

EFFECTS OF RADIATION ON POPULATIONS OF *DROSOPHILA MELANOGASTER* WITH DIFFERENT GENETIC STRUCTURES

II.* TEMPORAL CHANGES IN THE SEX RATIO

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Abstract

The effect of 1500 r X-rays, delivered for several generations to populations of *D. melanogaster*, on the sex ratio of the flies emerging has been examined. The results have been compared with those from similar populations living in the same environment but not given any radiation treatment. The mean values of four irradiated populations, when compared with their unirradiated counterparts, showed an initial drop in sex ratio followed by a marked recovery particularly between generations 7 and 12. When composite populations were set up, combining males from one irradiated population with females from another at each generation, this recovery in sex ratio was not observed. It was concluded that this latter result ruled out chromosomal phenomena and indicated a biometrical explanation as the most likely one for the observed rise in sex ratio with accumulated ancestral radiation in integrated gene pools.

I. INTRODUCTION

Although there are many reasons why the sex ratio of most Mendelian populations is expected to change as a result of irradiation, a large number of studies, both on the sex ratio of offspring of irradiated parents (Russel 1954; Neel 1963; Searle 1964; Schull, Neel, and Hashizume 1966) and of changes as ancestral radiation accumulated (Chapman *et al.* 1964; Sugahara 1964), have given results which are rather contradictory and in many cases completely negative. The experiments to be reported here allow comparisons between the number of generations irradiated and the nature of the gene pool. They show that sex ratio can and does alter under the stimulus of continued irradiation, although the extent of the change may be dependent on the genetic structure of the population being irradiated. Throughout this paper sex ratio is defined simply as the percentage of males hatching.

II. MATERIALS AND METHODS

The experimental situation is that described in Part I (Dyer 1971). The three series of experiments are those described in that paper with the exception that subpopulations RF and CF have not been analysed for sex ratio changes.

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III. RESULTS

(a) *Series 1*

Previous work on the induction of dominant lethals and the accumulation of recessive lethals (Dyer 1966, 1969*a*, 1969*b*) suggested that the proportion of males emerging at each generation would be expected to decrease slowly from slightly in excess of 50% to about 47% as the frequency of sex-linked recessive lethals reached equilibrium. Table 1 shows that this was not the case. In the "irradiated" cage CR the mean sex ratio among the *straw* was in fact higher than in the *light*.

TABLE 1
SEX RATIOS FROM THE TWO CAGES OF SERIES 1 EXPERIMENTS

Generation	Cage CR		Cage CC	
	<i>straw</i>	<i>light</i>	<i>straw</i>	<i>light</i>
1	50.44±2.11	47.81±2.04	50.51±1.59	46.85±2.95
2	53.75±2.42	46.70±2.61	47.25±1.85	45.98±3.77
3	44.70±2.39	46.85±3.34	53.78±1.92	45.54±2.81
4	45.71±2.22	47.27±2.74	52.93±1.66	47.94±2.75
5	47.72±2.09	47.79±2.35	52.08±1.41	50.43±2.68
6	48.04±2.16	46.84±2.46	51.45±2.06	56.77±3.57
7	50.08±1.99	49.26±2.16	50.89±1.84	47.79±2.19
8	48.15±3.04	48.58±2.54	46.93±2.03	46.03±3.62
9	48.24±2.42	53.23±2.54	46.84±1.74	43.31±2.80
10	53.68±2.56	49.85±1.97	48.88±1.63	43.64±2.67
11	53.25±2.71	47.97±2.31	48.90±1.92	45.52±2.48
12	54.17±3.07	50.16±2.86	46.91±1.57	53.10±3.10
13	51.50±3.53	52.08±3.60	47.85±1.49	51.41±4.19
14	49.69±2.79	46.26±2.58	50.78±1.89	49.07±3.94
15	50.56±3.74	44.06±2.25*	52.16±2.32	49.69±3.97
16	52.86±2.26	45.90±2.20	58.15±1.75*	48.91±2.47
17	50.84±2.64	50.98±2.21	49.34±1.89	51.49±4.97
18	52.73±3.01	48.42±1.68	52.23±3.33	51.90±2.44
19	52.27±2.32	53.74±1.99	54.16±2.14	49.20±1.20
20	58.52±2.79*	55.61±3.55	51.33±1.82	43.92±3.61
21	45.22±3.28	49.47±2.56	44.59±1.80*	48.52±2.99
22	46.91±3.92	46.15±1.95	54.39±1.55*	46.52±2.81
23	44.60±3.41	43.75±1.96*	51.47±1.79	48.82±2.71
24	50.80±2.58	47.74±1.82	52.22±1.75	54.10±2.75
25	53.16±2.51	49.35±1.90	54.14±1.93	50.11±2.39
Mean	50.30±0.05	48.63±0.05	50.81±0.03	48.53±0.05

* Significant at the 5% level.

The results from both *light* and *straw* in this cage were apparently similar to their respective counterparts in the control cage CC. The size of the samples are given by Dyer (1969*c*, tables 2 and 3). The magnitude of the standard errors given, however, shows that it is difficult to discern any different trends in irradiated compared with unirradiated populations. It would appear from these results, therefore, that the overall effect of accumulated radiation on sex ratio is not very large. The levels of

TABLE 2
SEX RATIOS FOR *straw* AND *light* FLIES FROM THE THREE IRRADIATED AND TWO CONTROL CAGES OF SERIES 2 EXPERIMENTS

Gener- ation	Cage A		Cage B		Cage C		Cage D		Cage E	
	<i>straw</i>	<i>light</i>	<i>straw</i>	<i>light</i>	<i>straw</i>	<i>light</i>	<i>straw</i>	<i>light</i>	<i>straw</i>	<i>light</i>
	Irradiated Cages				Control Cages					
1	56.16±2.23	53.14±1.45	54.02±2.06	51.58±1.74	52.25±1.93	53.63±1.59	51.78±1.81	54.81±2.54	52.30±1.56	56.04±2.60
2	55.44±2.23	52.48±1.40	50.56±2.36	50.86±1.26	50.32±2.82	53.23±1.49	51.95±2.01	52.99±1.65	51.63±2.12	49.53±1.47
3	48.76±2.27	50.75±1.13	48.98±2.26	48.95±1.10	52.54±2.47	51.31±1.13	50.09±2.12	50.16±1.39	51.60±1.55	54.31±1.55
4	46.65±2.12	50.40±1.34	42.95±2.77	47.88±1.57	47.40±2.34	52.24±1.35	45.14±2.13	53.28±1.18	47.57±1.39	48.82±1.64
5	46.51±2.33	48.46±1.03	44.75±2.48	51.80±1.21	50.13±2.51	49.94±1.19	48.30±1.36	51.16±1.79	52.22±1.62	50.83±1.43
6	48.94±2.10	50.22±1.17	47.51±2.62	51.81±1.49	47.34±2.52	50.12±1.25	50.74±1.63	51.34±1.70	50.97±1.80	51.01±1.32
7	43.93±2.84	49.48±1.24	46.60±2.17	46.86±1.49	49.05±2.71	49.41±1.35	48.70±1.95	53.89±1.67	54.95±2.42	51.21±1.88
8	46.98±2.40	50.94±1.34	43.01±3.00	50.28±1.13	51.71±2.52	48.02±1.30	49.92±1.94	47.29±1.66	46.91±2.01	49.67±1.53
9	48.14±2.15	48.42±1.28	46.83±3.14	50.82±1.36	48.51±2.92	47.20±1.27	51.68±1.57	51.79±1.46	52.95±1.35	48.98±1.40
10	49.08±2.15	49.12±1.19	48.62±2.77	50.60±1.16	45.13±4.95	48.93±1.11	52.34±1.56	55.49±1.26	52.50±1.45	51.47±1.34
11	50.54±2.07	49.08±1.25	51.09±2.69	50.37±1.22	47.66±3.66	49.64±1.22	50.76±1.59	49.61±1.41	48.37±1.59	50.63±1.41
12	50.99±2.03	48.17±1.26	52.67±2.44	49.86±1.12	49.28±3.39	49.95±1.12	49.84±1.62	48.71±1.58	46.20±1.75	50.05±1.57
Mean	49.34±0.6	50.06±0.3	48.13±0.7	50.14±0.7	49.28±0.8	50.30±0.4	50.10±0.50	51.71±0.44	50.68±0.48	51.00±0.44

competition in the two cages were inevitably slightly different, since there was a reduced number of larvae in the irradiated cage due to the induction of dominant lethals; this further complicates the task of determining any long-term trends. The apparent similarity in the results from both cages of the unirradiated *light* flies can be interpreted as a demonstration of the lack of cumulative effect of differences

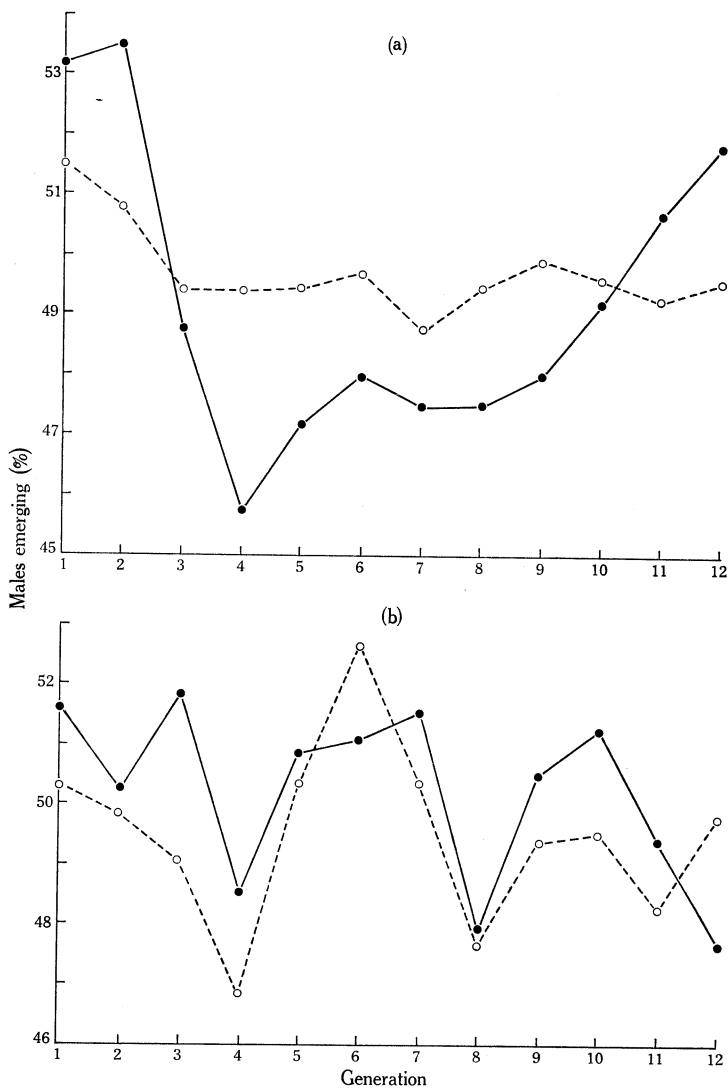


Fig. 1.—Mean sex ratio at emergence among the *light* and *straw* flies of (a) cages CR, A, B, and C and (b) cages CC, D, and E of series 1 and 2 experiments. In (a) the *straw* flies were irradiated at every generation in all cases; in (b) none of the populations was irradiated. ○ *light*; ● *straw*.

in competitive levels on sex ratio; it is therefore possibly an extension of the findings of Miller (1964) who found this lack of effect after one generation of differential competition.

(b) Series 2

Table 2 shows the results from the irradiated and control cages respectively. There was a marked internal consistency in this series of experiments between replicates A, B, and C and replicates D and E, respectively; i.e. at each generation the results for the *straw* and *light* flies respectively from both the irradiated and control cages were in each case statistically homogeneous. The overall means at each generation, for both *light* and *straw* flies from the four irradiated and three control cages respectively of series 1 and 2 combined, were therefore calculated and the results are plotted in Figures 1(a) and 1(b).

The contrast between *light* and *straw* from the irradiated cages is now very marked. The *light* populations show no significant departure from a 50:50 ratio and the results are constant from generation to generation. The successive sex ratios among the *straw* are more heterogeneous. After generation 4 there was a pronounced rise in sex ratio—a trend which was reinforced after generation 9—and

TABLE 3
CORRELATION BETWEEN THE SEX RATIOS OF *light* AND *straw* FROM THE SAME POPULATION CAGES

Series	Cage	α	β	Test for $\beta = 0$	
				n	p
1	CR†	-0.15 ± 0.09	0.41 ± 0.18	2.24	0.025*
	CC	-0.10 ± 0.05	0.31 ± 0.10	2.97	0.003**
	CR†‡	-0.03 ± 0.003	0.68 ± 0.01	0.11	0.91
	CC‡	-0.09 ± 0.08	0.31 ± 0.17	1.82	0.10
	A†	-0.78 ± 0.48	2.54 ± 0.96	2.65	0.008**
2	B†	-3.12 ± 3.27	7.18 ± 6.52	1.10	0.27
	C†	0.21 ± 0.23	0.57 ± 0.45	1.28	0.20
	D	0.71 ± 0.15	-0.40 ± 0.29	1.36	0.17
	E	-0.11 ± 0.10	0.32 ± 0.20	1.60	0.11
	A+B+C†	-0.06 ± 0.03	0.21 ± 0.06	3.45	0.0006***
1+2	D+E	-0.09 ± 0.08	0.27 ± 0.15	1.83	0.067
	A+B+C+CR†	-0.35 ± 0.48	0.79 ± 0.96	0.82	0.41
	D+E+CC	-0.06 ± 0.09	0.23 ± 0.19	1.21	0.23

* Significant at the 5% level. ** Significant at the 1% level. *** Significant at the 0.1% level. † *straw* irradiated. ‡ First 12 generations.

it seems clear that some process became established whereby the proportion of males emerging gradually increased. Combining the data from series 1 and 2 in this way demonstrates no such trends in either the *light* or the *straw* from the control cages, although, as Figure 1(b) shows, there does appear to be some random environmental or competitive effects on the sex ratio from generation to generation. The combining together of series 1 and 2 therefore brings out a difference between irradiated and non-irradiated populations not apparent from considering each alone. An appropriate means of comparing these sex ratio changes in irradiated populations, while allowing for any possible effects due to differences in the environment between the cages, is to examine the correlations between the sex ratios among the *light* and *straw* flies emerging from one cage. If the differences in sex ratio change are due primarily to environmental effects, there should be a fairly close correlation between

the sex ratio changes among the *light* and *straw* flies from a particular cage, irrespective of whether the *straw* flies were irradiated or not. It was assumed that, out of n flies

TABLE 4
SEX RATIOS FOR *straw* FLIES FROM THE COMPOSITE POPULATIONS OF SERIES 3
EXPERIMENTS

Generation	Sex Ratio for <i>straw</i>		
	Cages A and B	Cages A and C	Cages B and C
1(2)	43.40 ± 2.33*	50.90 ± 1.81	48.46 ± 2.59
2(3)	50.40 ± 2.09	44.69 ± 4.27	48.05 ± 3.38
3(4)	45.27 ± 3.48	38.69 ± 2.20*	46.75 ± 3.20
4(5)	46.42 ± 5.50	44.22 ± 4.87	49.17 ± 4.2
5(6)	44.78 ± 5.63	46.20 ± 2.44	52.83 ± 3.53
6(7)	50.08 ± 4.70	49.61 ± 2.90	51.09 ± 3.96
7(8)	47.90 ± 2.51	44.06 ± 5.97	49.43 ± 8.33
8(9)	42.38 ± 4.22	52.42 ± 2.84	42.98 ± 3.95
9(10)	40.82 ± 3.96	48.85 ± 3.08	41.95 ± 4.11
10(11)	41.41 ± 3.07	47.83 ± 3.47	42.15 ± 4.49
11(12)	40.21 ± 4.98	47.95 ± 4.13	40.40 ± 4.93
Mean	44.83 ± 1.05	46.81 ± 0.8	46.66 ± 1.14

* Significantly different at the 5% level.

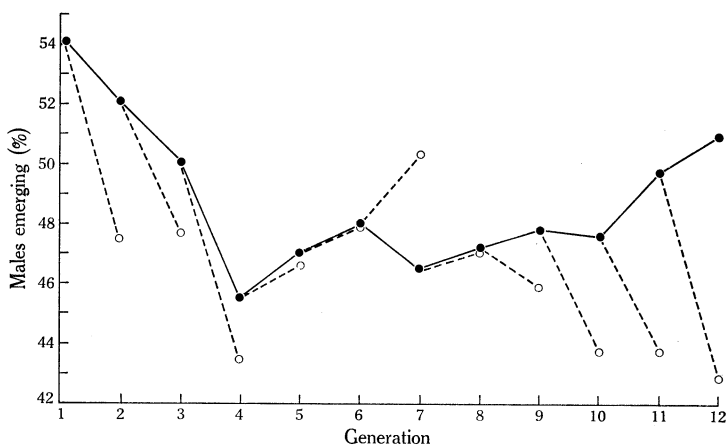


Fig. 2.—Mean sex ratio at emergence among the *straw* flies of cages A, B, and C of series 2 and the composite populations formed from them comprising series 3. ●—● Cages A, B, and C. ● - - - ○ Cages A and B, A and C, B and C.

emerging from generation i , the number of males emerging was binomial (n, p_i), where

$$\begin{aligned}
 p_i &= \pi_i \text{ (for } \textit{light} \text{ flies)} \\
 &= \alpha + \beta\pi_i \text{ (for } \textit{straw} \text{ flies).}
 \end{aligned}$$

The parameter β is, in effect, the slope of the regression of *straw* on *light* and is therefore a measure of the correlation between *light* and *straw*. Maximum likelihood estimates of the parameters α and β , with tests of significance, are shown in Table 3.

In all cases, except the result from control population D of series 2, β is positive, indicating an increasing proportion of males emerging in later generations among the *straw* populations. This increase is significant in the case of three irradiated populations and only one control population. These tests therefore provide qualified support for the hypothesis of a recovery in the sex ratio of irradiated populations.

(c) Series 3

It is clear from the results shown in Table 4 that these composite populations did not show any recovery of sex ratio. Figure 2 shows the mean sex ratio at each generation of these three populations, together with the mean sex ratio of the parental populations from which they were derived. The mean sex ratio was lower in the composite populations for 9 generations out of 11, significantly so in the last four, and the largest absolute difference occurred at the last generation.

IV. DISCUSSION

The importance of the sex ratio in evolution and the mechanism by which it is maintained have been the subject of a very great amount of work, both practical and theoretical. The ratio we have observed in these experiments might be termed a tertiary sex ratio, in contrast to the normal primary and secondary sex ratios, since we are observing it after a period of very marked larval competition and after pupation. There are several possible factors which can disturb the sex ratio at each of these stages and under the present circumstances we can make only indirect inferences as to the causes of the phenomena we have observed.

The radiation itself is obviously one of the main disturbing influences on the sex ratio at hatching, i.e. before it is altered by any environmental effects. But a large number of investigations of the offspring of irradiated parents from a variety of organisms suggest that there is still uncertainty regarding the effects of radiation on sex ratio. For instance, Searle (1964) found a reduction in sex ratio among the progeny of irradiated female mice but concluded that it was "due mainly to other unknown causes" rather than the action of sex-linked recessive lethals. On the other hand, Russel (1954) reported a decrease in sex ratio among offspring of irradiated male mice. Most of the investigations into the sex ratio of children from parents exposed to radiation (Neel 1963; Schull, Neel, and Hashizume 1966) disagree with Russel in showing an increasing proportion of males when the father was irradiated and agree with Searle in showing a decreasing sex ratio when the mother was irradiated.

One of the most important factors changing the sex ratio is chromosomal non-disjunction and, although this phenomenon is well known in *Drosophila* as a consequence of irradiation, accurate quantitative relationships in this field have still to be established (Traut 1964).

Certain other projects have failed to find any effects of ancestral radiation on sex ratio. Havenstein *et al.* (1968) found little change in sex ratio in the rat over 12 generations of irradiation and Charles *et al.* (1960) obtained a similar result in the mouse, as did Kohn (1960) and Luning (1963) after irradiating male mice.

There is, then, a good deal of uncertainty as to what the outcome of accumulating ancestral irradiation on sex ratio might be. While this might preclude discussion of absolute differences and the magnitude of changes in sex ratio, we have demonstrated

consistent comparative differences in these experiments. Our results rule out a number of possible explanations. In particular, one would expect any consistent bias due to chromosomal phenomena, such as chromosome non-disjunction or segregation distorter (Hiraizumi and Nakazima 1966), to be present in all irradiated populations. We have observed, however, a lower than expected sex ratio in series 3 experiments, and progressive changes in sex ratio leading to very high values in series 1 and 2.

One possibility suggested by these results is that sex ratio is a biometrical character susceptible to gradual change under selection. Falconer (1954) endeavoured to test this but found that selection for either higher or lower sex ratio was largely ineffective in both mice and *Drosophila*. In each case the heritability was lower than 5%; sex ratio therefore appeared to differ from normal biometrical characters which are, of course, susceptible to artificial selection. On the other hand, Weir and Wolf (1959) did succeed in changing the sex ratio of mice by selection, in this case as a by-product of successful selection for blood pH changes. They suggest that the change was "a function of the sperm source", i.e. a change in the primary sex ratio. There are numerous reports of variability regarding sex ratio within *Drosophila* populations (Wallace 1948; Malagolowkin and Carvalho 1961; Faulhaber 1967) and also in mice. Howard *et al.* (1955), for instance, showed significant heterogeneity between the sex ratio of six different hybrid genotypes. Even in man there is geographic and racial variation in sex ratio, with stable values of 94 and 120% male births encompassing the normal spread of 104–107% (Dyer 1969*d*). On the whole it does appear possible that there would be sufficient genetic variability among loci controlling sex ratio to account for the changes which occurred. Furthermore, the different results from series 2 and series 3 experiments—integrated versus non-integrated gene pools—suggest that the nature of the genetic background is important.

A possible mechanism producing these results is suggested by the work of Magalhaes *et al.* (1965), who showed the development in *Drosophila* populations of modifier genes which allowed the survival of homozygous recessive lethals. As they say in their paper "The destiny of lethal genes in a natural population does not depend only on the effects of the genes themselves but also on their interactions with the rest of the members of the gene pool". In this particular case we might postulate modifying genes active on genes detrimental or lethal in the hemizygous state. If such modifiers were not active in females and also inactive in the non-integrated genetic background of series 3 experiments, we have a measure of explanation of the observed results.

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