# ARE INDUCED MUTATIONS IN DROSOPHILA OVERDOMINANT? II. EXPERIMENTAL RESULTS[1]

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IN the first paper of this series (MULLER and FALK 1961) the background of the problem of overdominance has been explained as well as the general organization of our experiments, designed to test to what extent induced mutations are overdominant. This paper will deal *in extenso* with one series of the experiments carried out and will discuss the question whether many overdominant mutants may be induced and maintained in a population. It will be shown that in spite of the very stringent design of the experiment, which gave every chance for "overdominant" mutants to show up, no evidence for their production at noticeable frequency was found. In fact the opposite proved to be true: although the differences found were too small to be statistically significant in consideration of the variance expected under the circumstances of the experiment, the results turned out to be in good agreement with those expected on the "neo-Mendelian" hypothesis, in that they indicated the average viability to be reduced in the flies heterozygous for induced mutations.

## MATERIAL AND METHODS

For the present experiment pairs of third chromosomes of *Drosophila melanogaster* were prepared that carried the marker *ve* (veinlet, 3–0.2) and that were coisogenic except for the alleles *st* (scarlet, 3–44.0) and $st^+$, and the immediate neighborhood of this locus. The gene *st* had been introduced into only one of these two chromosomes by obtaining crossing over nearby *st* on each side of it successively: between *th* (thread, 3–43.2) and *st*, on its left, and between *st* and *cp* (clipped, 3–45.3), on its right. These pairs of chromosomes were the "prototype" and "marker" respectively as described in the previous paper (MULLER and FALK 1961). Males carrying these respective chromosomes balanced over a *ru h D InsCXF ca* chromosome were mated to their sisters of similar constitution and their progeny scored to determine the viability of the homozygotes. One pair of these coisogenic chromosomes which proved to be of good viability as homozygotes was chosen and males containing them were then crossed repeatedly to a

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large number of females from a stock homozygous for *cn bw*; *ri e*. All other pairs of chromosomes were discarded.

The scheme of the crosses is described graphically in Figure 1.

Several of the $F_1$ *cn bw*/+; *ri e*/ *ve st* males were mated singly to females having dominant markers and crossing over inhibiting inversions in their three large chromosomes, such as e.g. *y* $sc^{S1}$ *B In49* $sn^{x2}$ *v* $sc^{s}$ /+; *dp* $^{tx1}$ *CyIns05 pr* $cn^{2}$ *T23 Me Ins ri* $Sb^{1}$/+ (the X-chromosome balancer having been prepared by MULLER and the autosomal translocation and the modification of the "Curly" inversions in the second chromosome by OSTER). By inbreeding the properly marked progeny of these crosses it was hoped that a viable stock isogenic for all genes in the normal X, normal second and *ve st* third chromosomes of an $F_1$ male could be obtained in $F_4$. During this procedure, however, it proved advisable to abandon the effort to isogenize the X chromosome because of the low viability of the $F_2$ males loaded with all the markers necessary for this. There resulted a contrast between the females and the males that were finally scored, in that the former were heterozygous, the latter only hemizygous with regard to invisible alleles in the normal-appearing X chromosome. The final stock, obtained in $F_3$, homozygous for its unmarked second chromosome and its *ve st* marked third chromosome, will be called the *ve st* stock. The stock was kept and multiplied separately during the rest of the experiment.

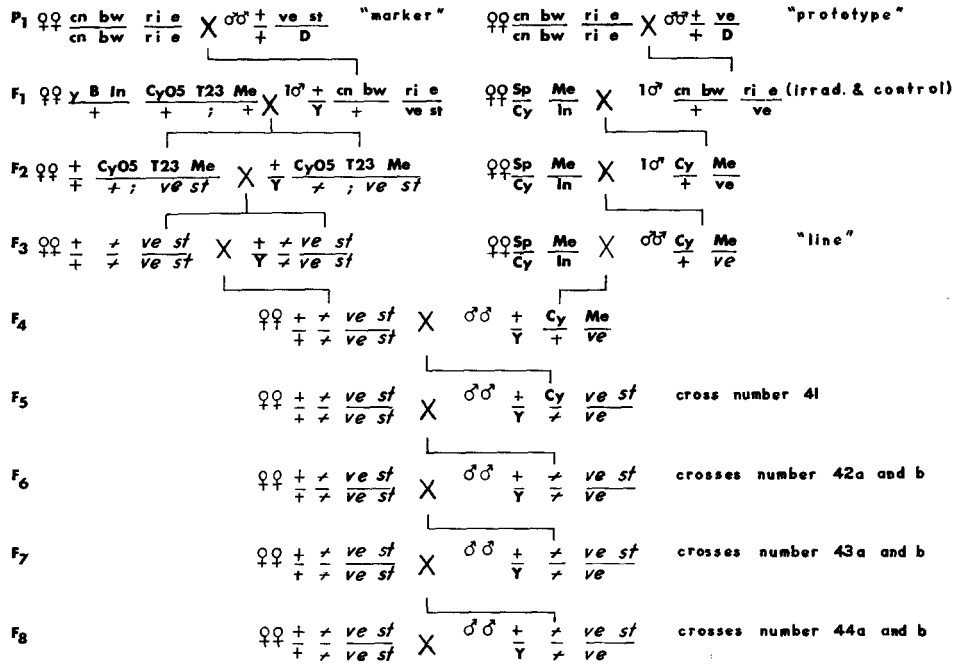


FIGURE 1.—Scheme of crosses. Abbreviations used: Cy05 T23 Me=*dp*$^{tx1}$ *CyIn05 pr* $cn^{2}$ *T23 Me Ins ri* $Sb^{1}$; D=*ru h D InsCXF ca*; In=*sep In ri* $p^{p}$ *Sb*; Me=*Me InL InC e* $l_{y}e$; Sp=*dp*$^{ix}$ *Sp cn*; y B In=*y* $sc^{S1}$ *B In49* $sn^{x2}$ *v* $sc^{s}$. Isogenic chromosomes are in italics.

Simultaneously with the preparation of this *ve st* homozygous stock a great number of lines carrying individual irradiated *ve* chromosomes, derived from the *ve* chromosome that had originally been coisogenic with the given *ve st* chromosome, were obtained in the following manner. Nearly a thousand *cn bw* / + ; *ri e* / *ve* males were collected and were then divided at random into one group of over 500 and another of less than 500. The larger group was exposed to six doses of 4,000r, with an interval of five days at 18°C between the individual exposures. After the flies had received a total dose of 24,000r they were aged at 26°C for another 15 days. The smaller group of males were kept in the same way except that they were not irradiated at all. Both groups of males were transferred to new vials every 4–6 days and a group of virgin females, approximately equal in number to the males, was added once a week during the irradiation period and each 3–4 days during the aging period after the irradiations. The females were used in order to stimulate spermatogenesis.

The four first irradiations were given with the X-ray machine used for the past 15 years in our laboratory, operated at 200 kvp, 20 ma, with a 1 mm Al screen, at a rate of 160r/minute. The last two irradiations were given with a newly obtained "Maximar-100" machine, operated at 100 kvp, 5 ma, with a 1 mm Al screen, a target distance of 15 cm, and a rate of 220r/min.

At the end of the aging period when it could be inferred that sperm then being ejaculated had been irradiated in a spermatogonial stage, the males of both groups, which we shall design as $F_1$, were, as shown on the right-hand side of Figure 1, mated to females of stocks, previously constructed for such purposes by MULLER, that were properly marked and balanced as follows in their second and third chromosomes: *dp$^{tx}$ Sp cn* / *Cy, Ins cn$^2$ sp$^2$* ; *Me, InL InC e l$_3$e* / *sep,In ri p$^p$ Sb* (or the equivalent). From any given $F_1$ male only one $F_2$ male, heterozygous for the two marked balancer autosomes and for the unmarked second and *ve*-marked third chromosome, was backcrossed to the marked and balanced stock. Male progeny from each such cross established a "line," in which males like the father and females like the mother were selected (*en masse*) for breeding. In this manner it was arranged that each irradiated male would contribute not more than one third chromosome to the experiment, and the possibility was avoided of clusters of identical irradiated chromosomes biasing the results.

By the time the progeny of the backcross $F_3$ were ready the *ve st* stock $F_3$ on the left-hand side of Figure 1 had been synthesized and multiplied. Virgin females from the *ve st* stock were now mated to *Cy cn$^2$ sp$^2$* / + ; *Me,InL InC e l$_3$e* / *ve* males of each irradiated and each nonirradiated line. Males derived from this $F_4$ cross which had the unmarked second chromosome from the *ve st* stock (here designated as $+_{iso}$) were of composition *Cy cn$^2$ sp$^2$* / $+_{iso}$; *ve st* / *ve*. These $F_5$ males were backcrossed to virgins of the *ve st* stock (cross number 41). Note that the unmarked second chromosome of these males was coisogenic with those of the *ve st* stock and that their pair of third chromosomes was homozygous and coisogenic with that of the *ve st* stock except for the *st* region and except for any mutations that had been induced in the *ve* chromosome (or that may have arisen

spontaneously since their common origin). In the case of each line, attempts were made to take for this cross (number 41 of $F_5$) 4–5 females from the *ve st* stock and about the same number of males from the previous backcross, but the number of parents could not be kept strictly constant. This was especially the case with lines which produced few offspring of the right type; every effort was made to establish such lines even if only one male could be used. The parents were allowed to remain in the vials for three days, then transferred twice successively to fresh vials for another two days each, after which they were discarded. All the crosses were kept in a 26°C incubator.

The progeny of cross number 41 were classified into four phenotypes of females and four phenotypes of males and counted separately for each line until the vials were exhausted. Males ($F_6$) of the phenotype veinlet derived from this cross and thus of genotype + / Y; $+_{iso}$ / $+_{iso}$; *ve* / *ve st* were kept separately for each line. Note that they were homozygous for the second chromosome (which was coisogenic with the second chromosome of the *ve st* stock) and that their X chromosomes were entirely derived from those of the *ve st* stock. Fifteen females of the *ve st* stock were then crossed to 15 phenotypically *ve* males of the above mentioned constitution from each line; where possible two crosses were made per line (crosses $F_6$ numbered 42a and b in Figure 1). In the case of many lines only one vial could be counted, either because not enough males of the right type for the cross had been secured from the previous generation, or because there was a suspicion of contamination in some of the vials to be counted.

Crosses 42a and b were kept for only one day in the 26°C incubator, after which the parents were transferred to fresh vials for two days for deposition of eggs, and the first-day cultures were discarded. It was assumed that, because the prior day had allowed mating, differences in numbers of eggs laid during this two day period were only negligibly due to differences in mating time. After the two days of egg laying the parents were discarded and the vials were transferred to an incubator kept at 18°C. The offspring were classified and counted twice: on the 27th day after beginning of egg laying and again on the 30th day. All progeny, except males of phenotype *ve*, were discarded. The males of the phenotype *ve* were backcrossed to females of the *ve st* stock, in a manner similar to that described above (crosses $F_7$ numbered 43a and b).

According to this plan it was intended to have counts of four vials for each line. In the majority of vials of crosses 42 and 43, 15 males could be provided, but there were a few in which only 14 or 13 males were available. It was found that this did not make any difference in the results so long as the full number of females was used. Furthermore, in a very small number of crosses even fewer males were available, some being done with only six or eight males. Here again, however, there seemed to be no indication for excluding them from the final calculations. It should be noted that not all lines were crossed simultaneously, and that two crosses of the same line were often prepared on different days.

In order to find out how far conditions at the 18°C temperature influenced the results one more cross ($F_8$ numbered 44a and b) was carried out with most of the

lines in the same manner as described above, except that the vials were kept in the 26°C incubator all the time and the flies counted on the 16th day after the beginning of egg laying.

It was obvious throughout the counts that there had been crowded conditions during development, especially since many flies were dwarfed and many others had a concave abdomen.

Throughout the experiment an effort was made not to select for the stronger lines. There is no doubt that in spite of this the lines lost did not represent a random sample. Out of 255 males which survived the irradiation and aging period, progeny were secured for the next step from 168; thus only two thirds of the males gave progeny. In the parallel group of controls only six out of 122 failed to give progeny, the success here being 95 percent. Since it was assumed that we would not be able to do counts on more than a hundred lines it was decided to keep some 70–80 lines from the irradiated group and a similar number from the controls, in the hope that at least 50 lines of each would be available for the final counts. Cross number 41 was started with 84 lines of the irradiated group and 71 lines in the nonirradiated group. Counts were in fact carried out on 73 and 65 lines, respectively, of these groups. Later there were only three losses of lines and a few cases of suspected contamination that caused six control lines to be lost. In general the procedure of the experiment was such that as soon as a vial was suspected of being contaminated it was discarded; this was necessary since in most cases there was no possibility of proving contamination by examination of the phenotypes present in the vial.

From the outset of the experiment, special consideration was given to the problem of accumulated spontaneous mutations. For this reason attempts were made to decrease as far as possible the number of generations between the isogenization of the chromosomes and the counts. The *ve* chromosomes were irradiated in the third generation after their preparation; the first count was done five generations after the irradiation and the three following counts in the three following generations. The *ve st* chromosome was re-established from a single heterozygous male three generations after its preparation. At the same time a single second chromosome for the stock was obtained. Five generations later the first count was done. The females for the cross number 42 were collected from the same generation as those for the cross number 41, and for the following generations they were obtained from successive generations of the *ve st* stock. Thus, not more than 12 generations elapsed between the time of separation of the *ve* and the *ve st* coisogenic chromosomes from one another and the end of the experiment.

In the case of most lines (but, because of technical difficulties, not all of them) the *ve* chromosomes had their viability determined not only in the heterozygous state but also in the homozygous one. For this purpose the chromosomes were transferred in $F_5$ or $F_6$ again to *Me / ve* males and females which were mated with one another and the proportion of *ve* among the total offspring of each of the lines determined.

## CONSIDERATION OF RESULTS

The viability of the *ve* flies in each culture was estimated by comparing their frequency with that of the *ve st* flies of the same culture, assuming that the genotype of the *ve st* flies was identical in all cultures, and taking them as a reference group. For most of the calculations the proportion of *ve* females (or males) in a culture out of the total of the *ve* and *ve st* females (or males) in that culture was used as the measure of viability of the former. In cross number 41 there were in addition four phenotypes with Curly-wings, their viability was calculated separately, but with the same non-Curly *ve st* reference group. In this manner the effect of induced mutations in heterozygous condition on the viability of flies otherwise either homozygous or heterozygous with respect to their background could be estimated from the viability of the *ve* and *Cy ve* classes respectively. The corresponding viability calculations for the *Cy ve st* phenotypes gave another estimate of viability variations which were independent of radiation effects. There are two possibilities for choosing the *ve st* reference groups: (a) taking the *ve st* females as a reference group for the *ve* females and the *ve st* males as a reference group for the *ve* males, or alternatively (b) taking the same group, viz. *ve st* males, as a reference group for both *ve* females and *ve* males of a given culture. Usually the first method was used but in some calculations both possibilities were utilized.

Another estimate of viability can be obtained by the ratio of the *ve* flies of a given sex (and in cross number 41, also of a given wing shape) to the corresponding *ve st* flies in a given culture. As shown by HALDANE (1956) this ratio is a biased estimate. Still, the ratio of this ratio in the treated group to the same in the untreated group ("ratio of ratios") is a good and practically unbiased estimate of the effects of irradiation on the treated group (GREENBERG and CROW 1960). It is approximately equal to the number of lethal-equivalents induced. Analytically considered, this "ratio of ratios" is equal to $e^{-KR}$, where R is the number of roentgen units and K the number of lethal-equivalents per r-unit.

Table 1 gives the results of the series of crosses numbered 41. The number of flies counted and the mean viability of each genotype over all lines is given. In this series eight different genotypes were classified. Four genotypes were heterozygous for the *ve* chromosome but had a varying degree of heterozygosity of background. The two *Cy ve st* classes, like the two *ve st* classes, were presumably identical in all lines, irradiated and unirradiated, at least insofar as their third chromosome was concerned. In this table all calculations were made with the (non-Curly) *ve st* phenotype as reference group. As explained earlier, this gives an estimate of the viability of the treated groups with the contribution of the background heterozygosity practically eliminated as a cause of difference from the reference group in two cases (*ve* females and males) or present in the other two cases (*Cy ve* females and males). The number of flies counted in this cross was limited. Yet, the average viability of the flies heterozygous for irradiated chromosomes is lower than that of the controls though the difference is not sig-

TABLE 1

*Numbers of flies counted and the mean viability of lines with standard error for series 41**

| Genotype | Degree of background heterozygosity | 62† treated lines | | 61‡ untreated lines | |
|---|---|---|---|---|---|
| | | Totals | Mean viability of lines ± standard error | Totals | Mean viability of lines ± standard error |
| + + *ve st* / — — ——— / Y + *ve st* | — | 2070 | 0.500(arbitrary) | 1919 | 0.500(arbitrary) |
| + + *ve st* / — — ——— / + + *ve st* | ± | 2326 | 0.500(arbitrary) | 2138 | 0.500(arbitrary) |
| + *Cy ve st* / — — ——— / Y + *ve st* | + | 2136 | 0.503±0.008 | 1886 | 0.500±0.011 |
| + *Cy ve st* / — — ——— / + + *ve st* | ++ | 2257 | 0.489±0.009 | 2044 | 0.495±0.008 |
| + + *ve st* / — — ——— / Y + *ve* | — | 2062 | 0.491±0.011 | 1821 | 0.493±0.018 |
| + + *ve st* / — — ——— / + + *ve* | ± | 2413 | 0.511±0.008 | 2140 | 0.508±0.009 |
| + *Cy ve st* / — — ——— / Y + *ve* | + | 2175 | 0.508±0.009 | 2037 | 0.518±0.010 |
| + *Cy ve st* / — — ——— / + + *ve* | ++ | 2252 | 0.495±0.007 | 2110 | 0.501±0.011 |

* All viabilities are based on the proportion of the respective phenotype in each line in the total of this phenotype and the corresponding *ve st* phenotype.
† Eleven lines with less than 20 flies for at least one phenotype were excluded.
‡ Four lines with less than 20 flies for at least one phenotype were excluded.

nificant. It should, however, be noted that a number of lines which gave too few progeny (less than 20 flies in at least one of the eight phenotypic groups) were eliminated from the calculations. There is reason to believe that these included low viability lines, which, if included in the calculations, would increase further the difference between the treated and untreated groups. (A similar result is obtained when the *Cy ve* groups are compared with the *Cy ve st* as reference groups. This comparison eliminates the factor of the background heterozygosity and is parallel to the comparison of the *ve* groups with the *ve st* as reference groups.)

Three out of the four comparisons show a nonsignificant lower mean viability of the treated groups than that of the corresponding untreated groups and one shows a small increase in the average viability of the treated group. There is no indication of any correlation between the degree of background heterozygosity

and the differences in viability associated with irradiation. As a matter of fact, in the two groups with the higher background heterozygosity the differences between the means of the treated and untreated groups are greater than in any other comparisons. This together with the fact that the means of the less heterozygous groups had somewhat higher standard errors stresses the contribution of many minor deleterious mutants, which on being made homozygous caused both the lower initial untreated viability means and the higher variance observed in these groups (see also later). A more meaningful test for the radiation effects in this connection might be obtained by taking the difference between the mean viability of the *Cy ve st* phenotypes (as compared with the *ve st* phenotype, see Table 1) in the treated and untreated groups as a measure for the variations due to background and environmental factors. It can be seen that the average reduction of the mean viability of the four genotypes in the lower part of Table 1 in the treated groups, as compared with the untreated groups, amounts to 2.5 times the environmental and background variation.

Series 42 and 43 with their subseries were carried out in an identical manner. Series 44 was made in a similar manner to series 42 and 43 but raised at 26°C instead of 18°C as 42 and 43 had been. Calculations were done for the 27th day counts and the 30th day counts. The 30th day counts include all flies counted up to this day, i.e. including the 27th day counts.

Table 2 shows the results of the analysis of variance for the counts of the 30th day, taking into consideration all proportions available (a) for the whole material of series 42, 43 and 44, and also (b) separately for the untreated and treated lines. This analysis makes possible a comparison of the variance between lines with that calculated from the average variance among the cultures within the same line, on the assumption that there are only random differences between lines. In a similar manner it makes possible a comparison of the variance between lines of the treated and the untreated groups (and also between the average of the lines raised in one temperature and that of lines raised in the other temperature within the treated and untreated groups, respectively, as well as between the averages of lines for males and for females within each temperature and treatment group) with that calculated, on the assumption that there are only random differences between treated and untreated means, from the average variance found among the lines within each treatment (or temperatures or sexes, as the case may be). Thus, we were testing whether the variance between the averages of two compared groups was larger than that calculated from the variance within the groups.

Table 3 gives the number of flies counted in these series and the mean of the proportions of *ve* flies in the different lines. The proportion for each line was a weighted mean of its proportions in its subseries.

A glance at Tables 2 and 3 would indicate that for most comparisons the differences were not significantly larger than expected by chance from the variance found within the groups compared. Although this might seem disappointing it was not unexpected. It should be kept in mind that all flies were homozygous for most of their genes, thus many recessive viability-reducing genes, present in the

TABLE 2

*Analysis of variance for viability calculations based on counts of the 30th day and with the* ve st *females as reference class for* ve *females and* ve st *males as reference class for* ve *males*

a. Analysis of variance for the whole material of series 42, 43 and 44.

| Source of variance | Degrees of freedom | Mean squares | F | P |
|---|---|---|---|---|
| Between treatments | 1 | 0.002152 | 0.616 | .... |
| Between temperatures | 2 | 0.067084 | 19.205 | <0.005 |
| Between sexes | 4 | 0.001760 | 0.504 | .... |
| Between lines | 486 | 0.003493 | 0.944 | >0.05 |
| Between repetitions | 808 | 0.003701 | .... | .... |

b. Analysis of variance for the untreated and for the treated lines for the series 42, 43 and 44.

| Source of variance | Degrees of freedom | Mean squares | F | P |
|---|---|---|---|---|
| *Untreated* | | | | |
| Between temperatures | 1 | 0.046905 | 15.038 | <0.005 |
| Between sexes | 2 | 0.001736 | 0.557 | .... |
| Between lines | 214 | 0.003119 | 1.102 | .... |
| Between repetitions | 350 | 0.002829 | .... | .... |
| *Treated* | | | | |
| Between temperatures | 1 | 0.087264 | 23.196 | <0.005 |
| Between sexes | 2 | 0.001784 | 0.474 | .... |
| Between lines | 272 | 0.003762 | 0.861 | .... |
| Between repetitions | 458 | 0.004368 | .... | .... |

original chromosomes, expressed their effect. In other words, from the outset our viability values were in the rather subnormal part of the viability scale. Since subnormality is connected with developmental instability (MULLER 1950b; LEWONTIN 1957; SPASSKY, SPASSKY, PAVLOVSKY, KRIMBAS, KRIMBAS and DOBZHANSKY 1960) it is not surprising that great variance between repetitions of similar lines was found and that this practically overrode any variance due to other sources. We are therefore in a somewhat paradoxical situation. On the one hand we have to keep the environmental variance component low in order to be able to detect small differences in genetic components, but simultaneously, for the possibility that overdominant mutations with only small effects are induced, we are trying to keep the lines highly homozygous so as to have a better chance (according to advocates of the overdominance hypothesis) of detecting on this genetic background small effects of viability due to heterozygous induced mutations. Keeping this in mind the analysis seems to be rather unequivocal.

The variance between lines is about as high as that calculated from the variance within lines (i.e., between repetitions of the same lines) (Table 2). This is what one would indeed expect to find in the untreated group since, if isogenization has been effective, the difference between two lines should not be greater than that between two repetitions of the same line. On the other hand, if irradiation affected

TABLE 3

*Numbers of flies counted and mean viability of lines with standard error for series 42, 43 and 44**

| | Temperature raised | Series and days of count | Number of lines tested | Females I | | Females II | | Males | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Totals | Mean of lines ± standard error | Totals | Mean of lines ± standard error | Totals | Mean of lines ± standard error |
| Untreated | 18°C | 42 & 43 27th | 58 | 14,476 | 0.526±0.007 | 13,751 | 0.551±0.006 | 13,163 | 0.531±0.005 |
| | | 30th | 58 | 16,592 | 0.517±0.007 | 15,808 | 0.537±0.007 | 15,223 | 0.521±0.005 |
| | 26°C | 44 16th | 51 | 8,846 | 0.498±0.007 | 8,371 | 0.530±0.008 | 7,875 | 0.497±0.005 |
| Treated | 18°C | 42 & 43 27th | 71 | 17,577 | 0.525±0.004 | 17,098 | 0.541±0.004 | 16,300 | 0.516±0.004 |
| | | 30th | 71 | 20,916 | 0.514±0.003 | 20,269 | 0.531±0.004 | 19,419 | 0.510±0.004 |
| | 26°C | 44 16th | 67 | 10,612 | 0.496±0.005 | 9,911 | 0.530±0.007 | 9,170 | 0.488±0.007 |

* Female viabilities taken with *ve st* females as reference group (I) and *ve st* males as reference group (II).

TABLE 4

*Radiation effects in series 42, 43 and 44 expressed as "ratio of ratios" and lethal-equivalents**

| Temperature | Series | Day of counting | Females I | | Females II | | Males | |
|---|---|---|---|---|---|---|---|---|
| | | | Ratio of ratios | Lethal-equivalent per $r \times 10^6$ | Ratio of ratios | Lethal-equivalent per $r \times 10^6$ | Ratio of ratios | Lethal-equivalent per $r \times 10^6$ |
| 18°C | 42 & 43 | 27 | 1.018 | —0.82 | 0.918 | 3.57 | 0.866 | 5.99 |
| | | 30 | 1.021 | —0.82 | 0.985 | 0.63 | 0.975 | 1.06 |
| 26°C | 44 | 16 | 0.982 | 0.76 | 0.940 | 2.58 | 0.981 | 0.80 |

* Females taken with *ve st* females as reference group (I) and *ve st* males as reference group (II). "Ratio of ratios" = ratio between the ratio of the *ve* to *ve st* in the treated group and the corresponding ratio in the untreated group. Average effect: 3.5 percent reduction in viability or $1.5 \times 10^{-6}$ lethal-equivalents per roentgen unit.

the viability of heterozygotes for irradiated chromosomes, we might expect a change in the variance of the treated group. If heterozygosity for induced mutations increased viability it would have increased also developmental stability ("homeostasis"), so that one would expect a decrease in variance between repetitions in the treated group as compared with the untreated group. If irradiation caused mutations which affected the viability of different lines to different extents, it would increase the variance between lines. It seems clear that, if anything, the irradiation caused rather an *increase* of variance between lines and even more so within lines; thus indicating a detrimental rather than a beneficial effect on viability.

The absence of significant differences between the means of the sexes (Table 3) indicates that the smaller background heterozygosity of males had little influence on either the viability of the treated group or that of the control group. This is also borne out by the agreement of the estimate of variation between sexes with that calculated from the variance between lines within each sex (Table 2).

A highly significant difference was found between the series raised at 18°C and those raised at 26°C (Table 2). All means, of the treated as well as of the corresponding untreated groups, were *lower* in the higher temperature series (Table 3). The reason for this seems to be at least partly that there is a difference in speed of development between *ve* flies and *ve st* flies, the latter being slower. This is borne out by the data of Table 3; within the series raised at 18°C the means of the 27th-day counts were higher than those of the 30th-day. Thus, it seems that 16 days at 26°C correspond to more than 30 days at 18°C, unless temperature affects these classes unequally.

The variance of the differences between the averages of the treated and untreated groups is only of the same order of magnitude as that calculated from the variance between lines (Table 2). Thus the change in the viability caused by introducing one irradiated chromosome into the genotype was too small to be statistically significant in conjunction with the other components of variance. The same conclusion can be drawn from Table 3. The differences between the means are too small when compared with their standard deviations. Still, comparing any two corresponding values in the table, the mean viability is in all but one case lower in the treated group than in the untreated group. These differences in the comparisons made with females average about 0.5 percent decrease in the mean viability of the treated group and in the males reach about a three percent decrease in the 27th day count at 18°C (the percentage here given being that which the difference forms in relation to the value for the untreated group).

Furthermore, for the males, the earlier the counts were made the greater the difference between the treated and untreated group (it has been noted above that the 16th-day counts of the 26°C series probably correspond to counts later than the 30th-day counts of the 18°C series). Thus, for the earliest count, that of the 27th-day of the 18°C series, the difference between groups is on the verge of significance ($t = 2.29$, $P = 0.02$). The selective importance of speed of development

in Drosophila populations has already been pointed out (as by MULLER and FALK 1961).

Table 4 shows the effect of the irradiation on the heterozygotes expressed as lethal-equivalents. The average decrease of viability is 3.5 percent; that is, $1.5 \times 10^{-6}$ lethal-equivalents per roentgen unit were induced. The order of magnitude of the lethal-equivalent per roentgen is about the same as that obtained by FRIEDMAN and CROW (1960) in their experiment; but since they applied the radiation to mature sperm our induced effects in spermatogonia would be expected to be lower.

The question might be raised whether the difference between the treated and untreated groups is not too small in comparison with the expected according to the "neo-Mendelian" hypothesis. Since recessive lethals have an average dominance of somewhere between three percent and five percent (STERN, CARSON, KINST, NOVITSKI, UPHOFF 1952; MULLER and CAMPBELL 1950) is it possible that these results reflect simply the net effect of heterozygotes for lethals in decreasing the mean viability taken in conjunction with net effect of heterozygotes for nonlethal mutations in increasing the mean viability? However, a little consideration shows that quite apart from the *a priori* improbability of a qualitative difference between lethals and nonlethal detrimental mutations, the data do not support such a possibility. As shown earlier, analysis of variance did not reveal so large an increase of variance between the lines in the treated groups, as compared with that in the untreated groups, as would be expected on such an explanation.

More evidence regarding this possibility is included in Table 5. Most of the *ve* chromosomes were tested for the presence of lethal mutations. Out of 61 treated chromosomes 20 carried recessive lethals, while only one out of 53 control chromosomes carried a recessive lethal. Thus irradiation induced recessive lethals in about 30 percent of the treated chromosomes. This is in good agreement with the expected frequency (MEYER, EHRLICH and MULLER 1959). Thirty-seven treated chromosomes and 42 untreated chromosomes were tested further to determine the viability of homozygotes, expressed as the percentage of *ve* flies in the cultures. The mean viability of the homozygotes for the treated chromosomes was only about 2/3 that of the untreated ones. Of this reduction over 90 percent was due to lethals and somewhat less than ten percent was due to nonlethal mutations induced by irradiation.

It should, however, be noted that if the effect of the mutants was dominant to some degree, which appears to have been the case, this is a minimum estimate of the proportional part played by nonlethals in the reduction of viability. That is because the viability estimate was based on the ratio of homozygotes for the relevant chromosome to heterozygotes for the same chromosome and for a tester chromosome. Any dominant effect would reduce the frequency of the heterozygote class to some extent in the same direction as the homozygote class, and thus would tend to cause an underestimation of the deleterious effect of the nonlethal mutations (see GOLDSCHMIDT and FALK 1959). The lower part of Table 5 gives the mean viability of the heterozygotes for those chromosomes the mean

TABLE 5

*Radiation effect on homozygotes and heterozygotes*

| | Untreated | Treated |
|---|---|---|
| Test of Homozygotes | | |
| Number of tested lines | 53 | 61 |
| Number of lethal lines | 1 | 20 |
| Percent of lethals | 1.9 | 32.8 |
| Mean Viability of Homozygotes | | |
| All tested lines | 0.333 | 0.207 |
| Lethals excluded | 0.342 | 0.333 |
| Mean Viability of Heterozygotes | | |
| All lines | 0.518 | 0.512 |
| Lethal lines | . . | 0.516 |
| Nonlethal lines | . . | 0.510 |

Note: The scale of viability for homozygotes was different from that of heterozygotes—see text.

viabilities of which were also determined as homozygotes. (These are means of the estimates of lines, each line based on the weighted values of all its subseries done at 18°C and counted on the 30th day, females and males pooled.) It is clear from this table that the effect of 41 nonlethals in reducing the viability of the heterozygotes was as intense as that of 20 lethals. The viability of heterozygotes for lethal mutations seems, as a matter of fact, to be too high when compared with that found by STERN *et al.* (1952) and by MULLER and CAMPBELL (1950), but this might be due to the large inherent error. Actually, the difference between the mean viability of the treated and the untreated groups ranges from practically zero to about three percent and is well within the limits of the 3–5 percent reduction of viability expected of heterozygotes for recessive lethals. This result, although statistically not significant, is in line with the suggestion that the degree of dominance of nonlethal mutations is greater than that of lethals (MULLER 1950; GREENBERG and CROW 1960; JAMES 1960) and rules out the possibility of an effect on the mean of lines heterozygous for nonlethals acting in the opposite direction to lines heterozygous for lethals.

Since the results do not show a *significant* decrease in viability of the heterozygotes for induced mutations it is of some interest to calculate how much *increase* in their average viability would be consistent with these results. If we reduce by 1.4 times its standard deviation the mean of the untreated group where the smallest difference between treated and untreated groups was observed (series 44 females when *ve st* males are taken as the reference group) and also increase by 1.4 times its standard deviation the mean of the corresponding treated group, we get the maximum increase in viability compatible with our results. This amounts here to about four percent. WALLACE (1959) got an increased viability of 1.5 percent by irradiating the spermatozoa with 500r. The effect of that dose corresponds to about a seventh of the effect of the 24,000r dose given here to the spermatogonia (MULLER and FALK 1961). Thus, if the increase in viability is

proportional to the number of mutations induced, the maximum possible increase in our experiment would correspond to an increase of about 0.6 percent for WALLACE's experiment. From WALLACE's paper it is possible to calculate that the 1.5 percent increase in viability had a standard error of about 0.6 percent, and hence the lower limit of the effect he got is larger than the maximum increase at all possible in our experiment. Therefore, even when the most extremely favorable assumptions are made one cannot reconcile our results with those of WALLACE.

WALLACE (1958) lists a number of factors which could possibly have caused an increase in the frequency of a given class of flies so as to produce an effect simulating an increase in viability. He believes he excluded, among others, the possibilities that the effect is due either to misclassification or to gametic-ratio distortion. These points, however, deserve some re-examination. In the presence of an additional Y chromosome the marker Plum will be classified as wild type (BRIDGES and BREHME 1944), and genic modifiers can also normalize it to some extent (MULLER, unpubl.). Since some nondisjunction as well as gene mutation was probably induced by irradiation it is possible that there were more misclassifications of *Pm* as non-*Pm* in the irradiated series than in the controls. At least some of these misclassifications might not be eliminated even after another testcross.

The original flies for WALLACE's experiments were taken from a population cage which had an irradiated history. A small test was done to determine the possibility of a segregation-distorter being present. Recently NOVITSKI and HANKS (1959) found at least two Segregation-distorter genes in the flies originating from the population cages of WALLACE. If indeed there were segregation-distorter genes present and if they had such capricious behavior as the *SD* gene described by SANDLER and HIRAIZUMI (1959), then it is very possible that such a gene (or genes) was missed in WALLACE's search. Of the various series of experiments carried out by WALLACE only two (2–9M and 15–22F) showed a significant increase in viability of over two percent (WALLACE 1959). All other experiments had an increased viability of the +/+ group of the same order of magnitude as that of the *Cy*/+ or *Pm*/+ group, respectively. This heterogeneity might indeed be due to the occurrence of segregation-distorters in some cultures and not in others. Furthermore, in his earlier paper WALLACE (1958) reports an average increase of viability of 2.5 percent while in the later paper (1959) an increase of 1.5 percent was mentioned. This would suggest that the claimed increase in viability was found only in the earlier experiments while it was absent from the later series. One wonders therefore if in spite of the large scale of his experiments, a chance deviation was not responsible for the results.

## DISCUSSION

It was the purpose of this paper to obtain some evidence regarding the mechanism underlying heterosis: in particular, whether it is due in any considerable degree to interallelic interactions giving overdominance or, on the other hand, to

simple dominance-recessivity relations between genes concerned with viability. The experiment presented here supports the notion that induced mutations are essentially deleterious, whether in the homozygous or the heterozygous state. Even under the most favorable conditions for their detection, mutations increasing the viability of the heterozygote, whether due to dominance or overdominance, were, to say the least, extremely rare.

It is unfortunate that with a few exceptions—notably WALLACE's papers (1958–1959)—much of the work taken to support the "neoclassical"-overdominance hypothesis of heterosis is not crucial since it is compatible with both theories when considered at the level of the gene. It may be of some interest to review shortly the possibilities for overdominant gene interactions and their chances of becoming established in the population, as well as to examine under what assumptions one can interpret the heterosis observed in natural populations as due to overdominant gene interactions and the load of the population as due to the segregation of the less adapted homozygotes (segregation load, CROW 1958).

Studies done by a great number of workers have made it clear that for many genes which seemed to be recessive, the heterozygote was somewhere intermediate between the homozygotes. In other words, they were dominant to some degree. This has been shown in many organisms, but mainly in Drosophila, especially for genes affecting viability (MULLER 1950b; STERN *et al.* 1952; MULLER and CAMPBELL 1950; FALK 1955 for newly induced mutations, and CORDIERO 1952; PROUT 1952; GOLDSCHMIDT and FALK 1959; HIRAIZUMI and CROW 1960 for mutants in natural populations), and also in yeast (JAMES 1960) and in man (LEVIT 1936; NEEL 1949). It may be noted also that Table 2 of DOBZHANSKY *et al.* (1960) suggests that nonlethal viability-reducing mutations on chromosomes free of lethals had on the average a higher relative degree of dominance than lethals (however, these authors regard the table as evidence for a more or less opposite argument, due to their taking the "average heterozygote" as the "normal" which serves as the reference point for their comparisons). On these grounds it is reasonable to assume that even those genes tested which did not show deviation from complete recessiveness were for the most part dominant, to a degree too small to be detected by present techniques. But it is also plausible that besides some fully recessive genes there are some which show overdominance (STERN *et al.* 1952; ALLISON 1955 for examples; and MUKAI and BURDICK 1959).

Many attempts have been made to classify the possible mechanisms for overdominance at the gene action level. Essentially they can be reduced to three types as suggested by CROW (1952) and ALLISON (1959).

1. One allele is responsible for the production of a certain material while the other allele does not produce any, or only a small amount of it. If only an optimal amount of material is required the heterozygote may produce an amount nearer to the optimal than either homozygote, as is the case in the sulfanamide-requiring Neurospora (EMERSON 1948) and perhaps also for cases like the absence of catalase from erythrocytes of dogs (ALLISON, APREES and BURN 1957).

2. The alleles each produce a different effect, both of which are important for

the individual, as in the case of sickling in man (ALLISON 1955) and in some cases of rust resistance in flax (FLOR 1947).

3. The heterozygote produces a primary product different from that produced by both homozygotes. This might be the case with some antigens, such as the AB antigen in man (MORGAN and WATKINS 1956) and the "hybrid antigen" in pigeons (IRWIN 1947) and rabbits (COHEN 1956).

It should be noted that the difference between types 2 and 3 may be mainly a theoretical one, because it is usually impossible to eliminate the possibility that the "hybrid product" is not the primary product of the alleles. SMITHIES and co-workers' study with haptoglobins is a case in point for, although they found a hybrid haptoglobin produced by heterozygotes (CONNELL and SMITHIES 1959), further analysis demonstrated this haptoglobin to be a secondary derivative of two products similar to those produced by each of the homozygotes. Thus the third type, if it exists, is the only one which comprises real interallelic interactions, while the first two confirm the rule that most genes are not completely recessive; in them, heterosis is present only on the phenotypic level from the point of view of fitness.

Since genes and their alleles are under the pressure of natural selection it is to be expected that "the exceptional cases of the heterozygote being superior are probably represented for the most part . . . by adaptations that have not yet stood the test of geologic time. For we should expect natural selection in the end to find mutations giving gene combinations which can achieve in homozygous form the same beneficial effects that at first could be attained only by the unstable, wasteful means provided by the selection of heterozygotes" (MULLER 1956).

Different authors (cf. MULLER and FALK 1961) have pointed out simple, known mechanisms which would turn the loci showing overdominant interactions into genes—or gene complexes—showing a regular dominance-recessivity relationship. The selective pressure for such a change is very considerable as has been shown by HIRAIZUMI and CROW (1960).

Thus, all evidence from the level of gene action makes it highly improbable that there exists on a large scale a system based on more or less nonspecific interactions of pairs of individually ill-adapted alleles giving adaptive combinations.

Turning to the populational level, an elementary notion must be borne in mind when dealing with overdominant allelic interactions: "The loss of fitness of the population (with a superior heterozygote) is of the order of magnitude of the selection coefficient, as HALDANE (1937) has shown, whereas with a detrimental recessive the loss is of the order of the mutation rate. Hence a single overdominant locus has a tremendously greater effect on the population fitness than a single locus with dominance and intermediate heterozygote" (CROW 1952). This in itself might raise the suspicion that a model based on a high proportion of loci having heterotic alleles will actually lead to conflict with observational evidence on the structure of the population, since the effect of many overdomi-

nant genes should consist in a much greater loss of fitness than any population might withstand.

MORTON, CROW and MULLER (1956) estimated the total mutational damage in human populations to be about 3–5 lethal-equivalents per zygote. They showed that it was impossible to attribute the load of the population to a "segregation load" from adaptive heterozygotes for a single pair of alleles per locus concerned. If it was assumed, however, that there exist many alleles at each locus, all of them (or at least a great proportion of them) interacting heterotically, the observed load could still be mainly a segregational one (CROW 1958). Studies by MORTON (1959, 1960) showed that for some malformations one has to assume the presence of some dozens to a few hundred alleles in each locus considered, all of which interact heterotically in virtually all combinations, in order to be able to attribute the observed load to "segregation load." Essentially similar results were obtained by GREENBERG and CROW (1960) when this type of calculation was extended to data available from Drosophila populations of different species. As shown earlier and as will be shown also below this huge number of alleles per locus interacting heterotically is a most improbable assumption.

A more direct demonstration of the absence of any considerable degree of overdominant interactions in determining heterosis was given by SIEGEL (1958) for *Paramecium aurelia*. In this species inbreeding predominates, although occasional advantage is taken of hybridization. Heterosis is demonstrable in the laboratory but exists also in natural populations. He demonstrated that some $F_2$ which were produced by autogamy from $F_1$ hybrids showed nearly as much vigor as the heterotic $F_1$'s. Since these animal clones were completely homozygous, this result could be due only to recombinations of advantageous genes—as expected according to the "dominance" hypothesis and in sharp contrast to the expectation for the "overdominance" hypothesis.

Another difficulty makes a model based on many alleles at given loci interacting heterotically quite improbable. At each locus where there are two or more alleles present there is a probability of fixation of one of the alleles merely by chance. In an organism the adaptiveness of which is based to a great extent on keeping many alleles at each locus there would during each generation be a large loss of variability due to chance fluctuations, especially when it is noted that the frequency of each allele cannot be very high. This has been noted by WALLACE and DOBZHANSKY (1959). In that case, as they also note, mutation should have a constructive value in compensating for the chance losses of fitness caused by this elimination of alleles, and the induction of more mutations by radiation might in that case have an over-all directly beneficial effect. CROW (1960) pointed out that the theoretical question of the effect of mutations on fitness in a system like that in a finite population is a difficult one. In view of the evidence accumulated over so many years (to which DOBZHANSKY and his group contributed so much) as to the importance of selection in determining the fitness of populations, it is hard to believe that fitness is due in a great extent to a balance between random drift and random mutation.

It would seem to follow from the above results and considerations that the variability, polymorphism and heterosis of natural populations is not due to a large extent to many loci with overdominant alleles. As shown, this view is not only improbable on the basis of considerations based on gene function and because of the contradictions to which it leads when the structure of populations is analyzed critically but also in view of the results of direct experimental attack.

## SUMMARY

The experiment described was intended to investigate whether induced mutations when in heterozygous condition and unselected can increase the average viability of an otherwise homozygous genotype. The design of the experiment (the general lines of which were given in the previous paper of this series by MULLER and FALK 1961) was such as to afford every chance for an induced increase in viability to be detected.

More than 60 lines were prepared, each having an irradiated third chromosome marked with the gene *ve*, carried along with a *ve st* marked chromosome (coisogenic—except for the *st* locus and its vicinity—with the *ve*-marked chromosome before its irradiation), and homozygous as well as isogenic with respect to the second chromosome genes. A similar number of control lines, identical to those irradiated in all details except for the irradiation, were also secured.

Males from all lines were repeatedly outcrossed to females from a stock homozygous for second and third chromosomes which were also coisogenic with the unmarked second and the *ve st* third chromosomes of the treated and control lines.

The irradiation delivered to the treated lines comprised six doses of 4000r each, given to males at five-day intervals. The irradiated males were kept for 15 days after the last irradiation before deriving from them the offspring studied, so that practically only sperm that had been irradiated at spermatogonial stages was utilized.

In each line the viability of the *ve* flies heterozygous for induced mutations, or the viability of the corresponding controls, was determined from the proportion of *ve* females or males out of the total of the respective *ve* and *ve st* phenotypes, the latter serving as a reference group for determining the viability of the former. In one backcross, where the male parents were heterozygous for the *Cy* marked chromosome and the isogenic second chromosome, viability was estimated for the *Cy ve* phenotypes separately but in a similar manner to that of the *ve* phenotype.

The lines were raised under standardized crowding conditions at 18°C or at 26°C on different occasions.

As could be expected from backcrosses of lines made homozygous for so many genes, including minor deleterious genes, the variability between repetitions within lines was rather high. This variability overrode most differences—which were expected to be rather small—between the treated and the untreated-control groups. Nevertheless, in nearly every possible comparison the treated lines had a lower mean viability than the untreated lines. The reduction of viability caused

by the presence in heterozygous condition of radiation-induced mutations ranged from zero to three percent.

The reduction in viability was more pronounced in earlier counts than in later ones of the same crosses, a result indicating that not only viability but also rate of development was adversely affected by the irradiation.

The reduction of viability found among heterozygotes for nonlethal-bearing chromosomes was about the same in extent as that expected for the lethal-bearing heterozygotes (the observed reduction of viability caused by the heterozygous lethals was smaller than that found for heterozygous nonlethals and than that expected for heterozygous lethals). In the homozygotes, on the other hand, 90 percent of the reduction of viability was due to lethals and only about ten percent to nonlethal detrimentals. This result may be taken as support for the notion that nonlethal deleterious mutants are on the average relatively more dominant than lethals.

The reported results were analyzed to find the maximum increase in viability statistically compatible with them. This was found to be even lower than the minimum increase possible according to WALLACE's (1959) experiments, in which he found an average increase of 1.5 percent in the viability of lines heterozygous for radiation-induced mutations.

The possibilities of inducing many overdominant mutations and maintaining them in a population are discussed.

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