ON THE GENETIC NATURE OF INDUCED MUTATIONS IN PLANTS[1]

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It is generally considered that the hereditary variations induced by irradiation are due to two fundamentally different types of change produced within the irradiated cells, (1) breakage and rearrangement of the chromosomes, and (2) modification of the composition of individual genes. The possibility that the induced gene mutations may be due to mechanical changes analogous to those causing the grosser chromosomal aberrations is rejected chiefly on the basis of the induced reverse mutations in Drosophila which have been convincingly demonstrated by MULLER (1928), TIMOFÉEFF-RESSOVSKY (1930), and others. PATTERSON and MULLER (1930), after a comprehensive experimental and theoretical analysis of this question, conclude that the induced point mutations are changes in the chemical composition of the genes, and that they probably are "endless in their eventual possibilities." This interpretation is applied to the point mutations in general, which are considered a group fundamentally distinct from the variations due to chromatin displacement.

The results of genetic experiments with X-rays in plants are not entirely in harmony with this view. Radiation induces gene mutation as well as grosser chromosomal variations in plants as in the fruit fly, and the induced mutations meet all tests of typical gene mutation. The evidence from plants considered alone, however, does not permit any sharp differentiation between the induced gene mutations and various extra-genic alterations which may be expected to accompany the types of chromosomal derangement brought about by the treatment. This does not imply that mutation as a whole is purely a mechanical process, for it is possible that the mutations affected by radiation are a special class not representative of mutation in general. This special class may be wholly or largely made up of mechanical or extra-genic changes.

There is no reason to assume that irradiation could not produce chemical changes within the gene, changes which might well be representative of those involved in the natural evolution of the gene. But since chemical changes in the gene are beyond direct investigation the conclusion that the genes are chemically transformed must be based almost entirely on negative evidence. To state that an induced variation is a gene mutation is not

[1] Contribution from Department of Field Crops, MISSOURI AGRICULTURAL EXPERIMENT STATION, Journal Series 351.

to explain it but merely to label it. We do not demonstrate that a chemical change has occurred; we simply infer, since no mechanical explanation can be found, that the variation must be due to this invisible mechanism. The implications of this conclusion are so far reaching, both for genetical theory and for breeding practice, that it is essential to examine thoroughly any possibility of accounting for the presumed mutations as phenomena subject to direct investigation.

In this paper I shall consider the evidence from experiments with plants, chiefly for its bearing on two questions: (1) Are the induced mutations partly or wholly the result of mechanical alterations, and (2) are they representative of natural mutation in general?

WHAT IS MUTATION?

I shall use the term mutation exclusively in the restricted sense of "gene mutation."

Even in the restricted sense the term is rather indefinite in meaning. We may define mutation as a transmissible change in the gene. But we identify mutations by experimental tests, and these tests are not such as to establish conclusively, in specific instances, that a change within the gene has occurred.

In effect, any Mendelizing variation which can not be shown to be due to a change involving more than one gene is a mutation. In Drosophila the gene variation is distinguished from variations of higher order chiefly by the use of closely linked genes. In plants few closely linked genes are available for use, but the inviability of deficient genomes in the haploid generation serves to some extent as an alternative distinction between mutation and deficiency. In either case the distinction is more or less arbitrary. Deficiencies may occur which do not involve the loci of two known genes, and which therefore cannot be distinguished from mutations. On the other hand, it is possible that variations involving changes at neighboring loci may in some cases be due not to the loss or addition of a chromosome segment but to simultaneous changes within the two genes affected. Similarly, in plants, there may be minor deficiencies which have no lethal effect when haploid, and which therefore are inherited as mutations, while in other cases transformations of single genes may be lethal to the gametophyte and thus may be eliminated with the deficiencies.

Thus the working definition of mutation necessarily differs somewhat from the ideal definition. It is this working definition which must be considered in generalizing from the experimental evidence. The mutations ex-

perimentally known may include not only variations due to alterations within the gene but also variations due to losses of genes or groups of genes, to additions of genes or groups of genes, and possibly also to changes in the spatial relations of genes to one another.

It is easy to say that the latter groups are excluded by definition and should not be considered mutations. But in practice it is ordinarily impossible to distinguish them from mutations of the ideal type. Many variations previously classed as mutations have been removed from that class as mechanical explanations were found. It is safe to assume that other types of variation still classed as mutation are the result of extra-genic alterations not yet recognized.

RADIATION-INDUCED MUTATIONS IN PLANTS

The induced mutations found in barley and maize are typical gene mutations in the sense that they show normal Mendelian inheritance and normal linkage relations. They are fully viable variations recognized by their visible phenotypic effects, and in many instances they appear to be identical with previously known mutants of spontaneous origin. In the course of experiments on the relation of various factors to mutation frequency in barley, many hundreds of these mutations have been observed.

If these mutations are due to the loss of germinal material rather than to some change within the gene, the losses are so slight as to have no lethal effect on the haploid gametophyte. Most of the mutants are the progeny of fully fertile plants, and the homozygous mutants which reach the flowering stage are also fully fertile. All of the induced mutations found thus far are recessive.

The most striking difference between the results in barley and the results of comparable experiments in Drosophila is the absence of induced dominant mutations in the plant. Why do X-rays induce dominant and recessive mutations indiscriminately in the fly, and induce recessive mutations only in barley?

This apparent difference is due at least in part to the different technique used in distinguishing mutations from grosser chromosomal changes in the plant and in the animal. In the plant the alternation of diploid and haploid generations subjects all of the induced variations to the test of haploid survival before they may appear in the diploid progeny. For example, if the treatment causes the loss of a chromosome segment essential to cell survival (that is, a deficiency lethal when haploid or homozygous), the diploid individual in which the change was induced may survive. But

half of its spores receive a deficient set of chromosomes, and these are unable to develop through the gametophyte generation to the production of functional gametes. Thus the gametophyte generation filters out the variations due to lethal chromosomal derangements, and the variations observed in the progeny are only those which can survive two or three cell generations in haploid form. These may be assumed to be mutations, though it is possible that even these may include variations due to actual losses of genes which are not essential to the gametophyte.

There is no reason to suppose that dominant variations are not induced in plants as commonly as in animals. Their occurrence in maize may be demonstrated in progenies produced by the use of irradiated pollen, which are heterozygous for any changes induced in the male germ cells. Among such progenies defectives of many distinctive types are found. In these plants at least half of the pollen and ovules are aborted, and the defective type is not reproduced in the progeny. By the use of dominant genes as markers in the treated pollen it may be shown that many of these plants are deficient for some part of the normal chromosome set, and that it is the deficient spores that do not function. The characteristic type of defective development associated with deficiency of certain chromosomal regions is striking.

These characteristic defects, associated with specific deficiencies, are typical induced dominant variations of maize. In an organism in which deficient gametes are functional, they would be transmitted to three-fourths of the progeny. One-fourth, being homozygous, would commonly be inviable; the other two-fourths would be heterozygous like the parent and would show the characteristic effect of the deficiency. Thus the defect would be inherited in the same manner as the familiar "dominant mutations, lethal when homozygous," which make up the majority of the known dominant mutations of Drosophila.

These are dominants of a different type from those with which we are ordinarily concerned in the cultivated plants. Numerous dominant variations are known in cultivated races of barley, entirely free from lethal effect and inherited in simple Mendelian fashion. These include various plant colors and other characters which could be detected in sectorial chimeras. The approximate distribution of the sectors to be expected is known from chimeras produced by the irradiation of heterozygous seed; and it is certain that they could be detected. The variety of barley used is recessive for a number of such genes. All treated plants have been minutely searched for evidence of the dominant mutation of any of these known genes or of un-

known dominant genes. Some 80,000 heavily treated plants have been thus examined. Each tests the occurrence of dominant mutation not in a single treated cell but in each of several treated cells. No case of dominant mutation has been found.

This result is in sharp contrast to the experience of TIMOFÉEFF-RESSOVSKY (1930) who tested several mutant races of Drosophila for dominant reverse mutation, and, though he used much smaller populations, found one or more cases of dominant mutation in about half of the genes tested. Whether or not the mutant races of barley will yield reverse mutations has not yet been determined.

It is not necessarily true that all of the dominant variations which are lethal to the gametophyte are due to gross chromosomal alterations. As mentioned above, the gametophyte lethals removed in the normal process of reproduction in plants may include some intra-genic as well as extra-genic alterations. If the recessive mutations are due to some change within the gene, it is reasonable to suppose that corresponding changes producing a dominant effect will sometimes occur. But the surprising fact remains that when the variations lethal to the gametophyte are removed only the recessive variations remain. Any change sufficiently extreme to show its effect in the presence of its normal allelomorph appears to be sufficiently deleterious to be inviable in haploid cells. This apparently quantitative relation between the dominant and recessive variations suggests the possibility that both may be made up chiefly of deficiencies, and that only the deficiencies of very minor extent may survive the haploid generation.

But it is very improbable that all types of induced mutation are the result of deficiency—particularly those involved in the reverse mutations of Drosophila. It is possible however that the extra-genic variations of non-lethal effect, which are brought about by the treatment, include types other than mere losses. In order to consider effectively what types may reasonably be expected we need as clear a picture as possible of the way in which irradiation affects the chromosomes. A study of the grosser changes within reach of cytological technique may yield a basis for inferences regarding events on a genic scale.

THE MODE OF ACTION OF RADIATION

Cytologists have long recognized certain typical reactions of the chromosomes to X-rays. There was no basis for a mechanical theory of the phenomenon, however, until the results of combined genetic and cytological investigations became available during the last five years. The evidence is

not yet sufficient to provide a clear picture of the mode of action of radiation, but it does permit the formulation of definite working hypotheses subject to experimental test.

In the genetic investigations with X-rays it soon became evident that breaks and rearrangements of the gene-string were a characteristic effect of irradiation, and cytological investigations showed that these corresponded to actual derangements of the chromosome material. The chromosomal alterations reported have included the loss of a segment of a chromosome (either terminal or internal), the removal of a segment to a new position in the same or in another chromosome, the inversion of a segment in its original position, and the interchange of segments between chromosomes. It was at once recognized that all of these effects might be accounted for as results of the random breakage and reattachment of the chromosomes, and that additional deficiencies and duplications might be expected as a result of genetic recombination of affected and unaffected chromosomes.

SEREBROVSKY (1929) has suggested as a mechanism for such chromosomal alterations (and for gene mutations) a tendency of chromosomes to become attached and later to break apart at a different point. Presumably, on this hypothesis, the effect of X-rays is due to an increase in the tendency of the chromosomes to become attached. Mutations are considered minor deficiencies, and they with other deficiencies are assumed to be a by-product of translocation. Other investigators also have noted the possibility that the characteristic genetic effects of radiation may be traced to a basic effect on translocation.

The chromosomal alterations induced by X-rays in maize are similar to those found in Drosophila. Although the genetic analysis of the induced changes is exceedingly crude in comparison to that which may be made in Drosophila, these alterations are of interest to us at this point because they have had the benefit of critical cytological study at the period of synapsis. At this stage in the prophase of the first meiotic division, because of the length of the chromosomes and the pairing of homologous parts, aberrant chromosomal conditions may be identified with a precision impossible in the study of the condensed chromosomes.

The cytological investigation is entirely the work of Doctor BARBARA MCCLINTOCK, and was made possible by her development of the technique for the study of prophase chromosomes in maize and her determination of the distinctive morphology of the 10 chromosomes in normal material. The cytological observations have been described in detail (MCCLINTOCK 1931).

In certain important respects, the chromosomal alterations found dif-

fer from those which might be assumed on the basis of genetic evidence or of the cytological study of condensed chromosomes. They include deficiency, inversion, and segmental interchange, and various modifications and combinations of these phenomena. No case of simple translocation of a fragment to a whole chromosome has been found. Deficient segments may be either terminal or internal, but inversions are always internal. Many deficiencies are associated with translocation, but there are also some deficiencies which occur in cells otherwise normal.

The absence of simple translocations and terminal inversions, if later investigation shows it to be general in irradiated material, will greatly simplify the interpretation of the effects of radiation. Among the induced chromosomal derangements which have been reported, these are the only types which involve the attachment of a fragment at an unbroken end. If they do not occur it is unnecessary to assume any increased "stickiness" of the chromosomes under treatment. All of the chromosomal attachments observed may then be ascribed to the tendency of chromosome fragments to become attached at their broken ends, a tendency already familiar in the normal process of crossing over. It is true that many cases of simple translocation "are known," but the study of meiotic prophase material indicates that cases giving both genetic and cytological results typical of simple translocation may actually be the result of segmental interchanges in which one of the segments is very short. BURNHAM (1932) has recently described a particularly instructive case of this kind.

All of the induced chromosomal derangements which have been found in maize are consistent with the hypothesis that the effect of the treatment is merely to break the chromonema at various points, and that the fragments tend to attach themselves to one another by their broken ends. Among the reconstituted chromosomes and fragments, those which include the spindle node continue to be distributed normally at mitosis, while those which have no spindle node are sooner or later lost.

On this hypothesis the occurrence of segmental interchange is due to breaks in two chromosomes, followed by an exchange of partners in the reattachment of the distal fragments. Similar interchange of two ends of the same chromosome produces an internal inversion. If the distal fragments exchange places not with each other but with the proximal fragments (transverse interchange), the result is the loss of both distal fragments and the formation of a new chromosome with two spindle nodes. (Such chromosomes are usually eliminated in the course of the first few cell generations, but occasionally are saved by the loss of a segment in-

cluding one of the spindle nodes). An analogous interchange within the single chromosome will produce either an internal deficiency or a ring chromosome with both distal segments deficient, depending on the location of the breaks with reference to the spindle node. Displaced internal fragments tend to become inserted in an internal position, since the attachment of one end to a broken chromosome leaves the other end free for the attachment of another fragment. Finally, simple deficiency results from a break followed by the loss of the distal fragment.

The hypothesis as stated is vague at one important point, the length of the possible interval between breakage and reattachment. As long as the broken ends retain the capacity for reattachment, translocations may occur freely after treatment, as fragments come in contact in the course of intracellular movement. But even if this property is a permanent characteristic of the broken ends, a large proportion of the induced translocations must occur before the next cell division, for the fragments without spindle nodes tend to be eliminated in mitosis. If the tendency to reattachment is assumed to be transitory the opportunities for deferred translocation are reduced accordingly. At the extreme in this direction we may assume that translocations take place only when the chromosomes affected are in actual contact at the points of breakage. This possibility is not positively excluded, but the evidence now available is more favorable to the assumption of a mechanism permitting delayed attachment.

Thus all of the known chromosomal derangements induced by irradiation may be viewed as secondary effects of one primary process, chromosome breakage. It is possible that even the known effects of X-rays on the frequency of crossing over and of non-disjunction may be accounted for, at least in part, as consequences of induced translocation and deficiency.

In what ways would the mechanism postulated lead to changes inherited as mutations? The frequent occurrence of mutations at points of chromosomal interchange suggests that deficiencies or other changes incident to chromosome breakage may be a frequent cause. But there must be many points of breakage at which the two fragments broken apart reunite, instead of changing positions with other fragments. Thus the resulting type of mutation, though frequently associated with translocation, should also occur readily at other points. But this is not the only type of mutation that may occur at a point of breakage, though probably much the most frequent type. Insertion of deleted segments may also occur, and the insertion of short segments, inherited as units because of the absence of crossing over, may simulate mutation also. Further, the extent to which gene displacement

alone, without gain, loss, or transformation of genes, may cause modification of the phenotype is as yet quite unknown. Consequently the fact that some of the induced mutations behave in a manner inconsistent with a deficiency interpretation is not positive evidence that the mutations in general are intra-genic transformations.

EXTRA-GENIC ALTERATIONS AS A CAUSE OF MUTATION

If the induced mutations are due to extra-genic alterations, a certain parallel might be expected in the relation of mutation and chromosomal derangement to factors affecting their frequency of occurrence. The correlation will not necessarily be close, for different types of chromosomal alteration may respond differently to the factors applied, and mutation may not correspond to all types of chromosomal change induced by irradiation. Particularly inconclusive are those comparisons which may introduce complications due to differential survival, for we may expect derangements of different types and degrees to differ widely in survival. Nevertheless, the parallel between induced mutations and induced chromosomal derangements is striking.

The frequency of induced mutation is directly proportional to dosage (STADLER 1928, 1930a). So are the frequency of deficiencies induced by treatment of pollen (STADLER 1931) and the frequency of endosperm mosaics induced by treatment shortly after fertilization (GOODSELL 1930). The frequency of induced mutation is unaffected by temperature variations between wide limits (STADLER 1931b). This is true also of the deficiencies involved in endosperm mosaics (GOODSELL unpublished).

These similarities may be merely the result of similar but independent response to factors which are without significant effect on the occurrence of either mutation or deficiency. A more convincing parallel is found in the relation to dormancy.

Dormancy greatly reduces the frequency of induced mutation per unit of radiation intensity, but even in dormant cells mutations are induced at an appreciable rate. If induced mutations are due to chemical changes in gene composition, and induced chromosomal derangements to mechanical causes, we would not expect to find this peculiar relation of dormancy to mutation duplicated in the case of chromosomal changes. We find, however, that the frequency of the chromosomal derangements resulting in partial sterility is reduced by dormancy to about the same extent as is the frequency of mutation. Presumably, though the material has not been cytologically checked, the chromosomal derangements involved include both

translocations and deficiencies. Further, the frequency of deficiency alone, as measured by sectorial loss of dominant plant characters, apparently responds to dormancy in the same way. Whatever the cause of the peculiar relation between dormancy and mutation frequency, it apparently applies as well to induced chromosomal derangements.

Deficiencies simulating mutation may be cytologically demonstrable in maize. Deficiencies for specific chromosome regions may readily be obtained by treatment of pollen carrying dominant genes as markers. By the observation of the location of the chromosome regions lost in such plants, the regional location of marker genes may be established. Among the genes which have been used extensively those most frequently lost are *A*, *J* and L_g, which are located near the ends of three different chromosomes. Under heavy pollen treatment each of these genes is lost in about 2 percent of the progeny.

In plants deficient for any one of these markers, the length of the deficient segment varies widely. Occasionally a plant is found which has lost so small a segment that the pollen receiving the deficient genome is partly developed. McCLINTOCK (1931) has described a plant deficient for the gene L_g that had lost only a terminal segment of 4 chromomeres. The deficient pollen was partly developed and contained some starch. Plants that have lost the marker gene *J* are in some cases normally vigorous, and some of these have defective pollen fairly well developed. This was true of one case in which the deficient region comprised about one-fourth of the long arm of the chromosome. Among a large number of plants which had lost these markers, however, none with wholly normal pollen was found.

Among about an equal number of plants which had lost the marker gene *A*, 3 were found with half of the pollen recognizably defective but approaching the normal in development, and 2 with pollen apparently fully normal. All five of the plants bore ears showing distinctly less than 50 percent sterility.

If, as seems probable, the loss of *A* in these cases was due to non-lethal or incompletely lethal deficiency, these plants are connecting links between induced deficiency and induced mutation. A deficiency of a segment including *A*, with no gametic elimination, would be inherited as a mutation of the dominant *A* to the recessive allelomorph. The two plants with apparently normal pollen may be of this class. An alternative interpretation is that the change involved in these cases was actually an intra-genic mutation of *A*. This seems improbable in view of the partial gametophyte development

of plants deficient for fairly large, readily visible segments, and the series of intermediate grades of gametophyte development ranging to the apparently normal. A positive determination of deficiency may be possible in these cases by cytological examination in the meiotic prophase, although it is not certain that deficiencies of as little as one or two chromomeres can be cytologically detected in maize even at this favorable stage.

The chief reason for questioning the mechanical interpretation of the induced mutations is the occurrence of reverse mutations in Drosophila. If the original mutation is due to a loss, how shall we account for the return of the lost gene in reverse mutation?

Apparent reversion may also be found however in cases of typical genetic deficiency. In deficiencies in the endosperm, induced by irradiation of pollen, small areas of tissue showing the recovery of the lost dominant characters are not uncommonly found. If we assume that a mutation which later reverts can not possibly have been due to deficiency, we might with similar logic state that these losses of linked genes in endosperm tissue, since they revert, cannot be considered deficiencies.

The notion that a variation which reverts cannot be due to deficiency is based on the assumption that the loss involved in deficiency is brought about by some sort of instantaneous destruction or elimination of a chromosome or fragment. Apparently deficiency is not produced in this way. As I have previously pointed out (1930b, 1931a) any change which prevents the reproduction or mitotic distribution of the affected segment may have the effect of deficiency, for even if this segment were never eliminated it might be present in only one cell of the mature individual. The simplest hypothesis to account for recovery in the endosperm is that the chromosome segment affected is a fragment without a spindle node, which in most cases would be eliminated in an early mitosis, but which may occasionally escape elimination through a few cell generations and may then be restored to normal distribution by becoming attached to some chromosome with the normal spindle mechanism. Proof that fragments may thus persist and that translocation may be so long deferred is still lacking. Other chromosomal mechanisms resulting in frequent but not invariable elimination, such as certain types of transverse interchange, also may produce results simulating segmental loss followed by reversion. But, whatever the mechanism of recovery, the occurrence in the deficient endosperms of sectors of tissue showing the dominant characters proves that the treated gamete carries with it the chromosome segment which is later found to be deficient.

Since induced deficiency does not necessarily involve the immediate and complete loss of the genes affected, and since these genes are able to resume their normal activity later when suitable conditions are somehow restored, we cannot wholly exclude the possibility of a mechanical explanation for even those induced mutations which are known to be reversible. The reverse mutations described in Drosophila are not explainable on the basis of the mechanisms mentioned above, but it is not obvious that a mechanical explanation of some kind is impossible. In this connection the important recent results of DOBZHANSKY (1932) with the induced mutant "baroid" should be cited. This mutation, apparently an allelomorph of Bar, arose in an irradiated fly at the point of breakage of a chromosome. Considered in connection with the induced mutations of Bar to wild-type reported by HANSON (1928), this might be considered a reverse mutation, and at a locus at which there is reason to suppose that the original mutation (Bar to wild-type) represented an actual loss. The analysis of such cases may discover a mechanical basis for the occurrence of reverse mutation.

POLYPLOIDY IN RELATION TO MUTATION

The interpretation of mutation in certain plant species is complicated by the possibility of polyploid origin. The identification of a Mendelizing variation as gene mutation or deficiency in plants is dependent on the assumption that deficiencies will have some lethal (or at any rate distinctly deleterious) effect on the gametophyte. In those species whose genomes include two or more distinct sets of chromosomes there is little ground for this assumption. Even though the different sets of chromosomes are derived from different species, and have undergone a long course of evolution since their union, they may still possess much in common. To the extent that they carry duplicate genes they may be protected from the effects of recessive gene mutation (and to a lesser extent of deficiency), for the loss of a gene is not likely to be very injurious in the presence of a duplicate gene.

The extent to which this effect will apply is likely to vary greatly in different polyploid species, depending on the amount of differentiation that has occurred in the corresponding chromosomes. From the time the polyploid combination is established it is free to withstand the loss of duplicate chromosome regions, and such losses, if not unfavorable to survival, may become established in the evolution of the species. Eventually some species of polyploid chromosome number may lose almost all of their duplicate genes, while others may have a high proportion of genes duplicated. Since we have as yet no evidence indicating the extent of gene reduplica-

tion in different polyploid species, it is well to be cautious in the interpretation of mutation evidence from any polyploid species.

The characteristic effects which may be expected in polyploid species are:

(1) A reduction in the apparent frequency of gene mutations, due to the presence of duplicate genes. For example, seedling mutations are common in *Triticum monococcum* with 7 pairs of chromosomes, much less common in *T. durum* with 14 pairs, and rare in *T. vulgare* with 21 pairs (STADLER 1929).

(2) A reduction in the frequency of induced partial sterility, due to the survival of deficient gametophytes. According to unpublished data of W. R. TASCHER, of the UNIVERSITY OF MISSOURI, irradiation induces partial sterility with high frequency in *T. monococcum,* but the frequency is much lower in *T. durum,* and extremely low in *T. vulgare.*

(3) The appearance of variations due to the phenotypic effects of deficiencies and duplications which have been transmitted because of polyploidy. If deficiencies and translocations are induced in *T. vulgare* as in *T. monococcum,* but sterility does not occur, we must expect types characterized by deficiencies and duplications in the progeny. Some of these may be inherited as if due to mutations, either dominant or recessive. This is the probable cause of some of the speltoid and dwarf types which are found in the progeny of treated plants of *Triticum vulgare.*

(4) The appearance of apparent recessive mutations, due to the loss of segments bearing a dominant gene which is present in only one set of chromosomes. For example, if a variety of wheat is homozygous for a dominant, say beardless, in only one of its 3 sets of chromosomes, a nonlethal deficiency of this region will behave as a recessive bearded mutation. In another variety with the dominant gene present in 2 or 3 sets this mutation would not appear. This effect of gene reduplication provides a basis for "premutation" in the polyploid species.

Typical Mendelian behavior therefore is not to be considered entirely convincing evidence of the occurrence of gene mutation in the polyploid species. Mutations may occur, but there is no genetic method now available by which they may be distinguished from viable deficiencies.

This effect is not limited to polyploid species, although they furnish the most convenient material for its demonstration. Gene reduplication probably has occurred in the evolution of other groups as a consequence of translocation or other chromosomal derangements. So far as the reduplicated genes are concerned, analogous effects would occur in these species.

Is maize a polyploid species? The indication that fairly extensive deficien-

cies may be viable in maize suggests the possibility that this species also may be polyploid. The genetic and cytological conditions found in maize have not been such as to suggest polyploid origin. There is no unexpectedly high frequency of duplicate factors; the 10 chromosomes are all morphologically distinguishable; chromosome pairing is normal in the diploid, and the chromosomes of haploids show no distinct tendency to pair. The chromosome number 10 and its multiples are common in species of the Maydeae and of the related tribe Andropogoneae, and (so far as basic chromosome numbers may be determined by comparative counts) it has seemed reasonable to regard 10 as the basic number for this part of the grass family.

However, in the course of his extensive investigations of the chromosomes of various species of the Andropogoneae, BREMER (1925) found one species, *Saccharum* (*Erianthus?*) *narenga,* with 15 pairs of chromosomes, suggesting the possibility of an ultimate unit of five chromosomes in the ancestry of this tribe. Recently LONGLEY has reported a 5-chromosome species of sorghum, *Sorghum versicolor,* and P. C. MANGELSDORF informs me that he has found *Coix aquatica,* an Asiatic species of the Maydeae, to have 5 chromosomes also.

It therefore seems probable that the 10 chromosomes of maize are derived ultimately from five, though the subsequent differentiation may have been extreme. If maize is polyploid we may expect that some deficiencies may not be lethal to the gametophyte because of chromosomal reduplication, and that apparent mutations may sometimes occur as a result of the occurrence of a viable deficiency including a dominant gene. Consequently even though it may be possible to demonstrate cytologically that the radiation-induced mutations are accompanied by deficiency in maize, there is no necessary implication that this is generally true of induced mutations in species which are not polyploid.

It is possible that a certain disparity in the effects of translocation in Drosophila and maize may be due to gene reduplication connected with polyploidy in maize. In Drosophila few of the induced translocations can be made homozygous, since most of them are accompanied by lethal mutations at the point of breakage of the chromosomes. On the contrary most of the induced translocations in maize may be made homozygous without difficulty. If translocation in maize were accompanied by lethal mutation, we should expect the translocations to be eliminated in the first gametophyte generation. The fact that they are not so eliminated and that they may be established in homozygous form may be due to the suppressing action of duplicate genes.

NATURAL MUTATION

The foregoing considerations indicate that the mutations induced in plants by X-rays may be of a special type not representative of typical gene mutation. The most direct way to determine whether this is the case is simply to determine whether the treatment affects the frequency of various typical gene mutations.

But what is a typical mutation? We do not know enough about the mutation of specific genes to be able to select the typical. Practically all of the available evidence on the mutation rate of specific genes applies to genes which were selected for study because of their known high rates of mutation. If mutation is a compound class of diverse phenomena, there is little likelihood that these selected cases are a representative sample. Even the use of genes arising as mutants in culture is somewhat selective, for these will tend to be the more mutable genes. This difficulty can be in part avoided in the cultivated plants by using the characters distinguishing the established races, since these are a sample, almost random as to mutability, of the genes appearing over a much longer period.

The determination of specific mutation rates in unselected genes is feasible in maize, at least in the case of genes for endosperm characters. By a simple technique it is possible to determine the frequency of mutation in several genes simultaneously. Since each seed tests one female gamete for mutation it is practicable to determine mutation rates based on many thousands or even millions of gametes. During the last several years we have accumulated data on the normal frequency of recessive mutation of eight genes determining endosperm characters in maize. The genes used are entirely unselected except in their one common quality of affecting the endosperm. None of these genes had previously been known to mutate. Each is entirely regular in genetic behavior.

All but one of the eight genes tested yielded recurrent mutations. The frequency of these mutations varied for different genes from approximately 1 to 500 per million gametes. Different families varied rather widely in frequency of mutation of the same gene. The most mutable gene, *R*, mutated in the most frequently mutating family at a rate of more than 0.1 percent.

The mutations of these genes have no lethal effect on the gametophyte. Among more than one hundred mutant plants examined, none was affected by genetic partial sterility. Closely linked genes do not mutate together. Even in cases which may be regarded alternatively as due to multiple allelomorphs or to completely linked genes, the natural mutations

seem to affect one unit independently of the others. For example, in the multiple allelomorphic series R^r-R^g-r^r-r^g, affecting aleurone and anther color (which may also be represented as being due to two completely linked genes, R for aleurone color and N for anther color) the mutation affecting aleurone color is not accompanied by a mutation affecting anther color. In other words R^r mutates frequently to r^r, or $R\ N$ to $r\ N$, but R^r never mutates to r^g, or $R\ N$ to $r\ n$. If the series is regarded as due to multiple allelomorphs, mutation of the extreme dominant is always to an intermediate member; if it is regarded as due to combinations of completely linked genes, the mutation which affects one gene never affects the other with it. Deficiency of varying extent, simulating mutation, would be expected usually to simulate mutation to the ultimate recessive.

Several interesting questions regarding the nature of the induced mutations may be investigated by determining the effects of irradiation on the mutation of these genes. Are all of the genes caused to mutate more frequently by the treatment, or is the increase in the general rate of mutation due to a relatively great increase in the mutation rate of some genes with no effect on that of others? In the case of genes affected by irradiation, are the mutation rates increased in more or less uniform proportion, as might be expected if the normal processes of mutation are somehow catalyzed by the treatment? Or do induced mutations of all of the genes occur at similar rates, regardless of their normal frequency, as might be expected if the induced mutations are due to mechanical changes induced at random in the treated chromosomes? Are the induced mutations of R^r, like the natural mutations, limited to one of the component genes or gene-fractions, or do they, like deficiencies, usually affect both components together? These and many similar questions require evidence from the mutation of specific genes; they are untouched by experiments on the general rate of mutation. In spite of the technical difficulties, an attempt was made therefore to determine the effects of X-ray treatment on the frequency of mutation of the unselected genes whose normal mutation rates had been determined.

EFFECTS OF IRRADIATION ON THE MUTATION OF UNSELECTED GENES

In order to determine whether the normal mutation rates of the unselected genes are modified by X-ray treatment, it is necessary to apply the treatment during the early development of the ears of the plants in which mutation is to be determined. This seriously limits the amount of material which can be handled, but during the past three seasons we have secured

evidence regarding mutation of seven endosperm genes in treated populations of from 40,000 to 80,000 each. The populations are large enough to yield several mutations of the more mutable genes *R* and *I* in untreated material of the families used. They are too small to yield more than an occasional mutation of the other genes, unless the mutation rates are much increased by irradiation. If the mutation rate for all genes were increased as much as fiftyfold, numerous instances of mutation of all seven of the genes should be found.

The treatments were applied in doses sufficient to induce seedling mutations with fair frequency. In the selfed progeny of the plants thus treated several seedling mutations have been found, indicating that the X-ray treatments used produced the characteristic mutational effect. The extent to which the spontaneous mutation frequency was increased is unknown.

Mutation of the endosperm genes under test occurred at about the same rate in the treated ears as in the untreated check. In the case of the more mutable genes *R* and *I* a considerable number of mutations was obtained in both the treated and untreated groups. Not only was the mutation rate not greatly multiplied by the treatment but it was not even significantly increased. The genes P_r and *Y* yielded only a few mutations scattered at random among treated and untreated ears, with no significant increase following treatment. S_h and S_u yielded no mutations. From the natural frequency previously determined the probability of finding mutations of these genes may be computed for different rates of increase in mutation rate. The chances are even that at least one mutation of each of these genes would have been found in the irradiated material if the treatment had resulted in a tenfold increase in their natural mutation rates.

For the seventh gene (W_x) a different result was obtained. Previous trials in untreated material had failed to discover a single mutation among more than 1,500,000 gametes tested. Among 50,000 irradiated gametes tested, two clear mutations of waxy were found. The mutant waxy gene was found to have no detectable injurious effect on the male or female gametophyte and was indistinguishable from the standard waxy gene in its phenotypic effect in endosperm and pollen. Although the previous tests of untreated material were made with various families, the stock used in the radiation experiment has been tested quite extensively for natural mutation of waxy. It has yielded no spontaneous mutations in more than 100,000 tested gametes. Waxy therefore may be a locus susceptible to induced mutation.

If the 7 genes used may be regarded as a representative sample of the

genes of maize they indicate that the specific mutations appreciably affected by radiation may be a rather small fraction of the whole. It would be hard to prove that the 7 genes tested are a perfectly random sample, and at best they would constitute an inadequate sample inadequately tested. But the results of this necessarily small experiment lend no support to the assumption that mutation in general is "speeded up" by irradiation. Induced mutation does not differ from natural mutation merely in its greatly increased frequency of occurrence; apparently there is a qualitative as well as a quantitative difference.

DISCUSSION

The mutations induced by X-rays are typical gene mutations, apparently identical in genetic behavior, and in many instances in phenotypic effect, with well-known mutations of spontaneous origin. Are the germinal changes involved in these mutations representative of those involved in the natural evolution of the gene? If this is true, we may substitute the easily obtained induced mutations for the much rarer natural mutations, and we may reasonably hope for rapid progress in the solution of fundamental problems of gene structure and behavior which have previously, because of the rarity of mutation, been beyond the reach of experimental investigation.

But an affirmative answer to this question requires something more than the demonstration that mutations are induced by the treatment. Mutation is not a single homogeneous class of germinal variations. It is a residual class, in which are included various types of germinal change whose physical nature is undetermined. These may include extra-genic as well as intragenic variations. A treatment affecting only some particular type of mutation may greatly increase the total yield of mutations without necessarily affecting any process involved in the normal evolution of the gene.

The induced mutations also may include various types of germinal change, for there are several conceivable ways in which irradiation may so modify a chromosome as to cause the appearance of a Mendelizing variation. The available evidence does not yet provide an adequate basis for final conclusions as to their physical nature, but it is probable, in view of the considerations discussed in the preceding pages, that most of the induced mutations in plants are due to various extra-genic alterations, chiefly non-lethal deficiencies. This is true in spite of the fact that the variations recorded as mutations in the experiments with plants are exclusively the "visibles," the "lethals" being automatically excluded by the interposition of the haploid gametophyte generation.

The possibility that chemical transformations of the genes also are in-

duced by the treatment cannot be excluded, for various chemical reactions are known to be energized by high frequency radiation. The difficulty here is that the assumption of a chemical change can not be directly tested. All germinal variations induced by X-ray treatment could be ascribed to chemical changes in the genes, by analogy with the known chemical effects of X-rays. Since the assumed chemical change in the hypothetical gene could not possibly be disproved, the only induced variations which could be removed from this class would be those for which some other mechanism could be demonstrated. It is hazardous to assume that the known mechanical causes of germinal variation are operative only in the cases in which they are specifically demonstrated, and the hypothetical chemical causes are responsible for all other cases—particularly since the genetic and cytological criteria of deficiency approach the limit of their range of effective application as the segments involved become small enough for their effects to be transmitted as mutations.

Nevertheless the occurrence of reverse mutations in Drosophila is a strong argument for the inclusion of chemical transformations of the gene among the mutations induced by X-rays. Mutation to different allelomorphs of the same series also adds weight to this argument, though our knowledge of the nature of multiple allelomorphism is not sufficiently definite to exclude alternative interpretations. These cases considered alone might plausibly be assumed to be the result of chemical, or at any rate intragenic, mutations. However, in view of the indications of a mechanical basis for the greater portion of the induced mutations, the possibility of some mechanical explanation of even the reverse mutations should be considered. The frequent occurrence in Drosophila of mutations at the points of breakage of the chromosomes suggests that the point mutations in this species as well as in plants may be largely of mechanical origin. This association of induced mutation with chromosome breakage does not necessarily exclude the possibility that the mutations are intra-genic changes, for it is conceivable that some change within the gene may be the cause of the break, and that in some instances the gene may continue to function as a gene after the change has occurred. But the possibility that some mutant characters may be due merely to the changed spatial relations of genes to one another ("position effect") cannot be disregarded. Moreover, as has been shown in the text, the occurrence of reversion is not proof that the original mutation could not have been due even to deficiency.

Although the possibility that the induced mutations may include instances of intra-genic transformation must be left open, the identification of the

induced mutations in general as a class distinct from the chromosomal abnormalities is not possible in the experiments with plants.

If the induced mutations are wholly or largely by-products of the induced chromosomal variations, other treatments which have similar effects on the chromosomes may yield mutations of the same type. SPRAGUE (1930) found that exposure of pollen to an electromagnetic field caused an increased frequency of translocations and endosperm mosaics in maize. It is interesting to note that in the selfed progeny of these plants "a few recessive mutations were observed." RANDOLPH (1932) found various chromosomal aberrations to be induced by high temperature treatment of ears, including chromosomal deficiencies and translocations. No seedling mutations were found among 113 selfed progenies examined, but a more extensive test would be required to detect an effect on mutation rate comparable to that of an equivalent X-ray treatment, if equivalence is based on the frequency of induced translocation and deficiency.[1]

Finally, the basic question, whether the phenomenon of induced mutation is representative of the phenomenon of natural mutation, can be answered only by the determination of the effects of the treatment on specific genes unselected as to mutability. The experiments in which the effect of irradiation is determined from its influence on the general rate of mutation automatically eliminate from consideration the genes which are unaffected by the treatment. The results of these experiments may be generalized only on the assumption that mutations in general are a homogeneous class, of which the induced mutations are a representative sample. Unfortunately, the experimental test of the validity of this assumption involves technical difficulties, for it requires the determination of specific mutation rates which may be very low. An attempt in this direction is reported in the present paper. The results do not support the assumption that mutation in general is affected by irradiation.

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