ARE "PROGRESSIVE" MUTATIONS PRODUCED BY X-RAYS?[1]

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I. THE PHYSICAL BACKGROUND OF THE PROBLEM

Do the X-ray mutations consist merely of losses and rearrangements of portions of the chromosomes? Or do they also include "progressive" changes—the kind of steps of which real evolutionary advancement has been composed? This is one of the most vital immediate questions confronting X-ray genetics, for upon the answer to it may depend in turn the answers to fundamental questions concerning the mechanism o natural evolution, the nature of the gene, and the means of bringing abo t artificial evolution. The issue, therefore, must be faced squarely.

[1] During the course of the radiation work in this laboratory both authors became interested in the problem in this paper and accumulated various data relative to it. It was then decided to present a combined report of all the data, and it was arranged that the senior author (PATTERSON) should conduct the major experiments described, those in sections IX to XIII inclusive. Except where otherwise specified, the other experiments described, and the discussion, are by the junior author.

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There are two main facts which tend to give plausibility to the suspicion that X-rays may not be constructive in their genetic action. One is the general fact that they do often cause "destructive" changes (especially noticeable when the treatment is heavy or long continued) both in the case of organisms and of non-living materials. The other is the more specific fact, discovered coincidently with the gene-mutation effect, that they cause breakages of chromosomes, sometimes accompanied by losses of the fragments and sometimes by their reattachment at a different point in the chromatin from before (MULLER 1927, WEINSTEIN 1928, MULLER and PAINTER 1929, PAINTER and MULLER 1929).

A little further inquiry into the manner in which X-rays exert their chemical effects serves to dispel the idea that the changes induced must always be of a destructive nature—no matter how we choose to define the word "destructive." At the same time it becomes evident why many of the changes so induced are judged to be destructive. The primary effect of the X-rays, as also of beta, gamma, and cosmic rays, on the structure of any material is the expulsion of an electron (sometimes more than one) from some of the atoms, either directly, by the absorption of a quantum of the radiant energy, or, in the case of far more of the atoms (some hundreds to one like the former), indirectly, through the electron's being forced out by another electron that was previously expelled through the (direct or indirect) action of the radiation. In case an electron is held by two atoms in common it may become detached from one of them, thus causing the molecule to become directly broken up. Sometimes, when the action is "indirect" in the sense above explained, instead of an electron's being completely ejected, it is merely forced to a position more distant from the atom nucleus. In the case of ejection, the atom remaining is usually incomplete or "ionized"; in the case of displacement of the electron, the atom is sometimes said to be "excited." No atoms are exempt from these effects. In any case, but especially in the case of ejection, the altered atom is in a state of higher potential energy than before, and of heightened reactivity. It is now often capable of releasing its former intra-molecular union with some other atom or atoms, and of forming a new union, the exact nature of which will depend upon what other atoms, and atom-groups, happen to present themselves in an appropriate manner.

Thus, in an organic mixture that is complex to start with, many different kinds of new compounds will come into existence in a chaotic fashion, lying in the form of isolated molecules scattered indiscriminately through the mixture. Of course any such hodgepodge of changes should in general make for disorganization and will thus be more and more "destructive" in

its effect, the stronger the treatment is, that is, the more multitudinous these random changes are. Nevertheless, many of the new molecules, individually, may be just as complicated, and some more complicated than the old. It is inevitable that many of the changes—though doubtless (because of the smaller number of chances for this) not the majority—should be of the nature of syntheses, and that a rather high proportion of the changes should be endothermic, involving intake of energy, as compared with the original substance, by virtue of the previous absorption of the energy of radiation.

There is no mechanism by which a gene, whatever its setting, could be protected from the occasional occurrence of such an individual molecular alteration when subjected to X-rays or related radiation. Mutant genes thus produced would therefore be expected to involve alterations of varied nature, not merely losses, breakages, and the sequelae of these (although it might well be the case that losses and partial or complete inactivations would be produced oftener than changes of other kinds).

It is true that not all the induced mutations need be supposed to result from any such immediate effects of the rays. It might be postulated that some particular substance, or physical condition, to which the genes were sensitive, was first produced in the protoplasm in some abundance, as a regular result of irradiation, and that this in turn then reacted upon the genes. If so, it might further be postulated that this latter action was a purely destructive one. There is now, however, an accumulating body of experimental evidence which militates against such an idea by tending rather to the conclusion that the induced mutations are in general the direct result of the local electronic "hits." These findings may be briefly passed in review here.

(1) There is, first, the general randomness in the occurrence of the induced mutations. (2) There is, more especially, the fact that, of two chemically identical allelomorphs present near together in a treated cell, only one becomes altered (p. 532–537; also MULLER 1928 a, c). (3) The degree of phaenotypic change (proportion of lethals to non-lethals; likelihood of the most extreme allelomorph being produced) is obviously independent of the strength of the treatment (MULLER 1928c, 1929a). (4) Attempts to produce gene mutations by the application of specific substances, without irradiation, have so far failed (MULLER 1923, 1928c, 1929a). (5) Most important of all, probably, is the fact that a direct and simple proportionality has been shown to exist between the frequency of the induced mutations and the amount (energy) of the radiation absorbed. This has

been proved by HANSON and HEYS (1929) for radium rays (beta, gamma, and mixed) and more recently by OLIVER (1930) for X-rays. There is no indication in the results of any lower critical intensity, or threshold value, beneath which there is no (or a relatively lesser) effect. All these facts and considerations converge to indicate strongly that the mutations are rather direct effects of individual quantum-absorptions and electron-hits, and that the changes involved in them are therefore probably most varied in their nature and rich in their possibilities.

II. EVIDENCE CONCERNING THE DUALITY OF THE X-RAY EFFECT ON CHROMATIN

What, now, is the bearing on the question at issue of the finding that X-rays produce not only such changes as would ordinarily be recognized as "gene-mutations" or "point mutations," but also clearly recognizable "chromosome abnormalities," which have obviously resulted from chromosome breakage, loss of pieces, and shifted attachments of pieces, and the further fact that these "abnormalities" are comparable in numbers, or perhaps even more numerous than the apparent "point mutations" (MULLER and ALTENBURG 1928, 1930)? Other things being equal, would it not be simpler to assume only one kind of effect rather than two or more radically different kinds of simultaneous effects, such as inter-genic rearrangements and intra-genic changes in composition would seem to be? Is it not for this reason probable that the apparent "point mutations" are really only smaller editions of the "chromosome abnormalities"?[2] Our tests for chromosome abnormalities are only (1) the cytological test (2) the finding of changed linkage values in previously known genes, and (3) the simultaneous "mutation," loss or duplication of several linked genes. It must certainly be admitted that pieces of a chromosome might at times become lost or displaced which were so small as to answer to none of these tests.

The argument concerning the question just raised will take two different forms, according to whether or not it is proposed that the natural mutations, as well as those artificially induced by radiation, consist merely of losses, attachments, and rearrangements of portions of chromosomes. This, however, is a thesis that can scarcely be seriously maintained nowadays, especially in view of our knowledge of the linear differentiation of the chromatin into hundreds, at least, of qualitatively distinct parts (the genes), and in view also of the now well-known objections to the

[2] This position has recently been stated in considerable detail by SEREBROVSKY (1928, 1929), and by DUBININ (1929).

theory of "presence and absence" as an explanation of all[3] Mendelian differences. It seems absurd to suppose that all the different genes now existing must in ages past have arisen, *de novo*, full-fledged, in their present form, from non-genic material, and that subsequent evolution has involved merely their loss, rearrangement, or change in proportionate numbers. There is no evidence forcing genetics into any such *cul-de-sac*.

On the contrary, it might with more reason be maintained that some mutations may consist of actual new "creations" of genes from non-genic protoplasmic material. It is conceivable that such an event occurred in the origination of bar eye, since the normal, non-bar, is merely the absence of bar (STURTEVANT 1925). The fact that infra-bar acts as if recessive to bar (WRIGHT 1929b) but not to normal genes at any other locus raises difficulties in supposing that bar arose by translocation or duplication of some other locus, and hence tends to support the interpretation of a *de novo* origin. If, however, an event of such a radical nature could be proved to have occurred, we should have even stronger grounds for supposing that new kinds of genes could also arise by the mere change in composition of a preexisting gene.

If, now, it be agreed that natural mutations include real changes in the inner composition of the genes, the most obvious objection to putting the X-ray mutations into a different category in this respect is the fact that they resemble the natural ones so closely. Those which may be classed as "visibles" give no more evidence of involving two or more contiguous loci (that is, of being losses of sections of a chromosome) than do the natural visible mutations. Many of them, in fact, are sensibly identical with well-known natural mutations, not only in phaenotypic effect, but also in locus and other genetic behavior. The clustering of the mutations in the genetic map of the X-chromosome (in which alone it has been studied adequately in the case of the induced mutations) is the same both in the case of the natural and the induced mutations (MULLER 1928b, HARRIS 1929). There is a similar excess of recessives over dominants found among both cases, natural and induced. The evidence so far indicates that the natural mutations which have been found to recur most often (for example, white eye, rudimentary wing) also recur with unusual frequency after irradiation.

[3] At the same time it is reasonable to suppose that some mutations involve losses even though in any given case we cannot determine whether or not a loss has occurred. It is even quite possible that a large proportion of mutations may consist of losses, or at least of inactivations,—a view recently redefended by WRIGHT (1929a), on the basis of the recessiveness of most mutations. This is a question which remains for future investigation, and which should not be confused with the question of whether *all* mutations are losses.

Thus a study of the induced "point mutations" themselves lends absolutely no support to their being viewed as different from the natural ones. It may further be observed that the apparent point mutations produced by X-rays are surprisingly abundant in comparison with the losses and displacements of large sections of the chromatin, if they represent only the extreme lower limit of size of the latter.

Especially noteworthy in the present connection is the fact that the proportion of the "point mutations" which are lethal (or otherwise deleterious) seems to be no higher in the case of the induced than of the natural mutations. Thus, in the first experiment in which mutations were produced by X-rays, there were in all 89 "point mutations" in the X-rayed chromosome; of these 57 were lethal, 14 were to be classed as "semi-lethals" (viability from 1 to 10 percent of the normal), and the rest, 18, were "visible" mutations of greater viability. With this there may be compared a previous experiment of MULLER and ALTENBURG (1919, 1920) on natural mutations in the X chromosome. Out of a total of 18 mutations in this experiment, there were 13 lethal, 4 semi-lethal, and only one which, under special conditions, had a higher viability. Other studies both on induced and on natural mutations have given similar results. If there are among the natural mutations enough of a "progressive" kind to allow of organic evolution, and if the induced mutations do not include changes of this and allied types, but only losses, then the induced mutations as a class should be more detrimental than they have been found to be, in comparison with the natural ones.

In the case of large section changes, many, at least, of the fragments became reattached elsewhere, so that translocations and "inversions" result. When individuals containing these are bred, some offspring are produced, by recombination, which receive the displaced section in addition to two doses of the same kind of chromatin, present in its normal location. These often show phaenotypic effects of the resulting genic disproportions ("unbalance"), even when only a comparatively small piece of chromatin is involved. If, then, many of the induced "point mutations" are due to losses of small pieces of chromatin, there should also be converse cases like the above, in which the phaenotypic abnormalities were the effects of *additions* of small pieces. These additions would more usually be placed at the ends of chromosomes, as is the case with the majority of known translocations. Thus there would be an excessive number of different non-allelomorphic "mutations" found, with apparently identical loci, at the ends of the chromosomes—a state of affairs which has not been known to arise. The same kind of addition could, how-

ever, be made on different occasions at the ends of different (non-homologous) chromosomes, and sometimes at still other points ("insertion"), and so we should have the paradoxical phenomenon of allelomorphs occupying different loci, as well as the above-mentioned phenomenon of non-allelomorphs occupying identical loci.

While it is true that such additions would sometimes fail to give phaneotypic effects visibly different from the normal, even when they were homozygous, yet it must be remembered that they would arise in connection with the losses of which they formed the converse. When a genically normally proportioned individual (F_1 from the treated parent) having such a displaced section (that is, "loss" plus "addition") is bred, the effect of the loss, at least, would often be detectable in those individuals of the next generation (F_2) which received this loss without receiving the displaced section, but the latter would behave as though it were a "suppressor" of this loss, lying at another locus, and thus peculiar ratios of the mutant character would be produced in this generation (F_2), the first generation in which the "mutation" would be distinguished. Such effects have not been found in the X-ray work to date, except of course in the case of the admitted translocations and inversions of large size. Many of the experiments, however, have been done in such a way that such effects would have been detected in them, had they occurred. Hence the data on these matters corroborate those previously referred to, in indicating that most of the induced "point mutations" do not consist of losses or additions of small chromosome sections.

III. A PARTIAL SEPARATION OF THE DIFFERENT GENETIC EFFECTS OF X-RAYS

That X-rays and related radiation should produce more than one kind of effect upon chromosomes and genes is not surprising; it is rather to be expected in the light of the indiscriminate metamorphosing influence which the rays have upon matter of all sorts. In the mixed medium of protoplasm, the effect of high-frequency radiation may be compared to the rampages of a bull, not so much in a china shop as in a pastry shop. Even the purely genetic effects are rather multiple than simply dual. This will be realized better when attention is called to the influence of X-rays in causing primary non-disjunction (MOHR, MAVOR, ANDERSON) and in temporarily altering the frequency of crossing over (MAVOR, MULLER), as well as in causing point mutations and losses and displacements of chromosome parts. It would obviously be far-fetched to contend that the

induced non-disjunction is brought about by essentially the same kind of interference with the genetic structure or mechanism as is the chromosome breakage; in fact, the stages in the germ cycle at which the two phenomena are most readily induced are different. Here at least, then, there are two genetic effects of irradiation that cannot be regarded as mere quantitative or spatial variants of the same genetic phenomenon.

The argument that the induced "point mutations" are only small losses and displacements because they and the large losses and displacements are produced by a common agent (X-rays) loses still more of its force in the light of some recent experiments of one of the authors, which show that changes of these two classes are not always produced with equal relative readiness by this common agent. That is, it is possible partially to separate the production of these two effects. This finding was made as a consequence of some experiments carried on during the past year (1928–1929) to study the genetic effects of irradiation upon female germinal tissue under various conditions. The point in question becomes evident when the results here obtained are compared with those from experiments in which spermatozoa were treated.

The females used for irradiation contained the dominant sex-linked gene for Bar eyes, but were otherwise normal. They were crossed in separate cultures to males containing as "markers" the recessive genes for "scute," vermillion eyes, and forked bristles, which lie scattered along the X chromosome at convenient distances. The heterozygous F_1 females were then bred in separate half-pint bottles (records of their relationships being kept) and the male offspring (F_2) of each were carefully examined in order that lethals, visible mutations, and inherited reductions of crossover frequency—the latter being indicative of displacements of chromosome sections—might be detected. In each case, except that of the two lethal point mutations marked "(?)" in the table, it was possible, by comparison of sister cultures, to make sure that these variations were newly arisen, not derived from some generation previous to the P_1.

There were three groups of the P_1 females, distinguished by their physiological states at the time of treatment. The females of the first group (A) had been kept virgin, and in a condition of semi-starvation (by having them crowded together in a small vial, upon old, partially dried food) for a week previous to the irradiation. Those of the second group (B) had been kept virgin and well-fed. Those of the third group (C) had been allowed to mate at will, and were fed well, for the week preceding irradiation. They were all given the "t4" dose (approximately the same as the

"D5")[4], and immediately afterwards put into fresh culture bottles, with

TABLE 1

Results of irradiation of Bar females (dose "t4") (P_1 cross: $\frac{B}{B}$ ♀ × $s_c vf$ ♂; F_1 cross: $\frac{B}{s_c vf}$ ♀ × $s_c vf$ and B ♂).

	CONDITION OF P_1 FEMALES AT TIME OF IRRADIATION	TOTAL NUMBER OF FERTILE CULTURES FROM F_1 FEMALES	NUMBER OF F_1-F_2 CULTURES WITH MUTATIONS IN:							
			X DERIVED FROM P_1 FEMALE				X DERIVED FROM P_1 MALE			
			POINT MUTATIONS		SECTIONAL DISLOCATIONS (CROSSING OVER REDUCED)		POINT MUTATIONS		SECTIONAL DISLOCATIONS (CROSSING OVER REDUCED)	
			Lethal and semi-lethal	Visible	Lethal	Non-lethal	Lethal and semi-lethal	Visible	Lethal	Non-lethal
A	Starved, virgin	214	5(+2?)	0	0	0	0	0	0	0
B	Fed, impregnated	249	6	1	0	(1?)	9	0	1	1(+1?)
C	Fed, virgin	298	6	1	0	0	1	0	0	0
Total		761	16(+2?)	2	0	(1?)	10	0	1	1(+1?)

[4] The factors of dosage for the so-called "t4" dose are as follows: filter, 1 mm aluminum; peak voltage, 50 K.V.; milliamperes, 5; distance, 16 cm; duration, 48 minutes. In some cases the milliamperage was doubled (that is, made 10) and the time cut in half; often, too, the distance was shortened and the time then reduced proportionately to the square of the distance; in all such cases the total energy is the same and the treatment is designated as "t4." Treatments in the earlier experiments (1926 and most of 1927) were, however, given with a different machine from the later ones, and we have found that the "t4" treatment, involving the above factors, on the old machine, belonging to Doctor DALTON RICHARDSON, was in reality about three times as strong as the "t4" treatments involving the same factors, given later, on the new machine of the same make (Victor, with Snook transformer) acquired by our own laboratory. In the present paper, whenever the treatment was given on the old machine, it will be so stated—for example, "t4(old machine)"—and when the machine is not designated, it may be understood that the new machine was used. "t4(old machine)," which is the "t4" of the earlier papers, must therefore be understood to be the equivalent to our present "t12."

In the experiments of one of us, a somewhat different series of time factors has been used, and the resultant dosages, all given on the new machine, have been designated in terms of "D," (PATTERSON 1929b). The dosage "D5" here referred to would be the same as "t6" (new machine), and, in general, t1 = D1.08, or D1 = t0.93.

We have found recently by means of dosimeter measurements that doses given only in terms of the above factors are far from accurate. The "t1" on the new machine may represent a dosage as high as 300 *r* units or as low as 150. Hereafter, where dosage is to be accurate, *r* units must be measured at the time.

males, and allowed to remain there for a week, laying the eggs that produced the F_1 females which were tested. The results of the tests of these F_1 females are summarized briefly in table 1. The minutae of the data from this experiment are not given here, since, as will be seen, the three groups showed no significant differences from one another, and since the chief interest of the work, from the standpoint of the present paper, attaches rather to the totals. These totals, for variations of different kinds, in the X from the treated female germ cells, are to be compared with the corresponding totals from experiments in which spermatozoa were treated.

Reference to table 1 shows that, of the 18 to 20 mutations there listed in the X from the treated female germ cells, all but one doubtful one behaved as point mutations. The exception was a case which was lost, due to sterility of the offspring, before it could be determined by subsequent breeding whether the chromosome abnormality was in the X of maternal or in that of paternal origin (the latter in this case having received the treatment also).[5] Of the 11 or 12 mutations in the X from treated sperm, on the other hand, the number which contained distinct chromosome abnormalities was 2, or 3 if the doubtful case above alluded to is to be regarded as having been in the X of paternal origin. Only about a third as many of the sperm used had received treatment as of the eggs.

As the data relative to mutations in the X chromosomes from treated sperm are meagre in the above experiments, they may be supplemented by citation of an experiment in which the P_1 cross was the reciprocal of the P_1 cross in the above experiment. Here Bar males were treated, and then crossed to untreated "$C_1 B$" females. The dosage (t13+) was, however, much higher than in the other experiment. Here, too, there were physiological differences between different groups of the treated flies,—in this case the difference was in temperature, one group being kept warm, the other cold, while treated—but as the two groups gave essentially similar results, the data have been combined in table 2. It will be seen from this table that the total number of sectional changes is comparable with that of the point mutations, and that if anything like this ratio of the two classes had existed in the treated female cells of the experiment reported in table 1, the results obtained would surely have been different, despite the small total numbers o mutations there dealt with.

[5] Chromosome abnormalities do sometimes occur in the chromosomes of irradiated females, however. In group A, for example, an F_1 fly was found which proved to have a duplication on one of its third chromosomes—a piece from the middle of the genetic map of the left arm, not including the end, having become attached to the right end of an otherwise normal third chromosome.

Further corroboration of the point in question may be obtained from the data of the original X-ray experiment, obtained in 1926 (MULLER 1927, 1928b), although these would, by themselves, hardly have been extensive enough, on the female side, to be conclusive. The figures pertinent

TABLE 2

Results of irradiation of Bar males (dose "t13"). P_1 cross: $\frac{s_c vf b_b}{\text{"ClB"}}$ ♀ × B ♂; F_1 cross: $\frac{s_c vf b_b}{B}$ ♀ × $s_c vf b_b$ ♂).

	TEMPERATURE OF P_1 MALES AT TIME OF IRRADIATION	TOTAL NUMBER OF FERTILE CULTURES FROM F_1 FEMALES	NUMBER OF F_1-F_2 CULTURES WITH MUTATIONS IN:						
			X DERIVED FROM P_1 FEMALE	X DERIVED FROM P_1 MALE					
				POINT MUTATIONS			SECTIONAL DISLOCATIONS		
				Lethal	Semi-lethal	Visible	Lethal	Visible	Invisible
A	6°±2°C	36	0	5	1(visible)	3	5	1	4
B	34°±1°C	62	0	12	2 (visible)	3	6		5
Total		98	0	17	3 (visible)	6	11	1	9

to the matter at issue are given in table 3. From the treated male cells, there is approximately the same proportion of sectional to point

TABLE 3

Results of treatments given October, 1926.

X-RAY DOSE (OLD MACHINE)	SEX IN WHICH CHROMOSOME WAS TREATED	TOTAL NUMBER OF FERTILE F_1-F_2 CULTURES	NUMBER OF F_1-F_2 CULTURES WITH MUTATIONS IN X DERIVED FROM TREATED PARENT.						
			POINT MUTATIONS			SECTIONAL DISLOCATIONS			
			Lethal	Semi-lethal	Visible	Lethal	Semi-lethal	Visible	Invisible
t2	♀	216	12	4	1	1	0	0	?
t2	♂	65	6	1	1	4	0	0	?
t3	♂	72	5	1	3	3	1	1	?
t4	♂	405	38	7	12	16	2	1	5(+?)
Sum ♂	♂	542	49	9	16	23	3	2	5(+?)

changes as in table 2, when allowance is made for the fact that the "invisible" sectional changes were not specially looked for, and that hence probably only a small fraction of those occuring were detected. But, again, the chromosomes from treated females show a far smaller number of

chromosome abnormalities, in proportion to the point mutations, there being only 1 to 17, whereas those from the treated males given the same dose show 4 to 8 and those from all treated males combined show 33 sectional to 75 point changes.

Taking these three experiments together, then, it seems safe to conclude that under certain conditions not nearly so many sectional changes can be obtained by treatment with X-rays, in proportion to the point mutations simultaneously obtained, as under other conditions. The decisive conditions seem, in the present experiments, to be somehow connected with the sex of the cells undergoing treatment, though whether the connection with sex is causal or accidental is as yet somewhat uncertain. It is possible that the different average dosages given the males and females may also have played a part. But, be the basic determining conditions what they may, the important point here is that, by their means, one of these two processes may be influenced largely separately from the other. Accordingly, there must be some real difference between the mechanisms whereby the sectional and point changes occur, and, though there may also be some feature or features common to the two mechanisms, as suggested by the fact that both can be initiated by X-rays, nevertheless there remains no reason to make the specific assumption that the difference between them is purely quantitative, rather than of some other nature.

It is easy to conceive of ways in which the two processes might be related so that they would be affected in the manner found. For example, the breakage of a chromosome, as well as the change in composition of a single gene, unaccompanied by breakage, might be due in the first place to the breaking of a single chemical bond, by the displacement of an electron, followed by rearrangement of the interatomic associations. When the rearrangement occurred within a gene, in such a way as to leave it still a gene (that is, capable of multiplication) in spite of its change, and still connected with its neighbor genes, a "point mutation" would have occurred. But when the rearrangement happened to be such as to destroy the gene (that is, to leave it no longer with the power of multiplication), or to break its connections with its neighbor genes, then it might be supposed that a break in the chromosome would result, though perhaps not until after the chromonema-envelope previously existing had become used up or replaced by a new one. In some cases, the broken chromosome-ends would probably join with each other again; in some other cases, we know that they become attached to other chromosomes, or at other places on the same chromosome. Now this latter phenomenon, the process of attach-

ment, might well be subject to different influences than the original process of inter-atomic rearrangement above postulated was subject to. It might, for instance, be much more likely to occur when the chromosomes were in a particular physiological condition, or when they were packed together tightly morphologically, as they are in the sperm-head. Under such circumstances, then, displacements of chromosome sections would be especially likely to occur, though the number of "point mutations" need not be correspondingly more frequent. We have sketched here, however, but one out of numerous possible interpretations. It would not, at the present stage of our knowledge, be profitable to speculate upon the matter in more detail, particularly since certain further tests bearing upon it can be made.

IV. THE PRODUCTION OF MULTIPLE ALLELOMORPHS

In the eyes of many geneticists, the most convincing line of evidence that has been brought against the "presence and absence" theory in general has been the phenomenon of multiple allelomorphism, with its attendant features. There is now evidence indicating that this same phenomenon, in all its details, can be induced by X-rays. If so, then the same series of arguments as have been found applicable in the case of this general question (MORGAN, STURTEVANT, MULLER and BRIDGES 1915, 1923, MULLER 1919, 1920) now apply similarly against the idea of "presence and absence," or "mutation by loss alone," in the more specific case of the changes induced by X-rays. The experimental evidence concerning this matter will therefore be in place here.

The locus in Drosophila at which the greatest total number of "spontaneous" mutations, and also the greatest number of different looking "spontaneous" mutations, have been detected, is that of white eye (MULLER 1920). Of the dozen or more different mutant allelomorphs of spontaneous origin known at this locus, white has been found by far the oftenest (over a dozen times, possibly two dozen), eosin several times, and most of the others just once in all the Drosophila work to date. In the X-ray experiments, likewise, it has been this locus in which the most mutations have been discovered, and it is likewise found that most of the mutations induced at this locus have given rise to the allelomorph white. The latter has been induced by irradiation as a mutation in a germ cell on more than a dozen different occasions in our laboratory. From the combined results (see table 4), it may be calculated that it arises in something like 1 in 1000 X-chromosomes treated with our heavy "t12" (or "D13") dose, in the mature spermatozoa. And, in addition to white, several other mutant

allelomorphs at this locus have been found in our laboratory, after raying (MULLER 1928 a, c).

One of these allelomorphs is eosin, found by a graduate student, Miss CAMPBELL, in May, 1927, in an experiment directed by one of the authors to test the frequency of production of translocations. The eosin arose from the normal allelomorph, in a chromosome carrying also the mutant genes for scute, vermilion, and forked. A male (P_1) bearing these genes had been heavily X-rayed (dosage, "t4, old machine") and mated to a female having only normal genes in the X chromosome. In the male progeny (F_2) of one of the F_1 females, there was the expected count so far as the characters that were supposed to have entered the cross were concerned, but the individuals carrying scute and vermilion were of a lighter eye color. By subsequent breeding, the gene responsible for this effect was separated from scute and vermilion, and was found to produce the peculiar color of eosin, in the sexually dimorphic fashion characteristic of the latter. Its locus also was detemined to be at about 2.0, with reference to scute, and when crossed with white it gave a light eosin color, as does the familiar eosin. As there had been no eosin stock in the university except one which carried no other mutant genes, it is unreasonable to suppose that eosin could have crept into this particular combination with the expected genes, scute, vermilion, and forked, by contamination. The evidence is therefore complete that in this case eosin arose from the normal allelomorph by mutation, after irradiation of the sperm.

A second allelomorph of white, found by one of the authors in the fall of 1928, is to all appearances identical with the known allelomorph, "apricot." It arose in the experiment summarized in table 2, in a culture descended from the flies that had been kept at the colder temperature (6° C) during treatment. The F_1 female among whose progeny it was found had received from her mother an unradiated X chromosome with the genes, scute, vermilion, forked, bobbed, and from her father, a radiated X, containing Bar, in which a lethal inversion had just arisen somewhere in the right region, and the gene for apricot in the left. The male progeny, therefore, included only one non-crossover class (s_c v f b_b), the other noncrossover class (B) dying. The former non-crossovers had the expected characteristics. There was, in addition to these non-crossovers, only one crossover male, due to the reduction of crossing over caused by the inversion, and to the fact that crossover males receiving the left end of the unradiated X and the right end of the radiated X died. The crossover male which appeared was of the contrary class to this; it carried the normal allelomorph of scute (therefore the radiated left end) and the mutant genes

for vermilion, forked, and bobbed (the unradiated right end), but it had a light lemon-like eye color, indicative of the new mutant gene (apricot) in the left-hand portion of it, derived from the radiated X. As a result of crosses between this male and normal females, numerous crossover males were obtained in F_2 which carried the new mutant without the genes *v f b* $_b$, and a pure stock was derived from these, in which it was evident that both males and females had the eye-color characteristic of the known apricot. Crosses with white resulted in an eye color of intermediate shade in the daughters; this showed that the new mutant was really an allelomorph at the locus in question.

A probable third allelomorph was obtained by PATTERSON in 1929, from a cross between a radiated (D10) eosin singed male and a yellow female with attached X's and a Y chromosome. The sons from such a cross, receiving their father's X and mother's Y, would ordinarily be eosin singed, like their father. The great majority were, but one appeared very much lighter in color than the rest. Through crossing, the new gene has been separated from singed, and a pure stock of it has been obtained, in which it is evident that the females are somewhat darker than the males, as is true of eosin, from which the new gene was derived, and of another known allelomorph called ivory; the flies are consistently lighter than eosins, however, and probably lighter than ivory. The fact that heterozygotes, carrying one dose of eosin and one of the new mutant, are of intermediate color indicates that the case is one of allelomorphism rather than of modifying genes.

A probable fourth allelomorph arising in somatic tissue as a result of raying the allelomorph apricot in an embryonic stage will be referred to subsequently, in connection with the account of reverse mutations.

Nine cases of mottled eyes have also been found by the authors (MULLER 1928 a c, 1930). These are recessive to red and give intermediates when crossed with white and the other mutant allelomorphs of this locus. They vary through all the colors known in this series, and more. However, the mottleds, unlike the mutants of uniform color above described, do not behave as simple point mutations, but, without exception, involve breakages and reattachments of chromosome parts; accordingly they do not furnish material for illustrating the principles here under discussion.

Another locus in which several "point mutations," including different allelomorphs, have been induced by X-rays is that of forked bristles (*f*). The normal allelomorph of forked has mutated on three different occasions

in experiments of one of us involving irradiation of mature spermatozoa, not designed specifically for studies of the mutability of this particular locus. In the first case (spring of 1927), males having the gene for small eye, but otherwise normal, were given the t4 (old machine) dose and mated to females carrying in one chromosome the "C_1B" combination and in the other $s_c\ v\ f\ b_b$. In a section of this experiment in which the males were held for six days after treatment, before mating, 243 F_1 females inheriting the $s_c\ v\ f\ b_b$ chromosome from the mother were produced. Among the latter, one was a typical forked which, on breeding, proved to be homozygous for the same, having a new gene for forked in the irradiated chromosome, with the gene for small eye, and no evidence of any X-chromosome abnormality. Pure stock of the new forked had good viability and fertility, and exhibited the character in typical fashion.

In another experiment (fall, 1928) males containing the gene for bobbed bristles (b_b) and an inversion designated as "$\delta 49$" were given a t16 dose and crossed to yellow attached-X females. Among the 615 male offspring, one was a typical forked, which transmitted its mutant character to its offspring as a sex-linked recessive. Crosses with the old forked resulted in forked daughters; this showed the new gene to be allelomorphic to the old.

A third mutation in this locus occurred in the experiment shown in table 2 in the "warm" series. Among the 120 F_1 females tested in this series, one which had received "C_1B" from its mother and an irradiated Bar-containing X-chromosome from its father yielded sons all of which were both forked and Bar. Further study proved the new forked to be in the same locus as the old, but it was noticeable that in the new stock the forked character was not nearly as well marked as in the typical stock of the old forked. As it is unlikely that a modifier had happened to arise in just the same chromosome as the gene for forked itself, it is probable that there was here a different, and "weaker," allelomorph, "f^w," such as has been found in some of the previous Drosophila work on material not artificially irradiated. In one of the following sections of the present paper, dealing with reverse mutations, another case is recorded of the origination of a "weakly forked" allelomorph (considerably weaker than the foregoing), and likewise of other mutations at this locus, after irradiation, in experiments especially intended for the study of changes at this locus.

The occurrence of two mutations from non-scute to typical scute, subsequent to irradiation, will also be described in a following section. In addition to these mutations to scute, there has been one giving rise to

a distinctly different mutant allelomorph (shown in figure 1). This arose in an experiment in which wild-type adult males were given a t13 (D14) dose, and then mated to females having in one X chromosome the s_c C_1B s_m v t_n complex and in the other s_c v f b_b. Four hundred thirty two fertile F_1 females that had received C_1B were bred in separate vials, the progeny (F_2) being examined through the walls of the vials under the low power of the binocular. In this method of examination, devised by C. P. OLIVER, the vial is held under the binocular in a horizontal position with the stopper towards the light; the flies then congregate against the upper wall of the vial, next to the stopper, and any conspicuous visible mutations present in the males as a group can be readily detected (as well as the absence of males, indicating a lethal). Among the 432 cultures of the

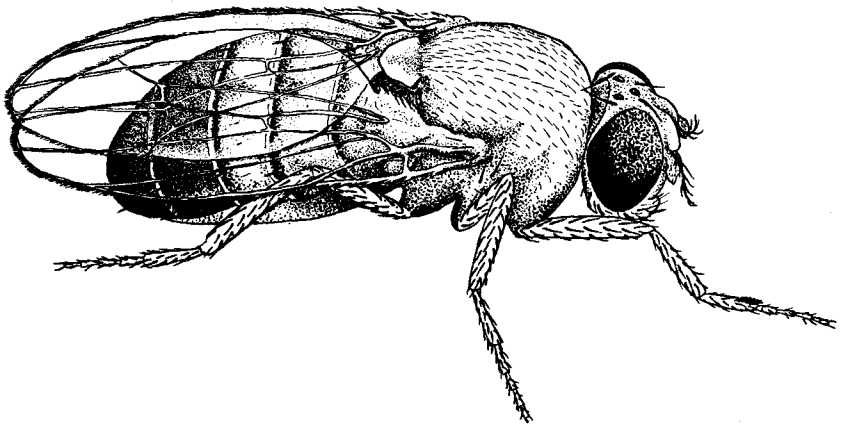

FIGURE 1.—The mutant "scutex" produced by irradiation.

type stated, one was found in which there were only a few males, and these were all of peculiar appearance, while all the females appeared to be scute. On anaesthetization and examination under higher power, it was found that the males were of a very extreme scute type, completely devoid of bristles on the dorsal surface of the thorax and scutellum. The body color also appeared to be somewhat lighter, and the wings more transparent looking than in the normal, so that the whole fly had a delicate, flimsy appearance.

The females in the above cultures, on being bred to scute (attempts to breed or keep alive the males with the new character proving unsuccessful) gave rise to offspring all of which showed either scute or the new mutant. Hence either a new allelomorph of scute had arisen, or scute and an intensifier had arisen simultaneously. The latter inherently improbable

assumption was made still less tenable by the crossover ratios, which showed the effect to be located at the left end of the X chromosome, like scute itself. The new allelomorph, which may be designated as "scutex" (s_c^x), was found to act as a semi-lethal, inasmuch as, in most cultures, fewer then a tenth of the flies expected to show it actually hatch. In combination with the old scute, the latter dominates, but *not completely:* that is, there is on the average somewhat more reduction in the number of thoracic bristles in the heterozygous s_c^x/s_c females than in the homozygous s_c/s_c. As shown in the figure, the artist found postvertical and subhumeral bristles present, although these are absent on scute flies, which have a greater tendency to produce bristles in the other positions. We have not been able to check up on this point in pure scutex, since the latter has recently been too inviable, but in compounds with scute we find the postverticals and subhumerals to be absent. If they were really present oftener in scutex than in scute it would be evident that these characters were not different merely in a quantitative way.[6] They would be related non-quantitatively in a somewhat similar manner to that first found by MULLER to hold in the case of the allelomorphs of the truncate series (vortex, oblique, dumpy, lopped, thoraxate, truncate; MULLER 1919, 1923).

Perusal of the above three cases of multiple allelomorphism, following upon irradiation, will bear out the contention that they exhibit all those characteristics which, in the case of the natural multiple allelomorphs, have been taken as evidence against the idea of mutations in general consisting of losses. For one thing (1), the allelomorphs are surprisingly frequent if they represent only the last term—the most extreme possible cases—of close linkage between genes at essentially different loci. Cases of merely very close linkage, though occasionally found, are on such a view relatively much less frequent than they should be, and the advocate of "presence and absence" is thereby forced to some such subsidiary hypothesis as the existence of intra-chromosomal groupings or "nests" of genes.

Secondly (2), the different mutant allelomorphs in any one of the three series affect the same general character (eye color, bristle conformation, or bristle distribution, as the case may be). There is no known or apparent

[6] Since the above finding was made on scutex, the authors' attention has been called to a certainly non-quantitative, and much more extensive series of allelomorphs of scute, arising after irradiation, in experiments of SEREBROVSKY, DUBININ and their colleagues (SEREBROVSKY, DUBININ, AGOL, SLEPKOV AND ALTSHULER 1928, DUBININ 1929). The reports on these, however, were not, at date of writing, available to us.

reason why this should be true if the different allelomorphs in a series are simply losses of different completely linked (neighboring) genes, but it is readily understandable if they are different changes in one gene, that has become specialized to react chiefly, so far as visible characters are concerned, in the development of this particular character.

Thirdly (3), whenever individuals with different mutant allelomorphs are crossed, instead of the normal type becoming reconstructed phaenotypically in F_1, as it is when individuals with recessive non-allelomorphic genes are crossed, the F_1 shows the characteristics common to the mutants that were crossed, and, in any respect in which they are different, and both abnormal, it is no nearer to the normal than the allelomorph that is more normal in this respect. To explain this non-appearance of the normal in F_1 on the "presence and absence" hypothesis (of different completely linked genes that become lost), requires the special assumption of the linked genes observing a precedence in regard to the order in which they are lost. On the presence and absence view, the less extreme allelomorph is considered to lose a gene, which may be designated as "*A*"; then the more extreme one, in order, on crossing with the other, to yield a hybrid showing at least as much variation from normal as shown by the less extreme allelomorph, must likewise lack *A*, but it must lack something else, "*B*," in addition, which distinguishes it from the less extreme allelomorph. Thus, *A* can be lost by itself, but *B* is never lost unless *A*, which has the precedence, is lost with it. This assumption must be carried to considerable lengths in the case of the series of X-ray allelomorphs of white, where apricot must be considered to have lost *A*, eosin to have lost *B* and *A*, the lighter mutant from eosin above mentioned to have lost *C*, *B*, and *A*, and white to have lost *D*, *C*, *B*, and *A* simultaneously (or many more, if the allelomorphs of spontaneous origin also are taken into account). On the other hand, the assumption of no such hierarchy of genes is necessary on the view that the allelomorphs are merely different changes in one gene, which alter its reactivity to various extents, and in various ways. On this view the usual lack of reconstitution of the normal type, in crosses between two mutant allelomorphs, ceases to be paradoxical.

To meet such objections, the believer in "presence and absence" would now have to resort to some additional postulates, such as that the completely linked genes formed a cluster of identical "elements" or parts, any one or more of which could be lost, and the number of which determined the degree of expression of the character. Among the numerous difficulties that would arise for such a view only two need be mentioned here. One is the difficulty that would be met with in accounting for the

consistent dominance of the normal allelomorph in these cases. (Apparent normals should arise, which did not have a sufficient number of elements to dominate.) A far more serious obstacle is encountered in the fact that, in the case of the white and probably the scute series, the members do not form a purely quantitative series at all. Apricot, unlike eosin and the lighter variant from eosin, is alike in color in male and female; scute, though having far less extreme bristle reduction than scutex in most respects, nevertheless probably lacks certain bristles more often appearing in the latter. If there has been loss, then, the parts lost have been somehow different from one another, and yet these various parts were obviously related to one another in their functioning, in a much more intimate way than that in which genes in different loci are ordinarily related. The readiest method of explaining their peculiarly intimate relationships is to assume that they were chemically united. But we cannot tear off a piece of a molecule without healing the broken bond, either by a rearrangement of the remainder, or, as is far commoner, by the addition (substitution) of something else, large or small, in the place made vacant. In either case, the idea of a pure loss becomes vitiated. In truth, there is no theoretical reason left for assuming that a loss would be the exclusive method of change of the gene.

In connection with these allelomorphic series, it is pertinent to put the question, "Were the different allelomorphs all really produced by the irradiation, or were only the commoner (usually the most extreme) allelomorphs so produced, and the others of 'spontaneous' origin in each case?" While this question cannot be given an absolute answer, it can be met in terms of strong probabilities. Thus, in the case of the white-locus series, in all the experiments on treated mature spermatozoa combined there was a total of somewhat less[7] than 22,366 flies that would have served for the detection of white or one of its allelomorphs (table 4). Seven thousand two hundred fifteen of these were F_1 males derived from treated males crossed by attached-X females, and 15,151 were F_1 females (derived from mothers with separate X's) which were bred in individual cultures, and the male progeny of which were examined. In this total there were 10 or 11 that carried white, and 1 that carried an allelomorph (apricot) as a heritable germinal mutation, and two males that carried white "fractionally" in some of the somatic and not in the germinal tissue.

The "fractional" white mutations that were confined to the soma should probably be counted at a value of at least $\frac{1}{2}$ each. It is likely that there were parallel cases in which white occurred in the germinal tissue but in

[7] Lethal-bearing flies are to be subtracted from the total.

which the male was not bred because there were no somatic indications of the mutation; the latter cases would, however, be less frequent than the former, since at least one of the two eyes is usually derived from the same nucleus of the two-cell stage as is the germinal tissue. The use of the factor $\frac{1}{2}$ will therefore serve to keep our results on the side of caution. Taking 13, accordingly, as the total number of whites, we find that white arose about once in 1800 sperm cells and a different allelomorph about once in twenty-three thousand sperm cells (in round numbers), in this work. In all the previous Drosophila work on non-radiated material, up to 1925, it has been estimated that upwards of twenty million flies have been examined (MORGAN, BRIDGES and STURTEVANT 1925). Nearly half of these must have been males in which white or one of its allelomorphs would have been pretty sure to be detected (and something like one one-hundredth must have been females subjected to the progeny test and similarly serviceable). Among the 10,000,000 thus available, white has been observed to originate only about two dozen times, at the most, and all other allelomorphs of white, combined, not much over one and a half dozen times. This makes a frequency for white of about one in four hundred thousand times, and, for the other allelomorphs, of about one in six hundred thousand times. In the total of less than 23,000 in the series of radiation experiments above referred to, there was, therefore, about 1 chance in 17 that white should have appeared at all, and 1 in approximately $(17)^{13}$ that it should have appeared as often as 13 times, unless there had been some peculiarity in the conditions of the experiment responsible for producing it. In like manner, the chances that one of the other allelomorphs, such as apricot, should have appeared once, would have been only 1 in 26 if the material had not been somehow made especially mutable.

It can readily be calculated from the above figures that, in all, the frequency of appearance of mutations at the white locus in the irradiated sperm was of the order of magnitude of 200 times the corresponding frequency in non-radiated material. If, now, we weight the flies in all the experiments according to the dosage of radiation used upon the sperm,—which is legitimate, in view of the direct proportionality between dosage and mutation rate found by HANSON and HEYS and by OLIVER—we find that there would have been 1 detectable mutation of the normal allelomorph of white to some mutant allelomorph or other among about 1,000 sperm treated with the heavy "t12" (or D13, or "t4, old machine") dose. The items in this calculation are shown in table 4. This result is to be compared with 1 in about 350,000 in the non-radiated material. It will be seen that the former frequency is about 350 times the latter—a

TABLE 4

Summary of results with regard to loci of s_c, w, and f, from treated sperm (except for experiments recorded in tables 9 and following).

1.	2.	3.	4.	5a	5b	6.	7.	8.	9.	10.
DESIGNATION	WORKER	DATE OF EXPERIMENT	METHOD OF DETECTION*	NATURE OF CROSS‡ (TREATED GENES IN HEAVY TYPES) ♀ PARENTS	♂ PARENTS	NO. OF FLIES TESTED OR INSPECTED	DOSAGE (IN "t")	FACTOR OF X-RAY MACHINE (OLD = 3; NEW = 1)	"UNITS TESTED" (PRODUCT OF 6×7×8)	MUTANTS AT ABOVE LOCI
"first"	M	fall, 1926	F_2	$(F_1)\frac{\mathbf{b}_b}{s_c vf}$	$(F_1)\frac{s_c vf}{Y}$	25 65 72 405	1 2 3 4	3 3 3 3	75 390 648 4,860	 1*w*
"aged sperm"	M	spring, 1927	"	$(F_1)\frac{s_y}{s_c C_1 Bv}$	$(F_1)\frac{s_c vf}{Y}$	619 567	4 2	3 3	7,428 3,402	1*f*
"fractional"	M	spring, 1927	F_1♂	$(P_1)\frac{yy}{Y}$	$(P_1)\frac{\mathbf{b}_b}{\mathbf{Y}}$	1,150 1,490	4 2	3 3	13,800 7,940	$\frac{1}{2}w$ 1*w*
"stability"	M	fall, 1927	"	$(P_1)\frac{yy}{Y}\frac{Cy}{}$	$(P_1)\frac{+}{\mathbf{Y}}\frac{\text{"Sv"}}{+}$	2,080	4	3	24,960	1*w* $\frac{1}{2}w$
"δ49 loci"	M	fall, 1928	"	$(P_1)\frac{yy}{Y}$	$(P_1)\frac{\boldsymbol{\delta 49}}{\mathbf{Y}}$	615	16	1	9,840	1*f*
"δ49 loci" No. 2	O	fall, 1928	F_1♂	$(P_1)\frac{yy}{Y}$	$(P_1)\frac{\boldsymbol{\delta 49}}{\mathbf{Y}}$	376 1,504	16 1	1 1	6,016 1,504	1*w* (died)

TABLE 4 (*continued*)

"translocation"	M	winter, 1927–spring, 1929	F_2	back cross for F_1 markers in II and III	282	9.3^x . .	1^x	2,616	
"temperature"	M	fall, 1928	"	$(F_1)\frac{\mathbf{B}}{s_c v f b_b}$ $(F_1)\frac{s_c v f b_b}{Y}$	187	12	1	2,244	$1w^{az};1f^w$ (from (from cold) warm)
"sex"	M	spring, 1929	"	$(F_1)\frac{\mathbf{b_b}}{s_c C_l B v}$ $(F_1)\frac{s_c v f b_b}{Y}$	402	8	1	3,216	
"inversion"	M	spring, 1929	"	$(F_1)\frac{\mathbf{\delta 49B+}}{s_c C_l B v}$ $(F_1)\frac{s_c v f b_b}{Y}$	856	14	1	11,984	
"aged sperm"	M	spring, 1927	F_1 ♀	(for loci of s_c and f only) $(P_1)\frac{s_c v f b_b}{s_c C_l B v}$ $(P_1)\frac{\mathbf{sy}}{\mathbf{Y}}$	$\begin{cases}619\\567\end{cases}$	$\begin{matrix}4\\2\end{matrix}$	$\begin{matrix}3\\3\end{matrix}$	$\begin{matrix}7,428\\3,402\end{matrix}$	
"metabolism"	M	winter, 1928–1929	F_2	(for locus of w only) $(F_1)\frac{\mathbf{s_c v f b_b}}{B}$ $(F_1)\frac{s_c v f b_b}{Y}$ and $\frac{B}{Y}$	260	4	1	1,040	

TABLE 4 (*continued*)

	2.	3.	4.	5a 5b	6.	7.	8.	9.	10
DESIGNATION	WORKER	DATE OF EXPERIMENT	METHOD OF DETECTION*	NATURE OF CROSS‡ (TREATED GENES IN HEAVY TYPES) ♀ PARENTS | ♂ PARENTS	NO. OF FLIES TESTED OR INSPECTED	DOSAGE (IN "t")	FACTOR OF X-RAY MACHINE (OLD = 3; NEW = 1)	"UNITS TESTED" (PRODUCT OF 6×7×8)	MUTANTS AT ABOVE LOCI
"dosage"	O	winter, 1928–1929	"	(for locus of *w* only) (F$_1$) $\frac{+}{s_c C_l B v}$ | (F$_1$) $\frac{s_c v f b_b}{Y}$	8,444	3^x	1	24,958	5*w*
"aging of males"	H	winter, 1928–1929	"	(for locus of *w* only) (F$_1$) $\frac{b_b\dagger}{s_c C_l B v}$ | (F$_1$) $\frac{s_c v f b_b}{Y}$	2,967	8	1	23,736	3*w*
Total for *w*	M+O +H				22,366	(6.6^x · · · ·	· · ·1^x)	150,657	$13w+1w^{az}$
Total for s_c and *f*	M+O				11,881	(9.0^x · ·	· · ·1^x)	111,753	$2f+1f^w+0s_c$

* F_1♂ = inspection of F_1 males; F_1♀ = inspection of F_1 females; F_2 = testing of F_1 females by inspection of F_2 males.

‡ Where detection is by inspection of F_1, composition of P_1 is given; where F_1 are tested, their composition is given. Symbols are as follows:

Y = *Y* chromosome.

yy = double X chromosome, homozygous for yellow body color.

+ = normal chromosome.

† From early broods of P_1 males.

^{x} = average

Controls to above experiments have totalled over 10,000 flies tested, without a single *w*, s_c, or *f* having arisen.

factor even greater than that expressing the increase in the frequency of lethals at the same dosage. (There is, however, more chance for errors in detection to affect the figures for white than those for lethals in untreated material). There can, therefore, be little doubt that the radiation was responsible for these mutations of the white locus.

Similar calculations can be made with respect to the mutations in the loci of forked and of scute. The results here will not be as accurate as in the case of the white locus because of the fact that these characters, being somewhat less conspicuous than those of the white series, are more apt to have been overlooked, especially in the non-radiated material. Nevertheless, the chances of detection of both forked and scute are distinctly good; once an investigator has worked with them, he is not likely to overlook them. In all our work on the progeny of radiated sperm combined, including that reported in the subsequent sections of this paper, there have been 29,402 F_1 flies in which a mutant gene for scute (received from treated sperm) would probably have been detected, and 5961 F_1 females in which, by progeny tests, scute would probably have been found. In the summarized Drosophila work to 1925, scute was reported 4 times among the 10,000,000 males examined, and scutex not at all, among some 200,000 females whose male progeny were examined. These results would have given a chance of only 1 in about 71 of finding one scute mutation, or 1 in about 5000 of finding two of them, in a series of experiments of the magnitude of the above irradiation experiments, while the chance of finding scutex in these experiments would be less than 1 in 33, and would really be too small to be reckonable from the data. In all, the rate of mutation to scute found in sperm given a t12 (or t4, old machine) dose can be figured to have been about 250 times the rate in untreated cells.

The case of forked will be considered in greater detail later, when additional data more specifically concerned with mutations at this locus will be presented. Aside from these subsequent data, however, the previous work on the progeny of irradiated sperm have considerable significance. There were in this work in the neighborhood of 11,881 flies from treated sperm, in which a gene for forked would have been detectable. (The dosages were such that these would have been equivalent to 8,911 at the "t12" or "D13" dose.) It was among these that the two mutations to forked and one to "weakly forked" were found. Now, in the summarized Drosophila work, there were approximately 9 mutations to forked (or to some one of the 4 known kinds of forked allelomorphs) observed among the approximately ten million flies available for such a discovery; this makes the chance of finding one in a series of experiments of the total

magnitude of the above irradiation experiments only 1 in 90, and the chance of finding two, one in 8,100. While the weaker allelomorphs are much more likely to be missed, still it can be seen that the odds must be greatly against finding one of these in our 11,881 flies either, unless some special influence were producing them. In all, when the figures are corrected to appear as of the t12 dose, the mutation frequency to some allelomorph of forked found in the treated is over 300 times that found in the untreated individuals.

It is of course realized that in the case of this as of the other loci (white and scute) the mutations detected and reported in the untreated material represent only a fraction of those which occurred, since a special attempt was not made in most experiments to find the mutations in question, and since, even when they did occur, they were often ignored on account of the possibility of their having resulted from contamination. This probably accounts for the observed frequencies for the t12 dose being several hundred instead of about one hundred times those reported in the summarized untreated material (in view of the fact that the lethal frequency is raised only about one hundred- to one hundred fiftyfold). Nevertheless the reported frequencies of visibles in the untreated material are doubtless of the right order of magnitude at any rate, and so long as this is true, the conclusions reached from the above calculations would still hold, so high are the probabilities there arrived at.

To sum up, then, if we take all the three loci into consideration at once, it is quite evident that irradiation is really the agent which has brought about the production of the multiple allelomorphs, and, since these induced multiple allelomorphs display the same series of characteristics (with regard to phaenotypic expression, dominance relations, etc.) as do the multiple allelomorphs of spontaneous origin, all the arguments against "presence and absence" and "mutation by loss" which can be based upon the induced allelomorphs must have the same validity as they have admittedly had in the past in the general theory of heredity and variation, when they were based upon spontaneous multiple allelomorphs. It is likewise apparent that the notion of "addition" of genetic material (supposing small pieces to have become torn out of other regions of the chromatin and attached at the loci in question) is even less capable of explaining the peculiarities of the results that have been discussed above, than is the notion of mutations by loss alone. Such small-scale displacement, supposing it were possible, could not help but result in a heterogeneous collection of dissimilar mutations at a given locus showing no characteristics of multiple allelomorphs except the inability to recombine by crossing over.

V. AN INDUCED MUTATION VISIBLY DIFFERENT FROM A KNOWN LOSS OF THE SAME LOCUS

In October, 1927, 84 fertile males carrying a normal X chromosome (and a mutant combination including Star eye in one of their second chromosomes) were given the t4 dose (old machine) and, after being kept in isolation for 16 days, mated in individual cultures to yellow attached-X females heterozygous for Curly wing. After 4 days, the parents were transferred to a second set of culture vessels (called "brood 2") and, after another 7 days,

FIGURE 2.—Dominant "eyeless" (in fourth chromosome) produced by irradiation. Below is given pair of legs with sex combs of normal size, for comparison with the enlarged ones of the eyeless fly.

to a third set ("brood 3"). Altogether, 2,080 F_1 males were carefully examined for mutations, and 437 F_1 females (namely, all those of brood 1). This count of males is shown on row 4, table 4. In the second brood, among the 18 F_1 males appearing in the last culture (No. 84), there was one non-Star, non-Curly male which had one eye misshapen as if furrowed, and the other eye normal. The 11 males in brood 1 from the same parent were all normal. When the aberrant male was bred back to yellow attached-X females, it was found that the variation was transmitted as an autosomal dominant, to the daughters as well as to the sons, appearing in approximately half the members of both sexes. It usually affected both eyes rather strongly, causing a change in size and shape rather similar to that seen in the dominant second chromosome mutants "Lobe" and "Lobe,[2]" in the dominant third chromosome mutant Deformed, and in the recessive fourth chromosome mutant "eyeless." Besides the eye abnormality, it produced, in the male, a hypertrophy of the sex combs, the latter being about doubled in size. Examples of the variation are shown in figure 2. Sometimes even more abnormal types are produced in this stock, the head becoming split or otherwise malformed, and developing peculiar protuberances and multiple antennae as is sometimes the case with the recessive eyeless.

Backcrosses of the males inheriting the abnormal eyes from one parent, and black body color (in chromosome II) from the other, to black females, then showed that these two genes underwent independent segregation, and that therefore the new mutant could not be in the second chromosome. Similiar crosses were thereupon made involving the new mutant and peach eye, which is in chromosome III, and it was found that the mutant was likewise independent of this chromosome in its inheritance. Accordingly, it was crossed to bent wings, a recessive lying in the tiny chromosome of the fourth pair, and the F_1 females that showed the eye abnormality were backcrossed to bent males. The count of 2202 flies consisted of two major classes: bent, normal eyes, and non-bent, abnormal eyes. There was also a considerable number (219) of apparent normals, which were to be expected owing to the overlapping of the normal type by both the bent and the abnormal eye classes; a number of these normals were tested and all these fell genotypically into one or the other of the two major categories above named. On the other hand, there were no recombinations showing both the bent wings and the abnormal eyes. It was therefore clear that this gene was in the fourth chromosome, and that its locus showed complete or nearly complete linkage with that of bent (no bent-eyeless crossover combinations to 1248 bent non-eyeless and 735 non-bent eyeless).

As it was now highly probable that the new gene was a dominant allelomorph of the known recessive "eyeless," it was crossed to the latter. The F_1 flies containing the two genes in combination proved to have, on the average, a greater reduction in the size of the eye than was characteristic of either the homozygous recessive eyeless or the heterozygous new mutant, although they were seldom completely eyeless. It will then be assumed that the two genes are allelomorphic, and the new mutant will be designated as "Dominant eyeless" ($e_y{}^D$).

As all Dominant eyeless individuals tested proved to be heterozygous, even though both parents had carried the gene in question, it appeared likely that it acted as a lethal when in the homozygous conditions. Crosses were made between it and the dominant minute bristle (M_{IV}), located in the same chromosome, which also is lethal when homozygous. The F_1 flies having the heterozygous combination $e_y{}^D/M_{IV}$ were next crossed to each other. This resulted in F_2 all resembling their F_1 parents. In other words, a balanced lethal stock had been established, and there could be no doubt that homozygous $e_y{}^D$ was lethal. The combination of the two characters results in individuals that tend to be somewhat abnormal in bodily shape (broad and squat) but with no evidence of the rotated-abdomen condition which M_{IV} produces when combined with the recessive mutant of that name with which it is allelomorphic. The $e_y{}^D$-M_{IV} combination flies have a low fertility, but, once numerous individuals are obtained, the stock can, with care, be perpetuated indefinitely.

Crosses of $e_y{}^D$ to bent and shaven show that it is allelomorphic to neither of the latter. Thus, of the four loci known in the tiny fourth chromosome—namely, that of bent, of shaven, of the allelomorphs M_{IV} and rotated abdomen (TCHETVERIKOFF), and of eyeless—$e_y{}^D$ shows allelomorphism to eyeless only. Hence it is unlikely that it involves a "deficiency" of a chromosome region, or any disturbance other than ordinary "point mutation."

A digression may be made at this point to call attention to the bearing of the above results on some previously published genetic maps of the fourth chromosome. In these (for example, in MORGAN'S *Theory of the Gene*, 1928, p. 23) the known loci of chromosome IV are shown separated by distinct intervals, covering in all nearly one unit, and reflecting the idea that several tenths of a percent of crossing over occurs between the loci. Examination of the experiments on which these conclusions were based shows, however, that the few apparent crossovers there observed may really have been caused in some cases by the phaenotypic overlapping of the genotypic classes present, and in other cases by non-disjunction,

instead of by crossing over. In the present experiment, where such errors were guarded against, no evidence of any crossing over appeared, either between $e_y{}^D$ and bent or between $e_y{}^D$ and M_{IV}, and the conclusion may thus be drawn that crossing over probably does not occur between the tiny fourth chromosomes, and that a valid genetic map of their contained genes cannot be made by the crossover method hitherto employed.[8]

The significance of the findings concerning $e_y{}^D$ for the present work lies however in a different direction: namely, in the contrast which appears between them and the already published findings of BRIDGES concerning the effect of a total loss of one of the fourth chromosomes. The "haplo-IV" individuals in which, through some mitotic abnormality occurring in a previous generation, one of the fourth chromosomes is completely missing, have been studied by BRIDGES in considerable detail, and their composition has been conclusively demonstrated both cytologically and genetically. Phaenotypically, such individuals show various abnormalities, as would be expected owing to the abnormal proportion existing in them between the number of genes of each kind in chromosome IV and the number in the other chomosomes. The most conspicuous abnormality is the reduction of size of the bristles; less striking are slight changes in wing shape and body build. But no reduction whatever in size of the eyes, or unevenness in their contour, is discernible, nor are the sex combs enlarged. The known loss of all the genes in one of the two fourth chromosomes, including the loss of the normal gene at the locus of $e_y{}^D$, thus produces no such effect as is found when the latter normal gene in one of these fourth chromosomes "mutates" to $e_y{}^D$ (the other fourth chromosome in each case remaining normal). Does not this indicate that the mutation of the normal allelomorph to $e_y{}^D$ is not a loss?[9]

There is only one possible objection to the conclusion above suggested, and though it does not seem a very plausible one it cannot be definitely

[8] Since the above was written, we have noted a reference to a somewhat similar test made by BRIDGES with M_{IV}, with similar results (see MORGAN, STURTEVANT and BRIDGES 1926).

[9] By a curious coincidence, the recessive eyeless gene has sometimes been especially pointed out as a good example of a mutation resembling in its effects, and possibly consisting of, a loss. The conclusion was based on the fact that certain haplo-IV flies, carrying eyeless (*ey*) in their only fourth chromosome, were found, which seemed of a more extreme eyeless type, it being reasoned that since the loss of one fourth chromosome seemed to increase the "eyelessness," the gene for eyeless itself might be of the nature of a loss. This explanation disregarded the fact that the haplo-IV condition, even in the presence of all normal genes, produces various phaenotypic abnormalities, and so it would be not surprising if it affected the variable eye size of "eyeless" flies in one direction or the other. The evidence of the above text, indicating that not even the more extreme allelomorph, ey^D, is probably a loss, now makes it likely at the same time that *ey* is not a loss either, but rather the product of another kind of change in the inner composition of the gene.

laid aside. That is, finding the effect of a loss of the whole of the fourth chromosome is not a perfect test of what effect the loss of a single gene in the chromosome might have, for the loss of the other genes at the same time might somehow exactly compensate for the effect of the loss of the particular one in question. That would be equivalent to saying that the effect (the so-called "eyeless" condition) was really due to the disproportion ("unbalance") of gene-quantities arising between the normal gene (E_y) at the locus of e_y^D and the other genes in the fourth chromosomes, when one E_y gene was lost, rather than to the genic disproportion then arising between the number of E_y genes and of genes in the other autosomes: that is, the intra-fourth-chromosomal genic disproportion in this case would have to be much more important than the inter-chromosomal genic disproportion. Since, however, the other autosomes probably contain at least a hundred times as many genes as the fourth chromosomes, the chance of the intra- rather than the inter-chromosomal disproportion being the source of the eyeless condition would, other things being equal, probably be less than one in a hundred.

It is true that, in the case of the X-chromosome, we have found the effect of inter-chromosomal disproportion to be relatively small, as compared with that of intra-chromosomal disproportion, but the reason for this is evident in the history of the X-chromosome, since the rest of the genetic complex has had to become adjusted to the presence of the X in either one "dose" or two without giving rise to abnormal phaenotypic manifestations. There could not very well have been such a process at work in the case of the fourth chromosome (unless it be supposed that part of the fourth chromosome has been derived from the X by a translocation). Hence it seems on the face of it far-fetched to ascribe the observed phaenotypic effect of "e_y^D" to a disproportion involving the relatively few genes in the fourth chromosome itself, rather than to that involving the hundred or so times as many genes in the other autosomes.

If, however, the genes directly adjacent to a given locus commonly exert an important effect on the expression of the gene at that locus, through some reaction dependent on their contiguity (as suggested by STURTEVANT'S work on Bar eye, 1925) then this would be a factor tending to make the effect of intra- as compared with inter-chromosomal disproportion much greater than it otherwise would be. On such a hypothesis the "eyeless" character could be formally explained, as the result of a loss at the "E_y" locus, with a consequent effect on the mode of manifestation of the immediately neighboring genes, and the loss of the whole chromosome, by removing these latter genes also, would then fail to pro-

duce such an effect. All this, of course, is a very "special" hypothesis, having little known factual background, but since there is at present no way of disproving its possibility, or even of readily evaluating its plausibility, it must be admitted that the case of dominant eyeless does not, in itself, furnish final proof of an induced "point mutation" not involving the loss of a gene. It should also be pointed out that since this mutation has been observed only once (though a number of similar looking cases were not tested out), the evidence for its having been induced by the treatment remains incomplete.

VI. FIRST ATTEMPTS TO INDUCE MUTATIONS IN BOTH OF TWO OPPOSITE DIRECTIONS

In addition to the above lines of rather indirect evidence converging from various angles, it was felt that it would be desirable to have some really clear-cut experimental evidence which would be capable of proving more definitely and directly, and with as little theorizing as possible, our contention that the induced mutations did not all consist of mere breakdowns, losses, or displacements of the genes. The difficulty usually encountered in arriving at any such evidence lies in the fact that the outward character can in itself give no clue concerning the nature of the gene which is responsible for the effect seen. The absence of a somatic structure does not imply the absence of a gene, nor does recessiveness necessarily imply absence, and dominance, presence, as was once claimed. Fortunately, however, there is a rather simple short-cut attack possible on our present problem, not necessitating the acquisition of any further knowledge concerning the complicated chemical processes whereby a gene attains its phaenotypic expression. The method in question consists in determining whether or not mutations can be induced in both of two opposite directions. The bearing of the occurrence of reversible mutations on the general problem of mutation by loss was discussed by MORGAN in 1913 and again by MULLER in 1921 (MULLER 1923). SAFIR (1920), MORGAN, BRIDGES and STURTEVANT (1925), and especially TIMOFEEFF-RESSOVSKY (1925, 1928) have since given us additional examples of its application, in the case of "spontaneous" mutations. It is only necessary for us here to apply this same idea to our present more specific problem of mutations induced by irradiation.

For the purposes of this method it may be granted in advance that in any given instance we can never determine whether or not the induced mutation under consideration consists of a loss, partial or complete, of a gene. Even if it be admitted to be a loss, if we can then take the resultant

mutant, and, by treating it, cause it to mutate back again to the original form, we have, in effecting the latter step, caused a change opposite to the loss, which must therefore have been a gain of some kind. Contrariwise, for all we know, it may have been the second step, the reverse mutation, which was the loss, but in that case, by the same reasoning, the original mutation must have been a gain. It is also possible, and, in the authors' opinion, more likely that neither of the two opposite reactions were losses, but that both involved substitutions or rearrangements of parts, that were reversible in character, after the manner of many chemical alterations. Certainly it is difficult to conceive of either of the mutations as a complete loss, since if it were it would scarcely be expected that at another time the same gene as that which previously was present at that locus would somehow become suddenly recreated. But, no matter which of these interpretations were really correct, it would none-the-less be clear that *both* of the opposite mutations could not be losses, and so the demonstration that both were really induced by the irradiation would settle the major question at issue, namely, that mutations which were not of the nature of losses could be induced. While a positive result would thus lead to a positive conclusion it must be borne in mind that a negative result (lack of success in being able to induce both opposite mutations) would not prove the negative conclusion, that the mutations which occurred were necessarily losses.

In a first attack on this question, it was thought desirable to be able to study the possibility of mutation in respect to a number of genes in each individual examined, so that the chance of finding some gene or genes that would respond in both directions might be increased. The so-called "IIIpl" stock was used for this purpose. This contains the following genes, all located in the third chromosome, in the order given: r_u(roughoid eye), h(hairy), s_t(scarlet eye), p(pink eye), s_s(spineless), e(ebony). These six genes are all sufficiently independent of one another in their expression that a change from any one of them to its normal allelomorph would be readily noticeable in a culture of IIIpl flies, and a change from the normal to any of these mutant characters would be readily noticeable in a culture of normals. In order to be able to detect the changes in both directions, it was necessary to treat both opposite types of flies—the non-IIIpl's[10] and the IIIpl's. Treated adult males were used, as these can be given a stronger dose than the females or immature individuals without becoming sterilized; they were given the heavy t4 (old machine) dose on October 26, 1927. The treated IIIpl males were immediately

[10] The males used here which contained the normal allelomorphs of IIIpl contained the dominant Curly wing in one of their second chromosomes.

crossed to untreated IIIpl females, and the progeny were examined carefully for flies showing any of the normal allelomorphs of the characters concerned. Any of these normal allelomorphs would manifest themselves if they were present, since they are all dominant to the recessive allelomorphs that would be received from the untreated parent. The treated "non-IIIpl" males were likewise crossed to (untreated) IIIpl females, because the mutations of the normal genes from these males would be expected to produce recessives that would be able to manifest themselves only if recessive mutant allelomorphs were received from the female parent as well.

In all, there were 2,318 offspring from the cross of IIIpl by IIIpl. Only two of these aroused any suspicion that they might contain a mutation of one of the genes in question to or towards its normal allelomorph. One was a female that looked as if it might possibly contain a non-pink gene, but which proved, on testing, to be germinally a pure IIIpl, and the other was a male whose eyes appeared somewhat non-roughoid, but which proved to be sterile. There were various other mutations, most notable among which was a Notch-wing female which proved to carry the "mottled-1" eversporting allelomorph of white, combined with a translocation, that has been described elsewhere (MULLER 1928c, 1930).

The cross of treated non-IIIpl Curly males by IIIpl females yielded 2,170 offspring. Among these there was one Curly scarlet female which, when crossed again to IIIpl, transmitted the scarlet character. Later tests proved the new scarlet to be non-lethal but in close proximity to a lethal. Another Curly female showed the spineless character, and transmitted it; the new spineless was allelomorphic to the old, and, like it, to aristopedia; but it was a lethal. A spineless-appearing male failed to transmit his variation, and another male, part of whose body was similar to spineless, failed to leave offspring. There were five flies having eyes of a somewhat rough appearance; of these, four were fertile and none of them proved to contain in their treated chromosome a mutation to roughoid. Three of them contained, instead, genes resembling Star eye, and of the latter, one was tested sufficiently to show that it was really at the locus of Star (in chromosome II) that the mutation had occurred; the fourth, in which only one eye suggested roughoid, the other being normal, transmitted no visible variation but proved to have a translocation involving chromosomes II and III. There were, in addition, other visible variations observed among the offspring of the treated non-IIIpl males, but none involving the characters of IIIpl. Altogether, then, there were two certain mutations (or losses?) involving the loci in question—one scarlet and one spineless.

It might also be mentioned that 376 offspring were examined from a cross of similarly treated Curly but otherwise normal males by females homozygous for a_r p_x s_p in chromosome II and t_h s_r e_s r_o c_a in chromosome III. In this cross, no mutations in the loci in question were observed, except for one possible arc winged (a_r) female that was sterile.

Since no "reverse mutations"—from the mutant to the normal type—had been observed in the above experiments, the point at issue still remained unproved. The work was then temporarily discontinued, to allow of certain other experiments, it being planned to continue such tests later, using other loci. The work had shown that some of the characters of IIIpl had certain disadvantages in detection, hairy and roughoid both requiring considerable care, and roughoid and spineless being easily confused with non-allelomorphic dominant variations that occurred rather frequently. Since then, TIMOFEEFF-RESSOVSKY (1929 c) in independent experiments has used the same method successfully (see p. 568).

In the winter of 1927-1928, Doctor F. B. HANSON examined in our laboratory a considerable number of progeny from irradiated *y w f B* B_x males crossed to untreated females containing attached X's, with a view to the discovery of possible reverse mutations. It may be recalled here that they showed 4 reverse mutations of *B*(Bar-eye) to non-*B*, and no reversals in the other loci, in a count of 4,662 male offspring (866 from a t8 and the rest from a t4 raying) (HANSON 1928). These results were of considerable interest in connection with STURTEVANT'S work on reversals of Bar (STURTEVANT 1925). Unfortunately, from the standpoint of the question at issue in our present paper, the opposite change, non-*B* to *B*, has not been observed as yet in any irradiation experiments, so that the question of loss versus other change still remained undecided. It seemed evident that very large counts were needed if positive results were to be obtained.

As a third step in the attack, one of the authors (MULLER), in the spring of 1928, undertook to irradiate the larval stages of white-eyed flies in order to see if any that hatched showed pigmented ommatidia. It had previously been shown (PATTERSON 1928) that when the larvae of red-eyed flies are irradiated, gene-mutations to white occur in some of the cells destined to form ommatidia, so that individual white facets or groups of them are found in the adult eye (the number in the group depending upon the stage of cellular subdivision of the eye anlage at which the treatment occurred). Thus, for the purpose of our present problem, it was only necessary to prove that the change from white to or towards red could be induced likewise. Such proof assumes that in a stock of white the appearance of pigment (which could of course not be subjected to the breeding test)

would necessarily be due to a mutation at the locus of white, but this would be highly probable since of all the numerous eye color mutations known at other loci in Drosophila, none have been found to cause any production of pigment when the gene for white was concomitantly present.

If large counts were the desideratum, there was a distinct advantage in using the present method, even though only one locus was under observation. The advantage lay in the fact that each group of facets derived from a single cell present in the eye at the time of treatment would show mutations independently of every other such group of facets. If the treatment was given at such a late stage that nearly every cell then present in the optic rudiment represented a separate ommatidium, and yet early enough for the effect still to be producible in most of the ommatidia, the majority of the facets would be independent in their mutations of the other facets, and would manifest their mutations independently. This optimal stage, as previous work had shown, was when the larva was between 3 and 4 days old (after the egg had been laid and kept at a temperature of 27° C).

Since there are on the average about 850 ommatidia in each eye, the examination of both eyes of one fly should therefore reveal the number of mutations to white occurring in something like 1700 separate elements—a result as significant as if so many separate flies had been tested for germinal mutations. In the previous work on mutations from red to white, it had been found by PATTERSON that there was about one such mutation in 10,000 elements (containing one X chromosome), when an average dose of approximately t4 was applied to somatic cells. This is equivalent to 1 in 3,300 for the t12 dose. Considering the different conditions of the experiment this is not so very different from the figure, 1 mutation to white in 1,000 with the t12 dose which we have seen was found for germinal mutations induced in sperm cells. If now there were a frequency of reverse mutation from white to red in the larval somatic cells similar to that from red to white (1 in 10,000 for t4) then there should be an average of about 1 red ommatidium observed in every 3 male flies examined, when the larvae had been treated with a t8 dose (the one used). The female flies from treated larvae of homozygous white stock should show red facets twice as often as this, since there are two X chromosomes in each cell of the female, the gene for white in either of which could mutate to red and, as a dominant, manifest itself independently of the other. Thus there would be something like two-thirds as many red facets found as flies observed, in the case of females, and in the total population there would be about half as many red facets as flies. (If some of the cells at this stage still represented

groups of facets, or if some were at too late a stage for the production of an observable effect, the number of cases of red facets observable would be correspondingly lowered, but probably to not less than half the above number, in view of existing data concerning the stage in question.)

The stock of white used first was one designated as *wvf*, in which the white had itself been produced by X-rays, being the mutation shown in in line 4, table 4. The object of using such a white was to test out whether the white of X-ray origin was itself reversible, for it was conceivable that this particular kind of white might be a loss and really different from whites of spontaneous origin. Vermilion (*v*) which was in this stock could not interfere seriously with the detection of a mutation in the white locus, while forked (*f*) was useful as a check against contamination.

A large number of flies of this stock were allowed to lay their eggs, during 24 hours, on a flat circular slab of banana-karo-yeast-agar of the same diameter as the field under the X-ray machine. Ninety-six hours after the parents had been placed on this food (and 72 after they had been removed) the slab was taken from the incubator at 27° C where it had been kept, and subjected to the t8 dose.[11] When the progeny had hatched both eyes of every fly were carefully examined under the high power of the binocular in the search for red or reddish ommatidia. Four hundred fifty-one were studied in this way, consisting of 232 females and 219 males. Multiplying the females by 2 in order to allow for their two X's and then multiplying the figures for both females and males by 1700 (the approximate number of facets per fly) we see that a total of about 1,160,000 X-chromosomes (or at least half that number, in view of the qualifications in the last paragraph but one) were here studied for the mutation of white toward red. Among them a few cases were found of isolated discolored (grayish or reddish) facets which, when studied under the high power of the compound microscope, proved to be due, not to red or yellow pigmentation of the cells of the ommatidium which normally are colored, but to an opacity of the lens forming the surface of the facet; these then were not representative of the phenomenon which was being sought for. There was in addition one male that showed a group of four discolored facets which appeared to be of just the same type as that which has just been described, but which was lost before the examination under the high power was completed. There were no other cases that even suggested a reverse mutation.

If reverse mutation had been as frequent as the red-to-white mutation

[11] In another publication (PATTERSON 1929b) the dosage in this experiment was reported as "D5" instead of t8 and the age as 64–72 instead of 72–96 hours.

has been found to be, from one to two hundred colored ommatidia would have been found. Thus, in spite of the comparatively small total number of individual flies observed, the multiplicity of ommatidia allowed us to be certain that the frequency of this reverse mutation, if it could occur at all, must be far lower—something like a hundredth as great, at most—than that of the mutation in the direction red towards white. This was somewhat surprising in view of the fact that there is a spontaneous mutation of the kind in question already on record—namely, a mutation of white (itself of spontaneous origin) to eosin, found by MORGAN (MORGAN, STURTEVANT, MULLER and BRIDGES 1915, 1923). Cases were also on record of individual red facets arising spontaneously in stocks of white eyed flies (SPENCER 1926). SAFIR in 1920 reported red arising from eosin. It seemed however, that for our present purposes still larger numbers or a still different technique would be desirable.

Subsequent to the above experiment, considerable numbers of flies carrying white or some mutant allelomorph of white—tinged, eosin, apricot—have been treated by PATTERSON at one or another pre-pupal stage, and examined after emergence. In all, among 1040 white, 245 tinged, 2424 eosin, and 501 apricot flies from experiments suitable for the present purpose, treated with doses of D4 to D10 at various stages of their larval life, there was just one case in which a darker color appeared on a lighter background. This was in an apricot male and will be referred to later. The darker color in question was not as dark as the normal red. It will be recalled that apricot or an allelomorph very similar to it has itself arisen from red in an irradiation experiment reported in an earlier section.

As will be seen on page 568, TIMOFEEF-RESSOVSKY, independently using the method of treating white and eosin flies in larval stages, has obtained a case of red facets in an otherwise white eye, another case of reddish (but not red) facets in a white eye, and one case of red facets in an eosin eye.

VII. REVERSE MUTATIONS AT THE LOCUS OF SCUTE.

In the section on multiple allelomorphs, evidence has been presented to show that the normal gene at the locus of scute can be caused to mutate to scute by means of X-rays. Before this evidence was obtained, results had been secured in an experiment having a different primary object, which indicated strongly that the opposite change also could be induced, namely, from scute back to the normal, non-scute (MULLER 1928 a, c).

The object of the experiment had been to ascertain whether induced mutations occurred in only one or in both members of a pair of allelo-

morphs present in a treated cell. In order to discover this, it was necessary that both members should be transmitted together to the next generation, and then tested; in other words, they had to undergo non-disjunction. A stock was therefore used which was known to have a strong tendency towards non-disjunction. The females of this stock contained in one of their X chromosomes the combination $s_c\ C_l\ B\ s_m\ v\ t$ (the order of the genes beyond scute is normally the reverse of this) and in the other X chromosome the genes $s_c\ v\ f\ b_b$. C_l indicates a lethal inversion; hence the abnormal gene arrangement in the chromosome containing it. Since the presence of this must prevent a complete point for point apposition between the X chromosome containing it and the other X, having the normal gene arrangement, there is a tendency to imperfect pairing and to resultant non-disjunction. It was therefore not difficult, in outcrosses of such females, to find some cases of primary non-disjunction in which both X's had been received from the mother and a Y from the father. These F_1 non-disjunctional females would then, on account of the presence of the Y, exhibit secondary non-disjunction; the latter would be particularly high in frequency because of the non-matching of the X's and the presence of b_b heterozygously.

A single Y-containing female of the composition just described was used to start the experiment proper. It was treated with the t2 (old machine) dose on October 26, 1927, and immediately crossed to $y^2 b_b$ males, being transferred through two cultures. From the count of progeny, given in table 5, line 1, it can be calculated that the two X's and the Y segregated at random from each other, with no preference to any particular kind of pairing and disjunction. It is also evident from the results that there was no lethal in the $s_c\ v\ f\ b_b$ chromosome of the mother, since the males bearing this chromosome are viable.

Forty F_1 non-disjunctional females from the above cultures, which necessarily had received both X's from their mother, were then tested for sex-linked lethal and other mutations, those which were certainly virgins being mated by $y^2 b_b$ males (the parental cross thus being repeated) and those of doubtful virginity by S/C_y males, whose dominant genes would make their progeny recognizable. In all 40 cases "regular" (not non-disjunctional) sons appeared, bearing the $s_c\ v\ f\ b_b$ chromosome; hence no new lethal had arisen in this chromosome. Nor did any newly arisen visible mutations make their appearance. The work was therefore carried further. Six of the above 40 F_1 females had themselves been irradiated with a t2 treatment from the new machine. Their progeny would therefore serve as well for testing as would that of the original female used

TABLE 5

Progeny of non-disjunctional females having (except for newly arisen mutations) the composition $\frac{s_c ClBs_m vt}{s_c v f b_b / Y}$ *crossed by* $\frac{y^2 b_b}{Y}$ *or* $\frac{S}{CY}$ *males.*

COUNT NO.	MOTHER OF FEMALE	DESIGNATION OF FEMALE	PECULIARITY OF FEMALE	COMPOSITION OF MALE USED	NUMBERS OF OFFSPRING					
					FEMALE				MALE	
					Regular		Non-disjunctional		Regular	Non-disjunctional
					+ and b_b	B	$(s_c)^* vB$	triple-X	$(s_c)^* vf$	y^2(or +)
1.	Primary non-disjunctional	Original P_1 ♀	Irradiated	$y^2 b_b$	41	56	45	0	41	53
2.	Original P_1 ♀	F_1 ♀ *a*	"	"	31	42 ($1b_b$)	21	0	25	26
3.	"	" *b*	"	"	34 (1A)	25	19‡ (1A)	1	22	29 (1A)
4.	"	" *c*	"	"	10	5 (1A)	2	0	9	5
5.	"	" *d*	"	"	44	30	15	0	38	25
6.	"	" *e*	"	"	56	65 (1M)	48	3	41 (1A)	52
7.	"	" *f*	"	"	27	17 (1A)	15	0	20	15 (2A)

TABLE 5 (*continued*)

8.	F_1 ♀ *a*(br.2)	F_2 ♀ *a*	s_c-reversal (somatically nearly non-s_c)	$\frac{S}{C_y}$ —†	16 (1M)	19	19 (all non-s_c)	0	20 (all non-s_c)	29
9.	F_1 ♀ *b*(br.1)	" *b*	s_c-reversal 2 (somatically s_c)	y^2b_b	4	1	6 (one non-s_c)	0	5	7
10.	F_1 ♀ *e*(br.2)	" *c*	lethal 1	$\frac{S}{C_y}$	16	24	35	0	0	38
11.	F_1 ♀ *d*(br.2)	" *d*	lethal 2	"	4	4	8	0	0	4
12.	F_2 ♀ *d*	F_3 ♀ *a*,*b*,&*c*	lethal 2		21	26	30	0	0	30

* Scute except where non-scute is indicated.

‡ Numbers in parenthesis refer to aberrant forms included in count above parenthesis; A, phaenotypically abnormal in some way; M, minute bristles.

† Many offspring lost from this culture after examination but before counting.

(except for the unintentionally smaller dosage of irradiation used). To show that they themselves carried no new mutant gene, the counts from each of the six (which had been crossed to y^2b_b) are given in table 4, lines 2 to 7. (As in all F_1 from irradiated parents, various isolated abnormalities appeared; most of them are simply marked "A" in this table.) It will be seen that the results are similar to those from the first female. Sixty-four of the non-disjunctional females ("F_2") from these latter cultures were then again tested as before (virgins crossed to y^2b_b; others to S/Cy). It was among these cultures that the mutations of interest were found.

TABLE 6

Tests of lethals recorded in table 5. Cross: $\frac{s_c vf b_b}{Y}$ ♀ × + ♂ (l_1 *between* v *and* f; l_2 *between* f *and* b_b).

COUNT NO.	DESIGNATION OF MUTATION	NUMBER OF OFFSPRING								
		Females	Males							$(n-d)$ +
		(all)	s_cvf	s_c	vf	s_cv	f	s_cf	v	
1	lethal 1	51	0	11	2	4	0	0	0	8
2	"	40	1	2	0	2	0	0	2	8
3	"	49	0	6	2	4	0	0	0	14
4	"	52	0	10	2	4	0	0	0	12
Sum	"	192	1	29	6	14	0	0	2	42
5	lethal 2	44	0	13	0	1	0	2	1	7
6	"	86	0	8	0	1	3	0	1	15
Sum	"	130	0	21	0	2	3	2	2	22

There were, among these cultures, two cases of lethals in the s_cvfb_b chromosomes. These are shown in lines 10 to 12, table 5. They must have been newly arisen, since in the mothers, counts from which are given in ines 4 and 5, respectively, they were not yet present. The tests given in table 6 show that they were different from one another, the first found being located about 6 units to the right of forked, and the second about half way between vermilion and forked. These lethals could not have originated simultaneously in the C_1B chromosome also, for in that case the non-disjunctional females of lines 10 and 11, table 4 would have been homozygous for their respective lethals, and would not have lived to maturity. There was thus no escape from the conclusion that these mutations had occurred in only one of the two homologous chromosomes present in the cell at the time of treatment, and the original objective of

the experiment was attained. In addition, however, to this result, which is aside from the theme of the present section, there was another finding, or pair of findings, quite unexpected and surprising at the time, which is of importance to us here.

The counts of the two cultures in which these findings were made are shown in lines 8 and 9, table 5. In both cases a reverse mutation from scute to non-scute had occurred. It is quite evident, moreover, that the two cases represent independent mutations, since in the first one (line 8) the non-scute is inherited in the non-Bar chromosome, and thus appears in the males, whereas in the second, the results shown in line 9, when coupled with later tests (table 7, cross III), show that the non-scute is in the C_1B chromosome. (Later tests on the C_1B chromosome of the first case proved that scute was still contained in it.)

The original (F_2) mutant female of the first case was still present with the F_3 in the culture of line 8, and was examined to see why she had not been recorded as a non-scute. It was found that she was in reality non-scute, phaenotypically, except in that she lacked one bristle on the scutellum and might hence have passed as a "minus" (towards normal) variant of scute. The mother of the culture of line 9 was also found, and she was seen to be a typical scute; this agreed with the fact that most of her Bar offspring were likewise scute. The latter case thus provided an illustration of the fractional effect (the first found from treated female cells): the C_1B chromosome in the treated cell was evidently split already at the time when the treatment was applied, so that part of the body, including much of the epidermis and germinal tissue, came to have an unmutated C_1B chromosome, and the rest received the newly mutated C_1B chromosome. This result, by showing how late in the germ cell history the mutation had occurred, also gave further proof that the two reverse mutations must have been independent of one another.

Although we have in the above account referred to these mutations as "reverse mutations from scute to non-scute," the data so far given do not preclude the possibility that one or both of them had occurred in some other locus than that of the familiar gene for scute. The mutant genes might, in other words, have been dominant "suppressors" of scute, non-allelomorphic to the latter, instead of simply dominant normal allelomorphs of scute. Their method of inheritance showed them to be sex-linked, but the data did not yet show in what locus they lay. If they were "suppressors," the chromosomes containing them still contained the original gene for scute at its usual locus, but contained, in addition, the suppressor (an abnormal gene not present in wild type flies) at some other locus. To

decide between these two possibilities, it was necessary to study the linkage relations of the new non-scute.

In order to test these linkage relations, a cross was made between stock of the first non-scute (S_c^{x1}) and flies from a stock of yellow scute (ys_c). The heterozygous females, containing in one X the combination of "non-scute" and vfb_b and in the other X,ys_c, were back crossed to ys_c males. The count of 357 flies is given in table 7, "cross I." If the non-scute chromosome had really contained s_c and a suppressor, then the females tested here would have been homozygous for s_c but heterozygous for the suppressor, and the count of scutes versus non-scutes would have reflected entirely the distribution of this suppressor; its linkage relations (locus) would therefore be disclosed directly by the crossover ratios. The table shows that scute here is linked completely (so far as these numbers can show) with the locus of yellow, just as scute ordinarily is. The lack of crossing over with yellow is, however, not due to any abnormal reduction of crossing over between the X chromosomes, since *y*, *v*, and *f* cross over in quite normal fashion. It must accordingly be inferred that the mutation, if not in the locus of scute itself, was so close to it that no crossing over between these loci occurred in a count of this size.

A second test of the first scute reversal is recorded in table 7, "cross II." This test was essentially similar to the first, except in that the true normal allelomorph of scute was present in the chromosome with yellow, instead of scute itself. Since the locus of this gene is, in all ordinary crosses, inseparably linked to that of yellow it would not be expected that any of the yellow offspring from this cross could show scute, no matter what sort of a mutant had been present in the non-yellow chromosome. The non-yellows were therefore not counted. The yellows were as useful for our purpose as the flies in "cross I." They would have contained s_c if the suppressor hypothesis had been correct, and those of them would have shown the scute character in which this suppressor had crossed over from s_c, leaving the latter to manifest itself. The count of 251 (yellows) again failed to show any crossovers between the new mutant gene and the locus of s_c, and the hypothesis of non-allelomorphism was thus made exceedingly improbable.

The determination of the locus concerned in the second case of apparent reversal presented the difficulty that the mutated gene was located in the C_1B chromosome, which, owing to the inversion in its right hand region, undergoes very little crossing over with other chromosomes. There is, however, very rarely a single-crossover near the left end of the chromosome and also an occasional double-crossover in regions further to the right.

As the small sample count in table 7, case III, shows, only non-crossovers are ordinarily obtained. In the attempt to obtain a crossover of the desired composition, over a thousand flies from crosses of this type were examined, without exact counts being made. None were found of the composition

TABLE 7

Tests of scute-reversals recorded in table 5.

Cross I: $\frac{S_c^{x1} v f b_b}{y s_c}$ ♀ × $y s_c$ ♂.

FEMALES		MALES								TOTAL
		CROSSOVERS IN REGIONS DESIGNATED BY NUMBERS								
+	ys_c	0	0	1	1	2	2	1,2	1,2	
		vf	ys_c	+	$ys_c vf$	*v*	$ys_c f$	*f*	$ys_c v$	
110	99	35	45	19	18	19	7	2	3	357

Cross II: $\frac{S_c^{x1} v f b_b}{y^2 b_b}$ ♀ × $y\ s_c$ ♂.

Numbers of non-yellow offspring (yellows not counted but all observed to be non-s_c.)

FEMALES	MALES				TOTAL
	CROSSOVERS IN REGIONS DESIGNATED BY NUMBERS				
	0	1	2	1,2	
+	*vf*	+	*v*	*f*	
148	47	38	8	10	251

Cross III: $\frac{S_c^{x2} C_1 B s_m v t}{s_c v f b_b}$ ♀ × $s_c v f b_b$ ♂.

NUMBERS OF OFFSPRING			
FEMALES		MALES	
$s_c v\ b_b$	vB	$s_c vf$	OTHERS
10	36	28	0

especially desired, containing the non-scute (or scute suppressor) without the C_1B, which would have enabled extensive crossover tests of the locus involved to be carried out in later crosses. Nevertheless, two females of the contrary class were found, which contained in the chromosome with C_1B the gene for scute and no dominant non-scute. Tests showed that t(tan) and s_m(small wing) were still present in this chromosome, so that

the result had not been produced by double crossing over but rather by single crossing over occurring near the left end of the chromosome. This proved that the dominant non-scute which had passed across must be located in this left hand region, that is, very close in the genetic map to the locus of scute itself. Taken in connection with the more extensive evidence from the other case, it thus became highly probable that a real reverse mutation had occurred here too.

Were these reverse mutations of scute really due to the X-rays? There had been no controls to this particular experiment, but scute has been bred extensively, both in homozygous form and also in crosses in which no crossing over from other markers (non-yellow; sex) can occur; if anything like the rate of mutation indicated in the present experiment were common without treatment, numerous such reversals should have been found in this previous work. For the two cases here discovered to have arisen in a total of only 104 F_1 flies tested was a surprising result for a single gene, even in an X-ray experiment. However, in all the previous experiments not involving X-rays, there have been a few positive cases of reverse mutation of scute observed, and while it seemed highly unlikely that two such spontaneous cases would ever be found in a count as small as a hundred, nevertheless apparent "runs" or "epidemics" of certain spontaneous mutations—maroon and purple (BRIDGES 1918, 1919), yellow (PATTERSON unpublished)—have at times been encountered (see, too, BAUR'S finding of "premutation" in Antirrhinum) (BAUR 1926). In view of this source of uncertainty, and of the fact that, when the present reverse mutations were found, no induced mutations in the opposite direction—non-scute to scute—had yet been observed, in spite of a rather considerable body of data, it was decided to continue the search for opposite mutations, using by preference some other character than scute.

VIII. A REVERSE MUTATION AT THE LOCUS OF FORKED

Several months later, in the course of another experiment which was being undertaken for a different purpose, another finding pertinent to our present problem was unexpectedly made. This, in turn, furnished a clue suggesting a profitable direction for further research on the subject, which, when followed up, finally led to the obtaining of really convincing evidence of the type desired.

The experiment which served as the starting point was primarily concerned with the securing of mutations in a special type of X-chromosome known, on account of the number of the culture in which it originated, as the "delta 49" chromosome. This chromosome contains an induced non-

lethal inversion in its middle region, and it was desired to secure mutations in it in order that the sequence of its loci might be subjected to study. To avoid the fractional effect which is produced when spermatozoa are treated, whereby many progeny are found which are somatically mutant

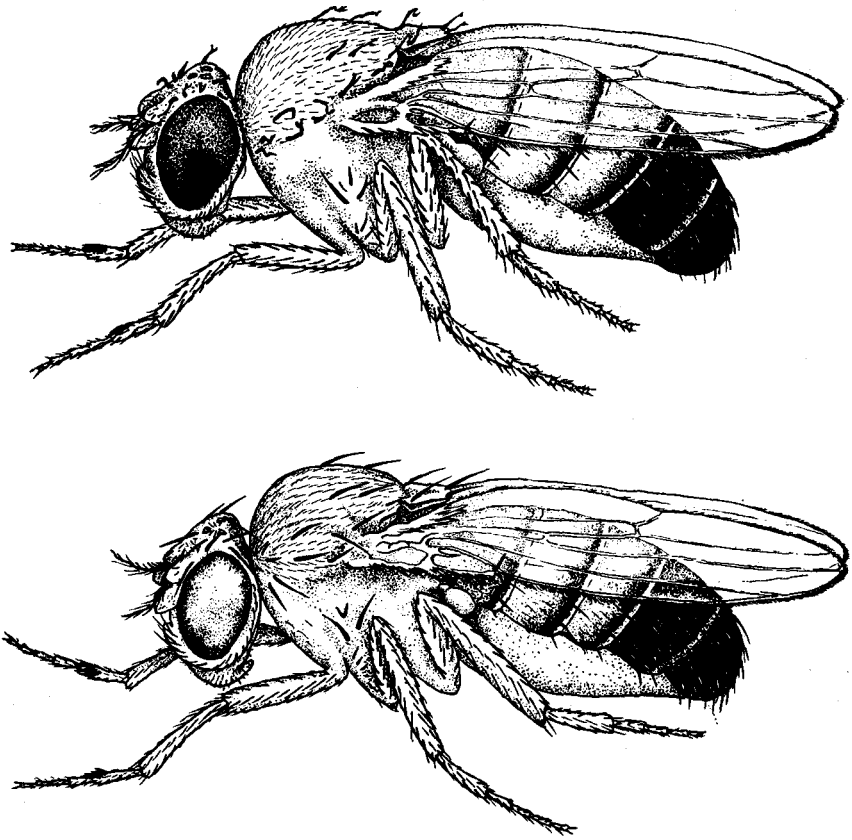

FIGURE 3. Above, forked-bristle fly with normal eye. Below, normal-bristle fly with "spectacled" eye, arising (after irradiation) from a race like that shown above. The normal bristle represents a *reverse* mutation of the mutant gene for forked to non-forked bristle. This is a case of double mutation.

but fail to carry the mutant gene in their germ cells, it was decided to treat larval stages. In flies treated previous to the maturation division, the sorting out of mutant and non-mutant genes into different cells should be completed by the time the stage of the spermatozoon is reached. Accordingly, flies of the $\delta 49f$ stock (containing *f*, forked, to the right of the inverted region) were given the t8 treatment 3 to 4 days after the eggs from which they were derived had been laid (they were kept at 27° C until treated). The male imagos which resulted were then separately crossed in 71

individual bottles to yellow attached-X females. After a week, these parents were transferred to a second brood (culture) and left there for another week.

In this cross, it was to be expected that the sons would show any abnormalities due to mutant genes in the δ 49 *f* X-chromosome, since they received this from their father and their Y from their mother. The daughters, not containing the treated X, might be disregarded. From previous crosses of this kind, in which, however, the father had been treated when adult, it was expected that a fairly high frequency of transmissible mutations (one-half to one percent) would be detected in the sons. In all, 2651 male progeny were examined, consisting of 1520 from the first brood and 1131 from the second. Among these there were several dozen with some kind of abnormality, of "nature" or "nurture." Most of these abnormal males were sterile; among the rest, the abnormality was usually slight or traumatic in apparent origin, and proved not to be inherited. In just two male flies was it possible to demonstrate any transmissible variation. One of these variants exhibited a slight disarrangement of the ommatidia, which was transmitted as an autosomal dominant. The other variant, which appeared in a culture different from the first, containing, in addition to it, 32 yellow non-variant females and 31 forked non-variant males, offered a considerable surprise. For it was a variant not in one respect alone, but in two separate and conspicuous respects. For one thing, it had eyes of a peculiar color and morphology, which we have termed "spectacled." Secondly, the same fly, unlike all the other 2650 males, was non-forked. Figure 3 shows above a fly of the forked type used in this experiment, and below, a spectacled non-forked fly of the stock derived by the double mutation from the above forked.

The doubly mutant individual, having lost its "marker," forked, was at once open to the suspicion of having arisen through contamination of the culture. This seemed highly unlikely, since there was no stock in the laboratory having eyes of the peculiar "spectacled" type, but as a double visible mutation in an experiment where so very few mutations at all were being found seemed unlikely also, it was important to reach a sure decision on this question. This was possible because of the inversion which had been present in the "δ 49" chromosome and which served as an invisible marker. The fly in question proved fertile, fortunately, and was crossed to females containing *s$_c$vf* in order that a test of the crossover properties of its X-chromosome might be made. Counts of the male progeny of the F_1 females heterozygous for *s$_c$vf* and for spectacled then showed no crossing over except for a very small amount of single crossing over near the right

end (between *v* and *f*), yielding the expected forked and scute vermilion flies. This result is characteristic of the behavior of the "δ 49" X chromosome when in combination with an X of normal configuration.

To get more specific evidence that the gene-rearrangement in the chromosome containing spectacled was the same as that in the "δ 49" chromosome, spectacled males were then crossed to "δ 49" forked females, and the resulting F_1 females were bred. The F_2 flies in this instance, unlike those in the previous cross, were found to contain a high percent of crossovers between spectacled and forked, of both contrary classes, spectacled forked and wild-type. This showed that the chromosomes of the two parental cultures "matched," that is, had their genes rearranged in an identical fashion. Now, the only δ 49-containing stock in the laboratory, besides the δ 49 forked stock, was one containing bobbed instead of forked. Homozygous females of the spectacled race proved, however, to be non-bobbed, so that the mutant chromosome was not derived from the δ 49 bobbed race by contamination, and the evidence was complete that it had originated from the δ 49 forked race through a double visible mutation.

Crosses with the previously known mutant, "lozenge eye," and with stocks containing other genes allelomorphic to lozenge then showed spectacled to be an allelomorph of lozenge, though, curiously enough, the combination, lozenge-spectacled, proved much more normal looking in our tests than either pure lozenge or pure spectacled was. Homozygous spectacled females were found to be extremely infertile, and yet, unlike most lozenge females, some of them produced a few offspring. In view of its allelomorphism, the symbol for spectacled may be taken as l_z^s.

It was of greater interest for our present purpose to study the genetics of the mutation that had abolished the forked character. Here, as in the case of the scute reversals, there was the possibility that a non-allelomorphic dominant sex-linked suppressor of forked had arisen, rather than a mutation in the locus of forked back to the original normal allelomorph. The test of the question was made by crossing the spectacled δ 49 non-forked flies to flies of the δ 49 bobbed stock. In this cross, F_1 females were produced both of whose X-chromosomes contained the δ 49 rearrangement, and in which, therefore, crossing over between the X's could occur freely. If the bobbed-containing chromosome really carried forked itself, and, in another locus, a mutant dominant suppressor of forked, then by crossing over between these two loci, a chromosome containing forked without the suppressor would be produced. From a backcross of such females, offspring which received such a crossover chromosome from their mother and a Y

chromosome or an X containing forked from their father would thus show the forked character. But if no forked offspring appeared, it would have to be concluded that the "suppressor" could not cross over with the forked locus, that is, that it was none other than the normal allelomorph of forked itself.

The heterozygous F_1 females were therefore backcrossed to forked males. These males were also provided with the gene for tinged eyes (w^t, an allelomorph of white) in order that forked sons produced by non-disjunction might be recognized by this marker and not confused with

TABLE 8

Test of locus of non-forked reverse mutation 1, originating with spectacled in chromosome carrying the δ49 inversion.

$$F_1 \text{ Cross: } \frac{\text{"}\delta 49\text{"}\ l_z{}^s\ \text{"}F\text{"}\ f?}{\text{"}\delta 49\text{"}\qquad b_b} \ \varphi \times w^t f\, \sigma^{\prime}$$

where "F" = the non-forked mutation. (Problem: is f present along with "F" or has F replaced f, being allelomorphic to it? If f is present, crossovers between F and f, showing forked character, will be produced.)

COUNT OF PROGENY (F_2) OF F_1 CROSS

FEMALES		MALES			
		REGULAR			NON-DISJUNCTIONAL
WILD-TYPE	FORKED	WILD-TYPE	SPECTACLED	FORKED	TINGED FORKED
528	0	273	199	0	4

forked due to crossing over. The counts were continued until 1000 of the F_2, exclusive of the non-disjunctional exceptions, had been counted. These counts are shown in table 8. It will be seen from inspection of this table that among these thousand "regular" offspring not a single fly showed the forked character. There could be no doubt, then, that a true reversal from forked to non-forked had occurred.

Granted, now, that the mutation had occurred at this particular locus, it still remained to be proved that it had been caused by the irradiation, that is, that its appearance in this radiation experiment was not a mere coincidence. It was difficult to believe that it was a coincidence in view of the fact that no such cases had been encountered by us previously in work with non-radiated forked. The further fact that it had arisen in conjunction with spectacled was suggestive of a microcataclysm, such as an electron passage, but the nearly complete absence of mutations in the other flies of the experiment seemed almost to "prove too much,"[12] and to suggest

[12] The relative scarcity of mutations was evidently connected with the fact that larval stages had been treated instead of the mature spermatozoa, an inference since substantiated by other

that possibly the X-rays had for some reason been ineffective this time. In view of that complication, and of the uniqueness of the case and of the lack of really critical determinations of the spontaneous reversal frequency of forked, it was evidently not at all a foregone conclusion that the radiation had really been the causative agent. What was needed, before the evidence could be regarded as unassailable, was large enough numbers to be sure of the effect, checked by equally abundant and critical controls in which the results were found to form a decisive contrast to those of the treated series. The above experiment did not meet these requirements, as it had been planned with a different object in view and the result in question had been only incidental to it.

A special series of experiments was therefore finally carried out in the months of January to June, 1929, on a scale of sufficient magnitude to allow the determination of the frequency of induced mutations from the mutant to the normal form. In the planning of this work the suggestions afforded by the preceding results were utilized.

IX. PROOF OF THE INDUCTION OF REVERSE MUTATIONS OF FORKED BY X-RAYS

a. Plan of the work.

It was decided that in these new experiments the forked character should be one of those used, since the above work had given us reason to believe that it might be caused to mutate back to non-forked, and since there was even better evidence that the opposite change could be induced (from non-forked to forked). Besides forked, the presence of at least one other mutant character was desirable, so that the latter could serve as a "marker" for the forked, and *vice versa*, in guarding against contamination. Moreover, such a plan would give opportunity to compare the mutation rates at two different loci. Tinged eye, a very light colored allelomorph of white, recessive to the normal red, was chosen for this purpose. It was already known that mutations from red to or towards white could be produced, and that mutations from white to or towards red

tests, although the larval stages are by no means completely insensitive to the irradiation effect. It is still a question whether the scarcity of mutations from germ cells treated when immature is really due to their insensitivity to the X-ray effect or to the non-mutated immature germ cells multiplying at a higher rate than the mutated ones (HARRIS 1929a).

It may also be noted in passing that the coincidence of two of the three heritable mutations having occurred in one individual, out of the 2561, is so great as to make it likely that mutations tend to appear in groups rather than independently of one another. Other cases of induced double mutations also have been found, apparently with greater frequency than would be expected on the basis of a random distribution of induced mutations.

were much less frequent, at best. Possibly, then, this locus would give us different results from the locus of forked. The presence of a certain amount of color in the case of the tinged allelomorph might, however, conceivably provide a somewhat better basis for mutations towards red than existed in the quite colorless white that had previously been tried. In order that the tinged might be used with the forked, a combination stock, tinged forked, had to be made up. We are indebted to Mr. C. P. OLIVER for making up this combination for us.

After it had been determined that reverse mutations could be produced, it was then decided to try to induce mutations back again to the previous condition.

During the course of this series of experiments on mutation in opposite directions 159,070 flies were reared and examined. The experimental lots included 91,405 individuals, derived from 3977 tested flies treated at larval or adult stages. The control lots included 67,665 individuals, derived from 2330 tested, untreated flies.

b. Reverse mutations from treated larval stages

In the first experiments of this series the larval stages were chosen for treatment. This was done because, in this way, the "fractional effect" which follows irradiation of the adult and prevents detection of some germinal mutations could be avoided, and also because the previous reverse mutation of forked had been found in progeny of treated larvae.

The method employed for handling larval stages was the same as that used in other work of the authors, and as it has already been described in detail (PATTERSON 1929b), a brief account will suffice here. About ten pairs of tinged forked flies were placed in one by four inch shell vials containing food on a cardboard spoon. The females readily deposited their eggs on the surface of the food. The spoon was exchanged for a fresh one twice a day, or every twelve hours. The bit of food containing the eggs was removed from the spoon and transferred to a small stender dish, also containing food, and fitted with a gauze cover. The stender dishes were placed in an insect box with tight fitting lid and kept in an incubator run at 27° C until such time as it was desired to give the X-ray treatment. After the culture was irradiated, the food containing the treated larvae was transferred to the half-pint culture bottle and the flies allowed to complete their development at room temperature. The controls for all such experiments were handled in exactly the same manner, except that the X-ray treatments were omitted.

The only exception to this method is the case of about two hundred

culture bottles handled in the month of May. While the work with these cultures was in progress, the outside temperature reached a point slightly above that at which the incubator was operated. The cultures were then removed and allowed to develop at room temperature. It may be added that all cultures from treated adult flies and their controls were run at room temperature.

Practically all of the cultures were given the D5 (= t4.6) treatment. This was applied by exposing the culture to X-rays for twelve and one-half minutes while the machine was operated at 50 K. V. peak, and 10 M. A., at 12 cm distance from the target, with a 1mm aluminum filter interposed. The only exceptions to this are two groups of larvae rayed with D8 dosage (twenty minutes exposure); (table 9), and one group of adult males treated with D10 dosage (twenty-five minutes exposure; table 10). All of the larval stages irradiated were at 72-84 hours of age except one group (table 9, rows four and five) treated at 48-60 hours.

Shortly after the flies began emerging in the culture bottles, they were removed and the males and females placed in separate vials. This was done from time to time until enough individuals were accumulated to make the breeding tests for germinal mutations. The treated flies were then mated, in all cases except one to be noted, to untreated individuals in various combinations. In the first set of these experiments (table 9), the interval between collections was sometimes so long that many of the treated females had already been fertilized by their treated brothers. In all subsequent experiments, efforts were made to secure virgin females, by collecting the flies every eight hours.

In examining the progeny of the treated and control flies for mutations, the following method was used. The etherized flies were carefully examined under the binocular microscope for mutations of the two sex-linked characters involved, and at the same time the males and females were separated into two groups. The flies were again examined when counts of the number of flies in each group were made. This procedure reduces to the minimum the chances of overlooking a mutant fly; for in work of this character it is highly important to detect every case, otherwise it would not be possible to determine with accuracy the frequency of mutation.

The results obtained from the counts of progeny of the tinged forked flies treated in the larval condition are recorded in table 9. The first column states the larval age (in hours subsequent to egg deposition) at which the treatment was given to the parent flies, the second column, the dose employed, the third, the number of cultures (bottles) in which the

treated flies were used as parents, the fourth, the nature of the cross used in the tests of the treated flies, the fifth and sixth, the numbers counted of female and male progeny, respectively, of the treated flies, and the seventh the number of progeny showing reverse mutations of forked to non-forked. In the fourth column, under "nature of tests of treated flies," the character of the treated fly (or flies) is given first, on the upper

TABLE 9

Reverse mutations from forked to non-forked, from flies derived from treated larvae.

AGE OF TREATED LARVAE	DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	FEMALE OFFSPRING	MALE OFFSPRING	REVERSE MUTATIONS
72–84 hours	D5	39	1 tinged forked female×1 tinged forked male	1,454	1,568	2 females; Nos. 2, 3
"	"	44	2 tinged forked females×2 tinged forked males	791	943	1 female; No. 4
"	"	58	2 tinged forked males× 1X-X female	..	910	0
48–60	"	37	1 tinged forked female× 1 tinged forked male	1,289	1,414	1 male; No. 7
"	"	14	5 tinged forked males× 1 X-X female	..	457	0
72–84	"	43	1 tinged forked female× 2 tinged forked males	2,246	2,632	2 females; Nos. 5, 6
"	"	26	2 tinged forked males× 2 X-X females	..	785	0
"	"	48	3 tinged forked females×3 tinged forked males, all treated	1,090	1,130	0
"	D8	46	1 tinged forked female× 1 tinged forked male	1,993	2,324	0
"	"	11	5 tinged forked males× 1 X-X female	..	264	0
Totals		366	834 tested flies	8,863	12,427	6 (5 females, 1 male)

X-X=attached X-chromosomes (each containing the gene for yellow body).

line of each horizontal row, while the character of the untreated fly (or flies) to which the treated fly was crossed is given second, on the lower line. In just one set of cultures, however, shown in the third to the last row, treated flies were mated with each other (three females by three males in each culture) instead of to untreated flies.

With the exception of the set of cultures just referred to, in which treated tinged forked males were crossed to treated tinged forked females, the treated tinged forked males were always crossed to yellow

females having attached X-chromosomes. The male progeny of such a cross carry their father's X chromosome and serve as an index of visible mutations in the latter, while the female progeny, carrying their mother's attached X's and their father Y's, are of no use for this purpose, and hence were not counted. (This is the main reason for the large excess of male over female progeny in the total count.) The treated tinged forked females, on the other hand, were crossed to untreated tinged forked males, and here male and female progeny were examined and counted, as both alike served to reveal any dominant genes that had arisen by mutation in the mother's X.

As the table shows, there were, in all, 834 flies bred which were derived from treated tinged forked larvae. They gave rise to 8,858 tinged forked females, 12,426 tinged forked males, and five females and one male which were *tinged* but *non-forked* and which therefore represented reverse mutations (or else mutant "suppressors" of forked).

No effort was made in this and the later experiments to search the material for other kinds of visible mutations because it was desired to focus the attention on mutations involving changes in eye color and in bristle structure. Nevertheless, many visible changes of other sorts were observed in the large number of flies examined. Some of the conspicuous mutations were recorded, and a few of these were isolated and developed into stocks. The records show that the following familiar variants were observed: apparently white eyes (five times; but these may have included cases of other eye-color mutants, like vermilion and garnet, which, with tinged, would produce a practically white effect), singed, scute (twice), dichaete-like, rudimentary-like wings (eight times), and miniature-like wings. The two mutations to scute have already been discussed.

The fact that five of the reverse mutations were found in the females and only one in the males has little statistical significance owing to the smallness of these numbers. It was to be expected that more cases of reverse mutation were to be found in the female offspring than in the males because the female offspring in many cases had received two treated X's (one from each parent) and therefore had two chances of mutation, whereas the male offspring had but one X in every case. Two treated X's were present not only in all the female offspring recorded in the third to the last row and known to have had both parents treated, but also in a large proportion of the female offspring in the other parts of the experiment. This resulted from the fact, previously stated, that the treated mothers were in many instances not virgin, that is, they had been allowed to mate with their treated brothers before they were placed with the untreated males for the

breeding tests. In consequence, the exact number of treated chromosomes which were present and subjected to the reverse mutation test, in the counts of daughters of treated females, must have been much greater than the total number of these daughters, but less than twice this number, and in any event, the number of these tested chromosomes in the daughters was probably considerably more than the number in the sons. This would, of course, tend to cause the discovery of a greater number of mutations in the female offspring than in the males.

There is also another fact which has some bearing on this point. The two reverse mutations numbered 2 and 3 came from the same bottle, that is, these two females were sisters. Since mutations of any visible kind are rare, the appearance of two identical mutants in the same culture, originating from a single pair of flies, suggests at once that they arose from a common germ cell which divided one or more times after the mutation had been induced. It is not uncommon to find two, or even more, identical mutants arising from a single fly treated during the larval stage (PATTERSON 1928). If this interpretation is correct, the figure 5, representing the mutations found in females, is subject to a greater probable error than that calculated by the ordinary rules of simple independent samples.

The question as to how the frequency of mutations in the treated female larvae compared with that in the treated male larvae is a different one from that of the frequency with which the mutations were found in female and male progeny respectively. The progeny which were known to carry X chromosomes derived from the treated male larvae were the sons from the crosses of treated males by yellow attached-X females and the daughters from the cross of treated males by treated females. These progeny totalled only 3546 altogether, and among them no cases of reverse mutation were found. The progeny which were known to carry treated X chromosomes derived from the female larvae *only* were the sons of the treated females. These numbered 10,011, and among these but one reverse mutation was found. The other 5 cases of reverse mutation all happened to occur in the daughters of treated females that may or may not have been mated with treated males—there being 7,773 such daughters in all. In these cases, then, the sex of the parent in which the mutation had occurred was uncertain.

In calculating the rate at which reverse mutations are produced from X-rayed larvae, we should count the case in which two mutations had occurred in the same culture as two distinct mutations, no matter whether or not they really had had a common origin, since the flies in which they

appeared would also have been counted separately, as non-mutants, if no mutations at all had appeared in them. The number of mutants being thus 6, what should our total number be, into which this is to be divided? The males, which constitute the total number of flies known to contain but one treated X, number 12,427. When we modify the numbers in the two last groups, rayed at D8, to make them equivalent to flies rayed at D5, the total number of males may be represented by the figure 13,980. The females in the third row from the last, which constitute all the flies known positively to contain two treated X's, number 1090. This latter number, then, should be doubled, to represent the number of X chromosomes here tested; it then becomes 2180. The remainder of the flies—all females of doubtful paternity—number 7,773 which, when modified to give the total number on the D-5 basis, becomes 8,969. If all these flies had been derived from treated fathers, the X's tested in them would be twice this number, or 17,938. Adding this number to those from the other two groups (representing progeny of treated males, and of both parents treated, respectively), we obtain 34,098 as the maximum number of X chromosomes tested. If, however, we take the smaller figure (8, 969) for the females of the uncertain class, thus assuming none of them to have had treated fathers, our sum becomes 25,129, which would represent the minimum number of X-chromosomes tested.

Dividing 6 into the above two respective totals, we find that the observed frequency of reverse mutations from forked to non-forked was somewhere between a minimum of 1 in 5,683 and a maximum of 1 in 4,188. On the basis of a t-12 dose, which was that used in computing the mutations in the previous sections, we have the frequency lying between a minimum of 1 in 2,186 and a maximum of 1 in 1612. It will be seen that this frequency is of a magnitude rather similar to that of mutations from red to white in sperm cells and in larval somatic cells, and is also similar to that for mutations of the normal allelomorphs of forked and of scute, so far as our previous figures will allow these to be computed.

c. Reverse mutations induced in spermatozoa

In order to have a basis for the comparison of the production of reverse mutations from flies rayed in larval and in adult stages, 855 tinged forked males were treated when adults, some with the D5 and others with the D10 dose. They were then mated to yellow females having attached X-chromosomes. As table 10 shows, a total of 11,298 male offspring bearing their father's X was obtained. Of these, 3,198 were derived from sperm

given the D10 dose; if the latter number is doubled to put the total count all on the basis of D5, the revised total is found to be 14,496.

There were two flies which showed mutations in the characters being studied. One of these had the left half of the thorax forked and the right half non-forked (forked reverse mutation No. 8). Cases like this are frequently found among flies arising from treated mature germ cells (MULLER 1927, 1928a, b, c); they are called "fractionals." In explanation of them, it is assumed that the chromosome in the mature germ cell may be precociously split, and that the mutation has taken place in the gene of one of the two halves. A fly developing from such a germ cell will usually have one half of the body bearing the mutation. These flies often fail to breed true to the mutant type. They breed true to it only if their germ

TABLE 10

Reverse mutations from forked to non-forked, from irradiated adult males.

DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	MALE OFFSPRING	REVERSE MUTATIONS
D5	176	2 tinged forked males × 2 X-X females	5,563	1 male; No. 8
"	49	3 tinged forked males × 3 X-X females	1,502	0
"	24	4 tinged forked males × 2 X-X females	603	0
"	22	3 tinged forked males × 1 X-X female	432	0
D10	97	2 tinged forked males × 2 X-X females	3,198	1 male; No. 9
Totals	368	855 tested males	11,298	2 males

cells have received the mutated gene. It will be seen from the tests to be reported in the next section that in the present instance the germ cells had received the non-mutated gene. The determination of the locus of this mutation by genetic means was accordingly precluded.

The second mutation involving the forked character resulted in a fly (mutation No. 9) which was tinged non-forked throughout except for the left posterior scutellar bristle; the latter was forked. Breeding tests, not yet completed, indicate that in this case a very "weak" allelomorph of forked, very nearly resembling the normal type, has arisen. We may then term the case one of "partial reverse mutation." The significance of of the induction of multiple allelomorphs has already been considered. The present case adds another bit of evidence to show that they really can be induced.

Assuming that the "fractional" mutation and the "weak" mutation both occurred at the locus of forked, and giving the fractional a value of

one-half in our count, we find that $1\frac{1}{2}$ partial or complete reverse mutations occurred in this experiment among 14,496 flies, on the basis of a D5 dosage. That is the same as 1 in 9,664 for a D5 dose, or 1 in 3,717 for a t12 dose. Considering the numbers involved, this frequency is not significantly different from the value, 1 in 1,612 to 1 in 2,186, for a t12 dose, obtained in the experiment on treated larvae, although it does suggest that mutations of this kind may be more difficult to obtain by treatment of spermatozoa than of immature larval germ cells. If we average the results from the two kinds of treatments together, we obtain a reverse mutation frequency, on the t12 basis, lying between 1 in 2000 and 1 in 2,500. This would make the frequency still more like that for other loci (treated in the adult male).

Though the reverse mutation frequency from treatment of the adult males cannot be said to be decisively lower than that from treated larvae, yet it certainly is safe to conclude from these results that the frequency in the adults cannot be much *higher* than it is in the larvae. This is a somewhat surprising fact, in view of the independent and unequivocal results of HARRIS (1929a, b) and of HANSON and HEYS (1929b), showing that at least five times as many lethal sex-linked mutations can be obtained by treatment of mature sperm as by treatment of the primordial germ cells of the adult male. It cannot yet be concluded from this distinct difference between these results and ours that the mutations from forked to non-forked are necessarily different in the mechanism of their production from the mutations more commonly dealt with. If true, this would be a fact of considerable importance. It is, however, at least as likely that the difference between the results from these particular mutations and the others lies in the greater viability or vigor of multiplication, in the immature gonad, of the cells bearing the reverse mutants, as compared with those bearing lethals.

It has been suggested by MULLER (see HARRIS 1929a) that one possible explanation of the infrequency of lethals from irradiated immature germ cells, as compared with those from treated spermatozoa, may be simply that a large proportion of the lethal-bearing immature cells die or fail to multiply at as high a rate as the non-mutated cells (that is, that a germ-cell selection occurs), whereas in the mature sperm the lethal changes would not be effective owing to the non-functioning of the genes in spermatozoa (MULLER and SETTLES 1926), and to the further fact that the chromosomes tend to be split in two halves, only one of which may contain the mutant gene. (In immature cells, the two halves would come to lie in different cells, through subsequent mitoses.) The fact that the pre-

sent case, dealing with "mutant' genes that do not lower vigor, shows no such difference between the frequencies observed after raying immature and mature germ cells as is found in the case of lethals and semi-lethals, tends to support the above explanation but the latter is still to be regarded as only provisional.

d. Tests of the loci of the reverse mutations

Though it has been tacitly assumed in the foregoing account that the non-forked individuals resulted from mutations at the locus of forked, it was essential to make perfectly sure of this point by means of breeding tests, since upon it depended the answer to our major question—whether or not mutations can be induced in both of two genetically opposite directions. The alternative to a real reverse mutation at the locus of forked was, it may be recalled, the origination of a dominant "suppressor" of forked, by a change at some other locus. Disproof of the latter idea and proof of the former would be equivalent to one another.

One way of testing whether or not a suppressor is present at a different locus from forked, and in addition to it, has already been described in connection with the tests of the reversion of forked accompanying the apparition of spectacled. In this method, the chromosome in question is crossed to one having normal genes, so far as the forked character is concerned. In the F_1 females, if a suppressor is present, with forked, in one of the chromosomes, crossing over between their two loci will occur in a certain percentage of cases, yielding chromosomes which contain forked but not the suppressor, and so forked offspring will appear. If the non-forked effect is on the contrary due to an allelomorph of forked itself, the F_1 females, being homozygous for non-forked, will give rise only to non-forked X-chromosomes. It is desirable, when making this test, to have some way of ascertaining the frequency of crossing over, since, if crossing over is for some reason prevented, females containing a suppressor accompanying forked would fail to yield the forked cross-overs, and the result would thus simulate that to be expected in the case of a true non-forked allelomorph. The danger of a considerable reduction of crossing over occurring is not negligible, since gene rearrangements having such effects have been found to be induced rather abundantly by X-rays (MULLER 1928b).

Mutants 2, 4, and 7 were tested by the above method. They, or flies inheriting their mutated X-chromosome, were crossed to flies which were normal in respect to the forked character but contained Bar eyes. The F_1 females, carrying in one X-chromosome tinged and the newly arisen

non-forked that was to be tested and in the other X-chromosome the gene for Bar, were then backcrossed to the triply recessive males (tinged forked non-Bar). Both male and female progeny (F_2) were of value for making the determination; their counts are given in table 11. The counts were continued in each case until just 1000 offspring had been examined; this would allow a chance for the existence of a suppressor to be revealed by crossing over even if it lay within a fraction of a unit of the locus of forked itself. It will be seen, however, that in none of these three cases were any forked flies produced. That this was not due to any reduction in the frequency of crossing over is indicated by the fact that in each of the three cases the percent of crossovers found between tinged and Bar was within a unit of 46.5, which is a normal value for the frequency of separations between these loci. Hence these mutations were real reversals at the locus of forked.

TABLE 11

Tests for the loci of reverse mutations No.'s 2, 4, and 7. The counts are based on the first 1000 flies.

Cross: $\frac{w^t F}{B}$ ♀ × $w^t f$ ♂ *(where* w^t *= tinged, F = non-forked due to reversal, f = forked, and B = bar).*

	NON-CROSS OVERS				CROSSOVERS BETWEEN w^t AND B				
NO.	BAR FEMALES	TINGED FEMALES	BAR MALES	TINGED MALES	WILD TYPE FEMALES	TINGED BAR FEMALES	WILD TYPE MALES	TINGED BAR MALES	PERCENT OF CROSSOVERS
2	139	127	135	139	106	111	122	121	46.0
4	126	81	172	156	99	105	131	129	46.4
7	142	144	118	123	126	117	114	116	47.3

A somewhat different method was used in determining the loci of reverse mutations 3, 5, and 6. The flies containing these were crossed to flies containing forked and Bar. In this way, females were obtained that, as in the preceding method, were heterozygous for tinged and for Bar; since they contained forked in their untreated chromosome, if the mutation in the treated chromosome had been due to a "suppressor" arising in another locus from that of forked, they would have had forked in both chromosomes and have been homozygous for it and heterozygous only for the "suppressor" (so far as this character was concerned). The percent of crossovers shown among their offspring, between the forked character and these other characters (tinged and Bar), would then depend upon the position of the "suppressor." It would not be the percent of crossovers expected between the forked locus and these other characters unless, indeed, the mutation had really occurred at the forked locus, that is, unless it had

been a true reversal, and not due to the origination of a suppressor. Since the locus of Bar is only about a fifth of a unit from that of forked, the Bar character in this cross serves very accurately to inform us of the exact locus of the gene in question, while the tinged-forked crossovers serve to show whether the general frequency of crossing over throughout the chromosome is normal.

The counts, as before, were contined until 1000 flies had been obtained in each case. Since the heterozygous females had been backcrossed to triply recessive males (tinged forked non-Bar), the daughters as well as

TABLE 12

Tests for loci of reverse mutations No's. 3, 5, and 6. The counts are based on the first 1000 flies.

Cross: $\frac{w^t F}{fB}$ ♀ × $w^t f$ ♂ *(symbols as in table 11).*

NON-CROSS OVERS					CROSSOVERS BETWEEN w^t AND f					
NO.	FORKED BAR FEMALES	TINGED FEMALES	FORKED BAR MALES	TINGED MALES	WILD TYPE FEMALES	TINGED FORKED BAR FEMALES	WILD TYPE MALES	TINGED FORKED BAR MALES	PERCENT OF CROSS OVERS	CROSSOVERS BETWEEN FORKED AND BAR AS INDICATED
3	155	103	155	130	107	98	145	105	45.5	1 Bar 1 tinged forked
5	164	124	115	119	119	113	125	120	47.7	1 Bar
6	137	120	148	118	105	121	122	127	47.5	1 Bar 1 forked

the sons were again of value. It will be seen that in cases 3 and 6, respectively, there were just two crossovers between forked and Bar in the entire thousand, and in case 5, just one. This result agrees with surprising nicety with the standard percent of crossovers between Bar and the locus of forked—0.2 (STURTEVANT 1925). At the same time, the percent of crossovers between the loci of tinged and Bar is again quite normal (ranging from 45.5 for No. 3, through 47.5 for No. 6, to 47.7 for No. 5), so that there is no reason to suppose that the low percent of crossovers between forked and Bar in these cases is due to reduction of crossing over. These mutations also are accordingly to be classed as reversals at the locus of forked itself.

In order to complete the account of the genetic behavior of the mutations in this experiment, a record is presented in the appendix of the crosses which were made of each of the original mutant flies derived from treated larvae, and the counts of progeny obtained from these crosses. These

are not given here, as in all these cases the results were regular, showing the features to be expected, respectively, of females homozygous for tinged and heterozygous for forked, and of the male containing tinged non-forked.

The "fractional" male derived from the treated sperm, on the other hand, gave the results expected of an ordinary (tinged) forked. When crossed to a virgin forked female (of the red-eyed "δ49" forked stock), it produced 52 red-eyed and typically forked females and 52 red forked males. Although the locus of the mutation could not be determined here, in view of the mutant gene's having been received only by the somatic tissue, nevertheless, in view of the fact that all six of the preceding apparent reversals had really occurred at the forked locus, it is highly probable that here too this was the locus which had undergone the change.

The "partially reverse" mutant male, having tinged eyes and but one forked bristle, was also mated to a virgin δ49 forked female. It produced 54 red males with typical forked bristles (their X being derived from their forked mother) and 57 red females which were very weakly forked, overlapping the normal type to some extent so that some appeared normal. Thus the mutation was proved to be sex-linked and partially dominant to the typical forked from which it arose. Further tests of it are being made to determine whether typical forked can be recovered from it by crossing over, but it is very likely, in view of the results with other "weakly forked" allelomorphs, that this mutation also was in the forked locus itself.

e. Radiation as the cause of the reverse mutations of forked

In order that definitive evidence might be obtained as to whether it was the radiation which was causing the above reverse mutations or some other peculiarities of the environment or the stock, control series of flies of the same stocks were carried along parallel to the experimental series and were handled in a similar manner, except that no X-ray treatment was given. This was especially important in the present instance, where reverse mutations were being looked for, since the general Drosophila work does not furnish nearly as extensive an indication of the frequency of reverse mutations, from the abnormal type back to the normal, as in the direction, normal to abnormal, owing to the fact that the vast majority of ordinary cultures contain flies which to begin with have the normal allelomorph of any particular gene. The counts from these control matings are all listed in table 13.

It was aimed to examine as many control flies as experimental, in order that the comparison might be adequate. As the totals show, 11,300 female

progeny were examined, representing 22,600 untreated X chromosomes, and 21,565 male progeny, making a total of 44,165 untreated X's altogether. Of these, it can readily be calculated from the table that 24,608 were derived from the mothers, and 19,557 were of paternal origin. In all the experimental series together, including those from treated larvae and from treated adults, there was a total of between 33,678 and 41,451 treated chromosomes (the exact number depending on how many of the females from treated larvae had been fertilized by their brothers). The number of untreated chromosomes subjected to the mutation test in the control series thus somewhat exceeded the number of treated chromosomes tested in the experimental series. Yet, in contrast with the 8 cases of reverse mutation found in the experimental series, not a single instance of this phenomenon was found in the controls.

TABLE 13

Controls for flies shown in tables 9 and 10. All from untreated flies.

NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS	FEMALE OFFSPRING	MALE OFFSPRING	REVERSE MUTATIONS
31	1 tinged forked female ×2 tinged forked males	1,347	1,576	0
153	2 tinged forked females ×2 tinged forked males	6,467	7,823	0
46	1 tinged forked female ×1 tinged forked male	3,486	3,909	0
244	2 tinged forked males ×2 X-X females	..	7,224	0
39	1 tinged forked male ×2 X-X females	..	1,033	0
Total 513	1,324 tested flies	11,300	21,565	0

This result may be examined from the standpoint of the theory of probability. In doing this, we may for simplicity take the control and experimental lots as having been of exactly equal size, although in so doing we do not weigh the results quite as heavily in favor of a significant difference as would be justifiable. What, then, would be the chances, in two lots of equal magnitude, of finding all the exceptional events to occur in a given one of the lots, if there were really no determinate cause tending to produce the events in this lot rather than in the other one? As the chance for any one event to be in the given (that is, the treated) lot is $\frac{1}{2}$, and 7 at least of the events are independent, the chance for all 7 to be in this particular lot is $(\frac{1}{2})^7$, or 1 in 128. It can therefore be concluded with consider-

able confidence that this was not a chance result, and, since the X-ray treatment was the only factor consistently differentiating the numerous identically handled cultures of these two series, this must have been the agent which was responsible. The conclusions based on the present statistics are rendered even more secure when taken in connection (1) with the prior case of non-forked spectacled arising after irradiation, and (2) with the mass of previous Drosophila work, in which, without treatment, no evidence of any such high incidence of reverse mutations had ever been noted, except in the special case of Bar crossovers and in DEMEREC'S special cases in *D. virilis*.

X. MUTATIONS FROM INDUCED NON-FORKED TO FORKED

The induction of reverse mutations does not in itself provide proof that other changes than losses can be induced, unless it is accompanied by sound evidence of the induction of mutations in the opposite direction also (that is, from the normal to the abnormal type). That point mutations from the normal to some abnormal type, can, in general, be induced by radiation is now a well-established fact; hence the major emphasis of the present work fell upon proving the induction of the reverse mutations. Nevertheless, it was important to have specific evidence that at the locus here involved, that of forked, such changes (that is, to the abnormal form, forked), could be induced, for it was *a priori* conceivable that this locus, like that of Bar, might constitute a special case of some kind.

Considerable specific evidence of the induction of changes at this particular locus in the required direction, non-forked towards forked, had already been gained before the reverse mutation experiments with this locus were begun. This evidence has been briefly reviewed in an earlier section. Three independent heritable mutations from the original normal allelomorph towards forked, following X-ray treatment of the sperm, were there described. Two of them resulted in typical forked, and one in a "weakly forked" allelomorph. This evidence, taken in connection with the fact that only 9 sure mutations from normal to or towards forked had been noted in all the rest of the Drosophila work, at COLUMBIA and elsewhere (MORGAN, BRIDGES and STURTEVANT 1925), despite the fact that the normal allelomorph had been present to start with in the great majority of the cultures, often checked by other markers, made it extremely probable that the present mutations owed their origination to the X-ray treatment.

It was decided, however, to conduct some special experiments on this point, in order to determine whether this production of forked could be

repeated, and also in order to take advantage of the opportunity thus afforded to run an experimental series having parallel controls, handled, except for the irradiation, in exactly the same fashion. In conducting these tests, it was thought to be advantageous to introduce a peculiar modification which the previous occurrence of the reverse mutations had made possible. This special feature consisted in using both for the experimental series and the controls, not the original normal allelomorph of forked, but one of the non-forked genes which had arisen from forked by reverse mutation under the X-ray treatment. If, then, forked was found to arise (with a frequency beyond that expected or found in untreated material), the case for reversibility of the mutation reactions would be made even stronger than if the mutations had been induced in material that had not originally been derived from forked. For then a cycle (forked⇌non-forked) would have been completed, all under the influence of irradiation.

For these experiments on the reversibility of the "artificial" non-forked, flies were used from a homozygous stock of tinged non-forked that had been established from reverse mutation No. 3. In the first set of experiments, larval stages of these tinged non-forked flies were treated at 72–84 hours with the D5 dose.

The males derived from the treated larval stages were tested in two ways. Some were mated to yellow females with attached X's and others to δ49 forked females. In the former test, only the male offspring would be able to show mutations to forked derived from the father. In the latter test, by δ49 forked females, only the female offspring could reveal such mutations. In this latter cross, in addition to forked females due to mutation, there was also the possibility of forked females due to non-disjunction, and to non-virginity of the mother, that might in rare instances have carried sperm from the δ49 forked stock. The forked mutants would, in the breeding test, prove to be heterzoygous for tinged, while the forked females of the other two classes would be homozygous for red. Females of the latter two classes could usually be distinguished from one another by whether or not they produced non-disjunctional offspring.

The test to the attached-X females yielded 9,738 male offspring. Among these, no mutation to forked was found (table 14, lines 1 to 3). In the test to δ49 forked females, 3034 female offspring were produced. Among these, there were six forked. Two of these forked females came from the same bottle, all the others from separate bottles. It was suspected that at least some of the six forked females were due to secondary non-disjunction, since eight exceptional males were found in the same series of bottles. Ac-

cordingly, the forked females were all mated to white forked males to see whether they were heterozygous for tinged, and whether exceptional males would be produced. The results showed that three of the females had been produced by secondary non-disjunction and two were non-mutants that were in all probability due to non-virginity, since no non-disjunctional offspring were produced. The remaining forked female proved to be a clear case of a mutation to forked at the locus of forked (forked mutation No. 4 in table 14), for, when crossed to white forked males, this female gave rise to 25 red forked and 25 light tinged forked daughters.

Three hundred and sixty-four virgin females derived from the treated larvae were also tested for mutations. This was done by crossing them to δ49 forked males. In this cross, both female and male progeny would reveal mutations to forked. The tests yielded 9,339 females and 8,886 males. No mutation to forked was found among them (table 14).

TABLE 14

Mutations from non-forked to forked, from flies derived from irradiated larvae.

AGE OF TREATED LARVAE	DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	REGULAR FEMALE OFFSPRING	MALE OFFSPRING	MUTATIONS
(hours)						
72–84	D5	113	2 tinged males×2X-X females	..	4,227	0
"	"	134	3 tinged males×2X-X females	..	4,304	0
"	"	48	4 tinged males×2X-X females	..	1,207	0
"	"	67	2 tinged males×2δ49 forked females	3,029	..	1 forked female; No. 4
"	"	182	2 tinged females×2δ49 forked males	9,339	8,886	0
Totals		544	1,318 tested flies	12,368	18,624	1

In addition to the tests of flies treated in the larval stage, another set of experiments of similar magnitude was carried out on the same tinged forked stock, the difference in method being that in this case the treatment was applied to the adult flies. The treated males were mated to attached-X females, and the treated females to δ49 forked males. As shown in table 15, there were 11,772 sons examined that were derived from the treated adult males. Three mutant forked males were found among these (forked mutations Nos. 5, 6 and 7).

Mutation No. 5 was tested by crossing it with a female containing the "C_1B" complex in one X chromosome and $s_c\ v\ f\ b_b$ in the other. There were produced 30 Bar females, 33 forked females, and 33 males showing $s_c\ v\ f$.

The fact that the non-Bar females (and not the Bar females) showed the recessive forked character proved that the variant male carried forked as a germinal mutation, and it was also clear that this mutation must have been at the same locus as the familiar forked. Mutation No. 6 was clearly a fractional, since one-half of the thorax (the left half) was forked and the other half non-forked. In matings with yellow attached-X females it proved to be sterile. Mutation No. 7 was tested, like No. 5, by crosses with females containing the "C_1B" complex in one chromosome and $s_c\ v\ f\ b_b$ in the other. The count of offspring showed 43 Bar females, 28 wild-type females, and 31 males showing the characters of $s_c\ v\ f$. Since the non-Bar daughters were not forked, it was highly probable that this mutation had been confined to the somatic tissue, that is, that it was a "fractional."

TABLE 15

Mutations from non-forked to forked, from irradiated adult flies.

DOSE	NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS OF TREATED FLIES	FEMALE OFFSPRING	REGULAR MALE OFFSPRING	REVERSE MUTATIONS
D5	312	2 tinged males ×2 X-X females	..	9,552	3 tinged forked males; No's 5, 6 and 7.
"	45	3 tinged males ×3 X-X females	..	2,220	0
"	211	1 tinged female×2 49 forked males	8,452	7,601	0
Totals	568	970 tested flies	8,452	19,373	3

The treated females in this series, mated to ♂49 forked males, gave 8,452 female and 7,601 male progeny. No mutations to forked were found among them.

All of the controls for the experimental series recorded in tables 14 and 15 are given in table 16. The total numbers counted in the experimental series dealing with treated larvae, in the experimental series dealing with adults, and in the controls, are all of similar magnitude. There was, in the controls, a total of 34,798 flies, including as many tested chromosomes, of which 14,450 were of paternal and 20,348 of maternal origin. There was one forked female among these flies, derived from a culture in which two tinged non-forked males had been mated by two ♂49 forked females. It was in the series from treated larvae carried on parallel to this that the two forked females produced by non-virginity had been found. Tests of the forked female here in question proved that she, too, had been produced in this way (by a previous mating of a ♂49 forked male

by a δ49 forked female), since she bred as a homozygous red-eyed forked fly. There were no other forked flies among the controls, Hence, there were no mutations to forked in the control series.

The present data on the production of forked from non-forked are not extensive enough to be very informative from a quantitative standpoint concerning the frequency of these induced mutations; nevertheless, some comparisons may be of interest. If we total all the data from tables 14 and 15 together, we find that 58,813 progeny of treated parents were examined; these would be represented by 22,646 on the basis of a t12 dose. Counting the two fractionals as each equivalent to $\frac{1}{2}$, we have a total of 3 complete mutations to forked among these, or 1 in 7548 on the t12 basis. This is about 150 times the frequency calculated from the figures 9 in 10,000,000

TABLE 16

Controls for flies shown in tables 14 and 15. All from untreated material.

NUMBER OF BOTTLES OF TESTS	NATURE OF TESTS	FEMALE OFFSPRING	MALE OFFSPRING	REVERSE MUTATIONS
266	2 tinged males ×2 X-X females	..	12,121	0
24	2 tinged males ×2δ 49 forked females	2,329	..	0
46	1 tinged female ×2δ 49 forked males	2,179	2,225	0
190	2 tinged females×2δ 49 forked males	7,911	8,033	0
526	1,206 tested flies	12,419	22,379	0

which roughly represents the results from previous Drosophila work on untreated material. It is considerably lower than the frequency, 1 in 2,000 to 2,500 at the t12 dose, for the lumped data on forked to non-forked mutations, but it is also lower than the frequency (1 in 3120) found for non-forked to forked mutations in the sum of all previous X-ray work. If we add together the present and the previous non-forked to forked data, we find an equivalent of 6 "complete" mutations to forked in 32,010 on the t12 basis, or 1 in 5,335, which is about 200 times the apparent "natural" frequency. This is still only half the frequency found for the mutations in the opposite direction; yet the difference is of doubtful significance, particularly since it is quite likely that the lumping of the data from both sexes and from two different stages of the life cycle is illegitimate.

The fact that all but one of the 4 (or "3") mutants to forked in the more-recent experiments were derived from treated sperm, although only a fifth of the progeny of treated flies were of such origin (the rest being from treated larvae), also merits some attention, for the opposite tendency appeared

to be at work in the mutations from forked to non-forked. While the figures on this point are not yet decisive, it is to be noted that this is the sort of result to be expected on the hypothesis of a "germ-cell selection," if the cells with the non-forked allelomorph be considered as having a tendency to multiply more quickly than those with the forked allelomorph. For, in that case, when forked larvae containing immature germ cells were irradiated, the non-forked mutant cells should be represented in the adult gonads in relatively greater abundance than that in which they actually originated, but when non-forked larvae were irradiated, the forked mutant cells should be present in the adult in relatively lesser abundance than that in which they originated, due to the higher rate of multiplication of their non-forked neighbor cells. In the adult sperm there would be no opportunity for such selective action.

The major interest of the present data lies, however, not so much in the exact mutation frequencies shown, but rather in the fact that mutations to forked occurred at all. The presence of two clear germinal mutations, and two somatic fractionals, among the treated series, and none among the controls, when added to the previously obtained data indicating the induction of forked, furnishes convincing evidence that irradiation can cause mutation in this direction. Taken by themselves, the data also make it probable that irradiation can cause a reversion of the very allelomorph which was itself so produced. Since it has been shown in the foregoing that mutation from forked to non-forked also can be induced, the links in our chain of evidence against the hypothesis of induced mutation being exclusively by loss have now been forged.

XI. VIABILITY OF THE INDUCED REVERSE MUTATIONS

An interesting point in connection with the question of the possibly destructive action of X-rays is whether the flies showing the induced reverse mutation are as viable as the non-mutated stock from which they arose. To test this point, tinged non-forked males from each stock of four of the reverse mutations (Nos. 3, 5, 6, and 7) were backcrossed to tinged forked females of the original stock. The F_1 flies were inbred, four pairs to the bottle, and the F_2 males examined and counted. Obviously, if the flies bearing the reversed genes of these four stocks are as viable as those bearing the original forked gene, then the two types of males (tinged forked and tinged non-forked) expected in the F_2 generation, should appear in equal numbers.

The results obtained in this test are recorded in table 17. The F_1 flies were kept in the culture bottles for six days and then removed. The

counts of the F_2 males were made daily for six successive days after they began emerging. This was done in order to determine whether there was any difference in the time of emergence of the two types of males. An examination of the table shows that the tinged non-forked males emerged in greater numbers at first then did the tinged forked males. This is made clear if we take the counts in two-day periods. The number of non-forked males of the first and second counts is 325 as against 240 forked males. Thus more than fifty-seven percent of all of the males of this period belong to the reverse mutation type. In the third and the fourth counts, there are 467 non-forked males and 521 forked males. About forty-seven percent of all of the males in these two counts were non-forked. Finally, in the fifth and sixth counts, there are 291 non-forked and 309 forked males. These counts show clearly that there is a strong tendency for the reverse mutation males to emerge earlier than the forked males. In this sense, they may be said to be more vigorous. This fact is of particular interest when considered in connection with the possibility previously broached, that the non-forked cells may multiply faster than the forked cells in the gonad, causing a "germ-cell selection."

TABLE 17

Showing results of tests for viability of four of the reverse non-forked mutations.

DAILY COUNTS	NO. 3		NO. 5		NO. 6		NO. 7		TOTALS	
	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES	TINGED MALES	TINGED FORKED MALES
1st	100	69	7	4	0	1	73	62	180	136
2nd	55	27	27	25	27	28	36	24	145	104
3rd	68	67	79	90	54	79	65	44	266	280
4th	77	107	30	53	26	18	68	63	201	241
5th	41	68	18	19	19	19	53	49	131	155
6th	20	26	48	31	48	47	44	50	160	154
Total	361	364	209	222	174	192	339	292	1,083	1,070

It is, however, to be noted that there is no significant difference in viability in the two types of males; when the counts from all periods are added together, it is found that there were 1083 non-forked and 1070 forked males in all. When we consider that either of these allelomorphs can be produced by X-rays from the other, we see that these tests lend no support to the view that the gene change produced by X-rays is necessarily of an injurious nature, even though it is to be expected that injurious

changes would, as a matter of chance, happen more often than beneficial ones, all loci considered (MULLER 1923).

XII. REVERSE MUTATIONS IN EYE COLOR

Throughout the examinations of all the flies in the experiments with forked, a careful search was made for possible reversions in eye color, from tinged to red or to some of the intermediate allelomorphs of white. It may be recalled that this was part of the original purpose of the experiments. As was reported in section IV, previous experience in trying to produce somatic reverse mutations in eye color had indicated that if such mutations could be induced by X-rays, they would occur at very rare intervals. In all the previous radiation work on larval or somatic mutations in which white or some other mutant allelomorph of white was used,—work involving altogether 4661 treated flies in which it would have been possible to detect reverse mutations at this locus,—only one certain case of a mutation to a more deeply pigmented condition had been found. This was a male apricot fly that had been treated in the mid-larval stage. The mutant area was composed of twenty-nine ommatidia which were distinctly darker than apricot, but slightly lighter than red (PATTERSON 1929b).

In the experiments on tinged forked, none of the flies showed areas or individual ommatidia of darker color. In these experiments there were however three males, two from one culture and one from another, that had red eyes and were non-forked. These came out of cultures in which the males had been treated during the larval stage and crossed to yellow attached-X females as a test for reverse mutations. The attached-X females came from a stock in which the males had the δ49 (non-forked) composition. It was therefore suspected that the three males might have come from females that had been fertilized before they were collected. Accordingly, each red-eyed male was mated to females containing one chromosome with the "C_1B" complex and one with the genes $s_c\ v\ f\ b_b$. The F_1 females containing the $s_c\ v\ f\ b_b$ chromosome were then tested for crossovers. In each of the three cases, practically no crossovers were found among the F_2 males; this showed that these males were not the result of reverse mutations, but were in reality δ49 males.

These results, combined, leave no doubt that reversions of tinged or white towards red cannot be induced with nearly the same frequency as reversions of forked towards non-forked, although mutations from red towards white can be induced about as readily as from non-forked towards forked. Similar conclusions have been arrived at independently by TIMOFEEFF-RESSOVSKY (1929b, 1930), in work to be referred to later.

Hence the mutations at different loci, as well as the different possible mutations at the same locus, have differing frequencies depending on rules of their own which doubtless are a reflection of their chemical composition.

XIII. THE TWO SCUTE MUTATIONS

All the male offspring of the treated tinged and tinged forked flies which carried an X-chromosome from their treated parent would be capable of showing scute if this mutation had occurred. As there was a particular interest attaching to this locus on account of the reverse mutations which had previously been found there, all such male offspring were carefully examined for this character. A summary of the tables shows that the following total numbers of male offspring were produced: 24,067 (27,265 at D5) carrying X's derived from treated adult males; 7,601 (all at D5) carrying X's from treated adult females; 12,154 (12,253 at D5) carrying X's from treated larval males; 18,897 (19,768½ at D5) carrying X's from treated larval females. The "grand total" is 62,719 (66,887½ at D5). Among these two scute flies were found. There were none in the 43,946 control males.

One of the scute males was a tinged non-forked, derived from a tinged non-forked male treated as an adult with the D5 dose, crossed by a yellow attached-X female. The other was a tinged forked derived from the similar cross of flies of this composition. It is worth noting that both were derived from treated mature spermatozoa, although the males of such origin did not form half of the total count. Both males were tested for germinal scute by mating them to virgin females homozygous for scute, carrying in one X chromosome the "C_1B" complex (which contains scute) and in the other X the genes $s_c\ v\ f\ b_b$. The offspring of the scute tinged non-forked male consisted of 8 scute females, 12 scute Bar females, and 14 scute vermilion forked males. The offspring of the scute tinged forked male consisted of 30 scute forked females, 22 scute Bar females, and 25 scute vermilion forked males. The presence of the scute in all the daughters in both instances proved that the mutation was germinal, and that it was allelomorphic to (that is, in the same locus as) the gene for the familiar scute character. Observation showed that in both instances all the bristles were affected in the typical manner.

XIV. DISCUSSION OF RELATED WORK, AND GENERAL CONSIDERATIONS

The demonstration that mutations at the locus of forked can be repeatedly obtained in both of two opposite directions in X-rayed and not in control populations of comparable magnitude affords convincing evi-

dence that high-frequency irradiation is capable of causing changes in the genes which are not of the nature of losses. Verification is thus provided of the inferences based on the less extensive experiments with the scute locus, and on the less direct evidence from other sources (nature of the physical action of radiation, partial separating of the induction of chromosome abnormalities from that of point mutations, apparition of the dominant eyeless not resembling the chromatin loss of the same region, induction of multiple allelomorphism).

The extensive work of TIMOFEEFF-RESSOVSKY (1929 a, b, 1930) in irradiating mutant stocks of Drosophila, which was carried out independently and came to our attention while our more recent experiments on reverse mutations were already under way, was undertaken with the same primary object and has given parallel evidence of the occurrence of reverse mutations. In his earlier experiments TIMOFEEFF-RESSOVSKY irradiated eggs and larvae of white and of eosin eyed flies, with the object of obtaining facet changes in opposite directions. Among 2986 flies from treated larvae having the gene for white eye one case of a group of 3 red facets was found, and one case of a single facet of intermediate color, while among 1407 flies from treated larvae of eosin stock, a case of a group of 3 red facets was found. Thus it was proved that mutations at this locus could be induced but it also became evident (as in our work) that mutations from white towards red must be much less frequent than those of red towards white.

In his later experiments TIMOFEEFF-RESSOVSKY irradiated adult males of white and of various multiple recessive stocks, namely, $s_c\ w^e\ e_c$, $y\ c_v\ v\ f$, and Xpl, IIIpl, and "rucuca" ($r_u\ h\ t_h\ s_t\ p\ s_r\ e^s\ c_a$). In all, 13 reversals to the normal character were found, distributed as follows: from scute (3 times), esin, echinus, crossveinless, vermilion, forked (twice), hairy, pink (twice) and sooty. In the case of all except the echinus, vermilion and sooty reversals it was found possible to breed the flies and establish the fact that the change was genetic, and the presence of the other characters as "markers" served to show that no contamination had occurred. At a number of the loci involved—those of scute, white, forked and pink,—one or more mutations in the direction, normal to abnormal, have also been observed by TIMOFEEFF-RESSOVSKY after raying. He has in his discussion emphasized the adverse significance of these results for the loss hypothesis.

It will be seen that TIMOFEEFF-RESSOVSKY's experiments on reverse mutations and ours are mutually confirmatory and complementary. His were carried out in such a way that reversals in a considerable number of loci could be found, ours in such a way that a considerable number of

mutations in opposite directions could be demonstrated to occur at a given locus, in both cases similar total numbers of mutations having been obtained.

In view of his results and ours combined there can be no doubt that the conclusions which we on the basis of our own work have reached in regard to the locus of forked and, secondarily, of scute can be generalized so as to apply to a large proportion of the existing loci.

Persistent adherents of the idea of mutation by loss would now be forced back into some very specialized modification of this hypothesis, such as that all mutations are merely quantitative—losses *and* gains of genetic material, of a kind previously present in the nucleus,—as postulated for instance by GOLDSCHMIDT (1927, 1928), but the burden of proof in regard to any such contention would now be clearly theirs. It is difficult to conceive a simple mechanism which would be able to actually increase the amount (or permanently increase the activity) of gene material of any kind that happened to be "struck." Such increase, on this hypothesis, would involve the transformation of neighboring non-genic material into genic material in a very particular way, specific for each case, and yet it would have to be supposed that the active agent was unable to cause any qualitative change whatever in the genic material that was already there. Addition of material at the given locus through some process resembling translocation, that is, a displacement, would be virtually ruled out by reason of the facts (1) that the supposed "addition" is usually found to be of a particular kind, for a given locus, (2) that there is never evidence of a complementary loss occurring in these cases, (3) nor of a complementary locus in which the reverse of these changes may, at other times, be found. Finally, there is the empirical evidence from the cases of multiple allelomorphism which have arisen following irradiation, showing that some of the different changes in a given locus show by no means purely quantitative relationships to one another in their phaenotypic expressions. From a theoretical standpoint, any "quantitative" hypothesis lacks what advantages the sheer "loss" hypothesis might have had, yet suffers from all the disadvantages of the latter and considerably more besides.

Since it is thus probable that irradiation can cause "random" changes of varied sorts in the inner composition of the genes, it becomes arbitrary to attempt to limit the kinds of changes which can be produced. The kind of change produced no doubt depends on the nature of the gene to begin with, the precise manner in which it was struck, and the arrangement, etc., of its own and surrounding atoms at the time. As in GALTON'S polygon

of variations, there would be certain more probable kinds of changes, but varied possibilities in all. Moreover, after one kind of change had occurred, not only reverse mutations but still different changes would theoretically be possible, which might in time accrue so as to result in a gene having a very different function from that which it had to begin with. In this manner, through the possibility of an *indefinite succession* of mutations in each locus, the way for continued evolution would be opened up.

At the same time, it should be borne in mind that many if not most of the characteristics of organisms have probably been developed to an optimal (and in some cases to a maximal) stage already, so that any further change would be likely to be somehow detrimental, disorganizing and often, in effect, reactionary. When some radical alteration takes place in the mode of living of the organism, through a peculiar change in outer conditions, or in some other character from the one primarily in question, then, perhaps, the optimal value for our given character becomes different and mutations previously detrimental are, in their new settings, progressive. Lacking such rare circumstances, really "progressive" changes might be of almost fabulous infrequency in organisms that had long become adjusted to their present mode of life. This would not mean, however, that changes chemically of the same general character as the "progressive" changes of the past were not still taking place nor that, at some time in the future when the outer or inner adjustments somehow become upset, such changes might not again have opportunity to cause a further advance in the organization of the race.

It might, to be sure, be a very difficult matter to study the further mutations possible in a given locus, following upon the initial steps with which we ordinarily deal in our mutation studies, since the familiar mode of phaenotypic expression of mutations at that locus might well fail to govern the further changes, and the investigator would then have little clue as to what variations to look for and to test (supposing they were visible to his superficial observation at all). Nevertheless, such studies may be undertaken eventually. The present work has corroborated the claim previously made that the X-ray treatment raises the frequency of mutations sufficiently to make practicable the study of changes in a particular locus, at any rate if attention is confined to certain types of changes at that locus. The work on the forked locus demonstrates this point. More work must be done before we can know whether the study of a succession of different changes in a given locus is practicable.

The above is one way in which the study of mutations produced by

radiation may be expected to have relation to the study of evolution. For, if our interpretation of the mechanism of production of the mutations based on our findings is correct, the varied and cumulative possibilities of the X-ray mutations would be like those existing for the natural mutations. The finding that the X-ray mutations are not confined to losses makes this conclusion considerably more probable. And the conclusion would also follow that in using the X-ray mutations in attempts at artificial evolution, we are employing an agent capable of causing an indefinite succession of changes, with as much chance of "progression" as there is in the evolutionary processes of nature.

SUMMARY

1. A consideration of the mechanism whereby X and related rays affect matter gives ground for concluding that changes of varied kinds in the composition of the genes are produced by such radiation.

2. Data are presented which show that in mature spermatozoa X-rays produce a much larger proportion of breakages and reattachments of portions of the chromatin ("displacements") in comparison with a given number of "point mutations" than they produce in immature germ cells (of females). This indicates that the "point mutations" are not simply breakages and reattachments on a small scale.

3. Other facts concerning the manner of origination of the induced "point mutations," the phaenotypic effects of those at different loci, and their relations of allelomorphism and dominance, lead to the same conclusion.

4. The origination of multiple allelomorphism at three different loci, following irradiation, is described. At the locus of scute, two mutant allelomorphs—scute and scutex— were found; at that of forked, three—forked, a "weakly forked," and a "very weakly forked" allelomorph, the latter derived from forked; at that of white, three, or more probably, four—(1) white, (2) an allelomorph resembling ivory and derived from eosin, (3) apricot, and (4) a probable allelomorph darker than apricot and derived from it.

5. The comparatively small total numbers among which these cases were found, as contrasted with the great rarity of similar cases in previous work with untreated material, make it highly probable that the irradiation was the causative agent in their production.

6. These multiple allelomorphs furnish the customary combination of evidences against the hypothesis of "presence and absence" as applied

to the mutations produced by X-rays, which has in the past served to undermine the same hypothesis when applied to mutations in general.

7. An account is given of the origination and characteristics of a dominant gene for reduced eyes ("Dominant eyeless") located in the fourth chromosome and probably allelomorphic to the known recessive "eyeless."

8. The fact that, when one fourth chromosome is absent and the other is present and normal (the "haplo-IV" condition), no such effect is produced as when one fourth chromosome contains this mutant gene and the other is normal (the heterozygous Dominant eyeless condition), indicates that this mutation did not consist of a loss of genetic material. Since the presence of an additional fourth chromosome (the "triplo-IV" condition) also fails to give the "eyeless" effect, the mutation does not seem to have involved the increase of a gene in the fourth chromosome. And the fact that allelomorphic eyeless changes have occurred at this locus on a number of occasions gives evidence that the change did not consist of an addition to the fourth chromosome of some genetic material from a non-homologous chromosome. It is, therefore, likely that what occurred was a change in the composition of a gene.

9. Experiments are described in which attempts were made to produce reverse mutations, to or towards red eye, in the gene for white and in other mutant allelomorphs of the white locus. Only one case of such a change was found (apricot to a darker color, lighter than red) although there would have been numerous cases in all the material examined if mutation towards red had been inducible with as high a frequency as the change in the opposite direction, or with as high a frequency as the reverse mutations at the locus of forked (to be referred to).

10. Scute or some other mutant allelomorph of this locus has arisen three times from the normal allelomorph in our X-ray work. On the other hand, among 208 X-chromosomes derived from X-rayed females, the mutant allelomorph scute reverted to non-scute on two independent occasions. Hence it is very likely that mutations can be induced in both of two opposite directions at this locus.

11. An experiment is described in which, following treatment of male larvae, a reverse mutation from forked to non-forked (at the locus of forked) occurred simultaneously with another visible point mutation, from normal to spectacled eye (at the locus of lozenge), in the same chromosome.

12. In order to determine definitely whether reverse mutations, from forked to non-forked, could be produced by irradiation, experiments were carried on on an extensive scale with flies carrying tinged eyes and forked

bristles. Both larval and adult stages, and both sexes, were rayed, in different divisions of the experiment, and an approximately equal number of non-treated flies were bred in the same way, as controls. In all, 8 cases of mutation to or towards non-forked (7 being of certainly independent origin) were observed among progeny of treated flies, and none among progeny of controls. In 7 of the cases, the mutation involved the germ tract of the progeny in whch it was found, and in the other case (derived from treated sperm) the mutation involved part of the soma only, being of a "fractional" nature.

13. Tests of the 7 heritable mutations showed all of these to be "point mutations" that had occurred at the locus of forked; they were therefore true reverse mutations of the forked gene.

14. The chance of obtaining all 7 independent reverse mutations in the treated and none in the control group would have been only 1 in 128 if the irradiation had not been effective in producing these mutations.

15. Similar experiments were then undertaken with stock of tinged non-forked that had been derived by reverse mutation from forked, to determine whether this could be caused to mutate again to forked. Two inheritable mutations to forked and two "fractionals" confined to the somatic tissue of the flies showing them (the latter derived in both cases from treated spermatozoa) were observed in a count of 58,817 progeny of treated flies. None were found in 34,798 controls. In previous irradiation experiments three other mutations to or towards forked had been detected among 12,482 progeny of treated males, and none in over 10,000 controls. The results suggested, though they did not prove, that mutations from non-forked to forked may be more readily obtained by treatment of the adult males than of the larvae, and that mutations from forked to non-forked may be more readily obtained by treatment of larvae than of adult males. Both these results would be expected if there is a "germ cell selection" among primordial and gonial germ cells, by reason of a faster multiplication rate of non-forked than of forked-bearing cells.

17. Tests of the above two heritable mutations from the later experiment, and of the three other mutations to or towards forked that had been found previously in the progeny of irradiated flies, showed all of these to be allelomorphic to the known forked.

18. The occurrence of these five heritable and two fractional (probable) mutations to forked in radiated material, when considered in connection with the extreme rarity of such mutations in non-radiated material, furnishes convincing evidence that the irradiation was the cause of the mutations in this direction also.

19. The above demonstration that mutations can be produced by irradiation in both of two opposite directions at the same locus, and that, in fact, a cycle of mutational change can be completed, is irreconcilable with the view that all mutational changes by X-rays consist of losses.

20. There are also grave objections cited in the text against interpreting these results as involving mere increases and decreases in the amount of genic material at the locus in question, or as involving displacements of portions of the chromatin. The non-quantitative relationships shown by the multiple allelomorphs produced after irradiation at the locus of white and possibly at that of scute bear witness to the same conclusion.

21. Comparisons of the viability of flies carrying forked with those carrying non-forked that were produced from this forked showed no significant difference in this respect. There was, however, a noticeably higher speed of development on the part of the non-forked flies. In this sense, the X-ray mutation to non-forked had caused an increase in vigor.

22. These results in general lead to the same conception as arrived at by consideration of the nature of the physical action of radiation, namely, that the induced point mutations are changes in the chemical composition of the genes, that they may be of varied kinds, and that they probably are, through the possibility of the accumulation of such changes, endless in their eventual potentialities. In other words, so-called "progressive" mutations can probably be produced by artificial irradiation in cases where there is the possibility of their occurring at all.

APPENDIX

Results of crosses of apparent reverse mutants derived from treated larvae.

Reverse mutation No. 2

This tinged female fly was mated to a forked Bar male. She gave 17 forked Bar females, 11 Bar females, 9 tinged forked females, 3 tinged females, 17 tinged forked males, and 21 tinged males. These results show that she was heterozygous for forked, and that she had been fertilized by a tinged forked male before the cross to the forked Bar male was made. Tinged (non-forked) males from the above count were crossed to virgin Bar females from stock of Bar, and yielded 31 Bar females and 32 Bar males. The resulting Bar females were then backcrossed to tinged forked males from stock, yielding the count shown in the top row of table 11.

Reverse mutation No. 3

This tinged female was mated to scute garnet forked outstretched males. She gave 20 tinged females, 10 tinged forked females, 25 tinged

males, 24 tinged forked males, 9 wild type females, and 4 forked females. These results show that she was heterozygous for forked and had been fertilized by her brothers. Tinged males (non-forked) from this culture were then crossed to virgin forked Bar females and yielded a count of 49 Bar females and 56 forked Bar males. The above Bar females were then backcrossed to tinged forked males, yielding the count shown in the top row of table 12.

Reverse mutation No. 4

This tinged female was mated to a forked Bar male, and gave 29 Bar females, 30 forked Bar females, 24 tinged males, and 18 tinged forked males. She was therefore heterozygous for forked, and virgin at the time of mating. Tinged males from the above count were then crossed to virgin Bar females from stock of Bar, and yielded 24 Bar females and 20 Bar males. These Bar females were then backcrossed to tinged forked males, yielding the count shown in the second row of table 11.

Reverse mutation No. 5

This tinged female was crossed to forked Bar males, and gave 31 Bar females, 32 forked Bar females, 11 tinged females, 6 tinged forked females, 27 tinged males, and 34 tinged forked males. She had been fertilized by her brothers, and was heterozygous for forked. The F_1 females, heterozygous for tinged, Bar, and forked, were backcrossed to tinged forked males, and gave the results shown in table 12, row 2.

Reverse mutation No. 6

This tinged female was mated to forked Bar males, and gave 28 Bar females, 28 forked Bar females, 28 tinged males, and 24 tinged forked males. She was therefore virgin at the time of mating, and was heterogygous for forked. The same test for crossovers was made as for No. 5 (see table 12, bottom row).

Reverse Mutation No. 7

This tinged male was mated to Bar females, and gave 82 Bar females and 98 Bar males. Females from this stock, heterozygous for tinged and Bar, were backcrossed to tinged forked males, giving the results shown in table 11, bottom row.

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