DOMINANT RADIATION EFFECTS IN MOUSE POPULATIONS¹

JACK LESTER KING

Donner Laboratory and Lawrence Radiation Laboratory, University of California, Berkeley

Received October 18, 1967

WELL established genetical theory requires that the net effect of induced random mutations in an outbreeding population must be to reduce the average fitness. Such an effect has not proven to be demonstrable in mammalian populations, perhaps in part due to the difficulties in measuring parameters of fitness.

It has not been difficult to demonstrate recessive mutational damage in mice or flies. Dominant lethal mutation—inferred from the reduced fecundity of irradiated individuals or from the rate of early death among their immediate offspring—has also been observed. The class of mutational damage that has not been observed in accordance with expectation is heritable net dominant deleterious damage.

The 1964 symposium "*The Effects of Radiation on the Hereditary Fitness of Mammalian Populations*," published as Vol. 50, No. 5, Part 2 of GENETICS, November 1964, consisted almost entirely of reports of negative findings. From the published summaries:

"No clear evidence of genetic effects of this cumulative irradiation is available in the first four generations of irradiation."

"There was no evidence of an accumulation of dominant detrimental effects in successive generations."

"No differences . . . were apparent in rates of embryonic losses prior to implantation, immediately after implantation, or long after implantation."

"Months of work, many dollars, and much effort has gone into a study only to find that the question of the extent of the damage, if any, is unsolved."

"The theory that ancestral irradiations . . . lead to cumulative detrimental mutations in future progenies is heavily discounted by this evidence."

"Further study will be required. . . ."

"The problem still exists that, so far, we have not shown in mammals a clear accumulation of genetic damage. . . ."

At that time it was generally felt that the expected dominant detrimental mutations were occurring, and that the failure to demonstrate losses in fitness was due to inadequacies of experimental procedure.

Studies of experimental irradiated insect populations have also generally failed to show heritable deleterious dominant effects, although SANKARANARAYANAN (1964, 1965) was able to show decreased fitness due probably to chromosomal damage as well as to accumulated harmful recessive mutations. AYALA (1966)

¹ This investigation was done under the auspices of the U. S. Atomic Energy Commission.

showed that radiation history markedly increased the fitness of populations subjected to intense selection in an unusually crowded environment. WALLACE (1956, 1958, 1963) found that the average heterozygous effect of new mutations on a homozygous background was a small increase in fitness; small general increases in fitness were also reported by CRENSHAW (1967) and FALK (1967).

It has become increasingly apparent that the net effects of heritable radiation-induced mutations in the heterozygous condition, whether positive or negative, if they exist at all, are rather small relative to recessive effects. If it is not possible to demonstrate significant effects in mammalian populations, it is still possible to place confidence limits on the maximum plausible magnitude of effect.

MATERIALS AND METHODS

From Cumberland View Farms 240 pairs of specific pathogen-free Ha/ICR random bred albino ("Swiss") mice were obtained. The commercial colony had originated with 33 pregnant females that had been randomly chosen from a large colony, and had been expanded and random bred for eight generations. For four additional generations prior to the first irradiation and for five subsequent generations, matings were by random number within groups, except that matings between sibs, cousins and second cousins were excluded. Matings were monogamous, and reproduction was limited to one litter per pair in discrete generations.

Selection of individuals for mating out of the population of weanlings was also quasi-random. It was desired that for each group the number of litters contributing to the subsequent generation should be the same; the number of eligible weaned litters was reduced to a common minimum by a random process. All remaining weanlings were ear-tagged, and the ear tag numbers for each sex and population were grouped by litter on a master list. According to the proportion needed for reproduction, a simple repeating number scheme was utilized for selection, usually every second individual. In this way at least one individual from each sibship larger than one was certain to be included, while the probability of selection was the same for each individual regardless of litter size; natural selection for favorable litter size was not eliminated, as it would have been if each litter was equally represented (KING 1965).

Two replicate control populations and two replicate experimental populations were treated for four generations, with approximately one hundred mated pairs per generation in each population. Four to six weeks old males selected for reproduction were irradiated once with 545 r Co⁶⁰ gamma rays at approximately 16 r/min. Both experimental and control males were then caged individually for three months prior to mating. Females and males were weighed just prior to mating at approximately 4½ months of age. Mating exposure was limited to 18 days, after which the males were removed and killed. Litters were counted on the first and second day and counted and sexed at weaning (25 to 35 days). Adult females were killed at the time of weaning of their young. In each generation a fifth and sixth group was made up of additional randomly chosen individuals from the irradiated populations. These were kept and mated as described but without irradiation. The relaxed-irradiation populations of the third and fourth generations, respectively, were each carried an additional generation without irradiation. Conversely, in generations three and four, additional males from the control populations were irradiated and mated to control group females, to produce new groups with only one generation of irradiation history each (Table 1).

Irradiation was discontinued after the fourth generation. In the fifth generation the number of matings was increased considerably. The replicates of each type were crossed to maximize outbreeding, and each male was mated to two females rather than monogamously. The litters born to this generation of adults made up three groups: the control group, without any irradiated ancestry (279 litters weaned); the first experimental group, with four generations of irradiated male ancestry but with unirradiated fathers (274 litters weaned); and the second experimental

group, with three generations of irradiated male ancestry followed by two generations of non-irradiated ancestry (272 litters weaned).

Throughout the experiment all groups were handled simultaneously and alike, except for the irradiation itself. Cages were coded and interspersed on the shelves. Although the windowless rooms were kept at nearly constant temperature, and caretaking procedures were not changed, subtle changes in such factors as caretaker personnel and season apparently were sufficient to bring about sizeable fluctuations in litter size from generation to generation.

First day litter size data proved to be unreliable. First and second day counts of the same litters varied considerably in either direction, because frequently additional offspring would be born subsequent to the first count, and about as frequently the mother would eat a few. In contrast, there was little fluctuation between second and third day counts of live young. Thus, second day and weaning litter sizes only will be considered.

RESULTS

Table 1 presents the litter sizes of live young on the second day and at weaning. Replicate data were similar in all cases and so have been combined. Only those matings which produced at least one weanling are included. Since some females ate their newborn without leaving a trace, it was not possible to distinguish accurately between sterile matings and those in which the young were born but

TABLE 1
Mean litter sizes on second day and at weaning

Irradiation history		zero	Litters born to parents of generation				
			one	two	three	four	five
unirradiated	No. litters weaned	91	100	136	182	104	279
control	2nd day mean	10.63	→ 9.58	→ 9.58	→ 8.82	→ 9.91	→ 10.19
	mean No. weaned	10.41	9.08	9.38	8.46	9.61	10.00
fathers	No. litters weaned		87		86	84	
irradiated	2nd day mean		8.83		8.47	9.52	
for only one	mean No. weaned		8.63		7.77	9.21	
generation							
fathers	No. of litters			127	79	96	
irradiated	2nd day mean			8.82	→ 8.54	→ 9.30	
every	mean No. weaned			8.62	8.43	8.85	
generation							
irradiation	No. of litters				70	73	274
relaxed	2nd day mean				8.50	10.29	10.45
for one	mean No. weaned				8.27	9.95	10.10
generation							
irradiation	No. of litters					41	272
relaxed	2nd day mean					10.49	10.36
for two	mean No. weaned						10.02
generations							

Data for the various populations and generations are arranged diagrammatically to show descent. Arrows show derivations; jagged arrows indicate spermatogonial irradiation of male parents.

failed to reach weaning age. There were no noticeable or consistent differences between groups with regard to the proportion of matings that failed to produce weanlings.

There is no evidence of a cumulative effect in the irradiated population. The litter sizes of the populations in which irradiation had been relaxed returned to control levels or slightly above. There was no clear evidence of heritable dominant deleterious effects. The differences between litters born to irradiated fathers and to unirradiated fathers, however, were generally significant and averaged about 5% (Table 2). This can be interpreted as an induction rate of approximately one dominant lethal gamete per 11,000 r, or $\sim 10^{-4}$ gametic lethals per roentgen at this dose and dose rate. Presumably most of this effect was due to chromosomal damage.

Because of somewhat larger numbers, the results of the last generation are of particular interest for the estimate of maximum heritable effect. The groups with ancestral irradiation actually show (non-significantly) larger litter size averages for both the second day and weaning, although they also show more preweaning losses.

Table 3 shows the differences between the control and the two experimental groups of the fifth generation, and the 95% confidence limits for these differences. If there is a net effect on early viability of nonlethal dominant mutation attributable to spermatogonial gamma irradiation at these doses, it is not more than about 3%, and apparently is more likely to be positive than negative.

In the fifth generation deaths between the second day and weaning occurred more frequently in the experimental groups. Under the favorable conditions of this laboratory, such deaths were relatively rare, claiming 1.8% of the control and 3.2% of each of the experimental groups. Thus, dominant deleterious effects

TABLE 2
Induction of dominant lethals

Irradiation history	Generations included	No. litters weaned	Mean litter size at weaning
Fathers not irradiated; no irradiation history	1,3,4	386	8.93
Fathers irradiated; no other ancestral irradiation	1,3,4	257	8.53*
Fathers not irradiated; mixed histories of other ancestral irradiation	1,2,3,4	665	9.06
Fathers irradiated; mixed histories of other ancestral irradiation	1,2,3,4	559	8.59*

* Significantly different from appropriate control at the 1% level.

The effect of paternal irradiation on litter size at weaning is seen in two comparisons. Where there had been no previous irradiation history, paternal spermatogonial irradiation of 545 r reduced litter size by 4.5%. When these groups are combined with others in which there is other history of ancestral irradiation, the reduction in litter size attributable to paternal irradiation is 5.2%.

TABLE 3

Generation 5 litter sizes on 2nd day and weaning, with t-test confidence limits

Irradiation history		No. litters weaned	Mean litter size	Standard error	Per cent difference from control	Lower 95% confidence limit of difference	Upper 95% confidence limit of difference
Control; no irradiation history	2nd day	279	10.19	2.41	..	..	..
	weaning	279	10.00	2.41	..	..	..
Four generations of irradiation; one with no irradiation	2nd day	274	10.45	2.76	2.5%	-1.7%	6.8%
	weaning	274	10.10	2.72	1.0%	-3.2%	5.4%
Three generations of irradiation; two with no irradiation	2nd day	272	10.36	2.36	1.7%	-2.2%	5.6%
	weaning	272	10.02	2.39	0.2%	-3.8%	4.2%
Last two groups combined	2nd day	546	10.40	2.57	2.1%	-1.4%	5.6%
	weaning	546	10.07	2.56	0.7%	-2.9%	4.2%

might account for the deaths of 1.4% of experimental group mice between the second day of age and weaning. This small effect is obscured by a somewhat larger opposing difference in second day litter size, which is not statistically significant.

From the standpoint of relevance to radiation risk to human populations, I believe that most persons would feel that death in infancy or childhood is more to be avoided than death *in utero*, and certainly cannot be compensated for by increases in fecundity.

It is not unlikely that the differences in preweaning deaths between experimental and control groups is spurious and due to an unusually small loss of preweanlings in the control group: in the previous four generations the proportion of preweanlings lost in the control groups was more than 3%, and in fact was usually slightly higher in the control than that in the experimental groups. Essentially, then, newly induced heritable mutations have not been proven to have any measurable effect in heterozygotes.

DISCUSSION

This author believes that it is no longer tenable that the net effect of heritable induced mutations in the heterozygous state is expected to be a reduction in population fitness. This is based not only on the rather narrow confidence limits of the negative results of the present experiment, which involved more than 2000 r ancestral irradiation, but even more on the rather uniformly negative results of a sizable number of similar experiments performed in various ways by different investigators.

Recessive spermatogonial mutation rates for individual loci at 16 r/min gamma have not been measured, but it can be presumed that the mean mutation rate is somewhere between 6 and 24×10^{-8} per roentgen per locus (RUSSELL, RUSSELL and KELLY 1958). With a conservative estimate of 12,000 mutable loci per gamete, the irradiation should have resulted in heterozygosity for something like

one to four new major mutations per mouse. Insofar as litter size at weaning is a valid parameter of fitness, these mutations, if present, appear to be entirely recessive. An inbreeding study of these mice is under way at this writing.

One cannot eliminate the possibility that large dominant effects might have been seen in a more stressful environment. The laboratory conditions are near optimal, and practically everything survives except unconditional dominant lethals or homozygous recessive lethals. The effects, if any, of other heterozygous mutations are of the nature of continuously distributed minor factors affecting fitness. Arguments have been given previously that the effects of individually minor genetic decrements might be expected to disappear under laboratory conditions, and that in any case the effect on populations of individually minor mutations might be much less than heretofore supposed (KING 1966, 1976).

The experimental procedure was similar to one that has been employed by LÜNING; the results also, with regard to the presence of dominant lethal effects and the absence of dominant detrimental effects, closely parallel those of LÜNING (LÜNING 1963; LÜNING and SHERIDAN 1964).

SUMMARY

Male random bred mice have been subjected to 545 r spermatogonial gamma irradiation and random bred for four consecutive generations, followed by a fifth generation of mating without irradiation. Other groups were withdrawn from the experimental population and bred without irradiation, or were withdrawn from the unirradiated control population and subjected to one generation of irradiation.—Litters born to irradiated fathers showed a decrease in size at weaning of between 4% and 5%, but litters in groups in which irradiation had been relaxed for one or more generations showed only a small and insignificant increase in litter size. It was concluded that dominant lethals were induced, but that there were no other net dominant deleterious effects.

LITERATURE CITED

- AYALA, F. J., 1966 Evolution of fitness. I. Improvement in the productivity and size of irradiated populations of *Drosophila serrata* and *Drosophila birchii*. *Genetics* **53**: 883-895.
- CRENSHAW, J. W., 1965 Radiation-induced increases in fitness in the flour beetle *Tribolium confusum*. *Science* **149**: 426-427.
- FALK, R., 1967 Viability of heterozygotes for induced mutations in *Drosophila melanogaster*. *Mutation Res.* **4**: 59-72.
- KING, J. L., 1965 The effect of litter culling—or family planning—on the rate of natural selection. *Genetics* **51**: 425-429. — 1966 The gene interaction component of the genetic load. *Genetics* **53**: 403-413. — 1967 Continuously distributed factors affecting fitness. *Genetics* **55**: 483-492.
- LÜNING, K. G., 1963 Studies of irradiated mouse populations. II. Dominant effects of productivity in the 4th-6th generations. *Hereditas* **50**: 361-376.
- LÜNING, K. G., and W. SHERIDAN, 1964 Dominant effects on productivity in offspring of irradiated mouse populations. *Genetics* **50**: 1043-1062.

- RUSSELL, W. L., L. B. RUSSELL, and E. M. KELLY, 1958 Radiation dose and mutation frequency. *Science* **128**: 1546-1550.
- SANKARANARAYANAN, K., 1964 Genetic load in irradiated experimental populations of *Drosophila melanogaster*. *Genetics* **50**: 131-150. — 1965 Further data on the genetic loads in irradiated experimental populations of *Drosophila melanogaster*. *Genetics* **52**: 153-164.
- WALLACE, B., 1956 Studies on irradiated populations of *Drosophila melanogaster*. *J. Genet.* **54**: 280-293. — 1958 The average effect of radiation-induced mutations on viability in *Drosophila melanogaster*. *Evolution* **12**: 532-556. — 1963 Further data on the overdominance of induced mutations. *Genetics* **48**: 633-651.