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Effects of X-rays on response to selection for a quantitative character of *Drosophila melanogaster*

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1. INTRODUCTION

Evidence on the effectiveness of induced mutation to provide increased response to selection for a quantitative character is rather conflicting. Scossiroli (1953) obtained appreciable response to selection following irradiation of plateaued lines of *Drosophila melanogaster* selected for increased sternopleural bristle number, although the response in lines selected downwards was only small. Scossiroli & Scossiroli (1959) found further evidence of the usefulness of irradiation when they obtained considerably greater progress in irradiated lines, for both outbred and inbred populations. On the other hand, Clayton & Robertson (1955) obtained only a small response to selection for abdominal bristles in an inbred line of *Drosophila* following irradiation. In later work, Clayton & Robertson (1964) used a selection procedure similar to that used by Scossiroli (1953) with plateaued lines selected for either abdominal or sternopleural bristles, but failed to obtain any significant response in most lines. At the same time they obtained only a small response to selection after irradiation of an inbred line.

In the experiments described here, the effects of irradiation of an outbred selected strain of *Drosophila* on the response and the nature of the variation produced were investigated.

2. MATERIALS AND METHODS

The Canberra strain of *D. melanogaster*, described by Latter (1964) was used. This strain has been maintained in our laboratory since March 1964 in a population cage averaging about 4000 adults. The character selected was the number of bristles on one abdominal segment—fourth in males and fifth in females.

Ten pairs of parents were used in each generation, the foundation parents being taken at random from uncrowded bottle cultures.

In each selection line, selection was imposed at an intensity of 5/25 in each sex, in each of two sub-cultures. The two sets of parents were then mixed and mated at random to provide the two cultures for the next generation. The parents remained for 3 days in 5-oz. cream jars, containing a dead-yeast fortified medium (medium F of Claringbold & Barker, 1961).

Three replicate lines (RA, RB, RC) were established, in which the parents were

exposed to 1000 r. each generation at a rate of 33·3 r. per min., just before they were set up in bottles. As very few of the eggs laid in the first day produced adults, the parents were transferred on the second day to fresh cultures. At any particular generation, the lines had been subjected to one more generation of irradiation than selection, because of irradiation of the foundation parents. Five replicates (12a, b, c, d and e) were set up as controls, with the same selection procedure but without irradiation.

After two and ten generations of irradiation, sub-lines were taken off RA, RB, RC, and selected without further irradiation. These are designated R2A, R2B, R2C, and R10A, R10B, R10C, respectively.

Every five generations relaxed lines were taken from each line. These were maintained in fairly crowded cultures with thirty pairs of parents transferred each generation. At the first, second and fifth generation twenty-five pairs were scored; taken from two cultures set up the previous generation with five pairs of parents.

Between the twenty-first and thirtieth generation the presence of second and third chromosome recessive lethals was tested, using a modification of the technique of Brown & Bell (1961). The first and fourth chromosome of their tester stock were replaced by wild-type chromosomes from the Canberra strain. The stock was thus +; In-SMI, al Cy sp^2/Pm ds^{33k}; $Ubx^{130}e^s$/CSb; +. The Cy and Ubx chromosomes contain crossover suppressors. All lethals detected were allelism tested to determine the frequency of each lethal gene.

3. RESULTS

(i) *Response*

The mean bristle number of the females at generations 0, 5, 10, ..., 30, is shown in Figs. 1 and 2. For the treated lines, those receiving a comparable treatment are indicated in the same way. Thus, RA, RB, and RC are all designated in Fig. 1 and later figures by a continuous line. Proportionately, the response in the males was similar. In general, by the tenth generation, the lines which had received some irradiation showed a greater response than the unirradiated lines. In RA and R10A, the mutant *scabrous* (identified by crossing to a *scabrous* stock) appeared at the tenth generation, and both lines soon became homozygous for this gene. A sub-line ($R10A^+$) was taken from R10A, in which only wild-type flies were scored. The frequency of *scabrous* remained high in this line as *scabrous* increased bristle number in heterozygous as well as homozygous condition. We have since shown that the *scabrous* gene increased bristle number by a factor of about 1·2 in heterozygotes and 1·65 in homozygotes for both females and males in the background of R10A and $R10A^+$ at generation 30. As we are chiefly interested in new polygenic variation the response of these lines does not contribute much information.

For both B and C replicates the response is fairly similar in the lines irradiated continually and those receiving only ten generations of irradiation. Both RB and R10B showed considerably more response between generations 10 and 25 than the

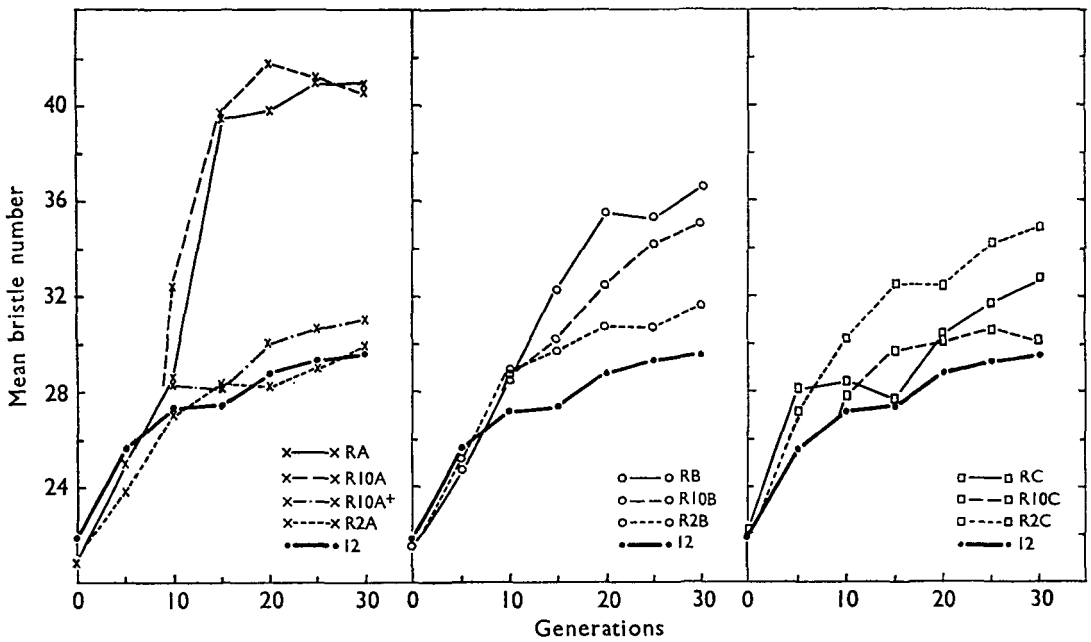

Fig. 1. Response to selection of the individual lines irradiated for 2 (R2A, R2B, R2C), 10 (R10A, R10A+, R10B, R10C) and every generation (RA, RB, RC) and the mean of the unirradiated controls (12).

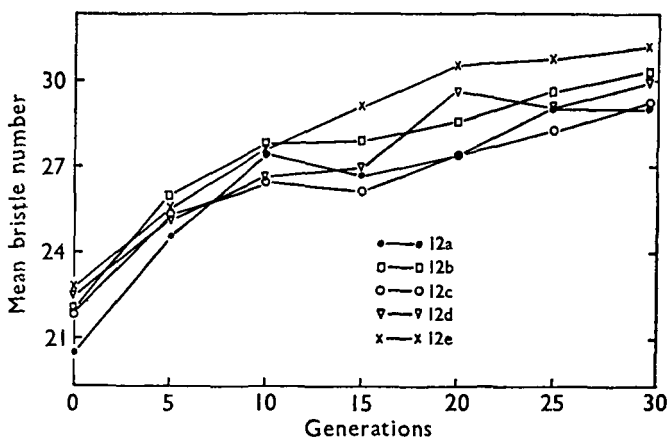

Fig. 2. Response to selection of the individual unirradiated lines (12a, 12b, 12c, 12d, 12e).

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unirradiated controls. Both lines showed a marked decline in fitness during this period and occasionally the selection intensity had to be lowered for RB due to insufficient numbers of progeny. After generation 15, only males were irradiated and there was less difficulty in obtaining enough progeny. R2B responded slightly more than the unirradiated controls.

In RC and R2C, there was a very rapid response during the first five generations. R2C continued to respond at a slightly lower rate, but RC showed little further progress and by generation 30 was only one bristle above the controls. R10C behaved in a similar manner to RC.

(ii) *Variance*

As male and female variances were fairly similar, their average is shown in Figs. 3 and 4. In RA and R10A, the variance was extremely high (23·1 and 27·7 respectively) at generation 11 when *scabrous* was still segregating in both lines.

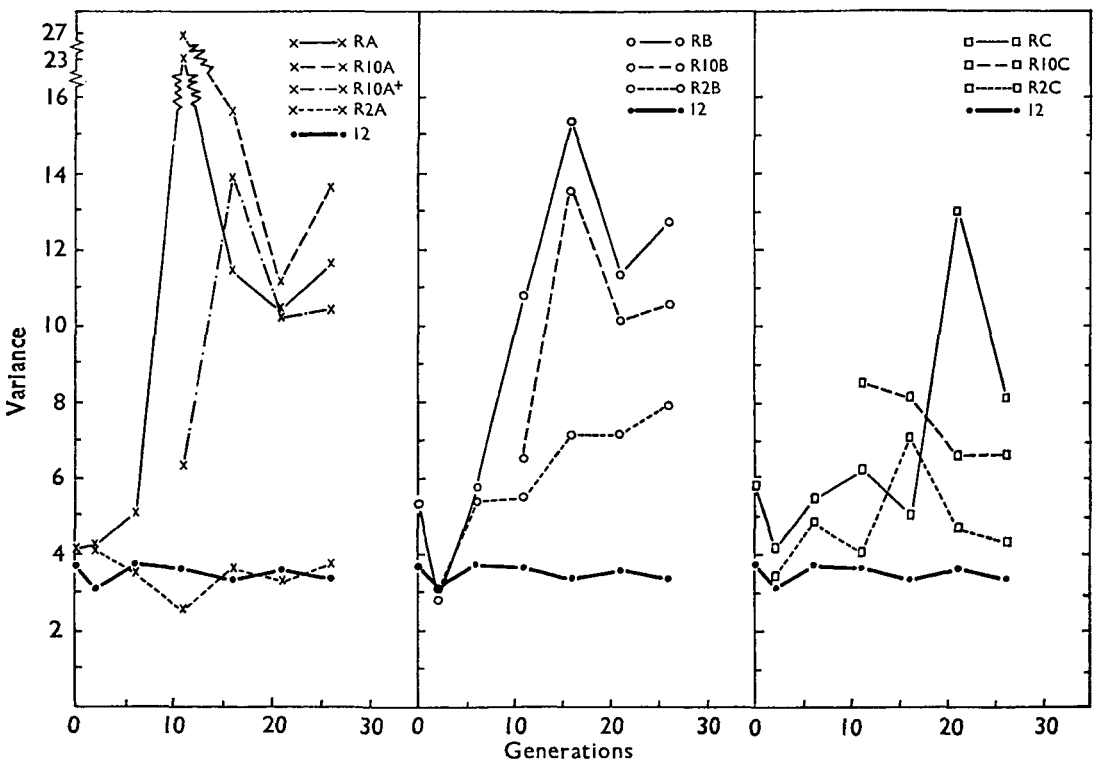

Fig. 3. The variance of the individual irradiated lines. Designation as in Fig. 1.

It remained at a fairly high level (between 10 and 16) even after *scabrous* was fixed. A *scabrous* line, taken from R10A shortly after this gene appeared, had a variance of approximately 9·8 after forty generations as a relaxed line. Thus *scabrous* appears to have been responsible for most of the increase in variance. The variance

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of R10A$^+$ from generation 16 onwards was at a similar level to RA and R10A. As noted earlier, *scabrous* continued to segregate in this line because of the effect of *scabrous* on bristle number in heterozygous condition. This gave rise to a bimodal distribution with modes at the mean of the heterozygotes and wild-type homozygotes. As these differed by a factor of 1·2 in both sexes, a large increase in variance resulted.

After generation 11, the variance of RB reached a similar level to RA. The variance of R10B, R2B, RC, and R10C increased appreciably, and R2C showed a small increase. Of the treated lines, only R2A showed no increase in variance.

The variance of the unirradiated lines showed little or no change.

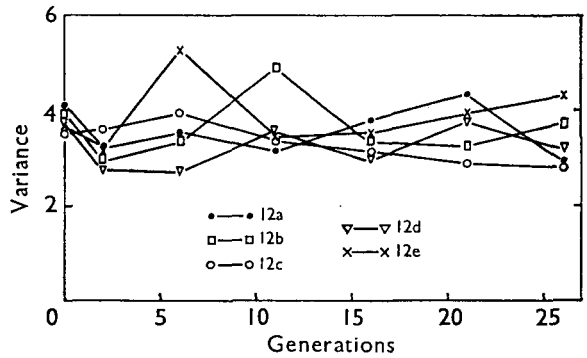

Fig. 4. The variance of the individual unirradiated lines. Designation as in Fig. 2.

(iii) *Relaxation*

The average decline of male and female bristle number after five generations of relaxation, of lines taken off the selection lines at generations 5, 10, ..., 30, is shown in Figs. 5 and 6. The effect of relaxation has been plotted as the decline in bristle number rather than the change in absolute value to allow ready comparison between lines. The lines were also scored after one and two generations of relaxation. There was little change in bristle number in the first two generations, so results for them are not included.

The mean of the line taken off RB at generation 5 fell 1·4 bristles, after being relaxed for five generations. Lines taken off RB at later stages fell much further on relaxation, the mean declining 6·6 and 7.2 bristles on relaxation at generations 20 and 25 respectively.

R10B declined considerably on relaxation, the decline becoming larger in later generations. R2B, RC and R10C declined fairly consistently by about two bristles. RA, R10A, R10A$^+$, and R2C declined only slightly, except for R10A at generation 10, at which stage *scabrous* was still segregating, whilst R2A did not decline. The unirradiated lines generally showed a small decline, and this decline became smaller in later generations.

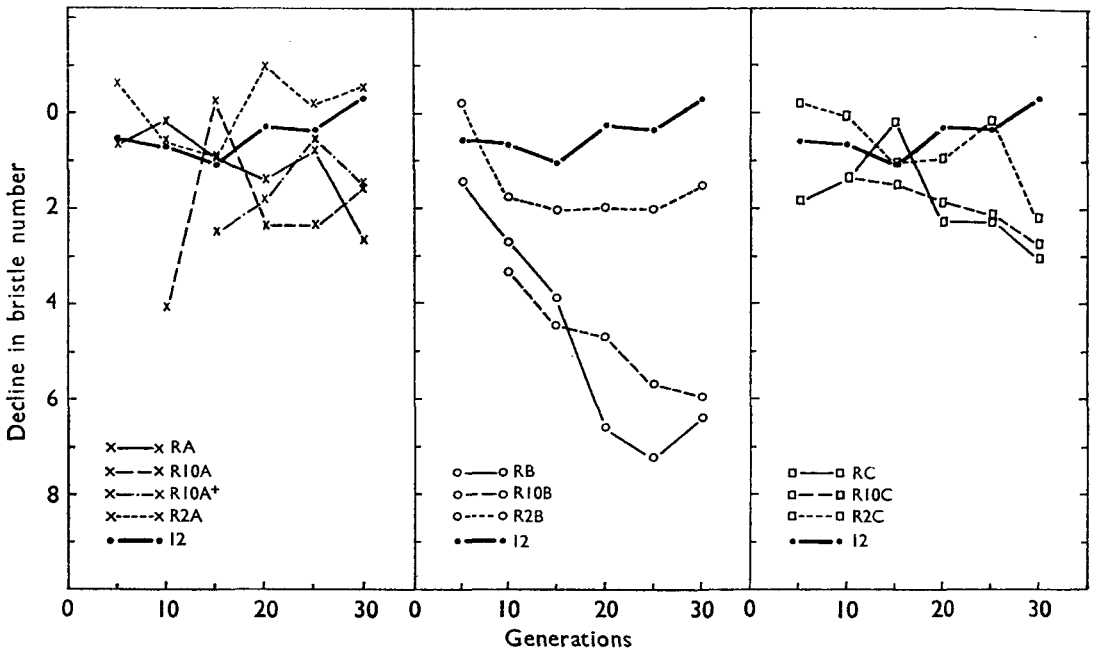

Fig. 5. The decline in bristle number of the individual irradiated lines after five generations of relaxation from generations 5, 10, ..., 30. Designation as in Fig. 1.

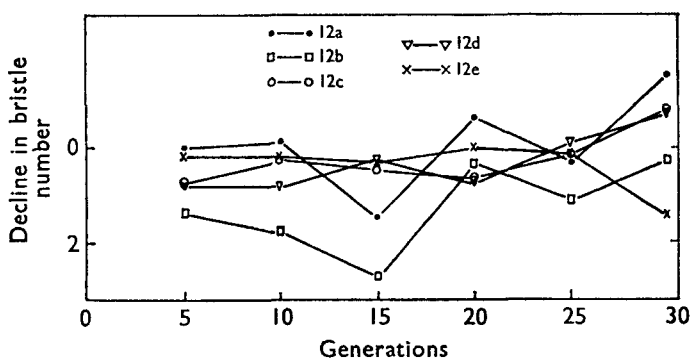

Fig. 6. The decline in bristle number of the individual unirradiated lines after five generations of relaxation from generations 5, 10, ..., 30. Designation as in Fig. 2.

(iv) *Lethal analysis*

The frequencies of lethal second and third chromosomes are shown with the number tested in each line in Table 1. All the treated lines except R2A had total lethal frequencies greater than 10% for at least one chromosome, whilst of the controls, only 12b (chromosome III) and 12c (chromosomes II and III) had frequencies above 10%. Table 2 shows the number of particular lethals in each line with frequencies greater than 10%. There was at least one lethal at a frequency

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greater than 10% in all treated lines except R2A. Of the unirradiated lines, 12b and 12c each had one chromosome III lethal at a frequency greater than 10%.

In several cases, lines from the same initial line carried identical lethals. Thus

Table 1. *The percentage of lethal chromosomes in each line and number of individuals tested* (n)

Irradiated lines

Treatment	R			R10			R2			R10+		
Chromosome	II	III		II	III		II	III		II	III	
	%	%	*n*	%	%	*n*	%	%	*n*	%	%	*n*
Replicate												
A	71·2	40·4	52	53·8	10·3	39	1·9	3·8	53	54·5	6·8	44
B	13·2	31·6	38	19·4	96·8	31	31·4	19·6	51			
C	84·2	50·0	38	36·2	59·6	47	35·1	0·0	57			

Unirradiated lines (12)

Chromosome	II	III			II	III	
	%	%	*n*		%	%	*n*
Replicate				Replicate			
a	2·2	0·0	45	d	4·0	6·0	50
b	2·0	28·0	50	e	0·0	4·3	23
c	16·7	14·6	48				

Table 2. *The numbers of particular lethals each at a frequency greater than* 10%

Irradiated lines

Treatment	R		R10		R2		R10+	
Chromosome	II	III	II	III	II	III	II	III
Replicate								
A	2*	2	2*	1	—	—	2*	—
B	1	2**	1	2**	1	1		
C	3***	3	2***	2	1***	—		

Unirradiated lines

12a, 12d, 12e	None on either chromosome.
12b	One on chromosome III.
12c	One on chromosome III.

* RA, R10A, and R10A+ had same two chromosome II lethals.
** RB and R10B had same two chromosome II lethals.
*** RC and R10C had same two chromosome II lethals, one of which was also in R2C.

RA, R10A and R10A+ each had the same two chromosome II lethals. Table 2 indicates where identical lethals occurred in more than one line. In no case, did the same lethal occur in lines from different initial selection lines. Besides the lethals at appreciable frequencies, there were lethals carried by only one or two individuals in most lines.

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4. DISCUSSION

At first glance, our results support the conclusion of Scossiroli & Scossiroli (1959) that induced mutation is a useful aid to selection for quantitative characters. A closer look indicates that much of the extra response is due to a few genes of large effect. Firstly, the spectacular response of RA and R10A was caused by a major gene, *scabrous*. This gene has since been found in other selection lines from the Canberra strain in our laboratory, and so may not have been induced by irradiation. In any case, the response of these lines cannot be ascribed solely to induced polygenic variation. The high variance of the *scabrous* lines was mainly caused by the *scabrous* gene, which also increased both mean and variance of other bristle systems, e.g. scutellars and sternopleurals.

RB and R10B, which showed a much greater response than the controls, had a higher variance, carried a number of lethals and the mean dropped rapidly on relaxation. This indicated that the lethals had a large effect on bristle number or were closely linked to genes affecting bristle number. The high variance of these lines could have been caused either by the segregation of the lethals, or by the lethal genes themselves in a similar manner to *scabrous*. Similarly, RC and R2C, which responded rapidly during the first five generations, carried a lethal which was detected in a preliminary test at generation 4. The regression on relaxation in R2C was generally small but RC declined considerably. The only irradiated line which failed to regress on relaxation was R2A, and this did not carry any lethals at high frequency. Of the controls, 12b generally declined the most, and carried a lethal on chromosome III. 12c, on the other hand, declined only slightly (about half a bristle) in the first twenty generations, and not at all since then. This line carried a lethal at a reasonable frequency on chromosome III, but it seems that this did not have much effect on bristle number and may have been lost in later generations. The other controls were almost lethal free and showed only slight regression on relaxation.

From the lethal analysis and relaxed lines, it appears that much of the extra response of the irradiated lines was due to particular lethals. Alternatively the lethals could have been linked to genes or chromosome segments which increased bristle number. Such lethals would prevent or reduce the rate of fixation of the linked genes affecting bristle number and these would continue to segregate. A decline on relaxation and an increase in variance would be expected. However, this would reduce the response of the irradiated lines in comparison with the un-irradiated lines, unless sufficient new variation was induced to compensate for this. Thus, it appears most likely that the lethal genes themselves increased bristle number. It is reasonable to assume that some of the response would be due to other genes with large effect on bristle number and less drastic effect on fitness. The fitness of RB and R10B declined appreciably from generation 10 onwards at which stage both lines were still responding to selection. In later generations the fitness of these lines improved only slightly on relaxation. This indicated that some genes which increased bristle number and had a deleterious effect on fitness became

fixed. However, no attempt was made to isolate any of these genes or to measure the fitness of the lines quantitatively.

Clayton & Robertson (1964) concluded that 500,000 r. of X-radiation is needed to produce, in an inbred line, a similar amount of genetic variation for abdominal bristles to that found in a wild population, but with Yamada & Kitagawa's (1961) figures this reduces to 60,000 r. Both of these are extremely high. Robertson (personal communication to J. S. F. Barker) has since shown that selection during the period of irradiation will make any significant difference only for genes with an average effect greater than one-third of a standard deviation. This appears to have been the case in these experiments where most of the extra response of the irradiated lines was apparently due to genes of large effect. Similarly, Harrison (1954) obtained response to selection under irradiation of an inbred line, only in a line in which a major wing mutant appeared.

In Scossiroli's (1953) high selection lines the response was spectacular but only lasted for a few generations in one line, and a decline in fertility upset selection in the other replicate in later generations. The response was very different in the two replicates, and reverse selection rapidly returned the mean to that of the unirradiated controls. Latter (1965) showed that a large gene initially at a low frequency would give a rapid response for a few generations. It appears that most of the response in these lines was due to a few genes of large effect. In later work, Scossiroli & Scossiroli (1959) obtained considerably more response to selection in irradiated inbred and hybrid populations than the corresponding unirradiated lines. The difference was marked and significant only in lines selected and irradiated every generation. In lines selected and irradiated every second generation the response was much smaller and the decline in fitness less marked. These results agree with the assumption that most of the extra gain to be expected with irradiation is due to a few genes with an appreciable effect on bristle number and a deleterious effect on fitness. Under the less intense selection procedure natural selection would have been too great for these genes to attain appreciable frequencies.

Hollingdale (unpublished) selected with irradiation in the same stock as mine as well as in an inbred line, but with a larger population (100 pairs of parents) and lower selection intensity (50%). There was little difference between the response of irradiated and unirradiated lines after twenty generations of selection. The lower selection intensity used by her would be less efficient in concentrating genes which increase bristle number, but which have a deleterious effect on fitness.

Similarly, Clayton & Robertson (1964) found that response to selection for high or low abdominal bristle number in an outbred Kaduna population, following twenty generations of irradiation, was similar to that of unirradiated lines. Their results were also complicated by the appearance of a *scabrous* gene in one line and this was known to be present in their base population. As the lines were irradiated prior to selection, any genes which increased bristle number but had deleterious effects on fitness would tend to be eliminated by natural selection.

Attempts to measure mutation rates for genes affecting bristle number are recorded by Yamada (1961), Yamada & Kitagawa (1961) and Clayton & Robertson

(1964). These have been based on increases in genetic variance in inbred or isogenized lines as measured directly or by response to selection. In our experiments, outbred lines have been used. Further, selection and irradiation have been carried out simultaneously. For genes of large effect initially at low frequencies, selection will cause an appreciable increase in variance and response will be rapid for a few generations (Latter, 1965). It does not seem possible therefore to obtain a valid estimate of mutation frequency from the response in our lines.

However, we may ask whether or not mutation rates for loci in *Drosophila* are sufficient to give the response obtained. Russell (1956) notes that estimates of mutation rates for visible mutations in *Drosophila* are of the order of 1×10^{-7} per roentgen per locus. With a dose of 1000 r. and 100 individuals scored per generation the chance of scoring an individual with a mutation at a particular locus would be 1/100.

We have little idea of the number and effect of loci likely to be involved. Falconer (1960) suggests that the variance in the Kaduna wild population was controlled by about 100 loci with proportionate effects of about 0·21 standard deviations per locus for total bristle number on two segments. Using these values, Yamada (1961) estimated that the mutation rate for abdominal bristles was between $3 \cdot 3 \times 10^{-6}$ and $4 \cdot 1 \times 10^{-5}$ per roentgen per locus. This is considerably higher than the estimate for visible mutations. Yamada suggests the difference could be partly due to underestimates of either the number of loci or the effects of the genes.

If we assume that there are 100 loci we would expect that, on the average, one individual would have a new mutation at one locus each generation. From the selective values of genes of large effect suggested by Latter (1965) genes with a proportionate effect greater than 0·5 standard deviation would be rapidly selected. The differences in response between the irradiated and unirradiated lines were only about 2·5 standard deviations, the mean of RB and RC being 4·8 bristles above the controls of generation 30.

Mutations at only five or six loci with effects of half a standard deviation could produce this difference. Over thirty generations, the probability of a mutation at any particular locus would be about one-third. There is therefore an appreciable chance of mutations occurring at a few loci. In view of the large errors involved in determining the number of loci and their effect, and realizing that the mutation rate could vary for individual loci by a factor of 10 or more, it seems reasonable to conclude that the mutation rate would be sufficient to produce the extra response obtained.

Irradiation may therefore have an appreciable effect on response to selection for a quantitative character, but only when the character is partly controlled by genes of large effect.

SUMMARY

1. Lines with ten pairs of parents and selected at an intensity of 20% were exposed to 1000 r. of X-rays for 0, 2, 10 or 30 generations.

2. Lines which received some irradiation generally gave greater response than the unirradiated controls. The phenotypic variance in the irradiated lines was much higher than in the controls. There was little difference in behaviour between lines receiving ten generations of irradiation and those irradiated every generation. Lines receiving only two generations of irradiation had lower variances than the other irradiated lines, but in one of three replicates the response was greater than the corresponding continuously irradiated line.

3. Lethal frequencies were much higher in irradiated than unirradiated lines. Particular chromosome II and III lethals were at high frequencies in most of the irradiated lines but in only two out of five controls.

4. On relaxation, the mean of the irradiated lines generally declined considerably, but in the unirradiated lines there was only a very small regression.

5. It appears that most of the extra response and increased variance in the irradiated lines were caused by a few genes with large effect on bristle number.

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