FURTHER STUDIES ON THE NATURE AND CAUSES OF GENE MUTATIONS

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RECENT WORK ON THE CAUSES OF MUTATIONS

The problem of the induction of mutations by irradiation

Possession of the capability of transmuting the gene brings with it the obligation of attempting to find an explanation of how this transformation takes place. At first it seemed quite an understandable result—even one to have been anticipated—that high-energy radiation should change the gene. For the atoms of genes cannot be immune from activation either by the X-ray quanta themselves or by the fast-moving electrons released by the latter, and such activation should, in the case of some of the atoms at least, be the prelude to chemical reactions which alter the composition of the molecules in which these atoms lie. It was tempting, especially for the physicist, to believe in this relatively simple explanation of the induced mutations, particularly since the work of HANSON, HEYES and STANTON (1929, 1931, 1932) and of OLIVER (1930a, 1932), corroborated by that of STADLER (1930), of SEREBROVSKY (1930), of TIMOFÉEFF-RESSOVSKY (1931b), of PATTERSON (1931), and of others, demonstrated so clearly that the frequency of the induced mutations is directly proportional to the total energy absorbed from the high-energy radiation, regardless, within wide limits, of its distribution in time and of the size of the individual quanta. These findings showed that the impacts of the released electrons must be the primary causative agents in producing the mutations, and it was easiest to think that the latter were always the direct results of the former. No doubt they are, in some cases; that is, it is difficult to believe that any gene can be so stable that no well-directed electron can produce a permanent change in it. However, later results give us reason to conclude that the induction of some at least of the mutations is a less direct matter which, though tracing back on the one hand to the electrons, also involves an intermediary course of events, dependent somehow upon the peculiarities of the biological system. The series of problems is as yet far from being solved, yet I believe it will be useful to present them at this stage, for consideration and further experimentation.

Interdependence of chromosome breaks

The clearest series of facts comes to light in connection with a study of chromosome breaks, which I believe may have some significance in the

study of gene mutations also. In my first work on induced mutations (1927), I showed that the "mutational" effects of irradiation fall into two main subdivisions, namely, changes of individual genes—"gene mutations"—and rearrangements of genes, due to breakage of the chromatin, often followed by reattachment of one or more of the pieces in a new order. It was natural to suppose that these two phenomena were interrelated. Since the genes form a chain, the chemical alteration of a gene might at times be a matter entirely confined to the individual link, and it might, on other occasions, by destroying or breaking the link or its connection with an adjacent link, result in a breaking of the whole chain. According to certain results of ALTENBURG and myself (1930), the two effects ordinarily occur with similar frequency, and OLIVER (1932) has obtained evidence that the frequency of the induced rearrangements, like that of the gene mutations, is proportional to the energy absorbed. Let us then examine further into the mechanism of production of these rearrangements in the hope of throwing further light on the problem of the gene mutations. In so doing, we come upon a significant series of facts in which, hitherto, the forest has usually been ignored on account of the trees.

In the study of the frequencies of induced translocations above referred to (MULLER and ALTENBURG 1930; submitted 1929) it was suggested that these translocations might frequently involve breakage of both chromosomes concerned, since otherwise it was difficult to explain why the smaller chromosomes should serve less often than the larger for the attachment of translocated pieces of other chromosomes. As more and more cases of translocations have been analyzed, this supposition has been confirmed. Since the finding of the first mutual translocation in Drosophila ("Swoop," in 1929—see MULLER 1930a and b), more and more of the induced translocations have been proved, on analysis, to belong to this category. Thus, of five translocations between chromosomes II and III, analyzed by DOBZHANSKY and STURTEVANT (1930, 1931), four (all the induced ones) were shown to be of the mutual type. OLIVER (1930b, 1932), GLASS (1932), working at the Texas laboratory, and VAN ATTA (1932), working with OLIVER at WASHINGTON UNIVERSITY, have made similar findings for induced translocations involving X with III, and II with III, and BURKART (1931, 1932), in STERN's laboratory, supplemented by OFFERMANN in Texas, has found a spontaneous mutual translocation of X with II (called "Blond"). In at least one of OLIVER's cases, where the translocation itself was not mutual, there was nevertheless evidence of a mutual breakage, since a piece broken off by one chromosome seemed to have become inserted into a gap made by breakage of the other chromosome.

It might be thought that at least those translocations which involved the tiny fourth chromosome with another chromosome might be cases where only one of the chromosomes—the larger—had been broken. As a matter of fact, breakage of the fourth chromosome would usually be very difficult to demonstrate in such cases, even if it had occurred, and so most translocations involving the fourth, reported by myself and PAINTER (1929), by DOBZHANSKY (1929, 1930b, 1931), and by PATTERSON (1932a) may or may not be of this type; the data so far given do not bear on this question. We have, however, at our laboratory, made several studies of the matter on translocations involving the X and IV, which are somewhat better adapted for a solution. Of the three cases studied, one, "X-IV 3," investigated by BOLEN and myself, is clearly a mutual translocation in which the little chromosome, IV, has itself been broken and a piece of it exchanged for a piece of the X, which was also broken (BOLEN 1931). Another, "X-IV 1," has been found by OFFERMANN (see OFFERMANN and MULLER 1932) to involve a breakage of the fourth chromosome together with an insertion, into the gap thus made, of a piece deleted from the middle of the X; the latter chromosome, which had been broken in two places, had its two terminal pieces joined together. The third translocation of X and IV studied, "X-IV 4," is as yet doubtful, though certain peculiarities of its behavior, found by STERN (1931), suggest a mutual breakage.

I do not mean to insist that all induced translocations involve mutual breaks; in fact, there are certain phenomena which indicate that this is not a universal rule. Thus, in the induced translocation accompanying scute-19, it has been found by LEAGUE and myself that although the left end of the X is attached at or near the left end of the genetic map of II, there has been no transfer of material from II to X (at least, not of any active region), since flies are viable that are homozygous for the chromosome II of this translocation but contain only a normal X or X's. If a piece had been removed from II, such flies would completely lack that piece. It is still possible, but dubious, to assume an insertion very near the end of II. A similar case is that of "II-III 26" (of PAINTER and MULLER 1929). BRIDGES' original spontaneous translocation (see HAMLETT 1926) and the spontaneous case described by DOBZHANSKY and STURTEVANT (1931) also appear to involve but one break, with attachment of the resulting fragment to the side of another chromosome. However, in the face of the above findings, including those concerning chromosome IV, in which they are to be least expected, it is evident that the great majority of translocations conform to this rule. Furthermore, in the great majority of cases of induced translocations in which we have been able to get any evidence on the ques-

tion, it has appeared that the fragments have become united together at their mutual points of breakage, rather than by any of their originally free ends, or by an attachment to the side of another chromosome.

To parallel the above facts concerning translocations, we have a similar series concerning inversions—that is, evidence that they usually involve two breaks, with a reattachment of the pieces at their points of breakage although in a different direction with regard to each other than the previous one. Of the five inversions (all spontaneous) analyzed by STURTEVANT (1926, 1931) and GRAUBARD (1932) four could be shown to involve two breaks; it is assumed that in the fifth (C_{IIIE}) one of the breaks is located near the end of the chromosome, beyond the farthest marker used. The comparison of *D. simulans* and *melanogaster* made by STURTEVANT and PLUNKETT (1926) also shows that a two-break inversion must have occurred in one of them in its evolution. We have analyzed one spontaneous and three induced inversions of the X (*"ClB,"* "♂ 49," *"ClB* reinversion," and "w^{m5}," respectively) sufficiently to determine this question in regard to them, and find them all to be double-break inversions, while the findings of SEREBROVSKY and of LEVIT on the induced scute-8 inversion show these also to have double breaks. Evidently the only difference between most inversions and translocations is the more or less accidental one that in the former the two points of breakage and exchange happen to be on the same chromosome, in the latter on different ones.

Deletions, including many of those less extensive genetic changes hitherto known as "deficiencies" of inner segments of a chromosome—which we now have every reason to regard as small deletions (see page 232),—conform to the same rule. That is, they too involve two breaks, with reunion of the pieces at the point of breakage, only it happens that in these cases, unlike the inversions, the new junction is between the terminal pieces. Apparently it is more or less a matter of chance which broken ends unite with which, so long as the junction is between one broken end and another one.

The above array of findings regarding translocations, inversions and deletions leaves only one alternative to the conclusion that the occurrence of one break tends to be associated with that of another one. That alternative is to suppose that the breaks occur independently of one another, but that only in cases where two breaks have happened to occur does reattachment usually take place. It is agreed that attachment must ordinarily be by the adhesive broken ends, and it is concluded that in the cases in which reattachment fails to occur the fibreless fragment is lost and the resulting aneuploid individual is usually inviable. This hypothesis would also require the assumption of

some as yet unknown mechanism to enable these four distant broken ends to find each other, two by two—either some force of attraction at a distance or a sort of very thorough groping movement.

The alternative hypothesis above depicted breaks down in the face of the evidence that the frequency of gene rearrangements varies approximately in proportion to X-ray dosage, and probably not as rapidly as the square of the dosage. The frequency would vary more nearly as the square of the dosage if the rearrangements required the coincidental occurrence of two independent events (the two different breakages), each of which separately varied as the dosage. Such a mode of frequency variation seems irreconcilable with the results of OLIVER (1932) above referred to; the results of MULLER and ALTENBURG (1930), though not so critical in regard to this question, also speak strongly against it. We would also expect some traces of such an effect on the general frequency of mutations (*including* those connected with rearrangements), if the square rule held true, whereas the studies of HANSON and of OLIVER on mutation frequency show a strict, simple proportionality with dosage, with certainly no excessive increase at higher dosages. As direct evidence against the idea of independent breaks we may also mention a study of LEVIT's (unpublished), which indicated that (although in some regions "simple" breaks occurred readily) in a certain region of the X (of scute-8 inversion) there were actually fewer simple breaks than breaks occurring concomitantly with breakage in another specified region.

If, now, the two breaks involved in a rearrangement are not independent, we must conclude either that one break somehow acts to induce another one, or—what seems *a priori* more likely—that both are due to a common cause. In either case, the localization of the effect (its confinement to two given spots) would practically require (in consideration of cell structure and mechanics), a spatial propinquity of the two chromosome threads concerned. This brings us, in essentials, back to the interpretation which SEREBROVSKY in 1929 offered for translocations and inversions, which was extended by DUBININ to deletions. SEREBROVSKY and DUBININ advocated the interpretation (which we also had had in mind as a possibility) that where the chromosomes crossed each other, no matter whether the same or different chromosomes, there, under the influence of radiation and sometimes of other circumstances, they were likely to stick together and then to become broken apart in such a way that the pieces came to lie in a different order than they had before. This would mean, in many cases, that a double break and exchange of parts occurred, the pieces becoming reattached at their broken, not their free ends.

There are still certain difficulties in this scheme, especially that of explaining how the insertion into one chromosome of a piece deleted out of another would take place, for this would seem to necessitate the rare coincidence of three strands meeting at exactly the same point. Moreover, we cannot at all agree with SEREBROVSKY's extension of this hypothesis to account for gene mutations as being simply deletions or additions of so minute a portion of chromatin as to be classified as a single gene. The reasons for rejecting this have been given in a previous publication (PATTERSON and MULLER 1930); and certain minor modifications may also be required—it may be, for instance, that union does not occur, or is not completed, before breakage, and it is highly unlikely that a piece can be mechanically cut out of the side of a chromosome, sausage-like, if the persistent structure in the chromosome is a thread-like chromonema, containing a single-file string of genes. Yet in view of the undoubted tendency for association of one break with another, far beyond what chance would allow, and for a union between the broken ends formed by these breaks, we are practically forced to adopt the essentials of SEREBROVSKY and DUBININ's general scheme, in so far as gene rearrangements involving reattachment are concerned.[1]

In adopting this scheme, we accept the principle that the rearrangements occur by a process which is virtually crossing over, except that it is between non-homologous regions and at a time other than the proper synaptic period. The irradiation has somehow (possibly by de-charging them) done away with the repulsion which normally holds chromosome strands apart from one another, and it has allowed the crossover mechanism to operate illegitimately.

It may be noted in passing that the existence of this effect renders quite unnecessary and improbable the hypothesis of BELLING, which seeks to ex-

[1] Certain further tests await the hypothesis. Among these is the determination of whether, in the case of two or more breaks, there is always an *exchange* of attachments. Suppose, for example, that the chromosome or chromosome-region containing the chain of genes *A B C D* broke between *B* and *C*, and that chain *L M N O* broke between *M* and *N*. If, now, sections *A B* and *L M* become attached together at their broken ends, forming a sequence *A B M L*, it is necessary, on the above hypothesis, that if *C D* becomes reattached at all, it becomes attached to *N O*, making the sequence *D C N O*. If *C D* or *N O* became attached at the side or at one of their free ends (forming *C D N O* or *D C O N* or *C D O N*), or if *C D* became attached by its broken end to the segment formed by a *third* breakage (forming *D C S T*, for example), some modification of the above hypothesis would be necessary. In the latter case, however, caution would be required in order to make sure that *C D* had not become broken again (say, between *C* and *D*), that is, that the junction with the segment *S T*, from the third break, was really at exactly the first point of breakage in question (forming *D C S T* rather than *D S T*). This matter is also being discussed by GLASS (1932). Such cases await future analysis.

plain normal crossing over without the assumption of breakage and reunion of chromonemata. To avoid the assumption that breakage and reunion are possible, he assumes instead that, in the propagation of genes, the daughter genes are at first unconnected by strands, and that the new strands, in growing out, may, in places, make cross connections between homologous chromosomes instead of intra-chromosomal connections. Our facts, however, show that the chromosomes do possess the potentiality of segmental interchange by breakage and reunion, since this can occur, under irradiation, even between non-homologous parts, at a period (the mature sperm) when there is no growth or synapsis. It becomes highly probable, therefore, that during the intimate union of homologous strands at synapsis this power of breakage and reunion is exercised normally.

For our present inquiry, however, the more important point in our conclusion is the following: The locus of breakage of a given thread, under irradiation, is determined somehow by its relation and its proximity to another thread, and the breakage of both threads accordingly has a common cause. On considerations of size it is easy to show (see discussion below on striking of genes by electrons) that there is no appreciable chance of a chromosome that is touching another one being struck and broken by the same electron as the latter. With all doses ordinarily given, the chance of its being struck by other electrons is much greater. But we have already seen that the breakage of the two chromosomes cannot be a result of two independent hits. Therefore the points of breakage (or at least of one of the breakages) were determined rather by the relation of the biological structures than by the exact paths of incidence of the electrons. The passage of the electrons in general, or of some one particular electron, must have provoked some more diffuse train of reactions, which in turn made possible, or facilitated, the breakage and reunion at the point of crossing of the threads. The occasional occurrence of similar phenomena at one jump even in non-treated material (observed in the case of the *ClB* inversion, the Blond mutual translocation, et cetera), where natural radiation was negligible, fits in with this conclusion.

Breakage, then (or at least the "second" breakage), is not determined by the fact that an electron has chanced to hit the broken chromosome thread particularly accurately. Now what bearing has this upon gene mutations? Simply this. If breakages can be proportional in frequency to the absorbed radiation, and can nevertheless be produced otherwise than by direct hits, then we have no right to assume that gene mutations, which likewise occur at more or less isolated points, and which obey the same frequency law in

relation to dosage as the breaks, are probably due to direct hits either. In fact, we should rather draw the contrary conclusion, that they, like the breaks, are probably *not* usually caused by direct hits but by a somewhat less direct action of the radiation.

On the relation between gene mutations and rearrangements

We have next to consider some evidence bearing more directly on the question of a relationship between the manner of origination of gene rearrangements and of gene mutations. Some years ago I noted the fact that the majority of induced translocations were associated with either dominant or recessive lethal or other phaenotypic effects; the same has proved true also of our induced inversions. The principle has held also in all the four cases of spontaneous rearrangements in which our knowledge of the time of origination of the condition was sufficient to indicate whether or not the two effects arose simultaneously. These comprise: the "Pale" translocation (BRIDGES 1919), the *ClB* inversion (MULLER 1922a), the "Blond" translocation (BURKART 1931), and translocation "II-III E" (DOBZHANSKY and STURTEVANT 1931). Spontaneous mutations are so rare that these cases cannot possibly represent coincidences. There are three more or less alternative possibilities to account for this association (MULLER 1930b, MULLER and ALTENBURG 1930), namely:

(1) That one or more genes directly at the point of breakage had become destroyed or altered in the process of breakage (this generalizes upon BRIDGES' proposal that in his "Pale" translocation not quite all of the piece that had been broken off became transferred, but that a small portion was lost [BRIDGES 1923].)

(2) That a gene mutation had simultaneously occurred at another locus, linked to that of the breakage.

(3) That the phaenotypic change was a result of a different mode of expression of the gene, dependent on its being in the proximity of different gene-associates, which influenced its kind or degree of activity. In this latter case, as I pointed out (MULLER 1930a), a reestablishment of the original gene order would automatically reestablish the normal phaenotype. (Likewise a recurrence of the same rearrangement would always reproduce the same phaenotypic change.)

My earlier inclination was rather toward what appeared to be the simplest view, which was comprised under (1), that is, that a gene directly at the breakage locus had been changed in the process of breakage itself. Now, however, evidence has accumulated to show that genes not directly at the breakage locus, even though very near by, can also become changed, while

at the same time loci *between* those of breakage and of the affected gene may not be changed perceptibly. This bespeaks either (2) a permanent gene mutation separate from, though no doubt somehow associated in its origin with, the break, or (3) a position effect.[2] I do not wish to deny the possibility of a position effect. Whether or not such an effect commonly occurs is a matter of great importance in its bearing on the mode of action of the genes and on evolutionary possibilities; it should, moreover, be capable of solution in the near future. I believe, however, that the third alternative, that of a real gene mutation, is more probable as an explanation of the phenomenon here in question, especially in consideration of related phenomena.

As an illustration of the phenomenon, I may refer to the case of a deleted X chromosome (MULLER 1930b), in which the left break was just to the right of the locus of facet, which is at 3.0. In this same deleted X there was a "mutation" of the normal gene at the locus of white (1.7) to a mottled allelomorph ("w^{m3}"). The genes that had mutated could be determined by getting the deleted X into flies that had in their entire X or X's recessive mutant forms of these same genes; if the dominant normal allelomorph was still present in the deleted X, the recessive allelomorph would then be "covered," that is, the phaenotype would be normal in that respect. In this case, facet, nearer the break, was "covered," but white, though further away, showed as a white-mottled compound.

Again, in another of my deleted X's (which I have called number "24"), the left break was found to be slightly to the right of the locus of a certain lethal ("l_{J1}"), but (by our usual tests) to the left of broad (locus 0.5). Now the deleted X "covered" the lethal; nevertheless, when tested with scute allelomorphs it proved to have a very pronounced "mutation" at the locus of scute, which is still further to the left of the break than the lethal is. The loci in question are, however, all so close together that their seriation would not have been discovered if a new method of analysis, involving the use of broken chromosomes, had not been available. The crossing over test would have been inapplicable, owing to the minute distance involved.

[2] DOBZHANSKY (1932) has recently espoused the last interpretation, that of a "position effect," mainly on the basis of GERSHENSON's (1931) supposed finding that an exact reinversion in the *ClB* chromosome abolished its lethal action. I have examined the data in question critically, and, in the light of a definitely analyzed prior case of my own (MULLER and STONE 1930) believe it far more probable that the former reinversion, like the latter, was not an exact reinversion, and that the apparent abolition of the lethal was caused later by ordinary crossing over. Coincident with the present paper, DOBZHANSKY and STURTEVANT (1932) adduce further data which they consider to support the "position" interpretation. A detailed study of such cases, for decision between the two possibilities, now becomes urgent.

It may be of interest in this connection to give a brief account of the method of analysis here used. A certain translocation which arose simultaneously with mutation scute-19 (found by LEAGUE and analyzed by LEAGUE and MULLER) involves a break close to the right of the scute locus, the left end of the X (containing sc^{19} itself) being attached at or near the left end of II. It is found that this left-hand fragment, when present as "an extra piece," fails to suppress the lethal action of lethal "l_{J1}." On the other hand a hypoploid female having l_{J1} in one X chromosome and having, as its other X, the right-hand remainder of the X from which sc^{19} was detached, but not the left-hand fragment, has l_{J1} covered (that is, it is able to live). Hence l_{J1} is to the right of the break of the sc^{19} translocation. Hence too must be to the right of scute. Now, deleted X 24, when present as an extra fragment, covers l_{J1}; that is, it allows males carrying l_{J1} in their entire X to live. Accordingly the left break of deleted X 24 is to the right of l_{J1}. Nevertheless, deleted X 24 carries an extreme scute allelomorph, which virtually fails to cover any part of the scute character except the so-called "left-hand" part (the dorso-central bristles).[3]

As in the case of the "w^{m8}" containing deleted X, then, so here also, we have an apparent gene mutation occurring at a perceptible, though very minute, distance from the break, with at least one gene between showing no noticeable change. Unless we accept the very improbable, and ascribe their simultaneous origin to mere coincidence, we must admit that two separated phenomena, a break and a gene mutation (or what is in effect a mutation), have both been engendered by a common cause, with a highly localized, yet by no means punctiform influence. Ordinarily, in such cases, we should not have the means of discriminating between the loci of the mutation and of the break, and we should say that the former happened "at" the locus of the latter. But the presumption now is that the ordinary cases of simultaneous mutation and breakage are in essentials like the cases here analyzed.

Consideration of various scute allelomorphs found by the Moscow geneticists and others is of interest in connection with the problem of the nature of gene mutations associated with breaks. Many of the scute mutations—

[3] See AGOL (1930, 1932) for a discussion of this deleted X in relation to the problem of scute. It was natural at first to suppose that the break of this deletion had probably occurred within the scute gene, and only after an analysis of its relations and those of the sc^{19} translocation with l_{J1} could the surprising finding of a double genetic change, separated by a distinct distance, be made. On the other hand, STURTEVANT and SCHULTZ'S criticism (1931) that the effect of deleted X 24 on the scute character could be due to hyperploidy in regard to other loci than scute was (even before the criticism) proved by AGOL to be incorrect.

like those of sc^{19} and of the deleted X 24 noted above—involve breakages at or near the scute locus. Some involve inversions, in which the scute locus has been removed from the genes normally to the right of it and has been placed near a portion of the inactive right-hand region of the X, that I have found to be homologous with the Y. Yet in these cases the scute mutations were different from one another, and some resembled closely, though not exactly, other definite scutes that had occurred without detectable gene rearrangement. Similar facts have been found in the cases of other loci.

If, now, we decide to consider the mutations arising in connection with breaks as true "gene mutations," then we must conclude that, since the position of a break is not decided by the direct hit of an electron, the position of the gene mutation is probably not so decided either. If it is decided by some other local disturbance—the same as that which produces the break—then it might sometimes happen that this disturbance, instead of producing a break and a gene mutation, produced two gene mutations (or a multiple group). This contingency makes it possible for our problem to be studied from another angle.

On the connection between one gene mutation and another

The question of the possible simultaneous occurrence of two gene mutations, that are expected often to be in close propinquity to one another, is rather difficult of approach, partly because of the fact that most detectable gene mutations in Drosophila are lethals. One means of partially avoiding this difficulty is to look for newly arisen visible mutations, by backcrossing the treated individuals to others which already have visible recessives at certain chosen loci, and then to test the visible mutations thus found to determine whether they have lethals near-by. In one such experiment (see PATTERSON and MULLER 1930), two visibles occurred at a locus under observation, one spineless and one scarlet, and both were somehow connected with lethal effects.

The mutation first named (spineless) proved to have its lethal effect inseparably connected with the visible. Many cases similar to this have been found, both before and since this experiment (see, for instance, PATTERSON 1932c), though such an extensive test for possible crossing over between the visible and lethal has not usually been made. Such cases, including the present case of spineless, are, on *a priori* grounds, open to any one of three possible interpretations. The first is that the lethality is one effect of the same gene as causes the visible result; this is undoubtedly true in some cases, such as, for instance, broad-lethal, and truncate. The second interpretation

is that a real deficiency, that is, a small deletion, has occurred, removing more than one gene; proof of this requires the condition rather seldom met that two or more *known* genes be involved. The third interpretation is that there has been a simultaneous mutation of two very closely or completely linked genes. This last possibility has usually been ignored.

That the last mentioned alternative has a just claim to consideration is indicated by the case of the second visible mutation found in the experiment in question—that of scarlet. This scarlet also seemed to act as a lethal, but after extensive trials it was found possible to secure homozygous stock of the scarlet separately from the lethal, and to establish the fact that one-tenth of one percent of crossing over occurred between the genes for these two effects. The small amount of crossing over was due to propinquity, not to some chromosome abnormality, since known third chromosome genes were proved in this case (and also in that of the spineless) to cross over with their normal frequencies. Now the chance that, with the dose of X-rays used, a lethal should have arisen at the same time as a given scarlet, in such close proximity to it, if the two mutations had been independent events (that is, the chance for a mere coincidence), is considerably less than one in a thousand. In other words, among over a thousand cases of scarlet produced in this way, only one should have another lethal so close by. Thus we should hardly find another such case among a thousand visibles detected by this method, if the case has no significance.

If, however, there is such a tendency for double mutations, and they are commonly in extremely close propinquity, it may be difficult to get evidence on the question, since the crossover test for their separateness may usually fail (see the possible case of spineless above). Fortunately, for the scute locus we have another test than that of crossing over available, a test which is sometimes capable of deciding this question with a finer discrimination. This method is the same as that which was employed in the analysis of deleted X 24, namely, the use of chromosome fragments to determine whether or not a given gene is "covered." It happens that in the scute-19 translocation the X chromosome is broken very close to the right of the scute locus (the left-hand fragment being attached to II). If now, in another scute mutant, having a lethal effect, the lethality is due to another gene, somewhat to the right of scute, then hyperploid males, containing this double scute-and-lethal mutation in their entire X, and possessing also the left-hand fragment from the scute-19 translocation, will have only their scute gene "covered" (by the sc^{19} allelomorph), but their lethal uncovered. They will therefore fail to be viable. On the other hand, females of the converse composi-

tion, having the scute-and-lethal in one X, and having as their other X only the right-hand part of the scute-19 chromosome, without the left-hand fragment, will have their scute quite uncovered, but their lethal covered. They will therefore be viable. The two results, checking each other, will prove that the lethal is at a separate locus, to the right of scute. If the lethal had been either at or to the left of the scute locus, the viabilities of both of these classes would have been reversed. If the lethal proves to be to the right, however, then by using deleted X's or translocated fragments of different lengths, and determining which ones "cover" the lethal, the locus of the latter may be ascertained more exactly, even though it be so close to scute as to give no appreciable crossing over.

An experiment was accordingly undertaken which had as one of its objects the finding of mutations of (yellow and) scute which might at the same time be accompanied by a lethal effect. This involved irradiating (non-yellow) non-scute males on a large scale and crossing them to (yellow) scute females; numerous female offspring were then examined for manifestations of the recessive genes and bred to determine whether a lethal effect was simultaneously present. (I wish to acknowledge the extensive help of JESSIE JACOBS-MULLER in this work. Scutes found by her are designated with the superscript *J*, as sc^{J1}, sc^{J2}, etc.)

It appears from the work that a fairly high proportion—perhaps more than a quarter—of scute mutations are lethal, or are connected with a lethal. The relation of the lethal to the scute gene has now been determined in several of them by the method outlined above. It appears that in the very first scute found in this experiment—scute-J1—a lethal arose simultaneously with the scute mutation, but at a different locus, just a little to the right of scute, but so close as to have given no crossovers. This lethal was in fact the lethal l_{J1}, previously referred to, which has been of help in the analysis of deleted X 24. Likewise in scutes-J4, J6 and J7 a lethal or semi-lethal arose simultaneously with scute, to the right of it. In some of these there was also a gene rearrangement; this part of the analysis, which is important because of the possible explanation of such results as "position effects," is not completed.

There are from a fifth to a tenth as many visible mutations as lethals induced by irradiation in the X chromosomes of Drosophila (see MULLER 1928b). Therefore, if there is a tendency for one gene mutation to be accompanied by another near-by, some of the induced scute mutations may have other visible effects besides those commonly ascribed to the scute locus, and even though closely or completely linked their genic separateness might

be demonstrated by the same test with scute-19 as was above applied in the case of lethals. To test this possibility, the scutes then available—about a score—were examined for other effects, and it was found that scute-10 (DUBININ), sometimes known as achaete-2, did exhibit another peculiarity—a disarrangement of the ommatidia—which behaved as completely linked with the scute. Tests with scute-19 and with deleted X's then showed that the eye abnormality was due to a different gene than scute, to the right both of scute and of the lethal, l_{J1} that had been found in connection with s_o^{J1} but to the left of broad. It can easily be reckoned that, on the principle of random sampling, the chance was less than one in a hundred that another visible mutation should have occurred in any of these scutes at all, in a locus less than half a unit away. When we consider this together with all the other evidence given above, there can then be no reasonable doubt of the tendency of induced gene mutations—or at least of mutational effects, if we still adhere to the "position" possibility—to occur in localized groups.[4]

On the chance of double hits by one electron

It is natural to conclude that these probable group mutations are due to some indirect effect of the radiation, dependent on certain peculiarities of the biological system and not to be explained merely on the basis of the general physics of ordinary materials. But we have first to dispose of an alternative possibility (MULLER 1928a). This is the possibility that two near-by mutations (including, in some cases, breaks) may be caused by the same electron, if the electron happens to have a course approximately parallel to the chromonema and makes an effective hit at two near-by spots in the latter. For in cases in which a point, A, is known to be hit, it is more likely that a point B, near-by, will be hit than in cases in which point A is not hit, for in the first class of cases we know positively that at least one electron did pass near to B, while in the second class of cases there is only the usual (or rather, somewhat less than the usual) chance of such electron passages.

This increased likelihood of a hit at a point a given distance away from

[4] The contingency must also be borne in mind that in some cases of double gene mutations (as of chromosome breaks) the two mutations may be causally related in their origin (owing to some transitory proximity?) and may nevertheless be far apart in the actual chromosome map. More than suggestive in this connection is the finding (see MULLER 1928c, and PATTERSON and MULLER 1930, pp. 591-593) of a double visible mutant—spectacled, and reversed-forked—involving two distant loci. There were only three demonstrable mutations (including these two) in a count of 2651 flies, yet two were in the same specimen! The chance of such a coincidence is only 1 in 883 if the events were unconnected.

a first hit is a matter that can readily be calculated to a sufficiently close degree of approximation, inasmuch as we know the approximate number of fast moving electrons released in a given volume of known material by a given dose, and also the approximate length of path of these electrons. It will be understood that the stronger the irradiation, the greater will be the chance that point B should be hit anyway, even when point A is not hit, and so the shorter will be the distance from A at which the fact of A being hit will cause a noticeably increased chance of B being hit. Doctor L. M. MOTT-SMITH, of the physics department of the RICE INSTITUTE, and I have made the requisite calculations on this matter, and we find that, with the heavy doses of X-rays used in genetic work, the increased likelihood of a second hit could extend noticeably for only a very minute distance, of the order of size of the molecules of relatively simple, inorganic substances. If, then, the group effect observed for gene mutations and breaks is simply an expression of the path of a single electron, the genes would have to be far smaller than any one had imagined, or else packed together like pancakes with their shortest dimensions length-wise of the chromonema. This would, however, probably make the chromonema too short, unless the genes were packed in groups, with connecting fibres between the latter. There would be additional difficulties in accounting for the fact that *effective* hits came so close together, for by no means every molecule that is hit, in the sense of having an electron pass through it, has its structure affected by that electron. The points along an electron passage at which the electron takes effect by tearing out other electrons from atoms, that is, by ionization, are much too far apart to allow of the group effect in question, and it would have to be supposed that between each two such ionization points there are usually a great number of other hits which, though not causing ionization, nevertheless can readily alter the physico-chemical structure of genes.

As a further test of the "two birds with one stone" hypothesis, I have been carrying on some experiments, in part suggested by Doctor HUGO FRICKE, physical chemist of the CARNEGIE INSTITUTION, Cold Spring Harbor, in which the gamma rays from radium emanation were used instead of X-rays. The electrons hereby produced are much faster. This greatly reduces the likelihood that a given molecule, traversed by an electron, will chance to be changed by it. Hence the distances between effective hits are much greater, under this treatment, and the group effect on mutations should be so reduced as to be imperceptible, if this effect really results from double hits by one electron. Analysis of the results is as yet incomplete, yet it is noticeable that about as many of the visible mutations in this experiment were accompanied by lethal effects, as when X-rays were used; the genes

in question were mainly yellow and scute as before. Hence it seems at present probable that the group effect occurs after gamma ray irradiation also, and that it is not due to "double hits." If, now, we conclude that one of the two mutations in a group mutation was not caused by a direct hit, there would seem to be little need to assume that the other one was caused by a direct hit either. Thus the idea of a somewhat less direct mechanism of mutation production would be strengthened. (We wish here to thank the RADIUM EMANATION CORPORATION, and Doctor SPERTI, for supplying us with this emanation free of charge, at the instance of the Committee on the Effects of Radiation on Living Organisms, of the NATIONAL RESEARCH COUNCIL.)

Incidentally, if the above is true, it follows as a corollary that we cannot use the likelihood of mutation in given genes at given doses to measure the size of these genes—or even of some portion of the genes, assumed to be sensitive to hits—as was done by BLACKWOOD, and in some independent calculations of MOTT-SMITH and myself (unpublished).

Mutations otherwise induced

If the X-ray effect upon the gene may be exerted via some intermediary chain of processes (perhaps one in which the touching of chromonemata plays some rôle), then it becomes more likely that other influences than radiation may also be able to induce gene changes by affecting this chain of processes at some point. This is in line with the calculations of MOTT-SMITH and myself (1930), and those independently carried out by TIMOFÉEFF-RESSOVSKY (1931b) and by EFROIMSON (1931), showing that most mutations in untreated material do arise otherwise than as effects of natural high energy radiation. Further, it is in line with the early findings of myself and ALTENBURG (1919, also MULLER 1928c), that a moderately raised temperature, applied over a considerable period, induces an increase in mutation frequency, and that certain unknown factors also cause considerable variation in mutation frequency.

In a later report on the production of mutations (MULLER 1928b)[5] I mentioned briefly that I had tried a new method of temperature treatment for this purpose—namely, the application of heat in semi-lethal doses, which I found to be attained in 40 to 64 hours at 36°C. For it was conceivable that under these abnormal conditions new and more drastically effective chemical

[5] The exact figures were given in a later publication (MULLER 1930a, p. 234), and here attention was called to certain peculiarities of the results, which indicated that factors were at work causing an especially high number of mutations to originate in certain particular individuals.

processes might be set into operation. In this case adult (newly hatched) males were treated and lethals were looked for by the *ClB* method. The work was done on a rather small scale (approximately a thousand F_1 cultures, equally divided among treated and control) but one large enough to show that there was no such enormous raising of the mutation rate as is produced by X-rays. On the other hand, there was a suspicion, not based on statistically significant numbers, that a lesser but positive effect had perhaps been produced, and it was stated that larger numbers would be desirable to settle this question.

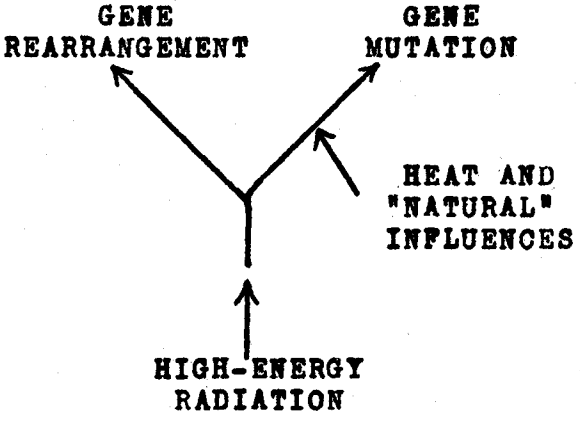
In the well known work of GOLDSCHMIDT, undertaken in the following year (1929), and in that of some workers following him (JOLLOS 1930, ROKITZKY 1930), the same method of treatment was followed, being, however, applied to the larvae. They believe that their results show that in certain cases—not in all—the frequency of visible mutations is raised enormously thereby. On the other hand, the published work of FERRY, SCHAPIRO, and SIDOROFF (1930), and the unpublished results of REDFIELD and SCHULTZ, of TIMOFÉEFF-RESSOVSKY, of DEMEREC and of STURTEVANT, are negative. The question thus arises whether the positive results first reported may not be due either to influences of some other kind, in combination with the heat, or to differences between the control and treated series in regard to the genetic composition of the stocks, the degree of inbreeding practiced, or psychological factors in the operators, affecting the detection of the visible variations.

The *ClB* method, applied to numerous P_1 individuals divided at random into the two series to be compared, is not open to these objections. This year MACKENSEN, in the Texas laboratory, has repeated on a far larger scale my experiment of applying an almost lethal degree of heat to adult males for from one to several days, and used controls which could differ consistently in no other factor than the heat application; as in my earlier work, the *ClB* method was used. His results thus far show a rise (3.6 times its probable error) in the lethal mutation frequency. Considering the relatively short duration of the treatment, this rise seems to be somewhat higher than that caused by an approximately equal amount of temperature difference when the latter occurs at lower temperature levels, more normal to the organism. The rise was, however, far short of that reported by GOLDSCHMIDT and his followers, and quite inadequate to account for the latter.[6]

[6] Decided effects from extreme heat, but effects of a far lower order than those of GOLDSCHMIDT, were also exhibited or otherwise communicated at the Congress by PLOUGH, EFROIMSON, TIMOFÉEFF-RESSOVSKY and GROSSMAN. Certain of these are not yet clear; others would lead to a conclusion similar to that above arrived at.

The above contradictions, as yet unreconciled, call for further research. It may be that, while temperature accelerates the mutation rate, it does so not merely as it accelerates many simple chemical reactions, but according to a curve that rises more sharply at higher temperatures. This would indicate a complicated set of reactions in which more than one process takes part. Thus it raises the hope that chemical treatments may yet be found that will affect mutation frequency despite the rather effective protection of the gene from outside influences shown by the negative results of the trials hitherto made.

In MACKENSEN'S experiments, the mutants were all tested for significant changes in crossover frequency, and it was found that neither those occurring in the controls, nor those in the heated series, were accompanied by gene rearrangements detectable in this manner. Among a similar number of X-ray mutants, a considerable number would have been associated with inversions or translocations that reduced crossing over markedly. Recent



tests of ALTENBURG, specifically designed to discover translocations, likewise show a dearth of these, as compared with gene mutations, in material not subjected to X-rays. It follows that heat and also the influences causing gene mutations in untreated material act differently from high-energy radiation, in that the former produce gene mutations with no where near as high a proportion of gene rearrangements. Yet the character of most of the gene mutations themselves is similar in all these cases, so that we must conceive a similar end-mechanism of mutation to be brought into operation.

Probably then, in the production of mutations by heat and "spontaneously," the influences impinge upon the chain of mutation-producing reactions at a more nearly terminal point than do the X-rays, as the accompanying diagram indicates. (It is a common error to suppose that all

the influences causing mutations are necessarily influences external to the organism. It is true only if taken in the most general and ultimate sense, inasmuch as certain conditions—warmth, food, oxygen, et cetera—are necessary in order that life, metabolism, and inner motion in general may occur at all. But whether or not a given mutation occurs must often be decided by a complicated set of internal "historical" processes, in part of a submicroscopic nature, in the determination of which "external influences," in the ordinary meaning of the term, play little or no part.) This again would imply that the mutation-producing reactions set into operation by irradiation may not always be so simple and direct as an alteration of a gene by an electron hitting it. What the processes involved are is another question. There would seem to be a field of research here which will some day prove fertile.

ON THE CHARACTER OF MUTATIONS

Methods of attacking the problem of whether mutations are merely quantitative changes

Probably some geneticists would welcome the problematical connection between induced gene mutations and rearrangements, and between the latter and chromosome contacts, as evidence for the view that gene mutations, or at any rate those produced by irradiation, are merely due to losses or transfers—the latter in some cases perhaps involving additions—of chromosome material of a type previously present. They would take it as evidence for a presence-and-absence, or at any rate for a quantitative, interpretation of mutational changes. Perhaps they might now extend the interpretation to parts of genes, or sub-genes, in order to account for cases like the scute or truncate series, but, so far as any given kind of gene material was concerned, they would see in the mutation process only a mechanical loss or diminution of the gene, by subtraction of material from the chromosome, or—as they would have to say in the case of some reverse mutations, for example—an increase of the gene, such as might be caused by its overgrowth or by the attachment to the chromosome of homologous material from a sister or homologous chromatid. Further plausibility is lent such a view by the fact that many allelomorphic series do give the phaenotypic appearance of being quantitative in their basis.

Fortunately X-rays provide us with a new tool which helps to shed light on these questions concerning the character of the mutations produced by them and by other influences. That is, we can induce gene rearrangements and so get fragments of chromosomes containing normal or mutant genes at given loci. We can then add or subtract such fragments, creating hyperploidy or hypoploidy, and can thus determine what the effects of changing

the quantity of a given gene material really are. These known effects of purely quantitative changes may then be compared with the effects that were produced by the mutations themselves.

It has sometimes been assumed that one can judge the phaenotypic effect of different quantities of a gene simply by comparison of the appearances of heterozygotes and of homozygotes of the two opposite types, or, as a greater refinement, by comparison of the different grades of heterozygotes in polyploids. However, the situation in these cases is hopelessly complicated by the fact that in the comparison of such types we deal not merely with a difference in the dosage of one allelomorph, but always with a simultaneous and opposite difference in the dosage of the other allelomorph, since we must always reckon with a *substitution* of one allelomorph for the other, when chromosome fragments are not added or subtracted. We cannot legitimately assume in advance of the evidence that either the one or the other allelomorph is a mere absence, and so we cannot tell to what extent the observed effects may be due to the changed dosage of the one, to what extent to that of the other allelomorph, or to an interaction process. For example, in a comparison of the homozygous eosin-eyed Drosophila, the intermediate colored eosin-white compound, and the homozygous white, it need not be assumed, *a priori,* that the eosin gene has the effect of producing color, and produces more in double dose. It might be assumed instead (or in addition) that the white gene inhibited color, and inhibited more strongly in double dose. It might even be conceived that both allelomorphs inhibited the pigmentation which genes in other loci tended to produce, but that white was a more effective inhibitor than eosin.

STERN (1929) used actual dosage differences of a given allelomorph in his determination that each additional dose of mutants of the bobbed series adds to bristle length, up to a certain limit. In his work, instead of a small chromosome fragment, the practically inert Y chromosome served to furnish the extra doses. MOHR and BRIDGES, in their studies on deficiencies, realized that they might be dealing with real dosage differences, but at that time other interpretations, such as a peculiar sort of chain mutation, were not excluded. In an attempt to answer this question, however, I have examined cases in which there were known to be actual losses of the same region as was involved in the above cases of deficiencies, and find the effects to be the same.

Thus, for comparison with the Notch-8 deficiency of MOHR (1919, 1923), in which a piece near the left end of the X chromosome, extending from the left of white (1.7) nearly to echinus (5.5), is "deficient," we have certain cases of PATTERSON'S (1932c) produced by X-raying. In these, a rela-

tively large piece was removed from the left end of the X chromosome, though at the same time the very left end, which he has found (1932b) to be necessary for the life of the fly, was provided in advance, in the form of a fragment (called duplication X1 or "theta") attached to the right end. These known losses of the w-e_c region result in Notch wings, and allow recessives of the w, f_a and e_c loci, present in the homologous chromosome, to manifest themselves just as they would in a compound having them in one chromosome and the most extreme possible allelomorph of that sort in the other. I find females having apricot in one X chromosome and either white, MOHR's Notch-8 deficiency, or one of these known losses in the other, all to be indistinguishable from one another in shade. Again, to parallel BRIDGES' forked deficiency (1917), I have obtained, by X-raying special stocks, known losses in the region of forked, which allow forked in the other chromosome to show to an exaggerated degree. And OFFERMANN and I, studying BURKART's (1931, 1932) Blond translocation, have been able to show that flies can be obtained from it which lack the right end of the second chromosome (this having been transferred to the X); in such flies the recessive speck, if present in the other second chromosome, manifests itself, and there is a plexus-like venation, as in BRIDGES' "Plexate deficiency."

There is now some evidence from Drosophila, but more especially from maize (MCCLINTOCK 1931), that the two breaks in cases of double breakage within a chromosome may be at any distance apart, not being limited in their proximity by any principle of interference as rigorous as that which applies to crossings over. In view of this, and the above parallelisms, there can now be no reasonable doubt that the original proved "deficiencies" were small deletions, that is, actual removals of small regions, and so the studies involving them may now take their place definitely with the dosage studies. Later, I shall again refer to the results from this source. In the meantime, before the status of these deficiencies was established, I undertook, with the assistance of MISS LEAGUE, purposely to produce fragments containing known genes, and to use these for studying the effects of dosage changes.

Hypomorphic mutations

The first locus which we undertook to study was that of white eye. We chose first flies containing the moderately pigmented mutant allelomorph of white called eosin, in which the color is considerably lighter than the normal red, and is distinctly sexually dimorphic, being much lighter in the male than in the female. By irradiation we produced a deleted X chromosome containing this gene. It was then found that the addition of this frag-

ment to a male or female which was otherwise an ordinary eosin caused the eye color to become darker, more nearly like the normal red. This shows that the actual effect of the eosin gene is not to inhibit color, as might have been thought by comparison of it with red, but to produce color, since the addition of more of it results in more color,—*only it does not produce as much color as the normal "red" allelomorph does.* In the male, the addition of the fragment raises the dosage to two, and results in a color like that of the ordinary eosin female, which of course has two doses, while adding the fragment to the female, and so raising the dosage to three, results in a still darker color. This shows that the sexual dimorphism of eosin is due to the difference in dosage normally existing between the two sexes, and not to a difference in the action of the gene in male and female.[7] That the above observed results were not to be explained as effects of the excess dosage of other genes than eosin in the extra fragment was shown by producing a slightly smaller deleted X chromosome, not containing the locus of eosin, and repeating the same tests with it. It was found to have no effect upon the eye color.

The allelomorph of eosin known as apricot, which has a similar coloration except that male and female are alike, was then tried in the same way as eosin. It was thought that it might not show a phaenotypic effect of dosage changes, since the female with two doses looks like the male with one dose, but it responded similarly to eosin, additional doses darkening the color. Two doses of apricot in the male, therefore, give a considerably darker color than two doses in the female. Evidently it is the difference in dosage of other genes in the X chromosome of male and female which, interacting with the effect of apricot, causes the color, for a given dosage of apricot, to be darker in male than in female, in fact, just enough darker so that one dose in the male gives about the same phaenotype as two doses in the female. The same is presumably true of most of the other members of the white series of allelomorphs, which, except for eosin and ivory, look nearly the same in the two sexes.[8] The important thing for us now, however,

[7] For this reason, eosin cannot legitimately be used as an indicator of sex in such experiments as those of BRIDGES, in which he sought to demonstrate the female character of haploid tissue. That the haploid tissue was dark eosin, as in a female, was doubtless due to the fact that one dose of eosin, with one dose of all other genes, involves the same ratio as two eosins in a diploid, and was not due to the tissue being female. In the present author's opinion haploid tissue of Drosophila containing but one X should in fact be female, but the matter cannot be demonstrated by the use of eosin as a sex marker.

[8] In a recent publication, MORGAN, BRIDGES and SCHULTZ (1931) include cherry among the strongly sexually dimorphic members of the white series. This was certainly not true of the original cherry (see SAFIR 1913). The present sexually dimorphic stock, labelled "cherry Abnormal," contains neither cherry nor Abnormal abdomen, but is doubtless an ordinary eosin that either displaced the cherry by contamination or was mislabelled.

is that apricot, like eosin, is a mutant gene which produces an effect similar to that of the normal allelomorph, but a lesser effect. That is, it works in the same direction (towards the same *superficial* end result) as the normal allelomorph, but not so strongly. It is, *in this sense,* like a lesser-normal. I therefore call it a "hypomorphic" mutant.

The above results agree perfectly with the findings of MOHR that if either apricot or eosin is in one X chromosome of a female, and the other X has Notch-8 deficiency, which includes a deficiency for this locus, the color is lighter than in the homozygous female. As mentioned above, the same result was obtained when this part of one X was known to have been removed by X-rays. Thus, one dose of this gene produces an effect less like normal than two, and two doses less than three.

Similar tests involving known additions or losses of fragments, or both, were then applied to genes in a number of other loci. A deleted fragment containing the gene scute-1 was first produced and was used to study the effect of increased dosages of scute-1, a gene which is said to "remove" certain bristles. (See, for example, STURTEVANT in these *Proceedings.*) As with apricot, eosin, and bobbed, so here, the addition of an extra dose of scute in male or female made the individual more nearly normal, in this case almost completely normal, while the presence of two extra doses tended to result in slightly more of certain bristles than are present in the normal. Scute-1 is therefore a hypomorph. It does not "remove" bristles, except by comparison with normal. It produces them, though not as efficaciously.

In line with this conclusion derived from hyperploids, AGOL (1932) found, by the use of a chromosome (from scute-19) from which we knew the extreme left end, containing the scute locus, had been removed, that a female with just one dose of scute-1 has fewer bristles than one with two. The test of the effect of underdoses, as seen in hypoploids, is obviously as valid and informative regarding these problems as the test involving overdoses in hyperploids. What MOHR has named the "exaggeration phenomenon" shown by deficiencies is, then, in our terminology, the lesser effect of one dose of a hypomorphic gene than of two doses. By this test the other mutant allelomorphs of scute, in which other groups of bristles tend to be absent, are also hypomorphic, as AGOL (1932) found; facet is hypomorphic, as shown by MOHR'S deficiencies and PATTERSON'S cases of known losses; and forked is hypomorphic, as shown by my experiment previously cited. In elucidation of the test for forked, it may be explained that in this experiment females were made up which possessed one entire X bearing forked and having attached to its right end an extra piece consisting of the region

from Bar to the right end; these females also possessed another X that had contained the scute-8 inversion but that had had the distal ("left") end of this chromosome removed up to a point between forked and scalloped. Hence all regions were present in double dose except a small region between scalloped and Bar, containing the forked locus. These haplo-forked hypoploids were markedly forked, phaenotypically.

Tests thus far indicate that most mutant genes (both spontaneous and induced) are hypomorphs, inasmuch as they show "exaggeration" with deficiencies, as MOHR has pointed out, or at least give a form having about the same degree of abnormality as the homozygous mutant. The latter relation would be expected in cases like white eye, where the mutant gene had nearly reached the bottom of the scale of effectiveness and hence itself had almost as little normal effect as the deficiency had. This latter type of mutant may, descriptively, be called "amorphic."

These hypomorphs and amorphs are just the kind of mutants which the few remaining advocates of the presence-and-absence hypothesis, and the advocates of purely quantitative mutation, require as evidence for their views. It should be noted, however, that their having a lesser effectiveness than the normal allelomorph by no means proves that they themselves involve material losses. They may consist of partial inactivations, or they may give rise to processes that lead in a somewhat different direction, and hence do not work so effectively in the observed direction, or they may involve conflicting tendencies. Moreover, a given mutant allelomorph (whether spontaneous or induced) may be very hypomorphic, or practically amorphic, in regard to one kind of activity of the normal gene, and normal or nearly normal in regard to another kind of activity. This is well exemplified in the scute series, in which each different allelomorph acts hypomorphically only in respect to its own peculiar combination of bristles, and is normal or nearly so in its action on other bristles. Since, in a comparison of different allelomorphs, the amount or intensity of effectiveness may vary separately from the types of effect, and both of these in turn may vary separately from the number or extensity of the effects, advocates of the quantitative view would here be driven to admit the existence of various parts of the gene, and to assume that these parts could vary quantitatively more or less independently of one another. This would be a distinct retreat from the simple hypothesis of quantitative variation of the gene as a whole.

Whatever the explanation of hypomorphism may be, it is of interest to observe that the finding that most mutant genes are of this type conforms to WRIGHT'S contention (1929; see also MULLER 1928b, pp. 259-260) that

gene mutations should in the majority of cases involve more or less inactivation of the processes governed by the normal gene, and that these less active genes should more often act as recessives to the normal than as dominants. This implies that one dose of the normal gene usually has an effect more nearly like that of two doses than of no dose. Whether the latter principle is a primary one, however, or is due to the past selection of modifiers, is another question.

On the compensation of the effects of dosage differences between the sexes, and on dominance

In the above connection, it will be worth while to make somewhat of a digression, to consider a curious fact that has emerged from the results concerning hypomorphs. That is, it appears that in the great majority of the cases of hypomorphic sex linked genes, one dose in the male produces about as strong or at times even a slightly stronger effect in the direction of normality than do two doses in the female. This must of course be due to the interaction of other genes in the X chromosome, whose simultaneous change in dosage affects the reaction.[9] In some cases at least it has been possible to show, by studies of the effects of different chromosome pieces, (a) that genes other than the genes for sex are acting as the "modifiers" in question, (b) that the modifiers responsible for the dosage compensating effect on different loci are to some extent different from one another, and (c) that more than one modifier may be concerned for a specific locus.[10] I base these conclusions on various results obtained in work of OFFERMANN, who has been especially active in the study, of PATTERSON, and of myself.

We may for convenience call these genes "modifiers," but with the reser-

[9] We arrived at our main results and conclusions regarding this phenomenon of dosage compensation in the spring of 1930. Although we communicated our results to Doctor STERN at that time (prior to the remarks made by STERN and OGURA 1931, upon this topic), we withheld our preliminary report (MULLER, LEAGUE and OFFERMANN 1931) until after certain checks had been carried through.

[10] Judging by certain results recently reported by MORGAN, BRIDGES and SCHULTZ (1931), the second-chromosome mutation Pale (associated with BRIDGES' original translocation) has, in addition to a "diluting" effect, an effect on the different eye colors of the white series similar to that produced by lessening from two doses to one the gene or genes in the X chromosome that are responsible for the dosage-compensation of most members of this series (thus, those allelomorphs of white that are lighter in the male are lightened by Pale, but the others are darkened somewhat). This means that the chemical process affected by Pale is the same as, or in its effect similar to, that affected by the dosage compensator (s) of the X; but, since we have seen that there is no reason to identify the latter with the gene or genes in the X that decide sex, we have no reason to agree with the suggestion of the above authors that "the translocation (Pale) may be closely connected with the sex-determining reaction."

vation that they may sometimes be as important in the causation of the phaenotypic effect as the "primary" gene whose mutations we have available for study. The essential relation is that, in so far as the amount of phaenotypic effect produced by this so-called "primary" gene depends on its dosage, it does not depend at all on the mere "concentration" of this gene in the cell, nor on the relation of its dosage to that of the other genes in general, still less to that of the autosomal genes, but solely on the ratio of its dosage to that of another specific gene or genes which lie in the same chromosome (the X). That a relatively high amount of intra-chromosomal interdependence in regard to dosage expression existed among sex linked genes was realized some time ago (MULLER 1930b) and denoted as "intra-chromosomal genic balance." In that work, however, we were dealing with those relatively rare normal genes, or gene-combinations, which have a quite different effect, visibly, in one dose than in two. The present findings go much further, in showing the existence of a far stronger interdependence, and one which applies not just to a relatively few scattered genes but to the great majority of the individual genes in the X which can be sampled.

Now this great system of "modifiers," all acting to give a similar sort of effect, and probably affecting most of the genes of the X chromosome, must have a function. It cannot be that of giving the male *mutant* as strong, that is, as nearly normal, an expression of its mutant gene as the homozygous female mutant has. It must therefore be a system which acts on the normal allelomorph similarly to the mutant, but the action of which is more readily apparent to our eye in the mutant type. In most cases the normal gene gives, so far as our eye can perceive, practically the same effect in one as in two doses. Nevertheless, there must be some difference which, though imperceptible, is important for survival; otherwise this system of genic interaction would not be thus maintained to keep the same optimum degree of effect in both sexes, despite the different doses. It follows that the dominance of the normal gene over its "absence" is really far from perfect, physiologically (that is, that one dose is not really as effective as two), though it may seem so to the casual genetic observer, and that by selection a system of interacting genes has become established such that the expression of the one dose in the haplo X type is like that of the double dose in the two X type. Bobbed, being present in double dose in male as well as in female, is, *as expected,* an exception to this rule of dosage compensation, in *Drosophila melanogaster.* In *Drosophila simulans,* on the contrary, bobbed does show dosage compensation, and here it is found, correspondingly, that the male carries only one dose of the "normal" allelomorph (the Y being

sometimes neutral and sometimes actually "antimorphic" in effect—see page 245—as shown by results of STURTEVANT [1929]).

The question may here be raised: Why were the normal allelomorphs of most of the sex linked genes other than the bobbed of *D. melanogaster* ever "lost" from the Y chromosome if their absence was so deleterious as to require the subsequent evolution of this complicated compensation system? The case of the Y of *simulans* shows that they can be thus "lost," or, better to say, changed in expression like a loss, and this would seem to point to the importance of *accidental* multiplication, not guided by selection, as an occasional evolutionary process. It may be, however, that most of the genes in the X, unlike bobbed, never were present in the Y, in anything like their present form, at least; that is, that the male has had but one dose of them from the beginning of their existence as such. In that case, they must have arisen either as duplications, as "neomorphs" (see page 246), or both, after the present sex-determining system had already become established. This question might be answered definitely by genetic analysis in a species in which we knew that a part of the X had been derived from an autosome (for example, *D. hydei* or *"obscura"*?).

The existence in the X of "modifiers" of such a specific kind that, by their change in dosage, they modify the amount of effect of other sex linked genes to the extent required to make the male and female alike, indicates that specific modifiers of gene action are plentifully available. In the case of some of the sex linked genes arising in the manner last suggested (so as to have existed in different dosages in the two sexes from the start) it is possible that the dosage compensation did not result from the selection of mutations in these modifiers but that the "primary" genes themselves were so selected at the time of their origination as to be, *ab initio, adapted in their action to* the other, preëxisting genes in the X which we now call "modifiers." And even when the compensation did not thus exist from the start, it is likely that the inter-adaptation of primary gene and modifier did not always occur through changes in the modifier alone but also through changes in the primary gene that made the latter sensitive to the modifier (such changes in the primary gene as would be involved in the mutation of eosin to apricot, for example). Thus, where there is only one modifier causing the dosage compensation of a gene that did not have this property to begin with, the chances seem *a priori* to be equal that the dosage compensation arose (if by one step) by a further change in the primary gene itself, or by a mutation in the modifier; the greater the number of modifiers, the larger the rôle that their mutations have probably played in the process, as compared with

mutations in the primary gene. We should also remember that mutations could also take place in other genes, for example, autosomal genes, which would serve to bring the primary gene and the modifier into the reciprocal relation with one another which they now have. But, however all that may be, the results do give evidence of the availability of "modifiers," or, to put it more precisely, of mutations which cause certain specific types of nicely adjusted genic interaction, favorable for survival, and not having this survival value too much obscured by pleiotropic effects.

The above conclusion would appear to lend support to FISHER's theory of the origin of dominance, inasmuch as on that theory, too, specific modifiers (albeit of a somewhat different kind), without important other effects of their differences from their own parent genes, are called for. It would also allow us to adopt to a certain extent the suggestions of HALDANE, concomitantly. There is, however, an important difference between the mechanism of selection for dosage compensation here studied and that postulated either by FISHER or by HALDANE for the modification of dominance. For in the former the selective moment, if I may call it so, exists throughout the population, while in the latter it is supposed to be limited to a comparatively small minority. Thus the difficulty is encountered that the pressure of the selection in question may be too small, as compared with that of mutation, or of the selection for even very weak pleiotropic effects.

I believe that the above difficulty can be avoided and a better case made out for the origin of dominance by selection if we assume that this selection has had a somewhat different mechanism from that previously postulated. I prefer rather to postulate that the mutations favoring dominance—the genes or genetic conditions which tend to make the heterozygote like the homozygote—have been selected and are maintained not so much for their specific protection against heterozygosis at the locus in question as to provide a margin of stability and security, to insure the organism against weakening or excessive variability of the character by other and more common influences—environic and probably also genetic. These modifiers must so affect the reaction set going by the primary gene in question as to cause this gene, when in two doses, to be near an upper limit of its curve of effectiveness,[11] that is, in a nearly horizontal part of the curve, not so readily subject to variation by influences in general, *including* reduction in the dosage

[11] That is, the curve expressing the relation of amount of phaenotypic effect (the ordinate) to the amount or concentration of gene material (the abscissa)—a curve which must usually, in its right-hand portion, rise with ever decreasing slope, approaching a horizontal limit, as seen, for instance, in STERN's studies on bobbed and in ours on scute and apricot.

of the primary gene. This does not mean that the phaenotype is necessarily made any more extreme, for counter-checks can be set up. That is, the level of the curve as a whole and its shape, as well as the region wherein it approaches a horizontal limit, are also adjustable, by means of modifying mutations that reframe the conditions under which the reaction takes place. Such modification (see FORD 1930), and not merely an increase in potency of the "primary" gene, will be necessary in the numerous cases in which the curve of effectiveness did not, originally, approach the horizontal within physiologically acceptable limits (or did not do so at all).

It should be distinctly understood that the crux of the above view of the origin of dominance lies in the proposition that, where a change in gene dosage causes a perceptible change in its phaenotypic expression (that is, when it is in a noticeably sloping part of its "curve of effectiveness"), it is likely that the degree of expression of the character will be modifiable to an unfavorable extent by environic and by other genetic changes. This seems reasonable, *a priori,* inasmuch as some of the disturbing influences would be expected to act by altering the reaction in a way similar to that whereby the change in gene dosage would alter it, and hence would tend to be similarly effective.

But we need not rely on *a priori* reasoning alone. There is a significant amount of experimental evidence already existing to show that there is considerably more phaenotypic variability in the expression of hypomorphic mutant genes than of their normal allelomorphs. Now, these hypomorphs evidently cause a reaction of a type similar to that of their normal allelomorphs, but a weaker or lesser reaction, one which, unlike that of the normal allelomorphs, is much affected by dosage changes. This variability is true of all the known hypomorphs yet studied: namely, all the hypomorphs of the scute series, the white series, the forked series, and the bobbed series (excluding the amorphs, which afford a converse test of the same proposition). The same variability applies also, as we should expect, to the effect of normal allelomorphs in single dose in those relatively rare cases in which the single dose has a perceptibly different (that is, lesser) effect than two: these cases comprise Notch wings, Plexate venation, and several Minute bristle conditions. All told, the evidence given above may be sufficient to show the truth of our proposition as a usual rule. It is not necessary to claim for it, nor do we believe that it has, the validity of a universal law.

In conclusion, we may call attention to the bearing of a further fact, derived from our study of dosage compensation, on the problem of dominance. We have seen that in all probability many of the normal genes in the X

chromosome have this dosage compensation, despite the fact that, even in the female, there is hardly a perceptible difference between the effect of one dose and of two. This indicates that, even though the normal gene produces its effect in what appears to us to be a nearly horizontal region of its curve of effectiveness (where changes in dosage produce little discernible effect), nevertheless there is a distinct influence, unfavorable to the organism and perceptible in its survival rate, if the effect is made either slightly stronger or slightly weaker. The disadvantage of a stronger effect is shown by the fact that, in the female, the strength of effect has become fixed at so low a level as to call for dosage compensation. For in a sense the dosage compensation may as rightly be regarded as a means of keeping the female from having too strong an action of the gene as a means of giving the male a strong enough action. If twice as high potencies in the female were biologically acceptable, this relatively simple change should often have been utilized (that is, have survived) whereby the male would automatically have been provided with a sufficiently high potency to obviate the need for dosage compensation. We must conclude, then, that in the fixing of the conditions determining dominance too, it was not feasible merely to increase the potency of the "primary" gene; instead, the characteristics of its curve of effectiveness had somehow to be altered.

Experimental evidence of a different nature, indicating that dominance is not a primary property of genes but must have become developed by selection, is given in the section on neomorphs (see page 248).[12]

Hypermorphic mutations

We must now return from our digression, which has perhaps helped us to understand why hypomorphic mutant genes usually show dosage changes better than do the normal genes from which they were derived, and are recessive to the latter. The question next arises: are all mutant genes hypomorphic? This can be answered categorically in the negative.

Since it has been found that there are reverse mutations of hypomorphic mutant genes, such as scute, apricot, and forked, both spontaneously and as a result of irradiation, we must regard the allelomorphs thereby resulting not as hypomorphic but as hypermorphic to their immediate progenitor genes. Whether or not such a change involves a real increase of material is a doubtful question, subject to the same considerations as applied, con-

[12] I am indebted to Doctor C. R. PLUNKETT for calling my attention to the fact that in a paper (1932) presented independently to the same congress he has espoused what is essentially the same viewpoint regarding dominance as that given in the above section.

versely, to the hypomorphic mutations. TIMOFÉEFF-RESSOVSKY (1929, 1931a), as well as PATTERSON and myself (1930), has discussed in some detail the varying frequency of such changes for different loci and allelomorphs.

Now if hypermorphic changes of already mutant allelomorphs may occur, resulting in partial or complete reverse mutations, there might well be hypermorphic mutations of normal genes also, resulting in changes of a type opposite to that of our ordinary mutations. Usually these would be difficult or impossible to detect, on account of the fact previously referred to that two doses of the normal gene are already at nearly the maximum point in the curve of effectiveness. Thus such changes would be apt to escape observation. Very likely, however, NASARENKO's (1930) mutant "abrupt" is a hypermorphic mutation of the normal allelomorph of Notch, for it is at or near the Notch locus, and it and Notch deficiency counteract each other instead of showing an exaggeration effect.

Antimorphic mutations

What evidence have we for other mutational changes than such as could be explained as mere diminutions and increases? The dominant (somewhat variegated) allelomorphs of brown eye in chromosome II are a case in point. When there is one dose of the recessive brown and one of the normal gene, the latter dominates and the phaenotype is red. But, as GLASS and I have found (see GLASS 1932), when to the above complex a dose of the dominant allelomorph of brown is added, the result is a brownish (somewhat variegated) color. It may be explained that this combination is produced by making up a fly that is a compound of recessive brown and dominant brown, and carries as excess a fragment of the second chromosome derived from BRIDGES' "Pale" translocation; this fragment contains the normal allelomorph of brown. The resulting brownish color shows us that the addition of dominant brown to a heterozygote of normal and recessive brown has a real effect and involves the addition of some kind of gene material different in its effect from the material in the normal gene. This effect, the color change, lies in the same direction from normal as does that of the recessive brown, as comparison of the colors indicates. This is shown more conclusively by the fact that while a hyper-diploid containing one dose of dominant brown and two of normal has practically normal red eyes, a hyper-diploid otherwise similar to the above but with a dose of recessive brown substituted for one of the normals has brownish (somewhat variegated) eyes—that is, the substitution of recessive brown in place of normal results in a

better manifestation of dominant brown. But the recessive brown itself acts practically as an amorph, since the addition of a dose of it, as an extra, has practically no effect either on the incompletely brown color of the heterozygote of dominant brown and normal or on the red color of the heterozygote of recessive brown and normal. Hence the dominant brown represents something that differs from normal, in its effect, in the same direction as a loss does, but more strongly. In a sense, it has an actively negative value. More accurately, it has an opposite action to that of the normal allelomorph, competing with the latter when both are present.

A similar conclusion may be drawn with regard to the mutant gene ebony, of chromosome III. For, starting with a hyperploid containing two ebony genes and one normal (derived from translocation II-III26—PAINTER and MULLER 1929), as a basis of reference, we find that the subtraction of one ebony makes the color lighter, while the subtraction of the normal makes it darker. I would term such antagonistic mutant genes, having an effect actually contrary to that of the gene from which they were derived by mutation, *antimorphic*.

Abnormal abdomen may now be interpreted to be a member of this class, as shown by results in MOHR's experiments with Notch-8 deficiency. In the first place, it is to be observed that the gene for Abnormal produces a change in the same direction as a loss of the normal gene. This is shown by the fact that if we start with a heterozygous fly having one Abnormal and one normal gene (this is somewhat Abnormal in appearance), the substitution of a real loss (Notch-8 deficiency) for the normal gene in it intensifies the Abnormal abdomen character. But the Abnormal gene, though thus producing a change in the same direction as a loss of the normal gene, acts more strongly in this same direction than a mere loss does. This in turn is shown by the fact that homozygous Abnormal flies are still more Abnormal in appearance than are the compounds of Abnormal and deficiency. That is, the degrees of phaenotypic Abnormality, as found by MOHR, were as follows:

$$\begin{matrix} Ab. \\ Ab. \end{matrix} > \begin{matrix} def. \\ Ab. \end{matrix} > \begin{matrix} norm. \\ Ab. \end{matrix} > \begin{matrix} norm. \\ def. \end{matrix} = \begin{matrix} norm. \\ norm. \end{matrix}$$

Since in the first three terms of the series the gene represented below was always the same, the observed differences prove the degree of abnormal effects to be in the order $Ab > def > norm.$

It may be mentioned that a recessive allelomorph of Abnormal has been produced by X-rays. It will be seen that in such cases the recessive mutant, though classifiable as an amorph or possibly a weak hypomorph, probably

involves no mere loss of material, since what is apparently a still greater change in the same direction gives a gene which again has a demonstrably active influence.

Unless we make the very improbable assumption that the Y may contain other active genes than bobbed influencing the same character, we may also include among antimorphs the gene existing in the Y of most races of *Drosophila simulans* (see STURTEVANT 1929) which (unlike the bobbed allelomorphs reported upon by STERN 1929) actually decreases the bristle length of males containing bobbed in their X. It is also possible that the genetic conditions designated as Minute 1^2 and Plexate include antimorphs—in fact, such is the conclusion which we should ordinarily draw from a recent report; on the other hand, an earlier report interprets these conditions as deficiencies (see MORGAN, STURTEVANT and BRIDGES 1927, and MORGAN, BRIDGES and SCHULTZ 1931). Possibly the apparent contradiction is due to the effect of dosage changes of other genes in the added fragments rather than to the genes in question (that is, an intraregional dosage interdependence). Fortunately, this possibility can rather easily be put to the test in these cases (in part at least), since a smaller fragment involving the region in question is available in the Blond translocation, and others can rather readily be manufactured. In the meantime, the "position effect" interpretation is not excluded here, nor is that of gene mutation accompanying breakage.

Neomorphic mutations

Somewhat different from the negatively acting, competing mutant genes, or antimorphs, is the class which I am provisionally terming "neomorphs." A good example is the dominant mutant, Hairy wing, near the left end of the X chromosome. The homozygous Hairy wing female is about twice as hairy as the heterozygous Hairy wing female or the Hairy wing male (this constituting an exception to the dosage compensation rule for sex linked genes). The relatively low grade hairiness of the heterozygous as compared with the homozygous female, in this case, is due solely to the single dose condition of the gene for Hairy wing and not at all to a possible influence of the normal allelomorph in the heterozygote. For if a small piece containing this region be broken off of a normal X chromosome, and added either to the heterozygous or homozygous Hairy wing female or to the Hairy wing male, there is no diminution of the hairiness. On the other hand, if a small piece containing a Hairy wing gene be added to an individual otherwise normal, Hairy wing will show. The normal allelomorph thus fails to compete. It itself acts like an *amorph,* so far as its detectable effect on the

character under consideration is concerned. Yet it is no mere absence; it has a material existence, for Hairy wing has arisen at the same locus several times (including at least twice by irradiation).

We must conclude from the above results that the mutation to Hairy wing does not result from an addition of material transferred from another locus (since the mutation always reappears at the same locus). It must rather be a change in the nature of the gene at the original locus, giving an effect not produced, or at least not produced to an appreciable extent, by the original normal gene. If the effect had been produced to some appreciable extent by the normal gene also, then the addition of a dose of the normal to the Hairy wing individual should have actually increased hairiness.

The fact that normal genes may thus act as amorphs with regard to a particular character affected by their mutations should serve as another warning against regarding mutant genes that seem to be amorphic or hypomorphic as really involving a mere absence or loss of material. The obtaining of reverse mutations from near-amorphs, such as eosin from white, gives further evidence for this conclusion.

The same kind of finding as above noted for Hairy wing—namely, lack of effect on the character when extra doses of the normal allelomorph are added—was observed by OFFERMANN in studying the spontaneously arisen dominant, Blond, of BURKART. This interpretation holds only if we regard Blond as having its locus in the X chromosome. This is uncertain as Blond lies near the break of a mutual translocation involving X and II (see BURKART 1932), but as Blond follows the sex linked rule of dosage compensation it is in all probability in the X. We are, however, making sure of its neomorphism by testing also the effect of adding an extra dose of the suspected region of chromosome II.

Bar eye is a third neomorph. It is well known that STURTEVANT has considered Bar as having no normal allelomorph, at least none at the same locus as itself. However, the recently reported finding, by DOBZHANSKY (1932), of a second Bar-like mutation ("baroid"), induced by X-rays at the same locus as the old, indicates to me that this locus normally contains a gene that is subject to this particular type of mutation, although DOBZHANSKY still believes that the normal allelomorph was somehow transported there from another locus, at the time of the mutation. BRIDGES' original Bar-deficiency of 1915 (published upon in 1917), which we may now interpret definitely as a loss, shows that the absence of the Bar-locus in the non-Bar chromosome of a heterozygous Bar female has the same effect on the Bar eye character as the presence of the normal allelomorph itself, and STURTE-

VANT'S work on chromosomes which have lost the Bar locus by unequal crossing over is an indication in the same direction. (There is a possibility that in the origination of Bar a gene became duplicated *in situ*, and that one of the resulting twins mutated at the same time. On this rather special hypothesis the mutation would have been of the neomorphic type. But in that case the normals formed from Bar by unequal crossing over would not represent complete "absence.") On the other hand, increased doses of Bar give the abnormal effect more strongly, just as we find for Hairy wing and Blond, and unlike the situation in the case of hypomorphs.

While THOMPSON (1929) has raised some objection that we may here be adding and subtracting only a part of the gene, in getting these effects, this possibility is ruled out in some recent studies of OFFERMANN using a strong allelomorph of Bar ("Super-Bar," B^s, found by STONE) that exists in a chromosome fragment. The addition of fragments containing the whole Bar gene had the expected effect of increasing the bar-like character of the eye in a clear-cut fashion. OFFERMANN likewise proved that this result could not be due to the excess dosage of other genes in the piece. Bar, then, is a mutation of a normal gene, giving a gene that produces a new effect, foreign to the original gene, and not competing with the latter. It is very probable, however, that the new effect is in some way related to that of the normal allelomorph. For it is evident that Bar obeys the usual rule of sex linked genes, having the male, with his one dose, much more nearly like the homozygous female, with her two doses, than like the heterozygous female (see also the case of Blond, and note the contrast with that of Hairy wing).

A recently published mention by MORGAN, BRIDGES and SCHULTZ (1931) of the lack of effect of changes in dosage of a fragment containing the normal allelomorph of Bristle on the degree of expression of this second chromosomal dominant leads to the conclusion that it also must belong in the class of neomorphs.

It might yet be possible to evade the obvious conclusion that gene mutations, including those produced by X-rays, involve qualitative changes, changes in the kind of structure and not merely in the quantity of the gene or its parts. For it might be postulated that in all cases of neomorphs there was an imperceptible rudiment of the part which produced the effect in question, already present in the normal gene, and that this part merely became vastly increased in amount by the "mutation." Or it might be postulated that all such changes were "position effects," caused by gene rearrangements. While there are an exceptionally large number of rearrangements both among known neomorphs and antimorphs, there are cases—Hairy wing, Bristle, Dominant eyeless, Abnormal abdomen—which do not involve

such changes, unless we suppose the rearrangement to be on such a *minute* scale as to escape detection. Both these paths of escape into the ultra-small would, however, be pure speculations, the burden of proof for which would rest upon the advocate thereof.

It does not seem to be a coincidence that more loci have yielded hypomorphs than neomorphs, and that even loci which have yielded neomorphs have done so with relative infrequency. These results, if corroborated by more extensive work, would speak for the correctness of the principle put forward by WRIGHT (1929; see also MULLER 1928b, pp. 259-260) that mutations having an effect in the direction of losses (that is, those that tend to be disorganizing and inactivating) should in general be more frequent than those causing increased or new effects. But while this principle is necessary as one basis for WRIGHT's theory of dominance, it is not, alone, sufficient for a derivation of the latter; neither is it contradictory to the general viewpoint put forward by FISHER that the usual dominance of normal genes has been developed through natural selection. It is to be noted, further, that the hypomorphs tend to be recessive, and the neomorphs "dominant." This again is in line with WRIGHT's view, but it is also in line with FISHER's (since any given neomorph originates so infrequently that there has been much less chance for selection to have affected its mode of expression), and it is still more in line with the idea previously offered (p. 240), that selection has worked primarily towards the stabilization of the reactions of the normal, homozygous genes. (In the latter case, even rather frequently recurring neomorphs would tend to be dominant.)

When, however, we examine into the type of dominance found, we obtain a result of greater apparent significance. For while the recessiveness of the hypomorphs is usually fairly complete, as generally expected, *the "dominance" of the neomorphs is in most cases far from complete, being of the "intermediate" type.* Now this result is exactly what we should expect if dominance of the nearly complete type has been developed by selection (especially, if by the type of selection advocated on page 240), but it is a considerable surprise, in fact, it seems contradictory to the idea that such dominance is usually a primary property of the gene. It will therefore be important to examine further cases with reference to this question.

While we have spoken above of the general trends of the results, it should be emphasized that no absolute rules can be made with regard to the dominance of the different classes of mutants. A known loss like Notch-8, Plexate, and at least three known Minute bristle conditions, may be dominant or semi-dominant in its effect, and therefore an amorph or a hypomorph may be likewise. In these cases one dose of the normal gene has dis-

tinctly less effect than two. On the other hand, neomorphic genes may be so "weak" in their effect that two doses are required before they rise to the level of visible manifestation. This was very nearly true in the case of a certain Hairy wing mutant, and in the case of baroid in the female; under certain genetic conditions (for example, in the presence of ZELENY's modifier, called "emarginate") it was true of Bar itself, and under certain environmental conditions it was true of Abnormal abdomen. For the same reason, we cannot make absolute rules regarding the exaggeration of recessives and dominants by deficiencies. If the recessive or near-recessive should be a neomorph, like baroid, it will not show exaggeration by a deficiency; if the dominant should be hypomorphic, as in the case of the absence of coxal bristles in some scutes, it will be exaggerated by a deficiency. But the more usual case is the recessive hypomorph (for example, eosin, facet), which shows exaggeration, the amorph (like white) which shows no effect, and the semi-dominant neomorph (for example, Bar) and antimorph (for example, Abnormal), which show instead an apparent inhibition by a deficiency.

On our interpretation of most gene mutations as qualitative structural changes, even the distinction into classes above outlined is not an absolute one, and reflects rather the gene's final behavior than its real structure. So we may expect to find genes, for example, that are hypomorphic in one respect and neomorphic in another. Possible examples of this are scute-8, scute-12, and scute-M-4 (in deleted X 24); the two latter show certain semi-dominant Hairy wing effects, as well as hypomorphic scute characters, but it is as yet uncertain whether these effects are really referable to the same locus or represent group mutation or possibly effects of changed position.

Multiple allelomorphs forming non-quantitative series

There are already numerous cases known in which it can be shown that a given mutation has markedly changed a gene only in regard to certain of the effects which the original gene produced, while another mutation in the same gene changed it more pronouncedly in some other respects. This has been shown *par excellence* with regard to the various hypomorphic changes possible in the scute locus in the studies on scute allelomorphs carried on by the Moscow geneticists. One of their most important contributions lies in showing the richness of the different patterns of change possible in a given gene, since thus far very few of the numerous allelomorphs are indistinguishable from one another. That the tendency to certain kinds of groupings of effects on the different bristles is partly an expression of cer-

tain real features of gene structure, and will help us to understand the arrangement of gene parts, is also a reasonable conclusion.

Attempts to explain the matter in a simple quantitative way, as in GOLDSCHMIDT'S criticisms, or by means of developmental relations, as in the Plunkett-Sturtevant-Schultz hypothesis of diffusion of influences from a center, fall in the face of the facts. We do not have time to mention the various logical difficulties which the latter hypothesis encounters in its actual working out. Suffice it here to say that a study of numerous gynandromorphs involving various scute allelomorphs has been carried out in our laboratory, chiefly by PATTERSON, and that the results show clearly that the development of bristles, in so far as it is under the influence of the scute gene, is not governed by one or a few centers, but is in its major features autonomous at the site of each bristle. On the other hand, later work throws grave doubt on the possibility of grouping all the effects into one exact line (this is equally against both the unmodified sub-gene hypothesis and the theories of GOLDSCHMIDT, STURTEVANT, et cetera). And the evidence that such a line, if it represents gene parts in a one-to-one correspondence, may be cut without destruction of either piece, is still to be found (see page 222).

This still leaves the locus of scute the most suitable yet found for the study of multiple allelomorphism and gene structure, and it leaves the sub-gene hypothesis, or some modification of it, as a possible interpretation, although the way is not as clear and easy as before. It will, I think, be profitable to follow the method there used, that of concentrating on intensive studies of the different kinds of mutations possible in individual genes, as induced by irradiation and otherwise.

Such studies as we have carried out on other loci than scute have shown somewhat similar phenomena, and in some respects amplify our view. For example, the cases now known are fairly numerous in which different recessive mutant allelomorphs of the same locus have effects which are to some extent, or almost wholly, different in their character or in their location on the organism. Thus, mutant allelomorph 1 may affect character A very much and B very little or not at all, while allelomorph 2 affects A little and B much. Such allelomorphs, when crossed, usually form a compound that is more normal than either. For, in respect to each character effect or body region, the more normal effect is usually the more dominant; that is, the compound is usually in each respect more like that allelomorph which has a more nearly normal effect on that character or region. This was evident, for example, in EMERSON'S (1911) allelomorphs giving different combinations (= patterns) of red *versus* white silk, cob, grain, et cetera, in corn. In Drosophila, the first case was that of the truncate series (MULLER 1919,

1922b), which concerns not only different regions but different characters, and obeys the same rule throughout. Thus, in this case, the cross of vortex bristles by oblique wings was found to give a compound that was sensibly normal. To explain those members of this series which showed two or more of the effects at once, the interpretation of group mutation of neighboring but physiologically entirely distinct genes was early considered but it was rejected, chiefly because studies on the action of modifying genes as well as of "chief" genes at other loci showed the different developmental effects in question to be physiologically related. In this case, it was also observed that the groupings of effects of different allelomorphs fitted in with no linear series rule. The normal-appearing compound of achaete and scute-1 (found by DUBININ to be allelomorphs) falls under the same category as the vortex-oblique cross. So too may the normal compound of split bristles and recessive notch wings (GLASS and MULLER unpublished), and also certain effects observed by DOBZHANSKY (1930a) in the Stubble series of allelomorphs. The list could be considerably extended.

There are, however, exceptional cases, in which the compound is not more like the normal in respects in which the two allelomorphs differ. The best case of this is the appearance of leg-like antennae in the compound between aristopedia, which has such an effect, and its allelomorph spineless, which does not, as found by STURTEVANT (1929). A few of the missing bristle effects in scute crosses show a similar tendency; so too does the extra bristle effect in crosses of split bristle and facet-eye (see below).

We now have to report exceptions of the opposite type also, namely, those in which the compound is more like normal in respect to effects in which both allelomorphs are similarly abnormal. One such case is that of lozenge-eye in combination with a particular spectacled-eye allelomorph of it. The compound has a practically normal eye but has the female infertility common to both, and their mutual allelomorphism is further shown by the fact each gives a distinctly mutant eye type when crossed with still other members of the series (see PATTERSON and MULLER 1930, AGOL 1930). Another case is that of the ommatidial disarrangement in split bristles and facet-eye. Both of these mutants cause ommatidial disarrangement, yet (as with spectacled and lozenge) the compound has a normal eye (MULLER unpublished). Their allelomorphism is shown not only by their linkage but by their behavior with other mutual allelomorphs (notches) and by the appearance of extra bristles in the compound, as in split bristles by itself (see above). In such cases as these, we must draw the conclusion that the two allelomorphs, although acting on the very same body region, and having superficially similar effects on that region, nevertheless attain these effects

through the intermediation of qualitatively different developmental processes. Further studies of the relations in such series are needed.

Ultimately, too, we must undertake the still more difficult study of the effects of successive mutations in the same gene, to discover, if possible, principles governing their continued evolution. Such evolution, as I see it, implies the possibility of qualitative change in the gene as a necessary condition. The foregoing illustrations, if taken together, afford, I believe, considerable experimental evidence for the existence of such a phenomenon, both as a natural occurrence and as a result of irradiation. And this conclusion remains likely no matter whether the mutational effects of irradiation are of a direct or an indirect nature.

For the rest, I fear that the present paper has raised far more questions than it has solved. But if some of these questions may thus have been opened to attack, our time may not have been wasted.

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