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Selection for Increased Abdominal Bristle Number in *Drosophila melanogaster* with Concurrent Irradiation

I. Populations Derived from an Inbred Line

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Summary. Replicate lines, each with one hundred pairs of parents selected at 50% intensity, were derived from an inbred line. For twenty generations three lines were selected without irradiation and five with 1000 *r* *X*-rays per generation given to both females and males. After adjustment for level of crowding in the cultures, the final mean was 1.3 bristles higher in females and 1.0 bristles higher in males in the irradiated lines than in the unirradiated lines. In terms of phenotypic standard deviations in each sex in the base population, these total responses were 0.74 and 0.59 respectively. Radiation can induce mutations useful in increasing responses in selection programmes, but the average response attributable to radiation is small, and heterogeneity between replicate lines is to be expected.

The amount of genetic variation for quantitative characters present in populations is important because of its implications for the evolution of the species and for practical breeding purposes in domestic species. In an artificial selection programme, if the variation amenable to selection were proved to be limiting, induced mutations may provide genetic variance useful for further selection response. However, in outbreeding species, the amount of variation present may make it difficult to distinguish the effects of extra variation induced by mutagen treatment. But a homozygous genotype should provide a favourable background for the detection of even small amounts of induced variation utilizable by selection. Using isogenic stocks of *Drosophila melanogaster* irradiated every generation, Buzzati-Traverso (1954) found that the adaptation and modification of expression of the mutant *spineless* phenotype under conditions of intense larval and adult crowding was faster than in unirradiated populations. Artificial selection with concurrent irradiation has been done using populations derived from isogenic stocks by Clayton and Robertson (1955), Scossiroli and Scossiroli (1959) and Kitagawa (1967). As the magnitude of responses obtained in irradiated lines varied considerably, further experimental evidence would contribute to determination of the value of irradiation in artificial selection programmes.

The experiment described here investigated the effects of artificial selection with concurrent irradiation in lines of large population size derived from an inbred line of *D. melanogaster*. However, apart from inducing new genetic variation, irradiation is likely to exert a direct effect in reducing progeny numbers. As a result of the reduced larval crowding, body size of adults and abdominal bristle number are likely to be increased (Rasmuson 1952, Reeve and Robertson 1954). Clayton and Robertson (1955) found that the lower degree of crowding in irradiated lines could cause a small increase in abdominal bristle number. An experiment was done therefore to assess the importance of crowding effects, and in particular, to provide estimates of selection response free from any possible bias associated with differential crowding in the unirradiated and irradiated lines.

Materials and Methods

Base population: The base population was an inbred line (designated N5), which had been maintained by full-sib mating for one hundred and sixty generations. Subsequently, two generations of random mating were used to obtain sufficient flies to initiate selection.

Selection programme: Mass selection for increased bristle number on one abdominal sternite (fifth in females, fourth in males) was carried out for twenty generations. The code names of the various lines, with their histories of irradiation and selection, are given in Table 1. In each selection (*S*) line one hundred pairs were selected out of two hundred pairs scored per generation (including generation 0), *i.e.* 50% selection intensity. The unselected control lines (*U*) were maintained with one hundred pairs chosen at random each generation, although only fifty pairs were scored. All unselected lines were discontinued at generation 10.

Culture conditions: Lines were maintained in 5 oz cream bottles on a dead-yeast fortified medium (medium *F* of Claringbold and Barker 1961) with a drop of live yeast suspension (1 g compressed yeast: 2 ml of water) on the surface. The one hundred pairs of parents were divided at random into five groups of twenty pairs to make up five bottles per line. Cultures were maintained at 25 ± 0.5 °C and 65–70% relative humidity in a room lit twelve hours per day (6 a. m. to 6 p.m.). Parents were discarded after two days to prevent overcrowding of larvae. Progeny collected over the first two to three days emergence were

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Table 1. *Details of selection regimes of lines derived from inbred line N5*

Line	*X*-ray dose (*r*/generation)	Scored at generations	Number of pairs scored per line in each generation
Selected (100 pairs of parents, 50% intensity)			
SO.1, *SO*.2, *SO*.3	0	0–20	200
SR.1, *SR*.2, *SR*.3, *SR*.4, *SR*.5*	1000	0–20	200
Unselected (100 pairs of parents, chosen at random)			
UO.1, *UO*.2, *UO*.3	0	0–10	50
UR.1, *UR*.2, *UR*.5	1000	0, 2, 4, 6, 8, 10	50
UR.3, *UR*.4, *UR*.6	1000	1, 3, 5, 7, 9, 10	50

* Random sample of 100 pairs irradiated and mated in generation 0.

scored and selected on a within-bottle basis. Selected flies from the five bottles of each line were bulked, irradiated if required, and then mated. Each generation cycle was kept to fourteen days for convenience and to reduce any effect of age variation on mutation frequency, due to differing sensitivity of stages of development of the gametes (Lefevre and Jonsson 1964 and Sobels 1965).

Irradiation: Radiation treatments, of 1000 *r* *X*-rays delivered over thirty minutes, were given to both males and females immediately prior to mating in each generation. After scoring, all flies to be irradiated were lightly re-etherized and placed in large (approximately 4.5 cm × × 1.5 cm diam.) gelatine capsules, one hundred males or females per capsule. The dose was delivered by an *X*-ray machine operated at 100 kv and 4.35 mamps with a 1 mm aluminium filter. A relatively low intensity of 33.3 *r*/minute was used to reduce the likelihood of occurrence of gross chromosomal aberrations. One-hit point mutations do not show a radiation intensity effect, but the frequency of two-hit aberrations is directly related to the intensity of irradiation (Wolff 1967).

Effects of crowding on bristle number: At the end of the selection experiment, the effect of cessation of irradiation was examined in selected sublines taken from the irradiated selection lines. Interaction of crowding effects with the radiation history of the selection lines was studied by increasing the range of larval crowding in unirradiated cultures from all selection lines. To do this, cultures were set up at generations 19 and 20 with either five pairs or twenty pairs of parents selected at 50% intensity. Parents were left in the culture bottles for one and a half days. The various lines, sublines and parental crowding treatments are given in Table 2. The three unirradiated selection lines (*SO* lines) were continued at 20 pairs of parents per culture (Code (*i*)), and sublines were set up with 5 pairs of parents per culture (Code (*ii*)). Separate sublines from the irradiated selection lines (*SR* lines) were set up with either 20 pairs of parents per culture (Code (*iii*)), or 5 pairs of parents per culture (Code (*iv*)).

Progeny number per culture and the culture means for male and female bristle number were used in analyses of results. Within culture sampling variance for bristle number was not considered, *i.e.* no attempt was made to weight the data to allow for differences in standard errors of means derived from samples of size twenty or forty. The variation between replicate cultures within lines was used as the error variance.

Table 2. *Lines, sublines and parental crowding treatments (the degree of replication in each line or subline is represented by r/s where r = number of replicate cultures and s = number of pairs scored for bristle number per replicate culture)*

Source	*SO* lines	Sublines*		
		SO lines	*SR* lines	*SR* lines
Code	(*i*)	(*ii*)	(*iii*)	(*iv*)
Parents per culture (pairs)	20	5	20	5
Progeny *r*/*s* at:				
Generation 20	5/40	5/20	2/40	5/20
Generation 21	2/20	5/20	2/20	5/20

* Sublines derived by 50% selection from previous generation of source lines; no radiation treatments were given.

Results

Response to selection: Detailed results for mean bristle number of females and of males in each generation of all lines were reported by Hollingdale (1969). The means for females for each generation of the unselected control lines within each radiation treatment and the differences between treatment means are shown in Table 3. In these lines, there was an indication that irradiation had affected mean bristle score. Over the ten generation period (excluding generation 0, which was scored before the initial radiation treatment), the average difference in mean bristle number (irradiated — unirradiated) was 0.25 bristles in females and 0.35 in males. The possible causes of this difference are considered later (*Effects of crowding*).

Mean bristle numbers for females of selected lines, radiation treatment means and the difference between irradiated and unirradiated treatment means for every fifth generation are shown in Table 4. Data for males were similar. In the unselected control lines (Table 3), the difference between radiation treatment means did not change over time, but the means of the irradiated and unirradiated selection lines (Table 4) gradually diverged and this divergence, after generation 11, was consistently greater than the

Table 3. *Mean abdominal bristle number of females in the unselected lines*

Generation number	Bristle number		Difference (irradiated-unirradiated)
	Unirradiated lines	Irradiated lines	
0	21.19	21.52	0.3
1	21.15	21.24	0.1
2	20.82	21.19	0.4
3	20.98	21.19	0.2
4	21.21	20.92	−0.3
5	20.65	21.41	0.8
6	21.53	21.60	0.1
7	20.93	21.43	0.5
8	21.21	21.11	−0.1
9	20.86	21.44	0.6
10	21.15	21.32	0.2

Table 4. *Mean bristle number for females of each selection line*

Generation number	Bristle number										Difference of means (1)−(0)
	Unirradiated lines				Irradiated lines						
	SO.1	*SO*.2	*SO*.3	Mean (0)	*SR*.1	*SR*.2	*SR*.3	*SR*.4	*SR*.5	Mean (1)	
0	21.23	21.38	21.35	21.32	21.29	21.34	21.44	21.50	*	21.39	0.1
5	20.82	20.86	21.14	20.94	20.99	21.39	21.01	20.97	21.39	21.15	0.2
10	21.64	21.48	21.29	21.47	21.57	21.98	22.17	21.33	21.54	21.72	0.3
15	20.93	21.39	21.32	21.21	21.72	22.09	22.27	21.35	21.85	21.86	0.7
20	21.59	21.19	21.65	21.48	22.59	22.62	22.48	22.05	22.41	22.43	1.0

* Not scored; random sample set up.

average difference between irradiated and unirradiated unselected control lines. The radiation treatment was therefore effective in promoting greater response to selection.

Regressions of line mean score on generation number were used to examine selection response patterns. Combined analyses of lines in the unselected treatments and in the selected unirradiated treatment showed no heterogeneity of regression coefficients within treatment groups. Pooled estimates ($\hat{b}$) of these regression coefficients are given in Table 5. However, there was significant heterogeneity between replicate lines in the selected irradiated treatment, so regression coefficients for each line of this treatment are given in Table 5. Separate coefficients are shown for each sex, as the rate of response was generally higher in females. This tendency was also apparent in the pooled estimates from the unirradiated selection lines ($\hat{b} = 0.0190 \pm 0.0046$ in females, 0.0077 ± 0.0050 in males). There was no response in the unselected lines – the regression coefficients did not differ significantly from zero. The response was small but significant in unirradiated selected lines and considerably larger in irradiated selected lines.

Table 5. *Rate of response as measured by regression of mean bristle number on generation number ($\hat{b}$ = estimated regression coefficient)*

Line	$\hat{b}$ (pooled)	Line	$\hat{b}$ (females)	$\hat{b}$ (males)
UO.1, *UO*.2, *UO*.3	0.0020	SR.1	0.0742**	0.0432**
		SR.2	0.0940**	0.0732**
UR.1, *UR*.2, *UR*.5	−0.0043	SR.3	0.0868**	0.0529**
UR.3, *UR*.4, *UR*.6		SR.4	0.0526**	0.0597**
SO.1, *SO*.2, *SO*.3	0.0133**	SR.5	0.0720**	0.0454**

** $P < 0.01$.

Cumulative selection differentials to generation 19 are given in Table 6, together with realized heritabilities for each sex, calculated as the regression of mean bristle number (within sexes) on cumulative selection differential (averaged over sexes). In general, cumulative selection differentials after twenty generations of selection were higher in the irradiated selection lines than in the unirradiated selection lines. The only exception was line SR.4, where the total selection differential was no greater than in the unirradiated lines. However, the response of *SR*.4 was larger than that of the unirradiated lines (Table 5), so that irradiation had increased the genetic component of the variance. Realized heritabilities generally were lower in males than in females but did not differ between lines within radiation treatments. The pooled estimates for females and males in the irradiated lines were significantly higher than those for the unirradiated lines.

Table 6. *Cumulative selection differentials to generation 19 and realized heritabilities for each sex*

Line	Cumulative selection differential	Realized heritability (%)	
		Females	Males
SO.1	24.7	0.9	0.4
SO.2	24.8	1.9**	1.0
SO.3	24.4	1.5*	0.3
SO (pooled)		1.5**	0.6
SR.1	25.3	5.6**	3.3**
SR.2	27.2	6.6**	5.1**
SR.3	26.3	6.3**	3.8**
SR.4	24.5	4.1**	4.7**
SR.5+	26.1	5.3**	3.3**
SR (pooled)		5.7**	4.1**

* $P < 0.05$.
** $P < 0.01$.
+ Cumulative selection differential to generation 20, as there was no selection in generation 0.

Changes in variance: The normality of the distribution of bristle number in the inbred line was tested at the first scoring (Table 7). There was no evidence of asymmetry but the distribution for males showed positive kurtosis.

The phenotypic standard deviations are shown in Figure 1 for irradiated selection lines. Linear regres-

Table 7. *Base population parameters for abdominal bristle number (fifth sternite in females, fourth sternite in males) for inbred line N5 at generation 0*

	Number in sample	Mean	Standard deviation	Skewness g_1	Kurtosis g_2
Females	1900	21.37	1.76	−0.01	0.04
Males	1900	16.89	1.70	0.05	0.26*

* $P < 0.05$.

Table 8. *Phenotypic standard deviations for females of each unselected line (means calculated from mean variances)*

Generation number	Phenotypic standard deviation										
	Unirradiated lines				Irradiated lines						
	UO.1	*UO*.2	*UO*.3	Mean	*UR*.1	*UR*.2	*UR*.3	*UR*.4	*UR*.5	*UR*.6	Mean
0	1.81	1.31	1.66	1.61	1.80	1.88			1.89		1.85
1	1.83	1.73	1.80	1.79			1.65	1.69		1.75	1.69
2	1.89	1.86	1.91	1.89	1.50	1.91			1.85		1.76
3	1.58	1.53	1.87	1.66			1.82	1.97		1.48	1.77
4	1.83	1.79	1.71	1.78	1.36	1.49			1.61		1.49
5	1.59	1.82	1.72	1.71			1.22	1.58		1.93	1.60
6	1.47	2.08	1.59	1.73	1.75	1.42			1.62		1.60
7	1.64	2.15	1.74	1.86			1.60	1.72		1.82	1.71
8	1.62	1.80	1.83	1.75	1.63	1.70			1.70		1.68
9	2.18	1.55	1.92	1.90			1.79	1.53		1.74	1.69
10	2.14	2.10	1.69	1.98	1.62	1.67	1.63	1.56	1.96	1.92	1.73
Mean				1.79							1.68[+]

[+] Mean of the irradiated generations, *i.e.* generations 1 to 10.

sion equations were calculated for each sex in each line, but the regression coefficients did not differ between sexes. Comparable regressions for the unirradiated selection lines are also shown. Although there was considerable generation-to-generation fluctuation, the general pattern was clear. Regression coefficients for the irradiated selection lines were all significantly different from zero ($P < 0.01$), but those of the unirradiated selection lines were not. The rate of increase of phenotypic standard deviation was highest for *SR*.2, the line which responded most to selection. In *SR*.4, the standard deviation did increase, but as noted previously, the total applied selection differential over twenty generations was no greater than the total selection differential in unirradiated lines. The standard deviation remained low during the early generations and most of the increase occurred after generation 16 (Figure 1). An analysis of the data to generation 16 showed no significant increase.

Phenotypic standard deviations for females in the unselected lines are shown in Table 8. As each standard deviation was based on a sample of only fifty individuals the accuracy of estimation was low. Large generation-to-generation fluctuations and the few generations available precluded any analysis of trends in individual unselected lines. However, the mean standard deviations over replicate lines within radiation treatments apparently did not change over the ten generation period. Phenotypic standard deviations in females were higher than in males, as expected from the base population analysis (Table 7). The mean standard deviation in irradiated lines was lower than that in unirradiated lines for both females and males. The difference was small, similar in magnitude to the difference between the sexes, and could have been due to reduced larval crowding or to increased stability of development in the irradiated lines. Wallace (1963) found that, in a homozygous background, heterozygotes for newly induced mutations had increased viability; this suggests that developmental stability may also be enhanced following irradiation of highly inbred lines. Whatever the cause, the lower phenotypic variance in the irradiated unselected lines indicated that reduction in environ-

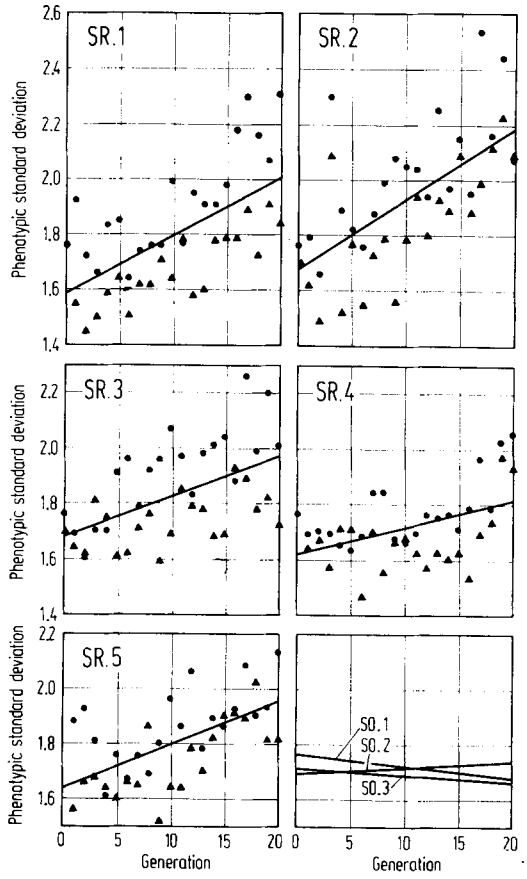

Fig. 1. Phenotypic standard deviations in the irradiated selection lines. Circles = values for females, triangles = values for males. Lines indicate pooled within-sex regressions. The regression lines for the unirradiated selection lines are also included

mental variance was greater than any increase in genetic variance. In similar unselected lines derived from a wild-type outbred base population, there were no differences between phenotypic variances of irradiated and unirradiated lines (Hollingdale and Barker 1971).

Effects of crowding on bristle number: The relationship between progeny number per culture and the culture means for bristle number was examined over all lines. There was a large difference in progeny number per culture between the two levels of parental crowding. However, progeny number varied greatly between lines within each level of parental crowding (Figure 2). An analysis of variance for progeny number was calculated at each level of parental crowding (Table 9). Degrees of freedom for lines were partitioned giving one degree of freedom for the comparison of unirradiated and irradiated lines (*SO*.1, *SO*.2 and *SO*.3 versus *SR*.1, *SR*.2, *SR*.3, *SR*.4 and *SR*.5), two degrees of freedom for comparing lines in the unirradiated group, and four degrees of freedom for comparing lines in the irradiated group.

Table 9. *Analyses of variance for progeny number — (a) data from cultures with twenty pairs of parents, (b) data from cultures with five pairs of parents*

Source of variation	d. f.	Mean squares ($\times 10^4$) (a)	(b)
Lines	7	2.76**	2.12**
Radiation effect	1	11.06**	1.00*
Unirradiated	2	0.51	0.08
Irradiated	4	1.82*	3.41**
Times	1	1.82	0.03
Lines × Times	7	0.66	0.15
Error: (a)	16	0.43	
(b)	64		0.20

* $P < 0.05$. ** $P < 0.01$.

In each analysis in Table 9 the main effect for lines was significant. Partitioning of sums of squares for lines revealed that progeny number was significantly higher for the unirradiated lines (radiation effect). Unirradiated lines agreed closely with each other, but there were significant differences between irradiated lines for progeny production at both levels of parental crowding.

By combining treatment codes (*i*) with (*ii*) and (*iii*) with (*iv*) (Table 2), and reducing at random the number of replicate cultures in generation 20 treatment code (*i*) from five to two, the data on bristle number were analysed as an equally replicated 8×2 factorial, with eight lines, two times (generations) and seven replicates (two replicates with twenty pairs of parents plus five replicates with five pairs). Exclusion of some of the results in treatment code (*i*) was done only to facilitate the analysis. The combination of twenty and five pair parental crowding treatments is valid as these treatments were imposed only as a means of increasing the range of the covariate, *i.e.* progeny number.

Table 10. *Analyses of variance for mean bristle number in progeny females and males*

Source of variation	d. f.	Mean squares Females	Males
Lines	7	8.427**	4.956**
Radiation effect	1	53.144**	31.169**
Unirradiated	2	0.409	0.196
Irradiated	4	1.256**	0.782
Times	1	0.243	0.048
Lines × Times	7	0.229	0.081
Error	96	0.344	0.406

** $P < 0.01$.

Analyses of variance for mean bristle number are given in Table 10. The lines main effect was the only significant source of variation with most of the between lines variance due to the significantly higher bristle number in irradiated lines (radiation effect). Of the residual between lines variation, the greater proportion was due to irradiated lines though this reached significance only in females.

Covariance analyses showed highly significant error regressions of female and male bristle number on progeny number (see Figure 2). The effect of lines adjusted for variation in progeny number remained highly significant in both sexes, and from the adjusted line means (Table 11), it is clear that the radiation effect (*i.e.* the comparison of lines *SO*.1 to *SO*.3 versus lines *SR*.1 to *SR*.5) remained the important source of between line variation in mean bristle number. After this adjustment for level of crowding, the mean bristle number of the irradiated lines following twenty generations of selection was higher than the mean of the

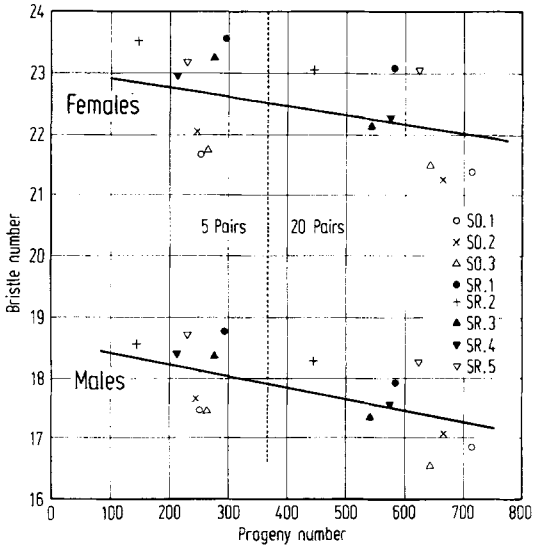

Fig. 2. Mean bristle number and progeny number per culture for selection lines cultured with five or twenty pairs of parents, and the error regression lines for bristle number on progeny number. None of the parents were irradiated

Table 11. *Regression over all lines of mean progeny bristle number on progeny number and adjusted means for each line*

Regression coefficient	Adjusted means of line:							
	SO.1	*SO*.2	*SO*.3	*SR*.1	*SR*.2	*SR*.3	*SR*.4	*SR*.5
Females								
-0.0015 ± 0.0003	21.57[+]	21.91	21.71	23.49	23.21	22.91	22.70	23.12
Males								
-0.0020 ± 0.0003	17.31[+]	17.44	17.23	18.59	18.26	18.09	18.11	18.60

[+] Standard error of an adjusted mean = 0.14.

unirradiated lines by 1.3 bristles (0.74 standard deviations) in females and 1.0 bristles (0.59 standard deviations) in males.

Discussion

These results for artificial selection with concurrent irradiation are in general agreement with previous work on lines derived from inbred or isogenic stocks. Response in the unirradiated control selection lines over twenty generations was 0.27 bristles (calculated from the pooled regression coefficient in Table 5) and response in the irradiated lines, measured as the average difference from the unirradiated selection lines over the last five generations, was 1.1 bristles in both sexes. This 1.1 bristle response included a bias of approximately 0.2 to 0.3 bristles due to the direct effect of irradiation (Table 3), so that an average response of 0.8 to 0.9 bristles is a more realistic estimate of the effect of the radiation treatment. The best estimate of response, free of crowding effect bias, was provided by averaging the adjusted line means in Table 11. Bristle number in irradiated lines was 1.3 bristles higher in females and 1.0 bristles higher in males than in the unirradiated lines.

There was some heterogeneity in the degree of response in our irradiated lines. Irradiated line means in females at generation 20 varied from 1.8 to 1.0 bristles above the level of the mean of the unirradiated lines (Table 11). This heterogeneity is not unexpected; Rokitzky (1936) selected over twenty-five generations for sternopleural bristle number in single-pair lines from an apparently inbred base population, and found that six lines out of the fourteen surviving in the irradiated group, and three lines out of twenty in the unirradiated group, showed responses of at least one bristle. All other lines in both groups showed little or no change in mean. In Rokitzky's experiment the small size of the individual lines would increase the likelihood of response occurring in only a few of the replicates. In larger lines there is a greater chance that useful mutations will occur in each replicate so that some response would be expected in all lines. Thus the results obtained with one-hundred pair lines should be more uniform and in fact all irradiated lines did show response above the level of the unirradiated lines.

Clayton and Robertson (1955), using a *D. melanogaster* stock inbred by full-sib mating for twenty-eight generations, selected for increased and decreased abdominal bristle number (total for two segments) for seventeen generations. Responses in the irradiated lines, measured as the differences from control means in the final generation, were 1.4 bristles per segment in the high line and 0.3 bristles per segment in the low line. No radiation treatments were given to parents of the final generation so that these values are free of bias due to lower crowding in cultures derived from irradiated parents. These results of Clayton and Robertson are quite similar to those reported in this paper.

Kitagawa's (1967) results for his strain derived from an isogenic stock are also in reasonable agreement with our results. In each line selected over twenty generations for high or low two-sternite bristle number, six pairs of parents were selected at 20% selection intensity. Radiation treatments of 1500 *r* *X*-rays each generation were given to males only, to females only, or to both males and females; there were also unirradiated selection lines and unselected control lines. There was no divergence between high and low lines selected without irradiation. Most of the irradiated lines showed some response to selection, although response patterns were rather irregular, due perhaps to the combination of high selection intensity and small population size leading to rapid increase in frequency of any effective induced mutations. Increases in the coefficient of variation were found consistently in lines which responded rapidly to selection, but not in the unirradiated lines or in lines which responded more gradually to selection. Using the mean deviations of the selected lines from the unselected control lines (Table 2 of Kitagawa), the increased response due to radiation treatments, averaged over all lines, was about 1.3 bristles per segment.

Allowing for differences in selection regime and radiation level, the results of all these experiments are in agreement and together provide strong evidence that irradiation can induce mutations useful in increasing the response obtained in selection programmes. Heterogeneity is a common feature and there is also general agreement that the average response is small.

Harrison (1954) selected for increased and decreased abdominal bristle number with and without concurrent irradiation using various base populations.

Irradiated lines from inbred base populations did not respond more than the unirradiated lines. According to Clayton and Robertson (1955), Serebrovsky (1935) selected for sternopleural bristle number in a presumed inbred stock and obtained greater divergence in the unirradiated controls than in the irradiated lines. Scossiroli and Scossiroli (1959), using an isogenic stock, selected at 15% intensity for increased sternopleural bristle number with concurrent irradiation of 3000 *r* *X*-rays each generation or every alternate generation. As compared with its unirradiated control selection line, rate of response was significantly higher in the line irradiated each generation. Also, the line treated every second generation had a mean bristle score at least one bristle higher than its unirradiated control from generation 3, although the rate of response over the full period of the experiment was not significantly different.

The large response of Scossiroli and Scossiroli's line irradiated every generation (the mean was about four bristles above its control in generations 9 to 11) is unusual in comparison with the other results discussed here. The relatively high radiation dose and selection intensity could be important factors, although the heterogeneity in response in irradiated lines makes the occasional spectacular response not unlikely. In one of Rokitzky's (1936) irradiated lines the mean increased by about four bristles over the last twelve generations.

Other evidence comes from artificial selection experiments using inbred or isogenic strains of *D. melanogaster* treated with *X*-rays before beginning the selection programme. Clayton and Robertson (1964), using three inbred lines, studied the divergence obtained in five generations of two-way selection for abdominal bristle number after varying periods of mass-mating in bottle cultures. *X*-radiation treatments of 1800 *r* per generation were given during the mass-mating period, but not during selection. The responses were considerably greater than those obtained using non-irradiated mass-mated or full-sib mated base populations, but even after one hundred and forty generations of irradiation prior to selection, the average divergence was less than half that expected in similar lines from an outbred population. Variation between replicates was also quite marked in this experiment.

Large responses following radiation treatment have been obtained in lines where some genetic variation was already present, *e.g.* in irradiated lines derived from hybrid stocks made by crossing two inbred or isogenic lines (Kitagawa 1967, Scossiroli and Scossiroli 1959). Some residual variation could have existed in Scossiroli's (1954) plateaued population — an irradiated line from this population gave a very large response to selection. The isogenic stock of Scossiroli and Scossiroli (1959) also may have retained a small amount of variation in spite of the isogenization process, as there was some tendency for the mean of the unirradiated lines to respond to selection. Epistatic interactions enhancing the effect of a new mutant on the character under selection may be important, and a variable population would be more likely to provide suitable gene combinations for their expression. There is increasing evidence that genes with large effects on quantitative characters can be important at least in some populations. For sternopleural bristle number, genes with large effects have been found to account for much of the response obtained in some selection lines (*e.g.* Spickett and Thoday 1966).

Fisher (1958) developed a mathematical relationship between the magnitude of the phenotypic effect of a mutational change and the probability of it causing improved adaptation. The chance for an improvement in adaptation increases with decreasing magnitude of change and at the limit, when the change is very small, the probability of improvement is one half. Gregory (1965) found that the frequency of artificially induced mutations increased as the magnitude of the mutational change decreased and in the class with the lowest magnitude of change (showing no detectable changes in phenotype and not segregating for visible mutations in following generations) the mean adaptation (as measured by yield in peanuts) was unchanged, although the variance was greatly increased.

However, in artificial selection experiments large responses to selection in irradiated lines generally were accompanied by loss of fitness and periods of relaxation of selection were often necessary to prevent extinction of selected lines (Scossiroli 1954, Kitagawa 1967). Deterioration in fitness is not uncommon in unirradiated selection lines even when the selected character is one which is not closely related to fitness in wild populations. Continued irradiation has the disadvantage of making reduced fitness inevitable, partly because of its immediate effects (such as production of dominant lethals) and partly because it causes a cumulative increase in the genetic load. Some mutants with effects on the selected character could affect fitness directly. Even if induced mutations affecting the selection trait were not in themselves detrimental, they could cause the increase in frequency of harmful mutants linked to them. Stone and Wilson (1959) showed that under natural conditions the genetic load of irradiated populations of *Drosophila ananassae* quickly returned to its normal level when exposure to radiation ceased, and the experiments of Buzzati-Traverso mentioned previously showed that adaptation may in fact be increased if irradiation is accompanied by strong natural selection.

For artificial selection, the usefulness of mutagenesis as a breeding technique would be improved by a method combining efficient selection of mutations affecting the character under selection with some form of selection to maintain a reasonable level of fitness.

The two-generation cycle procedure of Scossiroli (1954) was an attempt at overcoming this problem of loss of fitness. Gregory (1965) and others have suggested that major mutations should be recognised and eliminated before selecting for a quantitative character.

Irradiation can be useful in artificial selection programmes if it is realized that replicate variability will be high and average response small, and that simultaneous selection for fitness is essential if useable improved populations are to be obtained. These limitations restrict the practical usefulness of the technique to organisms with a high reproductive capacity, and to situations in which suitable genetic variation is not otherwise available. Better selection techniques are required to take full advantage of the potentiality for improvement that mutagenesis offers in these specialized situations.

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