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

III.* INTERACTION WITH POPULATION SIZE

By K. F. Dyer†

[Manuscript received October 30, 1970]

Abstract

A number of populations of *D. melanogaster*, varying in size from approximately 50 individuals to more than 5000, were chronically irradiated with 4000 r X-rays per generation and studied over a period of 40 generations to determine whether and to what extent the accumulation and equilibrium frequency of lethal chromosomes differed from one to the other. The overall fitness of these populations was studied by comparing the productivity of small samples taken from them, which were allowed to lay eggs and have their offspring develop under optimum conditions. The results of these experiments were as follows:

Lethals accumulated more rapidly and to a greater extent in large populations. Productivity varied systematically with population size. Samples from the larger populations, despite having a higher frequency of lethals, consistently produced a greater number of offspring.

Samples composed of males from one population and females from another were consistently less productive than the intrapopulation samples.

The conclusions from these results, including estimates of the proportion of partially recessive, recessive, and overdominant lethals present in the populations, and the effects of integrated versus non-integrated gene pools are discussed.

I. Introduction

In the extended analyses of the many irradiated populations which Wallace studied in the early 1950's (see particularly Wallace 1956) it was shown among other things that there was an important relationship between the equilibrium frequency of recessive lethal mutations and the size of the irradiated populations (Prout 1954). It was inferred from this, and the fact of greater fluctuations of lethal gene frequencies in small populations, that genetic drift was operating in these small populations. The populations analysed, however, were of only two sizes: 10,000 individuals, estimated to be of effectively infinite size, and 1000 individuals, with an effective population size of about 250.

* Part II, *Aust. J. biol. Sci.*, 1971, **24**, 575–83.

Department of Genetics, Monash University, Clayton, Vic. 3168.

However, recent theoretical work (Nei 1968, 1969; Curnow, Critchley, and Dyer 1969) on the frequency of recessive lethal genes, recessive lethal chromosomes, and the estimation of one from the other suggests that a more detailed investigation of this particular problem would be worth while. In particular, Nei has suggested that it may be possible to detect the presence of lethal genes showing either detrimental or overdominant effects by their rate of accumulation and equilibrium values in populations of different sizes. He suggests that the frequency for partially recessive lethals is independent of population size except in very small populations, while the frequency of completely recessive and overdominant lethals increases as population size increases. The present experiments, using chronically irradiated populations of six different sizes, were therefore carried out to investigate this particular possibility. In conjunction with this, it was also possible to obtain further information on the effect of population structure on the response to irradiation, since by hybridizing these experimental populations they could all be made of equivalent (infinite) size and the effects of the accumulated mutant genes could thereby be studied under fully comparative conditions.

II. Materials and Methods

The six populations to be analysed were each started with 50 pairs of flies derived from one F_1 hybrid cross of two inbred lines. They were kept in aluminium population cages approximately 1 cu ft in size and which had either 2, 4, 6, 8, 12, or 16 food cups inserted in the bottom. With regular changing of these food cups, it was estimated that the population sizes in these cages were approximately 50, 200, 1000, 2000, 5000, and 10,000 flies. These populations were exposed to chronic irradiation amounting to a dose of 4000 r delivered over slightly varying lengths of time every 14 days. This 14-day period was arbitrarily decided on as the generation interval, although it is possible that under these conditions and at the temperature at which the populations were maintained (23°C) the true generation interval might have been a little in excess of this. The dose rate was slightly below 0·4 rads/min and the minimum period of time over which this dose could be delivered was between 8 and 9 days. On most occasions the full dose was delivered within 10–11 days. Samples of newly laid eggs were taken during the intervals between irradiation by providing fresh food cups for 24-hr periods, removing them from the cages, and then allowing the eggs to hatch and develop under optimum conditions. The flies emerging were then tested for the presence of second-chromosome recessive lethals and for overall productivity.

In order to test the productivity potential of these populations, a total of 50 males and 50 virgin females hatching from the egg samples were put into a simple box population cage containing 150 ml of standard *Drosophila* food medium and left for 5 days before being removed. The total number of flies hatching between 12 and 20 days later were collected and counted. The second part of this study was to measure the productivity under the same conditions, except that the 50 males used were collected from one population and the 50 females from another; in other words, the genetic content of these test populations was the same as the normal ones but it was in an artificial gene pool of effectively infinite size. A complete set of interpopulation crosses was carried out in this way.

III. Results

The data on the accumulation of second-chromosome recessive lethals are shown in Table 1. The results from the first generation, together with a number of separate tests, indicate the net mutation rate was approximately 10% per generation.

This value has been used in subsequent calculations. When the combined values for generations 22–40 are considered, the populations are significantly heterogeneous, with an obviously greater frequency of lethals in the larger populations ($\chi^2 = 11{\cdot}434$, $P < 0{\cdot}05$).

The expected gene frequency of a recessive mutation in a small population is $(2\pi N)^{\frac{1}{2}}$ (Wright 1937). This can be summed over all the loci on a particular chromosome. Dr. A. Robertson (personal communication) has dealt with two extreme situations, one in which all the potentially lethal loci are segregating independently and the other in which they were all on one chromosome with no crossing-over at all between them. When all the loci are segregating independently we cannot tell whether lethality is due to homozygosity at 1, 2, or more recessive lethal genes (Curnow,

TABLE 1

ACCUMULATION OF SECOND-CHROMOSOME RECESSIVE LETHALS IN DIFFERENT-SIZED POPULATIONS

Generation	Percentage of Recessive Lethals in Populations of Size:					
	50	200	1,000	2,000	5,000	10,000
1	10·79	6·04	13·33	18·92	6·45	9·31
6	40·20	39·13	33·33	36·36	51·22	50·00
7	47·83	50·00	47·15	58·46		
8	48·05		52·08	56·04		55·90
9		54·01	63·15	60·78	54·67	58·96
10	49·32	53·06	62·75	62·82	55·29	68·67
16		57·75	72·73	82·14	74·29	60·40
17		65·69	71·72	74·42	62·04	70·59
22	60·32	69·12	70·93	86·05	73·80	77·97
30	67·39	57·69	72·50	70·69	89·71	89·71
32		69·17	82·98	86·00	78·13	84·85
36		76·19	78·95	80·00	87·01	91·86
40	64·50	71·43			90·38	88·68
22–40 inclusive	64·47 ±2·00 (352/546)*	70·05 ±2·23 (262/374)	75·00 ±3·00 (144/192)	82·28 ±2·23 (209/254)	80·04 ±1·73 (361/451)	85·47 ±1·41 (447/523)

* Actual number of lethals divided by the total number of chromosomes tested for these generations.

Critchley, and Dyer 1969). However, if it is assumed that the lethal loci are all independent, the probability that a given gamete has at least one lethal is $1-\exp(-KU)$, where $K = (2\pi N)^{\frac{1}{2}}$ and U is the total mutation rate. The likelihood that a random-bred individual is homozygous for at least one lethal is $1-\exp(-KU)$. This latter value is usually small and should, if there are no heterozygous effects, equal the rate of input of new mutations. The second expression divided by the square of the first, gives the value for allelism of lethals in the population.

If it is assumed that there is no crossing-over between lethal genes, new mutations which occur on chromosomes already containing a lethal can be ignored. The equilibrium frequency of lethal chromosomes is $KU/(1+KU)$ and at equilibrium the expected loss will equal the effective input. The different predictions of these two

models compared with the observed results are shown in Figure 1. The observed results are much closer to the model of no recombination and this means that the expected loss of chromosomes can be rather lower than the mutation rate. In other words, the expressed genetic load in the small population is lower than might be anticipated with such a high mutation rate.

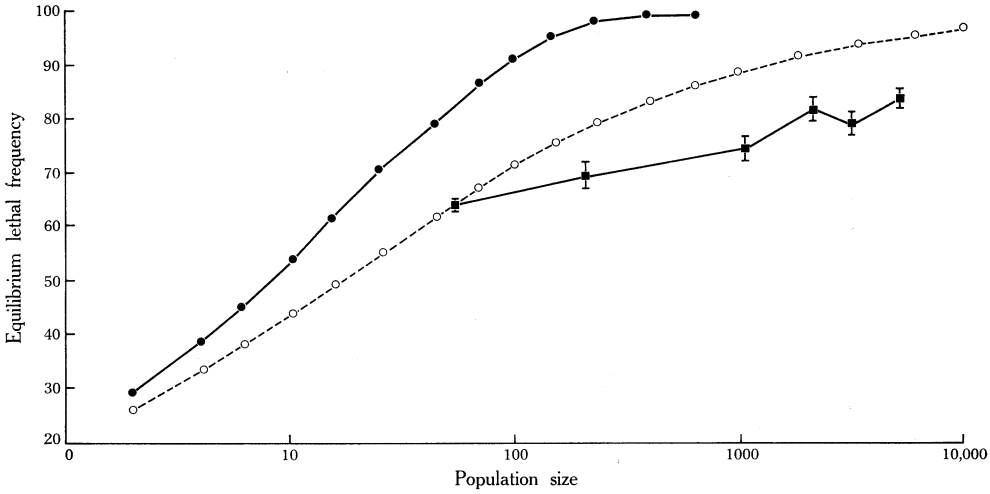

Fig. 1.—Equilibrium lethal frequencies for different-sized populations with a mutation rate of 10% per generation. ● ——— ● Free recombination. ○ - - - - ○ No recombination. ■ ——— ■ Observed values.

The measures of biological productivity which are measures of the effect of these induced mutations are shown in Tables 2 and 3. Examination of Table 2 allows the following conclusions to be drawn: firstly, that these populations, when tested under uniform equivalent conditions, show major differences in productivity positively correlated with population size; secondly, that biological productivity is inversely related to recessive lethal frequency. In other words, the populations with highest frequency of lethals are the least affected.

TABLE 2

NUMBER OF OFFSPRING PRODUCED BY 50 PARENTS FROM IRRADIATED POPULATIONS

	Number of Offspring from Populations of Size:					
	50	200	1,000	2,000	5,000	10,000
Intrapopulation matings	1,726	2,389	2,495	2,168	2,551	3,057
Mean of interpopulation matings	1,507	2,108	2,194	2,009	2,189	2,149
Mean of all matings	1,527	2,133	2,222	2,023	2,222	2,232

The results reported in Table 3, in which, by outcrossing, all the populations are of effectively infinite size, demonstrate two things: firstly, the differential effects are due to the presence of particular genes promoting productivity in the larger populations

and not just inbreeding depression in the small ones; secondly, there is a universal and significant reduction in productivity in all outcrossed populations compared with those retaining the continous integrated gene pool. This is perhaps more clearly shown in Table 2 where the means for inter- and intrapopulation matings are compared.

The conclusions from these results are that lethal gene frequency is dependent on population size and that the effects of these genes can be assessed in terms of the population's productivity. Previous work (Dyer 1969*a*, 1969*b*, 1969*c*) has suggested that it is only a proportion of newly induced recessive lethals which are advantageous. The detrimental effects of the remainder can be assessed in terms of h, the decrease in fitness of the heterozygous carrier.

TABLE 3

NUMBER OF OFFSPRING PRODUCED BY 50 PARENTS FROM IRRADIATED POPULATIONS OF VARIOUS SIZES

Males from Population of Size	Females from Population of Size: 50	200	1,000	2,000	5,000	10,000
50	1,726	1,816	1,601	1,429	1,488	1,503
200	1,497	2,389	2,716	2,625	2,821	2,557
1,000	1,376	2,089	2,495	2,626	2,335	2,487
2,000	1,545	1,428	1,743	2,168	1,503	2,160
5,000	1,493	1,714	2,840	2,604	2,551	2,781
10,000	1,320	1,815	2,130	2,425	2,312	3,057

If in fact we are dealing with a situation where there is a mixture of partially recessive, completely recessive, and overdominant mutations the net heterozygous effect, h, will vary with population size in a systematic way. We can calculate this net heterozygous effect according to the method of Nei (1969).

Since we are normally assessing the frequency of lethal chromosomes and h is the effect of lethal genes, the lethal chromosome frequency must be transformed by

$$Q_1 = \ln(1-Q),$$

where Q is the frequency of lethal chromosomes. The expected value of Q_1 in the tth generation is given by

$$Q_1(t) = \hat{Q} - [\hat{Q}_1 - Q_1(0)]e^{-ht},$$

where h is the selection against lethal heterozygotes. In the present experiments $Q_1(0)$ is zero so that

$$Q_1(t) = \hat{Q}_1(1-e^{-ht}).$$

Expanding e^{-ht} and neglecting terms higher than the second order gives us

$$\begin{aligned} Q_1(t) &= \hat{Q}_1[(ht) - \tfrac{1}{2}(ht)^2] \\ &= \hat{U}t - \tfrac{1}{2}\hat{U}ht^2. \end{aligned}$$

Therefore

$$Ut - Q_1(t) = \tfrac{1}{2}Uht^2.$$

If $y = \hat{U}t - Q(t)$, and since $U = 0{\cdot}10$, then $\hat{h}$, the least-square estimate of h, is

$$(1/0{\cdot}05)(\Sigma yt^2/\Sigma t^4).$$

The estimates of net heterozygous effects of these lethal chromosomes, calculated from the rates of accumulation between generations 1 and 22, are shown in the following tabulation:

Population Size	h	Population Size	h
50	$0{\cdot}05 \pm 0{\cdot}002$	2,000	$0{\cdot}008 \pm 0{\cdot}004$
200	$0{\cdot}044 \pm 0{\cdot}002$	5,000	$0{\cdot}034 \pm 0{\cdot}004$
1000	$0{\cdot}034 \pm 0{\cdot}004$	10,000	$0{\cdot}032 \pm 0{\cdot}004$

It would appear that the net heterozygous effect of these mutants is overall detrimental, but that there is a relationship with population size such that the lower values occur in the larger populations. This is compatible with the possibility that heterozygously favourable mutations are accumulating in the populations of larger sizes.

IV. Discussion

The problems of the effect of newly induced mutations in diploid individuals have hitherto been the subject of some controversy. This has been partly due to a lack of comparability of important experiments and also to analytical tools which are inadequate to utilize the data which have been obtained. Now, however, we have theoretical work by Curnow, Critchley, and Dyer (1969), Prout (1967), and Nei (1968, 1969), coupled with large-scale population experiments (Sankaranarayanan 1966; Tobari and Murata 1966; Ytterborn 1968; Dyer 1969*a*, 1969*b*, 1969*c*). It cannot yet be said that the different points of view can be fully reconciled despite the comprehensive nature of this recent work, nevertheless the broad conclusions arrived at by the present author underline many previous findings and suggest how some of the discrepancies might arise.

First it is clear that the population structure and the nature of the gene pool and genetic background have enormous influence on the expression of recessive lethal genes. Many genes favourable in some populations are much less favourable in others (Dyer 1969*c*). The most important influence in this respect is the continuity or otherwise of the gene pool. These differences are perhaps most clearly demonstrated on a large population scale, e.g. in the transformation of the discontinuous heterozygote population from being at a severe disadvantage to being hardly selected against at all by the addition of five pairs of heterozygote flies to provide the nucleus of a gene pool (Dyer 1971). The strikingly uniform reduction in productivity shown when the chronically irradiated populations were intercrossed is another manifestation of this phenomenon.

Secondly, it is apparent that, even under what might be termed optimum conditions, the majority of newly induced mutants are detrimental and it is, therefore, the relative size and importance of this minority which is under discussion. The present results suggest, in fact, that the heterozygously favourable mutants may form a biologically significant addition to fully integrated gene pools which are of sufficient size to accommodate them.

It was shown in the original experiments of Wallace that heterozygously favourable lethals could be selected in irradiated populations and achieve fairly high frequencies. Of course, many natural populations demonstrate the capacity to

carry a large proportion of detrimental or lethal mutations. The present results show that neither the testing of mutants in artificial situations nor theoretical analysis of the dynamics of these lethals considered as a uniform class is adequate to fully describe the initial nature of these mutants and the rate of their spread in different genetic environments. The differential responses described in the present series of papers are a contribution to the description of this heterogeneity.

V. Acknowledgments

Much of the experimental work on which the above reports are based was carried out at the Medical Research Council Radiobiology Unit, Harwell, England. I am grateful to Dr. M. F. Lyon, Mr. M. J. Corp, and Mr. P. Adams for help in many ways. I would also like to thank Dr. A. Robertson and Dr. M. Nei for their helpful comments.

VI. References

Curnow, R. N., Critchley, M. J., and Dyer, K. F. (1969).—Estimating the degree of allelism of chromosomes carrying lethal recessives. *Genetics, Princeton* **61**, 219–26.

Dyer, K. F. (1969*a*).—The effect of radiation on small competing populations of *Drosophila melanogaster*. I. The accumulation of genetic damage. *Genetics, Princeton* **61**, 227–44.

Dyer, K. F. (1969*b*).—The effect of radiation on small competing populations of *Drosophila melanogaster*. II. The changing frequencies of autosomal lethals. *Genetics, Princeton* **61**, 245–74.

Dyer, K. F. (1969*c*).—The effect of radiation on small competing populations of *Drosophila melanogaster*. III. Changes in competitive ability. *Genetics, Princeton* **61**, 275–91.

Dyer, K. F. (1971).—Effects of radiation on populations of *Drosophila melanogaster* with different genetic structures. I. Changes in fitness and productivity. *Aust. J. biol. Sci.* **24**, 565–73.

Nei, M. (1968).—The frequency distribution of lethal chromosomes in finite populations. *Proc. natn Acad. Sci., U.S.A.* **54**, 517–24.

Nei, M. (1969).—Heterozygous effects and frequency changes of lethal genes in populations. *Genetics, Princeton* **63**, 670–80.

Prout, T. (1954).—Genetic drift in irradiated experimental populations of *Drosophila melanogaster*. *Genetics, Princeton* **39**, 529–45.

Prout, T. (1967).—The theory of allelism between *Drosophila* lethals collected at different times. *Genetics, Princeton* **56**, 654–66.

Sankaranarayanan, K. (1966).—Some components of the genetic loads in irradiated experimental populations of *Drosophila melanogaster*. *Genetics, Princeton* **54**, 121–30.

Tobari, I., and Murata, M. (1967).—Further studies on the effect of X-rays on genetic load in a *Drosophila* population. Ann. Rep. natn Inst. radiol. Sci. 1966. p. 51.

Wallace, B. (1956).—Studies on irradiated populations of *Drosophila melanogaster*. *J. Genet.* **54**, 280–93.

Wright, S. (1937).—The distribution of gene frequencies in populations. *Proc. natn Acad. Sci. U.S.A.* **23**, 307–20.

Ytterborn, K. H. (1968).—The persistency in experimental populations of second chromosome recessive lethals obtained after irradiation of spermatogonia and spermatozoa in *Drosophila melanogaster*. *Hereditas* **60**, 33–71.