ARE INDUCED MUTATIONS IN DROSOPHILA OVERDOMINANT?

I. EXPERIMENTAL DESIGN¹

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Received November 11, 1960

Background of the problem

IT seems likely (as pointed out by TYLOR in 1865, cited by DARWIN 1868) that primitive peoples had observed the tendency to lesser fitness manifested by the children of more closely related parents or, stated conversely, the tendency to greater fitness of the more crossbred, and that these observations were a factor in the widespread establishment of rules of exogamy. Lacking the genetic concepts for interpreting this matter, DARWIN (1859) thought of it as an expression of a principle, earlier proposed by HERBERT SPENCER, that living organizations tend to undergo stagnation (or to approach "equilibrium," as he put it) if continually subjected to unchanging conditions. Thus, the rejuvenescence that seemed to follow fertilization was thought to be more intense when the uniting germ cells were more (yet not too extremely) unlike, as stated, for example, by JOST (1904, 1907). Similarly G. H. SHULL regarded heterozygosity as a sort of stimulus when he originated his famous proposal for getting improved corn yields by taking the F_1 between selected inbred strains (1909, 1911) and EAST and HAYES (1911) held the same opinion. It is this ancient view of heterosis that is rightly to be termed the "classical" one, contrary to the practice of those (DOBZHANSKY 1955, WALLACE and DOBZHANSKY 1959) who have recently offered a version of it in more modern terms, that we shall refer to here as the "neoclassical" one.

However, even in the first two decades of this century the idea had already become prevalent among geneticists (see DAVENPORT 1908, and more especially BRUCE 1910; KEEBLE and PELLEW 1910; and ELDERTON 1911) that inbreeding leads to degeneration by bringing about the homozygosity of recessive, relatively deleterious genes, and that crossing acts conversely. (Neither BRUCE nor KEEBLE and PELLEW, however, indicated that they considered the dominant alleles in their scheme to be merely normals and the recessives as mutants, but ELDERTON seems to have taken this point for granted, and she even anticipated FISHER's hypothesis of the evolution of dominance.) On this view vigor was increasingly

¹ These investigations were supported in part by U.S. Public Health Service Grant RG5286 and in part by U.S. A.E.C. Grant AT(11-1)-195. This paper is Zoology Department Contribution No. 694.

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regarded as subject to the principles of multifactorial inheritance that were already being recognized as pertaining to quantitative characters (YULE 1906; LOCK 1906; MULLER 1914; MORGAN, STURTEVANT, MULLER and BRIDGES 1915). As COLLINS (1921) later showed, where numerous separately segregating loci are involved the production of an all-normal genotype is not to be expected, even apart from considerations of linkage, and the phenotypic frequency distribution will tend to be symmetrical, just as had been found for vigor.

EAST and JONES in their "*Inbreeding and Outbreeding*" (1919) gave an extended exposition of heterosis and related matters along these lines. They included, in addition, the important point made by JONES (1917) that linkage further tends to maintain heterozygosity by impeding the formation of recombinants that have only dominant normal genes (though unlike COLLINS they represented linkage as essential for the effects). The most extreme examples of this situation were those provided by balanced lethals (MULLER 1917, 1918). In connection with the latter it had, moreover, been pointed out (MULLER 1917, 1918, 1921) that mutations are in fact in the great majority of cases both recessive and detrimental, even degenerative, and that they are probably for the most part of minute, quantitative expression. WRIGHT also (1921) expressed essentially this view of the inbreeding-outbreeding contrast.

It happened, however, that EAST himself later (1936) proposed as a supplement to the above "neo-Mendelian" interpretation of the matter (as we shall here term it) the proposal that heterozygosity in alleles at an individual locus could, as by complementary action of the alleles, give rise to a fitness higher than that of either homozygote. Some time afterwards JONES (1944), followed by others, presented cases that he believed were of this type. HULL (1945, 1946), terming the postulated phenomenon "overdominance," and urging that it was of major importance in heterosis, then cited a series of results in crop plants that he believed presented evidence of it. He proposed that it might involve more than mere complementarity and thus brought the view closer to the ancient one of stimulation through difference *per se*. Some of these examples, however, involved pairs of alleles both of which were clearly mutants for different defects. As for the rest, it was seldom if ever possible to meet the rigorous genetic criteria required for proving that the individuals homozygous for either one of the alleles in question were not at the same time homozygous for detrimental recessives at other loci, especially at loci linked to that under consideration. In fact, this situation was the very one to be expected on the neo-Mendelian view. Reviews of the alternative hypotheses have been given by CROW (1948, 1952).

For somewhat over a decade there has been a reversion to the overdominance postulate among some geneticists. LERNER in his book *Genetic Homeostasis* (1954) suggested that "fitness as a whole exhibits the phenomenon of overdominance." Although leaving open the possibility that there are also "polygenic blocks" patterned after JONES's model, he states (p. 67) that "developmental homeostasis" (by which he means the stability of equilibrium in developmental reactions) is based on the "buffering of development by nonspecific action of

heterozygous gene pairs," though these nonspecifically interacting genes are assumed to be in specific and different groups for different characters considered. This seems to be a return, in modern terminology, to the old idea that difference is *per se* stimulating or somehow conducive to viability, or as EAST and JONES in 1919 put the matter (p. 169) when describing this idea, that there is "a physiological stimulation arising from the interaction of *different* hereditary factors."

WALLACE and DOBZHANSKY go even further and believe that the high proportion of deleterious alleles found to exist in populations "is most reasonably explained by the selective superiority of individuals heterozygous for two different forms of such alleles" (WALLACE 1958). As one would expect with such a view these authors question the legitimacy of calling an allele normal in the sense of fitness even when the allele under consideration occurs at so high a frequency as to have been termed normal in the sense of abundance. They consider that the viability of such an allele, when in a given environmental and genetic setting, would usually be less when homozygous than when heterozygous.

There is no doubt that such a situation exists for occasional loci, such as those of some blood antigens; but there is ground for remaining skeptical of its applicability to many loci, or to most alleles arising at any given locus. The neo-Mendelian interpretation, well based in theories of gene functioning, has been supported by significant observations made long after it had been put forward. Among these are the findings of the hypomorphic nature of most mutant genes, their incomplete recessiveness, and the peculiarities of dosage compensation (MULLER 1932). It has been argued (MULLER 1950; CROW 1952) that in most cases a situation of higher fitness of the heterozygote of a given locus would tend eventually to be superseded, through natural selection, by one of homozygosity in which the given allele had been suitably mitigated in its expression, as by modifying genes or intralocus reorganization, or had even been replaced by a condition of duplication having both alleles homozygous at once (*ibid.*, and HALDANE 1937). Calculations of the load on the human population (the loss of fitness as compared with the optimum combination of existing genes), that would be caused by the existence of many overdominant loci, moreover, have given results inconsistent with the findings regarding the loss of fitness associated with inbreeding (MORTON, CROW and MULLER 1956; CROW 1958). Furthermore, studies by MORTON and CHUNG (1959) have shown that for some specific cases investigated by them one would have to assume an unreasonably high number of overdominantly interacting alleles if one were to interpret their frequencies in the population by overdominance.

Despite these considerations, the present authors have felt that, in view of the results recently reported by WALLACE (1957, 1959) which he interpreted as confirming and extending the overdominance interpretation, a further experimental attack was advisable, using similar biological material. In a very large-scale experiment (involving some three and a quarter million flies) WALLACE found that a group of *Drosophila melanogaster* homozygous for a given unirradiated second chromosome had 1.5 percent lower average fitness than a second

group that did not differ in any consistent way from the first group except in having had one member of the given pair of second chromosomes previously irradiated with 500r in a spermatozoon stage, and in being to that extent more heterozygous. WALLACE (1958) concluded from these results that "more than half the alleles actually present in a given chromosome were seemingly improved by heterozygosity for a *randomly* induced allele" (*italics ours*). That is, the majority of the alleles induced at a given locus act with overdominance ("heterotically"). It is thereby suggested that most mutants are perhaps of only slightly or imperceptibly deviant homozygous expression, being in effect wild-type "isoalleles," and that relatively few give deleterious reactions when heterozygous (BURDICK and MUKAI 1958).

Since this view is mainly concerned with isoalleles it cannot be directly tested by the use of mutants having detectable effects. Few mutants that could be discovered by present techniques would have been produced by irradiation of spermatozoa with 500r. Even if we included as detectable those having, when homozygous, viability effects of as little as five percent but no visible effect we should expect (extrapolating from results of MULLER and MEYER 1959) that not more than one irradiated second chromosome in six would have one of these "detectable" mutant genes. If they alone had been the cause of the 1.5 percent increase in viability the average heterotic effect of any one of them would therefore have been of the order of nine percent. Such an effect, it might be thought, would be observable even if the "genetic background" of the treated chromosome instead of having been homozygous had been heterogeneous and heterozygous but comparable (i.e. not consistently different) in the two groups contrasted.

As a matter of fact, WALLACE did find, in his experiment, that there was a viability difference between two such groups having a heterozygous genetic background, and that it was of the same sign as and of a magnitude not much smaller than that found between his two otherwise comparable groups which had a homozygous genetic background in regard to their second chromosome. Since this result was not expected on his view, his findings seem to be at variance with both his own concept and the neo-Mendelian one. This consideration provided us with an additional reason for conducting a further experimental investigation of these matters.

Basic plan of present experiments

In the setting up of the plan and the construction of the stocks for the present series of experiments, by MULLER in 1956-58, it was decided to have the work of testing avoid crosses (like those of most previous studies) in which the chromosome under investigation had to be passed in many replicas through females heterozygous for a homologous chromosome containing inversions and markers, since occasional undetected crossovers and misclassifications happening in the course of such crosses could cause significant distortions in the final data. In each of the three major experiments of the present series, all of which were conducted on *Drosophila melanogaster*, the chromosome investigated was the third. A given

wild-type or nearly wild-type third chromosome, the "prototype," was first allowed to cross over, through one or two generations, with a third chromosome provided with suitably spaced markers, and a single final recombinant, to be called the "marked chromosome," was chosen that throughout was derived from the prototype except for a small region, not more than some five units long, containing a given recessive marker not present in the prototype. The marked chromosome and a single prototype chromosome, both derived from the same grandmother (in which and/or in whose daughter the given crossovers had occurred), were then subjected to crosses with stocks having inversions otherwise marked. Two stocks were thereby obtained, as rapidly as possible, one homozygous throughout for the marked chromosome and the other continually selected so as to be heterozygous in the males only for the marked and for the prototype chromosomes while in the females it was homozygous for the marked chromosome. In a part of the experiments the other major chromosomes (X and/or II) were also caused to be homozygous and alike in these two stocks, while in other cases, as will be explained later, they were replaced in the course of the testing so as to remove any consistent genetic differences that might be caused by these chromosomes between the two groups of flies that were to be compared.

After the establishment of the two stocks having their third chromosomes coisogenic except for the specially marked region the procedure was comparatively simple. Some flies containing the prototype chromosomes were irradiated and a comparable number of others were held as controls. A series of lines of descent, some "experimental," some "control," were thereupon established by crossing between these flies and flies homozygous for the marked unirradiated third chromosome. In any given line all the males chosen for breeding were always heterozygous for a given prototype third chromosome ultimately derived from a single irradiated germ cell or from a parallel unirradiated germ cell of a control fly of the same generation, as well as for a marked third chromosome derived from their mother. Moreover, the prototype chromosomes of different lines were always derived from irradiated or control germ cells that at the time of irradiation had been contained in different individuals. The males of each such line were in each generation backcrossed to females homozygous for the unirradiated marked chromosomes and counts were made of the numbers of unmarked and marked offspring in each culture. Among these offspring the unmarked males were again heterozygous and genetically like their fathers. Thus the operation could be repeated *ad lib.* if it was desired to increase the count for any or all lines. Comparison between the ratios shown by the experimental and treated lines was the final step for ascertaining what detectable effect on viability, if any, the induced mutations had produced.

It will be seen that in this experiment it is possible to have a much more direct comparison between the viability effects of irradiated and unirradiated chromosomes than in previously reported work, since this comparison can be made between flies (the marked *versus* unmarked ones) of the very same culture. This possibility arises from the fact that the two chromosomes here in question ordi-

narily differ, except in their small marked region, only in regard to such heterozygous mutant genes as may have been induced by the radiation, and that the cross is so constituted as to have flies with these two chromosomes both manifest their effects in the same culture. It is true that the effect of the difference in the small marked region must here be corrected for, by use of the data from the control cultures. On the other hand, the control cultures also supply information regarding effects on the ratios caused by differences in cultural conditions and in uncontrolled genetic influences (such as those due to spontaneous mutations and to possible pre-existing differences in the fourth and Y chromosomes). However, in the present work these other influences tend to be exerted alike on the flies of both irradiated and unirradiated classes of the same culture, whereas in previous works the corresponding classes were always in different cultures. Thus if there was any effect of a heterozygous mutant gene on viability it could have become evident in earlier work only by a comparison between the ratio manifested by it in its own culture or cultures and the corresponding ratio manifested by a control gene in a different culture or cultures. An additional way in which the present more simplified genetic technique enhances the determination of relative viabilities lies in the fact that in each test culture *all* the zygotes produced, instead of merely one quarter of them, as in most previous work, represent the genotypes of primary significance.

In order further to increase the significance of results involving any given total number of flies, it was decided to raise the effective dose as much as practicable, but in such a way as to leave the distribution of mutant types like that ordinarily obtained with comparatively low doses. In preliminary trials carried out separately by HELEN U. MEYER and by EHRLICH, under the direction of MULLER (MEYER, EHRLICH and MULLER 1959), it was found that flies of some vigorous types, of both sexes, could still give a few offspring when crossed to nonirradiated flies even after having received as much as 24,000r, provided that this was delivered in installments of 4,000r spaced at intervals of four days (the flies being kept at 26°C during the intervals). By taking offspring derived from spermatozoa or eggs released at least 12 days (usually at least 15 days) after the last irradiation it was insured that the germ cells used had been at a gonial stage during all irradiations. At this stage only about a sixth as many lethals are induced by a given dose as at the spermatozoon stage usually employed for irradiation, so that the 24,000r in this case give rise to about the same results, quantitatively, as are ordinarily obtained after a dose of 4000r to spermatozoa, and each installment of 4000r to the gonidia gives results for lethals about like those of 700r applied to spermatozoa. However, far fewer minute rearrangements and incomparably fewer gross rearrangements of chromosome parts, in comparison with lethal and other point mutations, are induced by 4000r given to gonidia than by 700r to spermatozoa.

Moreover, the relative frequency of the rearrangements is not raised by the increase in total dose, in our experiments, as it is in treatments of spermatozoa, for the broken ends of chromosomes produced by one installment of radiation

have undergone fusion in the four days intervening before the next installment is delivered. Thus the mutations induced by our irradiations must have consisted in a significantly higher degree of gene mutations than those in WALLACE's experiments did, despite the fact that the dose we used was some eightfold his dose in its point-mutation-inducing effectiveness.

If, now, we assume that both in the case of mutations that act beneficially when heterozygous and of those that act detrimentally their frequency rises linearly with total dose, and that the detrimental ones are at this level of frequency not significantly more additive or synergistic in their effects than the beneficial ones are, we must conclude that an increase in dose will not change the sign of the over-all effect but only its amount, rendering it more conspicuous. We believe, therefore, that our results have a level of significance about the same as would have been obtained in otherwise similar experiments involving at least eight times the total number of counted flies recorded by us, provided that those other experiments had been based on flies derived from spermatozoa irradiated with 500r.

Another technique designed to enhance the significance of our results was the use, in a part of the work, of cultures crowded in a standardized way. It had been found independently both by KERKIS (1935, 1938), working with MULLER (1934), and by TIMOFÉEF-RESSOVSKY (1934, 1935), and confirmed by later work, that small genetic influences on viability are expressed to a more marked degree when the competition is increased by crowding. Moreover, in some of our work relatively early counts of offspring were made. For, as BONNIER and JONSSON (1957) have shown, there is a strong positive correlation between viability and developmental speed, and it is also evident that in populations in which much competition occurs (as is often the case in *Drosophila*) speed of development would itself constitute an important factor in fitness. Finally, in some of our work a given line was subjected to several simultaneous tests, in order to make the statistical analysis more efficient and to throw light on whether the developmental stability had been affected in the lines with treated as compared with untreated chromosomes.

Three separate series of experiments following the general procedures above outlined have been carried out by the *Drosophila* group at Indiana University during the past three years. A preliminary experiment was carried out in the summer of 1958 under the direction of MULLER and with the assistance of Miss ELLYN COSDEN (now Mrs. KORN), together with some supervision by Dr. I. I. OSTER. A second experiment, on a much larger scale, was gradually prepared for by MULLER in 1958-59 and has been carried through under his direction by Mrs. ELIZABETH EHRLICH, assisted recently by Miss CAROL BART. But although started earlier, it was not finished so soon, either on the experimental or mathematical side, as the third experiment. This large-scale experiment, which was performed by FALK with the assistance first of BART and then of EHRLICH, has already had some of its earlier results reported on in abstract form (FALK 1959). Because the calculations connected with it have already been completed while those of the

other two pieces of work are still being processed it will be reported upon next, and the other two experiments will be taken up in the third paper of our series.

SUMMARY

The background of the problem of overdominance and the plan of experiments to test for overdominance of induced mutations have been presented. The experimental work is in part presented in the following paper by FALK.

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