GENETIC EFFECTS OF X RAYS ON QUANTITATIVE
CHARACTERS IN A HETEROGENEOUS POPULATION OF
*DROSOPHILA MELANOGASTER*¹

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A number of studies have been conducted to investigate the genetic effects of ionizing radiation on quantitative characters from the standpoint of agricultural application (e.g. CLAYTON and ROBERTSON 1955; OKA, HAYASHI, and SHIOJIRI 1958; YAMADA 1961). From these studies it has been clarified that the means of so-called neutral characters are barely affected by radiation, while their variances are increased. It has then been suggested that the genes controlling such quantitative traits are mutable in both positive and negative directions.

These results, however, were obtained almost exclusively from studies on inbred lines or on populations having reached a plateau under continuous selection pressure. In order to clarify the evolutionary significance of polygenic mutations, it seems important to examine the genetic effect of radiation in a genetically heterogeneous population, since most natural populations are believed to be extremely heterogeneous. In such a population, alleles with positive and negative effects are present with varying frequencies. If the positive and negative alleles are equally frequent in a large population, the means and variances of neutral characters may remain unaffected by radiation. On the other hand, if the frequencies of the two kinds of alleles are not equal, both the means and variances may be affected.

The present investigation was conducted to examine these points by using two characters of *Drosophila melanogaster*, namely, abdominal and sternopleural bristle numbers. These two characters were chosen because they are known to be largely neutral (FALCONER 1960); furthermore, their genetic mechanisms seem to be much simpler than those of other traits such as viability.

MATERIALS AND METHODS

The base population: The base population employed in this investigation was originally made up of 20 males and 20 females taken from each of 24 different laboratory stocks, which were, in turn, sampled from natural populations. Before starting the experiments, this base population had been kept for about one year in a plastic population cage (30 × 40 × 13 cm) provided with 15 cups of medium in a constant temperature (25°C) room. The population was therefore considered to have reached an approximate genetic equilibrium. The population size (the number of adult flies) was approximately 8,000.

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Irradiation and analysis: Virgin females and males (1,000 of each) taken from the base population were exposed to 1,000r of X rays at the rate of 100r/minute. Immediately after irradiation the flies were allowed to mate at random in a population cage. A nontreated control of the same size was also set up from the same base population. In both the irradiated and control populations, random mating was allowed for two further generations to eliminate the majority of chromosomal aberrations. At the third generation after the treatment a biparental cross was made in each population to examine the effects of X rays on the means and variances of the characters concerned. For the biparental cross 50 males were taken at random and mated each with four random females. Thus, a total of 200 matings were made. The total bristle number on the fourth and fifth abdominal segments and sternopleural bristle number on both sides were recorded for ten female offspring from each mating. A sib-analysis was performed to estimate the components of phenotypic variances (cf. FALCONER 1960).

The results obtained from the sternopleural bristle number were rather unexpected. Therefore, the whole experiment was repeated for this character. This second experiment was designated as Experiment II, and the first as Experiment I. The irradiation and mating schemes in Experiment II were exactly the same as those in Experiment I; however, the total numbers of males and females used for the biparental cross were 102 and 306, respectively. Furthermore, in Experiment II, the measurements were taken on the female parents as well as on the female offspring.

All the experiments were carried out at 25°C and the medium consisted of water 1,000 ml, dry yeast 80 g, agar 10 g, sugar 100 g and Moldex 10 ml.

RESULTS

Means: Mean values of the two characters are presented in Table 1. It is clear that, in either character, the mean value is not significantly affected by the treatment of X rays. This suggests, as will be discussed later, that positive and negative alleles occur with nearly equal frequencies on the average.

Variance and its components for abdominal bristle numbers: The analysis of variance for abdominal bristle number is given in Table 2. Estimates of the observational components of variance (FALCONER's [1960] terminology), i.e., male component (σ_M^2), female component (σ_F^2), and within-female component (σ_W^2), are obtained from this table. The additive (V_A), dominance (V_D), and environmental (V_E) components of phenotypic variance (causal components in FALCONER's [1960] terminology) are then estimated by $\hat{V}_A = 4\hat{\sigma}_M^2$, $\hat{V}_D = 4(\hat{\sigma}_F^2 - \hat{\sigma}_M^2)$, and $\hat{V}_E = \hat{\sigma}_W^2 + \hat{\sigma}_M^2 - 3\hat{\sigma}_F^2$, respectively. The epistatic components are assumed to be zero. The standard errors of these components can be obtained by the method described by KEMPTHORNE (1957). The estimates of variance compon-

TABLE 1

Mean numbers of abdominal and sternopleural bristles for the irradiated and nonirradiated populations

Character	Control	Irradiation	t
Abdominal	44.16 $\pm$ 0.08	44.25 $\pm$ 0.09	1.05
Sternopleural			
Experiment I	19.35 $\pm$ 0.04	19.38 $\pm$ 0.05	0.48
Experiment II	19.46 $\pm$ 0.03	19.43 $\pm$ 0.04	0.61

t: t-value for testing the difference between "Control" and "Irradiation."

TABLE 2

Analysis of variance for abdominal bristles

Source	d.f.*	Mean square		Expectation of mean square
		Control	Irradiation	
Male parents	49(48)	108.42	138.31	$\sigma_W^2 + k\sigma_F^2 + kf\sigma_M^2$
Female parents	150(147)	33.76	33.11	$\sigma_W^2 + k\sigma_F^2$
Individuals	1800(1764)	9.72	11.52	σ_W^2

 $k=10, f=4.$

* The numbers of degrees of freedom in brackets refer to the mean squares for the irradiated population. Data on the offspring from one male parent were accidentally discarded.

ents and their standard errors thus obtained are given in Table 3. This table shows that the total phenotypic variance (V_P) is significantly increased by X-irradiation. This increase is attributed to the additive and environmental components, although these, taken individually, do not show any significant increase. The increase of environmental variance may be due to an enhanced sensitivity of the irradiated population to environmental conditions. The dominance component is smaller than its standard error in both the irradiated and control populations. This, together with the small value of $\hat{V}_D$ relative to $\hat{V}_A$, indicates that the genes responsible for this character show little or no dominance, as generally believed.

Variance and its components for sternopleural bristle number: The analyses of variance for sternopleural bristle number in Experiments I and II are given in Table 4. Estimates of the phenotypic variance and its components are obtained in the same way as in the case of abdominal bristles. Table 5 presents these estimates. Clearly, the phenotypic variance is increased by X rays in Experiment I. However, the increase does not reach the level of significance. Unlike the case of abdominal bristles, the additive and environmental components are decreased, while the dominance component is significantly increased. This result is unexpected since the dominance component in the control population is negligibly small, while it is the largest component in the irradiated population. In a genetic analysis of spontaneous mutations of this character, DURRANT and MATHER

TABLE 3

Estimates of the components of phenotypic variance for abdominal bristle number

Component	Control	Irradiation	t
V_A	7.4668 ± 2.1827	10.5200 ± 2.7927	0.86
V_D	2.1460 ± 2.6787	-1.8832 ± 3.1902	...
V_E	4.3807 ± 1.3271	7.6728 ± 1.4056	1.70
V_P	13.9935 ± 0.4409	16.3096 ± 0.5209	3.39**

t: t-value for testing the difference between "Control" and "Irradiation."

** Significant at the 1% level.

TABLE 4

Analyses of variance for sternopleural bristle number

Source	d.f.*	Mean square	
		Control	Irradiation
Experiment I			
Male parents	49(48)	20.577	24.227
Female parents	150(147)	6.848	11.410
Individuals	1800(1764)	3.029	2.904
Experiment II			
Male parents	101	23.856	20.973
Female parents	204	6.080	8.579
Individuals	2754	2.658	2.855

* The numbers of degrees of freedom in brackets refer to the mean squares for the irradiated population. Data on the offspring from one male parent were accidentally discarded.

TABLE 5

Estimates of the components of phenotypic variance for sternopleural bristle number

Component	Control	Irradiation	t
Experiment I			
V_A	1.3728 ± 0.4150	1.2816 ± 0.5022	0.14
V_D	0.1548 ± 0.5221	2.1204 ± 0.7303	2.18*
V_E	2.2265 ± 0.2782	0.6733 ± 0.4283	3.03**
V_P	3.7541 ± 0.1009	4.0753 ± 0.0977	1.83
Experiment II			
V_A	2.3702 ± 0.4504	1.6524 ± 0.4056	1.18
V_D	-1.0012 ± 0.5102	0.6372 ± 0.5290	...
V_E	2.2240 ± 0.2238	1.5509 ± 0.2847	1.90
V_P	3.5930 ± 0.0716	3.8405 ± 0.0769	2.32*

t: t-value for testing the difference between "Control" and "Irradiation."

* Significant at the 5% level.

** Significant at the 1% level.

(1954) and PAXMAN (1957) found little dominance. It is unlikely that the mutations induced by radiation exclusively show a conspicuous dominance. Note also that in irradiation experiments with isogenic lines by YAMADA (1961) and others, no significant change in mean value was observed. The unexpected result was then considered to be due to some accidental error involved in the experiment. Therefore, the whole experiment was repeated (Experiment II), increasing the number of individuals measured.

However, the results of Experiment II are similar to those of Experiment I (cf. Table 5). That is, the additive and environmental components are decreased and the dominance component is increased. The negative estimate of V_D in the control population is not significantly different from 0 and should therefore be

attributed to sampling error. However, this negative estimate appears to have made the estimates of V_A and V_E unduly large, since there are negative correlations among the estimates of V_A , V_D and V_E . If this is the case, then the decreases of $\hat{V}_A$ and $\hat{V}_E$ computed for the irradiated population may also be accidental. Furthermore, examination along this line suggests that the decreases of $\hat{V}_A$ and $\hat{V}_E$ in Experiment I might have also occurred through the accidental increase of $\hat{V}_D$ in the irradiated population.

The negative correlations between $\hat{V}_A$, $\hat{V}_D$ and $\hat{V}_E$ arise primarily through the negative correlations between observational components of variance. According to OSBORNE and PATERSON (1952), the covariances between $\hat{\sigma}_M^2$ and $\hat{\sigma}_F^2$ and between $\hat{\sigma}_F^2$ and $\hat{\sigma}_W^2$ are given by

$$\begin{aligned} \text{Cov}(\hat{\sigma}_M^2, \hat{\sigma}_F^2) &= -\frac{V(\hat{\sigma}_W^2)}{k} \\ \text{Cov}(\hat{\sigma}_F^2, \hat{\sigma}_W^2) &= \frac{V(\hat{\sigma}_W^2) - k^2 V(\hat{\sigma}_F^2)}{k^2 f} \end{aligned}$$

respectively. There is no correlation between $\hat{\sigma}_M^2$ and $\hat{\sigma}_W^2$. The symbols $V(\hat{\sigma}_W^2)$ and $V(\hat{\sigma}_F^2)$ stand for the variances of $\hat{\sigma}_W^2$ and $\hat{\sigma}_F^2$, respectively. The number of individuals measured per mating and the number of females mated per male are referred to by k and f , respectively. The correlation coefficients obtained from these covariances and the variances of the observational components are shown in Table 6. These correlation coefficients are considerably higher in some cases, especially with sternopleural bristle number. In view of these negative correlations it seemed more appropriate to partition the phenotypic variance into two components instead of three, i.e., the genetic and the environmental components.

TABLE 6

Correlation coefficients between observational components of variance

Correlation concerned	Abdominal bristles	Sternopleural bristles	
		Experiment I	Experiment II
Control			
$r(\hat{\sigma}_W^2, \hat{\sigma}_F^2)$	-0.083	-0.127	-0.120
$r(\hat{\sigma}_F^2, \hat{\sigma}_M^2)$	-0.177	-0.188	-0.175
$r(\hat{\sigma}_W^2, \hat{\sigma}_P^2)$	-0.010	-0.098	-0.078
Irradiation			
$r(\hat{\sigma}_W^2, \hat{\sigma}_F^2)$	-0.101	-0.074	-0.091
$r(\hat{\sigma}_F^2, \hat{\sigma}_M^2)$	-0.137	-0.339	-0.277
$r(\hat{\sigma}_W^2, \hat{\sigma}_P^2)$	-0.050	-0.066	-0.075

TABLE 7

Estimates of the components of phenotypic variance—recomputation

Component	Control	Irradiation	t
(i) Abdominal bristle number			
V_G	8.4836 ± 0.3352	9.4974 ± 0.3839	1.99*
V_E	5.4818 ± 0.5544	6.7717 ± 0.6418	1.52
V_P	13.9654 ± 0.3239	16.2691 ± 0.5196	3.38**
(ii) Sternopleural bristle number (Experiment I)			
V_G	1.4398 ± 0.2050	2.3320 ± 0.2942	2.49*
V_E	2.3091 ± 0.2368	1.7384 ± 0.3216	1.43
V_P	3.7489 ± 0.1009	4.0704 ± 0.0977	1.83
(iii) Sternopleural bristle number (Experiment II)			
V_G	1.8616 ± 0.1937	1.9655 ± 0.2053	0.39
V_E	1.7275 ± 0.2143	1.8724 ± 0.2275	0.49
V_P	3.5891 ± 0.0716	3.8379 ± 0.0769	2.34*

t: t-value for testing the difference between "Control" and "Irradiation."

* Significant at the 5% level.

** Significant at the 1% level.

Recomputation of variance components: In partitioning of the phenotypic variance into its two components, a joint mean square for parents was computed by combining the mean squares for males and females in Tables 2 and 4. If there is no dominance for the two characters in question (CLAYTON, MORRIS, and ROBERTSON 1957; DURRANT and MATHER 1954; PAXMAN 1957) then the genetic (V_G) and environmental components are obtained by $\hat{V}_G = 2\hat{\sigma}_p^2$ and $\hat{V}_E = \hat{\sigma}_w^2 - \hat{\sigma}_p^2$, respectively, where $\hat{\sigma}_p^2$ denotes the observational component for the parents. The estimates of V_G and V_E are presented in Table 7, together with the total phenotypic variances.

The data from Table 7 shows that the genetic component is increased by X rays in all experiments, though the increase of sternopleural bristle number in Experiment II is not significant. On the other hand, the environmental component does not show any significant change in any of the cases. These results suggest that the increase of phenotypic variance by X-irradiation is mainly due to the increase of genetic component.

Parent-offspring covariance for sternopleural bristle number: In Experiment II, the measurement of sternopleural bristle number was made on the female parents as well as on the offspring, so that the parent-offspring covariance could be obtained. Twice this covariance is an estimate of the additive genetic variance. The values are 1.6669 for irradiated and 1.2174 for control populations. Again, the genetic variance is increased by X rays. On the other hand, the phenotypic variance of the female parent population showed little difference between the irradiated and the control populations. The values are 4.6856 ± 0.3782 for ir-

radiated and 4.6971 ± 0.3792 for control. This may be due to the small sample size for the parental population (306 females for each population).

DISCUSSION

In a heterogeneous population both kinds of alleles with positive and negative effects on a quantitative character are expected to be present with varying frequencies. Consider a locus with two alleles, A_1 and A_2 , having positive and negative effects, respectively. Let p and q be the frequencies of A_1 and A_2 , respectively. Denote the mutation rate from A_1 to A_2 by μ_1 , and the reverse mutation rate by μ_2 . The change in A_1 gene frequency is then given by $\Delta p = -\mu_1 p + \mu_2 q$. It is generally believed that, in neutral characters, the frequencies of positive and negative mutations induced by radiation are almost equal (YAMADA and KITAGAWA 1961; YAMADA 1961; MUKAI, YOSHIKAWA, and CHIGUSA 1964). Thus, it may be assumed that $\mu_1 = \mu_2 = \mu$. The change in gene frequency is then $\Delta p = -\mu(p - q)$. This indicates that the gene frequency changes unless the frequencies of positive and negative alleles are equal.

The mean of a quantitative character in a random mating population is a linear function of the gene frequencies at the loci involved, provided that there is no dominance and no epistasis. Thus, if the average frequency of the positive alleles is not the same as that of the negative alleles, the mean value is expected to change, assuming the same gene effect for all loci. In our case, no significant change of mean was observed in either trait. This suggests that the frequencies of positive and negative alleles are nearly equal on the average, though at individual loci they need not be equal. The fact that the average frequencies of positive and negative alleles are equal supports the view of CLAYTON and ROBERTSON (1955) that the genetic variability of bristle characters is maintained mainly by the balance between mutation and inbreeding, although in the phenotypic extremes they must be subjected to natural selection.

Assuming no epistasis, the genetic variance can be partitioned into the additive and dominance components, which are equal to

$$V_A = 2\Sigma pq [a - (p - q)d]^2$$

$$V_D = 4\Sigma p^2 q^2 d^2$$

in a random mating population. The letter a denotes half the difference between the genotypic values of two homozygotes A_1A_1 and A_2A_2 , and d the dominance deviation of the heterozygote A_1A_2 . Σ indicates that the summation is taken over all loci. The amounts of first order change in the additive and dominance components by radiation are then given by

$$\Delta V_A = \Sigma \frac{\partial V_A}{\partial p} \Delta p$$

$$= 2\Sigma \mu (p - q) [a - (p - q)d] [(p - q)a - (1 - 8pq)d]$$

$$\Delta V_D = 8\Sigma \mu pq (p - q)^2 d^2$$

respectively. The above formulas show that the dominance variance always increases unless $d = 0$ or $p = q$, while the additive variance may increase or decrease, depending on the values of p and d . If, however, $d = 0$, ΔV_A becomes

$2\Sigma\mu(p-q)^2a^2$ and $\Delta V_D = 0$. Thus, the additive variance is expected to increase unless $p = q$ for all loci. The increase of V_A or V_G observed in abdominal bristles agrees with the theoretical expectation.

The decrease of V_A and the increase of V_D in sternopleural bristles might be explained if the degree of dominance is high and if the frequencies of the negative alleles in the original population were considerably larger than those of positive alleles. However, the genetic analysis of this character by DURRANT and MATHER (1954) and PAXMAN (1957) indicates that the degree of dominance is negligibly small. The small value of V_D compared with V_A in the control population of our experiment also suggests that the degree of dominance is small. Furthermore, the mean value was not affected by radiation; this suggests that the average frequencies of positive and negative alleles were nearly equal in the original population. Hence, it can be assumed that the decrease of V_A and the increase of V_D are somewhat accidental, as previously discussed. Re-analysis of this character revealed that the genetic variance (V_G), which is considered to be largely additive, is increased by radiation.

Assuming a linear relationship between the X-ray dose and variance increase, the amount of variance increase per roentgen is computed to be 10.1×10^{-4} and 4.98×10^{-4} (average of two experiments) for abdominal and sternopleural bristles, respectively. The value for abdominal bristles is three to six times larger than those obtained by YAMADA (1961) and YAMADA and KITAGAWA (1961); it is 50 to 100 times larger than that obtained by CLAYTON and ROBERTSON (1964). However, these authors studied both males and females and used experimental procedures which were different from ours. YAMADA tested the effect of mutations in heterozygous condition, while YAMADA and KITAGAWA tested in homozygous condition exposing a homozygous line to X rays. CLAYTON and ROBERTSON's estimate is based on the response to artificial selection. On the other hand, the value for sternopleural bristles is larger by a factor of 3 than YAMADA's (1960) estimate, and almost equal to the estimate obtainable from the published data on females of MUKAI, YOSHIKAWA, and CHIGUSA (1964), who examined the heterozygous effect of mutations, delivering X rays to a homozygous line. These authors obtained a different result in males. That is, the phenotypic variance was slightly but significantly decreased by X-irradiation.

The fact that the rate of variance increase is almost equal to or larger than that obtained from homozygous lines suggests that the increase of genetic variance in our irradiated population was caused mainly by mutations at fixed or nearly fixed loci rather than at loci with intermediate gene frequencies (cf. the previous formulas). Theoretically, the rate of variance increase should be larger in homozygous line than in heterozygous populations. Note, however, that the standard errors of the estimates of genetic variances are quite large.

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SUMMARY

An outbred population was exposed to 1,000r of X rays. The numbers of abdominal and sternopleural bristles were studied after three generations of random mating. The mean values of the two characters showed no appreciable change due to radiation. In view of the practical equality of positive and negative mutation rates in these characters, this result suggests that the frequencies of positive and negative alleles in the outbred population are nearly equal, on the average.

The phenotypic variance was increased in both abdominal and sternopleural bristles. Partitioning of the phenotypic variance has shown that this increase is mainly due to the increase in genetic variance. The increase in genetic variance in sternopleural bristles was also detected by the parent-offspring covariance, which was larger in the irradiated than in the control population. The amount of variance increase per roentgen has suggested that the increase of genetic variance in the irradiated population is caused mainly by mutations at fixed or nearly fixed loci rather than at loci with intermediate gene frequencies.

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