$). Each of the 16 subclass means per generation is based on the daily egg production of 12 pairs of flies over a ten-day period. Each is the mean of 120 observations and is based on approximately 4000 eggs. To express the means in terms of average daily egg production, square the mean and subtract $\frac{1}{2}$. For example, the transformed mean daily egg production in the 0 males $\times$ 0 females subclass of Generation 1 is $5.7 = \sqrt{X + \frac{1}{2}}$; thus, $X = 32.0$.

sented for each of the three generations in Table 2. It is clear that treatment of females with up to 1500r of γ -irradiation results in no significant deleterious effect on egg production and that treatment of the male does not significantly affect the egg production of the female to which it is mated.

That the exposure of female *Drosophila* to less than 2000r does not result in a significant decrease in egg production is in agreement with the findings of GOWEN and STADLER (1952) and also with previously unpublished data obtained

TABLE 2
Analysis of variance of egg production data

Source	Degrees of freedom	Mean square		
		Treated flies	Generation 1	Generation 2
Linear male	1	.64	.06	.16
Quadratic male	1	9.45	4.99	3.18
Linear female	1	.33	1.23	1.63
Quadratic female	1	.01	1.01	.75
Linear male $\times$ Linear female	1	.29	1.57	8.05
Linear male $\times$ Quadratic male	1	1.32	.00	1.82
Quadratic male $\times$ Linear female	1	7.88	3.04	1.99
Quadratic male $\times$ Quadratic female	1	.00	3.12	.01
Error		2.70	3.81	2.78
Degrees freedom for error		1344	1323	1260

TABLE 3

Mean egg number for a nine-day period

Level of X-irradiation on female parent (1000r)	Level of X-irradiation on male parent (1000r)					$\bar{X}$
	0	1	2	3	4	
0	600	484	646	563	626	584
1	511	593	427	615	556	540
2	432	509	612	203	520	455
3	73	42	111	168	330	145
4	107	158	211	52	37	113
$\bar{X}$	345	357	401	320	414	367

by the present authors and shown in Table 3. The data in Table 3 were collected in the same manner as were those in Table 1; however, in this small preliminary experiment, the flies were exposed to X rays, only randombred flies were used, the data were not transformed and the subclass means are the mean daily egg production of three pairs of flies over a nine-day period. The results of this preliminary study indicated that higher dosages in the female parent result in a decline in egg production, while there is no indication that higher dosages in the male parent result in a decline in egg production.

The above results suggest that any genetic damage induced by 1500r or less of γ -irradiation has little or no effect on the mean egg production of the progeny and grand-progeny of treated *Drosophila melanogaster*.

Regression of daily egg production on day of oviposition: The linear and quadratic partial regressions of the square root of egg production on day of oviposition were obtained for each pair of flies. An analysis of variance of these regression coefficients indicated no significant differences associated with levels of irradiation of males or females for the treated flies or for Generations 1 and 2. The differences between populations and experiments were likewise not significant but there was a significant difference between generations involving the linear partial regressions. Shown in Figure 1 are the pooled within-pair regression curves for the treated flies and Generations 1 and 2. The curve for each generation in Figure 1 is based on the daily egg production of 192 mating pairs for a period of ten days, or 1920 observations. Because there were no significant differences among the regression coefficients associated with level of irradiation, the small difference between generations is attributed to changes in environment.

Percent adult emergence: Percent adult emergence for Generations 1, 2 and 3 refers to the proportion of eggs oviposited by the treated flies and members of Generations 1 and 2, respectively, which resulted in viable adults. Thus, percent adult emergence is considered to be a characteristic of the emerging adults; Generation 1 was derived from matings of the treated flies, Generation 2 resulted from full-sib matings of Generation 1 and Generation 3 from full-sib matings of Generation 2.

The means for transformed daily percent adult emergence for Generations 1, 2 and 3 are presented in Table 4. Each of the 48 subclass means is the weighted

FIGURE 1.—The within pair regression of the square root of the number of eggs laid per pair per day on day of oviposition; $E = \sqrt{N + \frac{1}{2}}$, where N is the actual number of eggs and E is the corresponding transformed datum. The equations for these regression lines are:

Treated Flies, $E = 5.7 + 1.05 \pm .07(X-5.5) - .10 \pm .006(X^2-38.5)$;
 Generation 1, $E = 5.8 + 1.47 \pm .08(X-5.5) - .12 \pm .007(X^2-38.5)$;
 Generation 2, $E = 5.4 + 1.58 \pm .07(X-5.5) - .11 \pm .006(X^2-38.5)$;

FIGURE 2.—The within pair regressions of the percent adult emergence of offspring of treated males on day of oviposition. These daily percentages were transformed to arc sine and expressed in radian measure. The equations for the regression lines depicting the effects of treating males at the various levels on Generation 1 are:

0r, $\hat{P} = .84 + .0915 \pm .0028(X-5.00) - .00830 \pm .00024(X^2-32.24)$;
 500r, $\hat{P} = .65 - .1489 \pm .0029(X-5.00) + .00991 \pm .00025(X^2-32.24)$;
 1000r, $\hat{P} = .48 - .1598 \pm .0025(X-5.00) + .01089 \pm .00022(X^2-32.24)$;
 1500r, $\hat{P} = .38 - .1279 \pm .0023(X-5.00) + .00775 \pm .00020(X^2-32.24)$.

The regression equation for Generations 2 and 3, pooled across levels and generations, is:
 $\hat{P} = .80 - .0117 \pm .0011(X-5.68) + .00014 \pm .00003(X^2-39.64)$.

mean of 120 observations, each of the 120 being weighted according to the number of eggs ovipositioned per pair per day. The effect of radiation of both parents on the proportion of eggs oviposited which result in viable adults is clearly indicated from the data of Generation 1 in Table 4. The linear main effects of level of radiation on both the male and female parents are highly significant as shown in Table 5, although the magnitude of the effect resulting from treatment of the male parent is substantially higher than that resulting from treatment of the female parent. From the analysis of variance in Table 5, it can also be seen that the quadratic main effect of level of radiation on the male parent and the linear male $\times$ linear female interaction are both significant at the 0.05 level of probability. The deleterious effects of radiation as found in the data of Generation 1 can be attributed to genetic damage (dominant lethals, sex-linked recessive le-

TABLE 4
*Mean percent adult emergence**

Generation	γ -Irradiation on female	γ -Irradiation on male				Mean in radians	Mean in percent
		0r	500r	1000r	1500r		
1	0r	.93	.69	.52	.38	.63	59
	500r	.91	.65	.51	.43	.63	59
	1000r	.80	.63	.46	.37	.57	54
	1500r	.71	.61	.43	.34	.52	50
	Mean in radians	.84	.65	.48	.38	.59	
	Mean in percent	75	60	47	37		56
2	0r	1.02	.72	.81	.68	.81	72
	500r	.97	.88	.78	.64	.82	73
	1000r	.90	.83	.91	.68	.83	73
	1500r	.92	.94	.78	.57	.80	72
	Mean in radians	.95	.84	.82	.64	.82	
	Mean in percent	81	75	73	60		73
3	0r	.83	.93	.72	.71	.80	72
	500r	.86	.77	.79	.70	.78	71
	1000r	.83	.70	.85	.77	.79	71
	1500r	.84	.80	.82	.68	.79	71
	Mean in radians	.84	.80	.80	.72	.79	
	Mean in percent	75	72	72	66		71

* The percentages were transformed to arc sines and are expressed in radian measure. Each of the 16 subclass means in each generation is based on the adult flies emerging from the eggs deposited on each of the ten days by each of 12 mating pairs, or a total of 120 observations. Each of the 120 observations was weighted according to the number of eggs oviposited per pair per day.

thals, etc.), although the possibility of somatic damage may not be excluded. For example, if radiation of the male parent reduced sperm production sufficiently, a decrease in percent adult emergence could result.

Critical evidence for the presence of induced genetic damage can be found in

TABLE 5
Analysis of variance of percent adult emergence data

Source	Degrees freedom	Mean square		
		Generation 1	Generation 2	Generation 3
Linear male	1	5.673**	2.177**	.347**
Quadratic male	1	.103*	.055	.019
Linear female	1	.351**	.000	.002
Quadratic female	1	.017	.017	.003
Linear male $\times$ linear female	1	.088*	.003	.017
Linear male $\times$ Quadratic female	1	.001	.033	.044
Quadratic male $\times$ Linear female	1	.021	.257**	.000
Quadratic male $\times$ Quadratic female	1	.017	.017	.051
Error	.	.022	.031	.027
Degrees freedom for error	.	1212	1130	1068

* Significant at the 0.05 level of probability.

** Significant at the 0.01 level of probability.

the data of subsequent generations, however, since only the parents of Generation 1 were treated. From the marginal means presented in Table 4, a deleterious effect associated with level of radiation administered to the original male parents is evident in the data of Generation 2, i.e. grand-progeny of the treated flies. As mentioned previously, these data have been pooled across populations (inbred and randombred) and experiments. This linear depressing effect associated with treatment of the original males was noted in each of the four separate tables from which these data were pooled. From the analysis of variance of the data presented in Table 5, it is shown that this linear male effect is highly significant. In addition, a highly significant quadratic male $\times$ linear female interaction was found in the data of Generation 2.

Randomly chosen surviving members of Generation 2 were full-sib mated so as to yield percent adult emergence data regarding Generation 3. From Tables 4 and 5, it is evident that treatment of the original males has resulted in a highly significant linear depressing effect on the percent adult emergence of their great-grand-progeny, the magnitude of this effect being approximately half that found in the preceding generation. The partial recovery of the population is consistent with the results reported by STONE and WILSON (1958). In Generation 3, as in Generation 2, no effect is associated with treatment of the original females.

A reduction in the fertility of offspring of treated parents has been previously reported. SNELL (1935), RUSSELL (1950) and RUSSELL and WICKHAM (1957) found a decreased fertility among male mice conceived after irradiation of their parents. Part of the reduction in fertility was attributed to the induction of reciprocal translocations. No cytological examinations were undertaken during the course of the present investigation; thus, the role of chromosomal rearrangements in the reduction of percent adult emergence in the present study cannot be determined precisely. However, the data were examined to ascertain the frequency of "semi-sterility" in Generation 2. When the data were plotted according to the level of irradiation on the original males, no significant difference was found between the observed and expected numbers of pairs which were classified as semi-sterile (40 to 60 percent adult emergence). Nevertheless, reciprocal translocations may have accounted for some of the observed reduction in fertility. It is also possible that the reduced percent adult emergence in Generations 2 and 3 may be due in part to partial sterility resulting from the induction of inversion heterozygotes. Incomplete penetrance of lethal point mutations may also be a factor, as well as polygenic damage. The relative contributions of these factors to the observed results cannot be determined from the data of the present study.

Early in the history of radiation genetics, MULLER (1930) reported that a given dose produced both point mutations and chromosomal rearrangements at a higher frequency in spermatozoa than in oöcytes. More recently, GLASS (1955a, b, 1956) has discussed this phenomenon in some detail. It is evident from Tables 4 and 5 that irradiation of the original males has had a greater effect on the mean percent adult emergence than has irradiation of the original females. The effect is four to five times as large in the data of Generation 1. One cannot say that this is a direct comparison of irradiating spermatozoa and oöcytes because by irradiat-

ing adult flies and observing the percent of adults to emerge over a ten-day period, effects of exposure at different stages of gametogenesis are in the data. One cannot measure these effects by an analysis of the means alone. The partial regression coefficients were included in the analysis to help determine the effects of exposure at different stages of gametogenesis and to help measure the differences arising from exposing the different sexes.

Regression of daily per cent adult emergence on day of oviposition: Analyses of variance of the regression coefficients for percent adult emergence indicated that there were significant differences among subclasses for Generation 1 and that these differences were associated with the treatment of the mating pairs. There were no significant differences among the subclasses for Generations 2 and 3 and percent adult emergence in these generations followed the characteristic curve shown in Figure 2. This curve in Figure 2 for percent adult emergence is based on the percent of the eggs produced daily by 384 mating pairs for a period of ten days which resulted in viable adults, for a total of 3840 observations. It should be remembered that the parents of Generations 2 and 3 were not exposed to radiation, while the parents of Generation 1 were. As shown in Figure 2, percent adult emergence decreases significantly as the day of oviposition increases when the parents involved were not treated. This suggests that the percent of eggs resulting in viable adults decreases as the age of the parents increases.

To interpret the differences between the regressions for Generation 1, the linear and partial regressions for each of the 192 pairs were tabulated and various tests of significance made. From such a procedure, it became apparent that the differences were associated with the radiation treatment of the parental pairs. The partial regressions for those subclasses where the parents were treated alike were remarkably similar; consequently, the linear and quadratic partial regression coefficients of percent adult emergence on day of oviposition were grouped into 16 subclasses of 12 mating pairs each according to the treatment of the parents. Tests of the differences between these 16 subclasses indicated that the significant differences were associated with levels in males and levels in females but not with interactions of these levels. The curve for the 0 male $\times$ 0 female subclass was almost identical to that for percent adult emergence for Generations 2 and 3, as shown in Figure 2.

Curvilinear regressions of the transformed percent adult emergence data on day of oviposition were then determined for each level of exposure of male and female parents and are presented for Generation 1 in Figures 2 and 3, respectively. The regressions representing treatment of male parents have been pooled across all levels of treatment on female parents and vice versa; thus, these regressions reflect not only the treatment on the parent indicated, but also an average of 750r on members of the opposite sex. In general, offspring emerging from eggs oviposited on successive days after treatment of the parents are derived from gametes which are treated at successive stages of gametogenesis. Thus, the effect of γ -irradiation on the percent adult emergence of offspring of treated parents at the various stages of gametogenesis would be indicated when the data are plotted as a function of day of oviposition.

Data from Generations 2 and 3 as plotted in Figure 2 were utilized to obtain the regression of percent adult emergence on day of oviposition when neither parent was treated. From these data, it was found that percent adult emergence decreases slightly and linearly with age. By using this regression shown in Figure 2, it was possible to adjust the regressions of percent adult emergence on day of oviposition for level of radiation on members of the opposite sex.

The adjustments were justified on the basis that the interactions between levels in males and levels in females were not significant in the analyses of variance of the partial and quadratic regression coefficients. The adjustment of the curve for 0 males was accomplished by comparing the curve in Figure 2 for 0 males with the curve in Figure 2 for Generations 2 and 3, in which neither parent was irradiated. The amounts by which the respective points on the curve for Generations 2 and 3 differed from the points on the curve for 0 males in Figure 2 were then added or subtracted to the respective points on the latter curve to make its slope correspond to that of the curve for Generations 2 and 3. The curve for 0 males in Figure 2 is really a curve for an average exposure of 750r in the female parent; thus, the adjustment in this case is removing the effects of an average exposure of 750r in the female parent. The same differences as derived above were used to adjust the curves for exposures of 500, 1000 and 1500r on the male parent. The adjustment of the curves for levels on the female parent were accomplished in a similar manner except that the comparison of the curve for Generations 2 and 3 was with the curve for 0 females in Figure 3. The curve for 0 females in Figure 3 represents an average exposure of 750r on the male parent and the adjustments remove this effect. These adjusted regression lines for males and females are plotted in Figures 4 and 5, respectively, and represent the expected response to the treatment indicated when the opposite parent received no irradiation.

The curves in Figure 4 for 500, 1000 and 1500r males show a marked decrease in percent adult emergence from the first to the seventh day after exposure, at which point the slope becomes zero. Sperm deposited from Day 1 through Day 7 after exposure would have been irradiated primarily as mature sperm, late spermatids, early spermatids and secondary spermatocytes, respectively. The temporal relationship of successive broods to stage of spermatogenesis in *Drosophila* has been discussed by AUERBACH (1954). From Day 7 through Day 10 after exposure, the curves take on a positive slope, indicating some recovery from the treatment, but it is doubtful if the recovery is complete by the tenth day. Sperm deposited from Day 7 through Day 10 after exposure would have been irradiated mainly as secondary and primary spermatocytes, with probably a few on the tenth day having been irradiated as spermatogonia. It should be pointed out that this recovery occurs in opposition to the general decrease in percent adult emergence as the day of oviposition increases as shown by the curve for Generations 2 and 3. The slopes of the lines depicting exposures of 500, 1000 and 1500r on the male parent in Figure 4 are remarkably similar, the major differences being due to differences in the means. From these curves, it is clear that those male gametes produced on the seventh day after exposure to γ -rays have resulted in the lowest percent adult emergence. From our data, it was not possible to deter-

FIGURE 3.—(Top) The within pair regressions of the percent adult emergence of offspring of treated females on day of oviposition. These data were transformed to arc sine and expressed in radian measure. The equations for the regression lines at the various levels are:

$$\begin{aligned} 0r, \quad \hat{P} &= .63 - .1229 \pm .0030(X-5.00) + .00850 \pm .00026(X^2-32.24); \\ 500r, \quad \hat{P} &= .63 - .1260 \pm .0024(X-5.00) + .00786 \pm .00021(X^2-32.24); \\ 1000r, \quad \hat{P} &= .57 - .0383 \pm .0027(X-5.00) + .00102 \pm .00023(X^2-32.24); \\ 1500r, \quad \hat{P} &= .52 - .0328 \pm .0029(X-5.00) + .00081 \pm .00025(X^2-32.24). \end{aligned}$$

FIGURE 4.—(Bottom left) Adjusted regressions of the percent adult emergence of offspring of treated males on day of oviposition. These regression lines have been adjusted for level of radiation on females (See text for method of adjustment).

FIGURE 5.—(Bottom right) Adjusted regressions of the percent adult emergence of offspring of treated females on day of oviposition. These regression lines have been adjusted for level of radiation on males (See text for method of adjustment).

mine whether more mutations were produced at this stage of gametogenesis or whether some selective mechanism has caused this differential response. Ives (1963) has indicated that the *rate* of mutations actually changes during the vari-

ous stages of gametogenesis. Thus, it appears that at all three levels of exposure, secondary spermatocytes and early spermatids are more sensitive to radiation induced genetic damage than are spermatozoa, later spermatids, primary spermatocytes and spermatogonia. Since eggs were collected for only a ten-day period, no effort was made to determine the point at which the regression lines would begin to level off if they would. In general, these results agree with those of LÜNING (1952), ALEXANDER, BERGENDAHL and BRITTAIN (1959), and McDONALD (1961) who found similar results with *Drosophila melanogaster*, *D. virilis* and *Tribolium confusum*.

Eggs oviposited during the first seven days and the last three days were subjected to radiation mainly as oocytes and oogonia, respectively (GLASS 1955). Thus, in the female all gametes were treated as premeiotic germ cells, while those from the male were treated as both postmeiotic and premeiotic germ cells. As a consequence, during the ten-day period, the magnitude of the differential effect on percent adult emergence for treatment of the female parent should be much less than that for treatment of the male parent, since no meiotic or postmeiotic germ cells were treated in the female parents. The much reduced curvilinearity shown in Figure 5 for levels on female parents as compared to those for levels on male parents supports this contention. The major difference between the levels of treatment on the female parent as indicated from this figure is a small difference in mean percent adult emergence and a diminishing deleterious effect resulting from higher levels of exposure during the first few days of oviposition following exposure to radiation. It is possible that some of this early effect may be due to somatic damage, as well as to the induction of dominant lethals.

It is interesting to note that the curves for 1000 and 1500r in females start relatively low and slope upward until a maximum is reached around Day 5 after exposure. These curves represent the differentials in the effects produced by irradiating female gametes at stages from primary oocytes to oogonia. This segment of the curves for levels in females should compare with the segment of the curves from seven through ten days for levels in males. The slopes are remarkably similar. From Day 5 through Day 10, the curves do not depart appreciably from the downward trend exhibited by the curve for 0 females but are at a lower level. Exposure of the females to 500r did not produce a marked effect, as can be seen from Figure 5.

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SUMMARY

Newly emerged male and female flies from one inbred and one randombred population were subjected to 0, 500, 1000 or 1500r of gamma-radiation and then mated in all possible combinations within populations, resulting in a four $\times$ four factorial arrangement of treatments. Daily egg production over a ten-day period was determined from two replicated experiments for the treated flies, their untreated progeny (Generation 1) and grand-progeny (Generation 2). Generation

2 resulted from full-sib matings of members of Generation 1. Eggs laid each day which resulted in viable adults were determined for Generations 1, 2 and 3 (Generation 3 resulting from two generations of full-sib mating).

Mean daily egg production was not significantly affected by irradiating males or females with 1500r or less of gamma rays; however irradiation of the female parent with 2000r or more of X rays had a marked depressing effect on egg production. Irradiation of the male parent did not affect egg production appreciably.

The partial linear and quadratic regression coefficients of daily egg production on day of oviposition for each pair of flies indicated no significant differences associated with levels of irradiation of males or females.

Large linear depressing effects associated with irradiation of both male and female parents were found in Generation 1 for mean daily percent adult emergence. Treatment of the females had no appreciable effect on the percent of eggs deposited by their progeny or grand-progeny which resulted in viable adults; however, treatment of the males had a significant effect on descendants.

The weighted partial linear and quadratic regression coefficients of percent adult emergence on day of oviposition were determined for each pair of flies. Analyses of the differences of these coefficients indicated that secondary spermatocytes and early spermatids were more sensitive to radiation induced genetic damage than were spermatozoa, later spermatids, primary spermatocytes, and spermatogonia. Less differential effect was found for irradiation of females than males, probably because all female germ cells were in premeiotic stages when treated. Nevertheless, gametes treated as primary oöcytes were more susceptible to damage than those treated as oögonia.

Irradiation of male *Drosophila* resulted in four to five times as much genetic damage in the gametes as did irradiation of the female. Evidence of this greater damage is also present in the gametes produced by the progeny and grand-progeny of irradiated males, the extent of the damage being approximately halved each generation.

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