# THE EFFECT OF X-RAYS UPON MUTATION OF THE GENE *A* IN MAIZE*

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THE experimental evidence indicating that gene mutation is induced by X-rays comes almost wholly from experiments on the general mutation rate; that is, from experiments in which the total frequency of detectable mutations at all loci is compared in treated and untreated cultures. "Gene mutations" as experimentally identified in such studies, include all variations inherited as if due to a change in a single gene. If it may be assumed that such variations can arise only from the transformation of a gene to an alternative form, these experiments prove the effect of the treatment upon some process of genic evolution.

It has long been evident that this assumption is not valid. Various extra-genic alterations may produce effects which are distinguishable only with great difficulty, if at all, from the effects expected from qualitative change in the hereditary unit. These include alterations involving loss, reduplication, and rearrangement of unaltered genes. To avoid ambiguity, the non-committal term "point mutation" will therefore be used to designate apparent gene mutations as experimentally identified, and the term "gene mutation" will be used only to designate the hypothetical conversion of a gene to an allelic form.

The inference that gene mutation is induced by X-rays is founded upon the assumption that some among the induced point mutations are in fact due to intragenic change, although others may be shown to be due to extra-genic alterations simulating gene mutation. But there are no general criteria by which the observed mutations occurring at miscellaneous loci may be individually identified as intra-genic or extra-genic. Consequently it is not possible to determine the effect of X-ray treatment upon gene mutation by experimental modification of the general mutation rate.

The alternative is to study the mutational behavior of selected genes especially suited to the purpose, both in spontaneous mutation and in mutation induced by various treatments, in the hope of developing for these genes a sounder basis for interpretation. For this purpose the loci *A* and *R* in maize provide favorable material, for reasons which have been stated in an earlier paper (STADLER 1941).

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The present study is concerned with the nature of X-ray-induced mutations at the $A$ locus. A parallel study of ultraviolet-induced mutations at the $A$ locus will be published shortly. These studies were made several years ago and the summarized results have been published in abstract form (STADLER and ROMAN 1943), but various delays incidental to the war have prevented the completion and fuller publication of the work. Meanwhile, certain other studies concerned with the general problem have been reported (STADLER and FOGEL 1943, 1945; STADLER 1944, 1946, FOGEL 1946a, b; LAUGHNAN 1946).

## EXPERIMENTAL MATERIALS AND METHODS

The gene $A$, in the presence of appropriate complementary genes, affects the pigmentation of various tissues of the maize plant. In the presence of $A_2$, $B$, $Pl$, $C$, $R$, $Pr$, and $P$, the phenotypic effect of $A$ may be represented as follows:

| GENOTYPE | ALEURONE COLOR | PLANT COLOR | PERICARP COLOR |
|---|---|---|---|
| $A\ A$, $A\ a$ | Purple | Purple | Red |
| $a\ a$ | Colorless | Brown | Brown |

The purple color of the aleurone and plant tissues is due to an anthocyanin pigment, while the red color of the pericarp is due to an unknown pigment, not an anthocyanin. Various additional alleles of $A$ have been reported, including some with effects apparently intermediate between those of $A$ and $a$ (EMERSON and ANDERSON 1932; RHOADES 1941).

The populations from which the mutants here described were selected were $F_1$ progenies produced by the cross $aa \times AA$, the pollen of the male parent being X-rayed just before pollination. Plants showing loss of the $A$ effect, whether by mutation, deficiency, or other types of genetic alteration, will be referred to as "$A$ losses." These are readily identified in $F_1$, $A$ loss in the endosperm resulting in a seed with colorless aleurone, $A$ loss in the embryo in a seedling devoid of anthocyanin. Endosperm and embryo effects are produced independently in maize pollen irradiated at maturity, since the second microspore division occurs several days before the pollen is mature. Spontaneous mutations of $A$ to $a$, occurring previous to gene reduplication for this division, would result in $A$ loss in both endosperm and embryo. As determined from the frequency of mutation affecting endosperm and embryo together, the frequency of spontaneous mutation of $A$ is low (STADLER 1941 and unpubl.), and no spontaneous mutations would be expected in a population equal to the total number of plants examined in this study.

With X-ray treatment of mature pollen, loss of the $A$ effect is not uncommon, its frequency varying with the X-ray dose. $A$ losses in the endosperm cannot be tested to discriminate between mutation and deficiency. The anthocyanin-free seedlings of the $F_1$, which include all $A$ losses in the embryo, may include also maternal haploids or diploids resulting from development of the embryo without fertilization. The plants may be grown for further study, to distinguish these classes, and such alterations as are not lethal to the gametophyte may be carried on to subsequent generations for more critical analysis.

Haploids which survive the seedling stage are readily recognizable by their distinctive growth habit, reduced cell-size, and almost complete pollen abor-

tion, as well as by their wholly maternal phenotype. Maternal diploids would be recognizable only by their maternal phenotype. They would presumably be normal in development, and would be distinguishable from self-contaminations only by their failure to show the maternal phenotype in the endosperm of the seed from which the affected plant was grown. In cultures so marked as to permit identification of both maternal haploids and maternal diploids, more than 30 maternal haploids, but no maternal diploids, were found. The frequency of maternal haploids varies rather widely in different stocks used as female parent, and is not materially affected by X-ray treatment of the pollen parent, except for the slight increase in relative frequency expected from the elimination of a portion of the diploid progeny through lethal effects of the treatment.

The great majority of the $A$ losses found were distinctly defective plants, among which those which survived to flowering had at least 50 percent of the pollen aborted. Among the remainder, with approximately normal plant development, similar segregation of aborted pollen is found in almost all cases. In a few of these plants the segregating defective pollen was not aborted, but was distinctly subnormal in size and in starch content at maturity. Many of these plants show segregation for two or more factors producing defective pollen, and in some of these there is segregation for both aborted and subnormal pollen. Various grades of defective pollen development are recognizable in iodine-stained pollen specimens, and specific grades are associated with specific deficiencies. These range form aborted pollen containing little or no starch, characteristic of most known deficiencies, to types only slightly subnormal in size and starch content. In a few cases the pollen is wholly normal in appearance but fails to accomplish fertilization in competition with normal pollen. McClintock (1944) has described minute terminal deficiencies in chromosome 9 with pollen visibly normal and capable of normal functioning in competition with non-deficient pollen. The occurence of segregating defective pollen serves as a rather sensitive detector of segmental deficiency, but deficiencies without detectable effect upon the pollen may occur.

The purpose of the experiment was to select from among the $A$ losses induced by X-ray treatment those least subject to the suspicion of deficiency, in order to determine whether these were in fact experimentally indistinguishable from gene mutation of $A$. If $A$ losses free from pollen effect occur, a certain proportion of these may be missed in the $F_1$ plants because of the coincidental occurrence of alterations at other loci resulting in defective pollen segregation. The proportion of such cases would increase with increasing dosage, and would be roughly equal to the proportion of pollen-segregating plants in the entire progeny. For this reason relatively low X-ray doses (usually 400 r) were used at first, but when it became evident that a very large sample of $A$ losses would be needed, doses of 1000 r were used. Among a total of 126 $A$ losses found in these progenies, 76 failed to survive to flowering or were so defective as to give no pollen specimen, 49 showed segregation for aborted pollen, or for aborted and subnormal pollen, and one showed segregation for subnormal pollen only. None was a normal plant free from defective pollen segregation. Since the pro-

portion of *A* losses free from gametophytic effect was obviously extremely low, various progenies produced for other purposes were used as a supplementary source of *A* losses. These were mostly from doses of 1000 r, though some with higher doses were included. They included progenies not so marked as to permit regular identification of haploids, and in some cases the genotype was such as to give anthocyanin-free seedlings by *R* loss as well as *A* loss. Among the anthocyanin-free plants which did not survive the seedling stage the *R* losses and in some cases the haploids could not be distinguished from the *A* losses. The inclusion of this supplementary material made it impossible to determine accurately the total number of *A* losses observed, but it did result in the finding of two *A* losses with no visible effect upon the pollen. A total of 543 anthocyanin-free seedlings was observed in the course of the experiment. On the basis of the relative frequency of haploids and *R* losses in cultures properly marked for their identification, it is estimated that about 415 of these plants were *A* losses.

## THE MUTANTS

The genetic alterations responsible for the two *A* loss plants with normal pollen were designated respectively *a-X1* and *a-X2*, and were extracted for further study. In addition there was included for comparison the genetic alteration extracted from one of the *A* losses with segregating subnormal pollen. This was designated *a-X3*.

Alteration *a-X1* occurred in a plant of normal vigor in an $F_1$ progeny of the cross $a\ A_2\ R^g\ C\ b\ pl\text{-}y\ pr\ wx\ p \times A\ A_2\ r^{ch}\ C\ b\ pl\text{-}y\ Pr\ Wx\ p$, in which the pollen was irradiated (dose 1000 r) just before pollination. The genotype was such as to give green plants by either *A* loss or $r^{ch}$ loss. The ear, pollinated by $a^p$ *Dt Dt*, yielded a full seed set, showing that the alteration was haplo-viable and transmissible through female germ cells. All of the seeds were pale, showing that the case was due to *A* loss rather than $r^{ch}$ loss. Somewhat less than half of these seeds showed one or more dots, and the remainder were dotless. The plants grown from these seeds were crossed on and by an *a*-tester ($a\ A_2\ C\ R$). In crosses on the *a* tester stock all of the plants from dotted seeds gave approximately equal transmission of pale and colorless, while all but one of the plants from dotless seeds showed lowered transmission of the colorless seed type. Subsequent tests confirmed this indication, showing that the *a* "allele" derived from the treated parent was distinguished from the standard *a* allele by failure to respond to *Dt* (in any dosage) and by consistently lowered transmission through male germ cells.

Alteration *a-X2* was found in $F_1$ of the cross $a\ A_2\ C\ R^g\ b\ pl\ p \times A\ A_2\ R^r\ C\ b\ Pl\ p$, with treatment of the parental pollen at a dose of about 900 r just before pollination. The treatment was applied in the field with a mobile X-ray unit not calibrated in r units. The dose is estimated from the relative frequency of genetic effects commonly produced by this treatment, in comparison to the frequency of similar effects produced by treatments at measured doses. The plant was normal in vigor and produced two ears, both with approximately a full set of seed. Pollinations similar to those described for *a-X1* gave similar results, except for a greater reduction in transmission through male germ cells

and an indication in some ears of possible reduced transmission through female germ cells.

Alteration *a-X3* occurred in the $F_1$ of the cross *a* $A_2$ *C-Wx* $r^r$ *j lg*×*A* $A_2$ *c-wx* $R^g$ *pr su y*, in which both the parental ear and the parental tassel were X-rayed just before pollination. This treatment also was applied in the field. The dose applied to the tassel was approximately 1200 r. The original $F_1$ plant was normal in development, but about 50 percent of its pollen was clearly subnormal in size and starch content. A similar subnormal pollen type, associated with an *R* deficiency (*DfX-1*) has been illustrated and described in some detail in an earlier paper (STADLER 1933). The ear of this plant did not produce a full seed set, but in parts of the ear there seemed to be definitely more than 50 percent seed set. Cultures grown from the seed in these sections yielded a few plants with pollen segregation similar to that observed in the $F_1$ plant, and these were crossed with various testers to provide material for further study. Pollinations similar to those described for *a-X1* showed that the subnormal pollen segregation is regularly associated with *a-X3*, and that there is no transmission of the alteration through male germ cells. The subnormal pollen is slightly less defective than in *Df X-1*, but is always clearly detectable. Transmission through female germ cells also is distinctly reduced. Its frequency is readily shown in pollinations of the type *A/a-X3*×$a^p$, in which the seeds heterozygous for *a-X3* are pale, while their normal sibs are fully colored. The pale seeds in this pollination are usually less than one fourth as numerous as the colorless seeds, and in general are slightly smaller. The difference in seed size is slight, and would not be detectable in the shelled seed, because of difference in seed size due to position of the seed on the ear. The reality of the difference may be demonstrated objectively in ears of the type *A/a-X3*×*A*, in which the two classes are indistinguishable in color-phenotype. Small seeds, carefully selected by comparison of seed size in neighboring seeds, give with few exceptions plants showing subnormal pollen segregation. The plants grown from the small seeds are not visibly inferior to their sibs, either in seedling growth or in development at later stages.

All three alterations occurred in cultures lacking the complementary factors, *B*, *Pl*, and *P*. In subsequent extractions it was found, for all three alterations, that plants of genotype *a/a-X B Pl* are brown, like *a/a B Pl*, and plants of genotype *a/a-X P* have brown pericarp like *a/a P*. Several objective tests were made in an attempt to distinguish *a/a-X3* from *a/a* sibs on the basis of plant color phenotype at various stages of development, but these attempts were wholly unsuccessful.

## GAMETOPHYTE VIABILITY

In crosses of the type *A/a-X* ×$a^p$, the production of pale seeds demonstrably *a-X*/$a^p$ in genotype proves that the *a-X* alteration is viable in the haploid female gametophyte, for these seeds can be produced only by the normal functioning of the *a-X* gametophyte. Similarly, the production of $a^p$/*a-X* seeds in crosses of the type $a^p$×*a/a-X* proves that the alteration is viable in the male gametophyte. On the basis of these tests it is clear that *a-X1* and *a-X2* are viable in both male and female gametophytes, and that *a-X3* is viable at least in

female gametophytes. The failure of male transmission of *a-X3* does not necessarily mean that pollen bearing this alteration is inviable, since it could result from the slower pollen tube growth of this type in competition with pollen bearing the standard allele *a*, as well as from failure of the pollen to germinate.

The pollinations made to determine haplo-viability show also the relative frequency of fertilization accomplished by *a-X* gametophytes versus gametophytes bearing a standard *A* or $a^p$ allele. Differences in female transmission between two contrasted types presumably result only from differences in the proportion effectively fertilized or differences in survival of the heterozygotes produced. Differences in male transmission may result from these causes also, but in addition the factor of competition in pollen-tube growth may cause large inequalities in the number of ovules which may be fertilized by the two types of sperms. Trials of male transmission frequency are complicated by the fact that there are many unidentified pollen tube growth factors in maize, so that 1:1 transmission through male germ cells is not necessarily to be expected in the case of two alleles which are themselves without effect upon gametophyte functioning. A significant deviation from equality may be the result of other factors linked to those under study. For more critical evidence of the effect, it is desirable to make comparisons of male transmission between sibs secured from a cross of the type $A \times a\text{-}X/a$, the transmission tests being crosses of a number of the progeny plants, including $A/a\text{-}X$ and $A/a$ individuals, upon an $a^{dl}$-*tester* ($a^{dl}$ $A_2$ *C R Dt Dt*). The symbol $a^{dl}$ represents an allele similar to *a* except for failure to respond to *Dt*. Colorless seeds in the crosses by $A/a$ are dotted; those in crosses by $A/a\text{-}X$ are dotless. The relative frequency of colored and colorless seeds in the crosses by $A/a$ plants establishes a norm of transmission of the *A* chromosome for comparison with that shown in crosses by the $A/a\text{-}X$ plants.

Trials of this type were made in various extracted stocks of both *a-X1* and *a-X2*, and trials of female transmission in several extracted stocks of each of the three alterations. Alteration *a-X1* regularly shows reduced male transmission, but the extent of reduction varies in different cultures. For example, in tests of $a\text{-}X1/a^p$ plants, in comparison with $a/a^p$ sibs, seven $a\text{-}X1/a^p$ plants of one culture yielded a total of 803 colorless and 1390 pale seeds (58 percent of normal transmission), while in a closely related culture tested at the same time five plants tested yielded 317 colorless and 742 pale (43 percent of normal transmission). The individual plants did not differ significantly from the averages stated, and the $a/a^p$ sibs in both cultures gave normal transmission. Occasional plants heterozygous for *a-X1* have been found to give less than 25 percent normal transmission, but ordinarily male transmission of this alteration is not far from 50 percent of normal. Female transmission is usually equal to normal, with occasional exceptions showing slightly lowered transmission.

With *a-X2*, male transmission is commonly about 25 percent of normal, and female transmission about 65 percent of normal, again with occasional rather wide variations. One culture, in which seven plants of $A/a\text{-}X2$ and six plants of $A/a$ were all tested for both male and female transmission, gave a total frequency of 1334 *A* and 398 *a-X2* in male transmission (30 percent normal) and

931 *A* and 661 *a-X2* in female transmission (71 percent normal). Differences between individual plants were slight and transmission was normal in the *A/a* sibs. In other cultures male transmission has frequently been as low as 15 percent and occasionally as low as 5 percent. For example, in one culture in which three $a^p$/*a-X2* plants were tested rather extensively for male transmission, the total frequency was 1588 $a^p$ and 72 *a-X2* (4.5 percent normal).

With *a-X3*, male transmission has never been found, and female transmission is always low, ordinarily about 15–35 percent. Individual ears with no transmission of the alteration are not rare, but usually at least a few *a-X3* seeds are produced, and occasionally female transmission approaches 50 percent.

## VIABILITY OF HOMOZYGOTES AND COMPOUNDS

When *A/a-X1* or $a^p$/*a-X1* plants are selfed or intercrossed, the homozygote *a-X1/a-X1* should be recognizable by absence of anthocyanin in the aleurone and plant tissues, and should occur with an average frequency of about 17 percent (assuming normal female transmission and 50 percent male transmission of *a-X1*). Selfed or intercrossed ears of these genotypes yield no colorless seeds at all. The absence of colorless seeds cannot be accounted for by the assumption that the phenotype of *A-X1/a-X1* is other than as expected, for all of the seeds from *A/a-X1* selfs are full-colored, and all of the seeds from $a^p$/*a-X1* selfs are pale. The plants grown from these seeds show the corresponding plant color phenotype, and at least half of these plants are found to be heterozygous for *a-X1*. In many of these pollinations, the same pollen sample used in selfing was used also in outcrosses on an *a* tester, to establish the level of male transmission of *a-X1*, and sibs of the plants selfed were pollinated by an *a* tester to establish female transmission of *a-X1* in the cultures used. Male and female transmission tests of *a-X1* in the outcrosses gave results in agreement with the tests described in the preceding section. Assuming random fertilisation, the absense of colorless seeds thus shows that the homozygote is inviable. There was no class of aborted or defective seeds, or of empty pericarps, which could be considered to represent the ovules in which the combination occurred.

With *a-X2* the proportion of homozygotes expected in selfs and intercrosses is lower, due to the lower male and female transmission of this alteration. With female transmission at 65 percent and male transmission at 25 percent of normal, the expected average frequency of homozygotes would be about 8 percent. A large number of selfs and intercrosses of *a-X2* heterozygotes, including several with simultaneous tests of male and female transmission, failed to yield a single colorless seed, and it is clear that in this case also the absence of colorless seeds is due to the inviability of the homozygote.

The viability of *a-X3* homozygotes cannot be tested in this manner, because of the lack of male transmission.

The viability of the compound *a-X1/a-X2* is readily tested by crosses of the type $a^p$/*a-X2*×$a^p$/*a-X1*. The expected frequency of colorless seeds representing the compound is about 13 percent, or for the reciprocal cross about 10 percent. Numerous crosses of these types were made, and all of the seeds produced were pale, except for one colorless seed. The plant grown from this seed proved

to be heterozygous for a dottable *a*. The seed thus does not represent the compound, and presumable was the result of pollen contamination.

Viability of the compounds *a-X1/a-X3* and *a-X2/a-X3* may be tested similarly, but because of the low and variable female transmission of *a-X3* the tests must be made on a fairly large number of ears of the *a-X3* heterozygote. Crosses of the type $a^p/a\text{-}X3 \times a^p/a\text{-}X1$ were made on 11 ears, which produced a total of 985 seeds, all pale. With average transmission of the *a-X* alterations concerned, and with normal viability of the compound, the expected frequency of colorless seeds would be 55 (5.6 percent). Similarly, 13 ears of the cross $a^p/a\text{-}X3 \times a^p/a\text{-}X2$ yielded a total of 1130 seeds, all of which were pale except two colorless seeds which proved to be due to pollen contamination. With average transmission rates the expected frequency of colorless seeds would be 37 (3.3 percent). These crosses indicate beyond reasonable doubt that the compounds in all combinations, like the homozygotes, are zygotically lethal.[1]

## EFFECT ON CROSSING OVER

Short intercalary deficiencies in maize sometimes reduce crossing over much more than would be expected from the length of the deficient segment. For example (STADLER 1935) *Df 5-1*, a deficiency of a short intercalary segment of the longer arm of chromosome 5, includes the locus $V_3$, and not the neighboring loci *Bt*, to the left, and *Bv*, to the right. Its genetic length is therefore less than 3.8 crossover units, the map distance from *Bt* to *Bv*, The deficiency is haplo-viable in the female gemetophyte, and in heterozygous plants it is cytologically detectable at pachytene by the occurrence of a short "buckle" in chromosome 5, usually located on the longer arm near the centromere. In plants heterozygous for *Df 5-1*, crossing over in the region between *Bm* and *Bv* (the region including the deficiency) is almost completely inhibited, and crossing over in the adjacent region between *Bv* and *Pr* is greatly reduced. The total reduction in crossing over is considerably greater than the map length of the deficiency.

Presumably, the reason for the reduction in crossing over outside of the deficient region is the strong tendency toward non-homologous pairing in the chromosomes of maize. MCCLINTOCK (1933) has shown, in plants heterozygous for various chromosomal derangements, the frequent occurrence of close pachytene pairing of non-homologous regions. In plants heterozygous for *Df 5-1*, the buckle observed in the chromosome 5 pair at pachytene is formed by a segment of the non-deficient homolog equal in length to the segment missing from the deficient homolog. If pairing were always between homologous regions, the position of the buckle would be constant and would mark the location of the deficiency. In fact, however (STADLER 1935), the position of the buckle varies widely, and in extreme instances it may occur on the distal half of the longer arm, or in the proximal region of the shorter arm. When the position of the buckle is not identical with the locus of the deficiency, the chromosome re-

[1] Note added in proof: RHOADES (Maize News Letter, Mar. 1948), using *a-X2* in combination with a gametophyte factor *ga* distal to *et*, finds no production of homozygous *a-X2* seeds on selfed ears of the heterozygote *ga/a-X2*. Here the ratio expected is increased to about 30 percent, and the absence of visible sterility on the ear suggests that selective fertilization may be involved.

gions paired between these two points must be non-homologous. In cases in which the buckle is found on the shorter arm, the non-homology of the paired region is cytologically evident, for the centromeres of the two chromosomes are separated by a distance approximately equal to the length of the buckled segment.

If non-homologous pairing is a major cause of the reduction in crossing over, it may be expected that pronounced reduction in crossing over may usually be found with short intercalary deficiencies in maize. The cytological observations described above are typical of the observed behavior of short intercalary deficiencies. Deficiencies too short to cause the formation of a visible buckle, though they would not permit its cytological demonstration, could result in non-homologous pairing which might extend over a considerable length of chromosome and thus might lead to distinct reduction in crossing over.

The effect of the *a-X* alterations upon crossing over can be determined only with rather distant or otherwise unfavorable marker genes. The only known marker distal to *A* is *et*, an X-ray-induced mutant located about 12 units from *A* (STADLER 1940). The phenotypic effect of *et* is shown by both endosperm and seedling, the endosperm surface being characteristically scarred (etched), and the seedling being virescent. The endosperm effect is usually distinct enough for positive separation, though it may sometimes be confused with scarred endosperm resulting from the action of other genes or occurring sporadically with no simple genetic basis. In some matings the endosperm effect is very extreme, and many of the etched seeds are small and inviable. The virescent character of the seedling is always clear and readily separable, except at unusually high temperatures. Cultures which give a clear etched endosperm separation always give virescent seedlings from all etched seeds, and in ears with endosperm separation doubtful, the seedling separation is useful as a check. The alteration is readily transmitted through both male and female germ cells, though sometimes in less than normal ratio.

The nearest useful locus proximal to *A* is $lg_2$, about 34 crossover units distant. The gene *na* is probably a few units closer to *A* but is not well suited to seedling detection. The effect of $lg_2$ is clear in the seedling stage, permitting positive classification in the rather large numbers needed for the comparison.

The effect of the *a-X* alterations upon crossing over in the regions *Et-A* and $A$-$Lg_2$ was determined from sib plants produced in crosses of the type $Et$ $a$-$X$ $Lg_2/Et\ a\ Lg_2 \times et\ A\ lg_2$. This cross yields plants of two types, $Et$ $a$-$X$ $Lg_2/et\ A\ lg_2$ and $Et\ a\ Lg_2/et\ A\ lg_2$. Crossover frequency was determined in plants of both types, by pollinating them by $et\ a\ lg_2$. The results are shown in table 1. The relationship of the plants tested is indicated by the culture numbers. For example, the effect of *a-X1* was tested in two cultures, 49:763 (including two *a-X1/A* plants and three *a/A* plants tested) and 51:14 (including three *a-X1/A* and three *a/A* plants tested). The cultures 49:763 and 51:14 were progenies of two different crosses of the type $Et$ $a$-$X$ $Lg_2/Et\ a\ lg_2 \times et\ A\ Lg_2$.

With *a-X1* there is no consistent indication of crossover modification, although the average frequency of crossing over in the *Et-A* interval is slightly lower than in the normal sibs tested.

With *a-X2*, there is a pronounced and consistent reduction in crossover fre-

TABLE 1

*Effect of a-X1, a-X2, and a-X3 on crossing over. Crossover frequency in female gametes in backcrosses.*

| $F_1$ GENOTYPE | NON-CROSSOVERS | | CROSSOVERS | | | | | | CROSSOVER % | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | REG. 1 | | REG. 2 | | REG. 1, 2 | | REG. 1 | REG. 2 |
| | | | | | *a-X1* | | | | | |
| *et A lg/Et a-X1 Lg* | | | | | | | | | | |
| 49:763-3 | 44 | 57 | 7 | 8 | 22 | 26 | 2 | 0 | 10.2 | 30.1 |
| 49:763-12 | 106 | 85 | 11 | 16 | 36 | 52 | 4 | 2 | 10.6 | 30.1 |
| 51:14-6 | 98 | 110 | 10 | 14 | 55 | 48 | 6 | 5 | 10.1 | 33.0 |
| 51:14-13 | 88 | 70 | 19 | 20 | 41 | 47 | 2 | 2 | 14.9 | 31.8 |
| 51:14-19 | 69 | 67 | 13 | 13 | 21 | 14 | 2 | 9 | 17.8 | 22.1 |
| Total | 405 | 389 | 60 | 71 | 175 | 187 | 16 | 18 | 12.5 | 30.0 |
| *et A lg/Et a Lg* | | | | | | | | | | |
| 49:763-4 | 80 | 81 | 25 | 24 | 26 | 45 | 2 | 1 | 18.3 | 26.1 |
| 49:763-9 | 81 | 82 | 19 | 16 | 36 | 39 | 3 | 2 | 14.4 | 28.8 |
| 49:763-10 | 42 | 61 | 13 | 10 | 28 | 26 | 2 | 6 | 16.5 | 33.0 |
| 51:14-5 | 72 | 70 | 17 | 16 | 35 | 29 | 0 | 4 | 15.2 | 28.0 |
| 51:14-9 | 93 | 85 | 16 | 16 | 36 | 58 | 4 | 8 | 13.9 | 33.5 |
| 51:14-20 | 82 | 62 | 6 | 18 | 28 | 31 | 4 | 3 | 13.3 | 28.2 |
| Total | 450 | 441 | 96 | 100 | 189 | 228 | 15 | 24 | 15.2 | 29.6 |
| | | | | | *a-X2* | | | | | |
| *et A lg/Et a-X2 Lg* | | | | | | | | | | |
| 49:774-7 | 86 | 62 | 0 | 5 | 26 | 28 | 0 | 3 | 3.8 | 27.1 |
| 49:774-8 | 43 | 60 | 2 | 7 | 12 | 9 | 0 | 0 | 6.8 | 15.8 |
| 51:15-2 | 63 | 110 | 2 | 9 | 12 | 25 | 1 | 1 | 5.8 | 17.5 |
| 51:15-4 | 21 | 19 | 0 | 3 | 3 | 6 | 0 | 0 | 5.8 | 17.3 |
| 51:15-11 | 62 | 82 | 1 | 4 | 13 | 35 | 1 | 1 | 3.5 | 25.1 |
| 51:15-13 | 48 | 64 | 1 | 8 | 16 | 9 | 1 | 3 | 8.7 | 19.3 |
| 51:15-14 | 57 | 90 | 2 | 1 | 14 | 25 | 0 | 0 | 1.6 | 20.6 |
| 51:15-17 | 68 | 97 | 4 | 9 | 13 | 34 | 0 | 1 | 6.2 | 21.2 |
| 51:15-18 | 45 | 92 | 2 | 5 | 10 | 18 | 1 | 0 | 4.6 | 16.8 |
| Total | 493 | 676 | 14 | 51 | 119 | 189 | 4 | 9 | 5.0 | 20.6 |
| *et A lg/Et a Lg* | | | | | | | | | | |
| 49:774-9 | 16 | 54 | 3 | 10 | 15 | 31 | 1 | 0 | 10.8 | 36.2 |
| 49:774-11 | 28 | 67 | 7 | 11 | 13 | 28 | 1 | 2 | 13.4 | 28.0 |
| 49:774-14 | 40 | 42 | 7 | 15 | 19 | 32 | 1 | 4 | 16.9 | 35.0 |
| 51:15-3 | 55 | 100 | 7 | 19 | 35 | 52 | 2 | 1 | 10.7 | 33.2 |
| 51:15-15 | 60 | 113 | 16 | 12 | 31 | 77 | 2 | 6 | 11.4 | 36.6 |
| 51:15-19 | 55 | 120 | 10 | 19 | 25 | 65 | 1 | 2 | 10.8 | 31.3 |
| Total | 254 | 496 | 50 | 86 | 138 | 285 | 8 | 15 | 11.9 | 33.5 |
| | | | | | *a-X3* | | | | | |
| *et A lg/Et a-X3 Lg* | | | | | | | | | | |
| 49:751.1-1 | 93 | 7 | 0 | 2 | 37 | 4 | 0 | 0 | 1.4 | 28.7 |
| 49:751.1-3 | 122 | 25 | 0 | 1 | 45 | 12 | 0 | 0 | 0.5 | 27.8 |
| Total | 215 | 32 | 0 | 3 | 82 | 16 | 0 | 0 | 0.9 | 28.2 |
| *et A lg/Et a Lg* | | | | | | | | | | |
| 49:751.2-1 | 110 | 109 | 20 | 17 | 52 | 53 | 2 | 0 | 10.7 | 29.5 |
| 49:751.2-3 | 19 | 101 | 9 | 6 | 44 | 20 | 0 | 14 | 13.6 | 36.6 |
| 49:751.2-7 | 41 | 51 | 6 | 8 | 30 | 32 | 1 | 4 | 11.0 | 38.7 |
| 49:751.2-9 | 85 | 77 | 17 | 13 | 27 | 42 | 7 | 2 | 14.4 | 28.9 |
| Total | 255 | 338 | 52 | 44 | 153 | 147 | 10 | 20 | 12.4 | 32.4 |

quency, both in the *Et-A* interval, to the left of the alteration, and in the *A*-$Lg_2$ interval, to the right. There is considerable variation in the extent of reduction of crossing over in different plants, but in each of the nine *a-X2/A* plants tested, for both of the segments covered, the crossover frequency is lower than in any of the six *a/A* plants tested.

With *a-X3*, only two plants heterozygous for the alteration are available in the test for crossover effects in the entire *Et*-$Lg_2$ region, since in the tests made in the later series it was necessary to use cultures not marked at the $Lg_2$ locus.

TABLE 2

*Effect of a-X3 on crossing over (Et-A segment). Crossover frequency in female gametes in backcrosses.*

| $F_1$ GENOTYPE | NON-CROSSOVERS | | CROSSOVERS | | CROSSOVER PERCENT |
|---|---|---|---|---|---|
| | *et A* | *Et a*(−*X3*) | *et a*(−*X3*) | *et A* | |
| *et A/Et a-X3* | | | | | |
| 49:751.1–1 | 130 | 11 | 0 | 2 | 1.4 |
| 49:751.1–3 | 167 | 37 | 0 | 1 | 0.5 |
| 51:11.1–1 | 30 | 24 | 0 | 3 | 5.3 |
| 51:11.1–10 | 91 | 64 | 1 | 6 | 4.3 |
| 51:11.2–8 | 64 | 63 | 0 | 10 | 7.3 |
| 51:12.1–12 | 68 | 19 | 0 | 4 | 4.4 |
| 51:12.1–14 | 71 | 10 | 0 | 8 | 9.0 |
| Total | 621 | 228 | 1 | 34 | 4.0 |
| *et A/Et a* | | | | | |
| 49:751.2–1 | 162 | 162 | 22 | 17 | 10.8 |
| 49:751.2–3 | 63 | 121 | 9 | 20 | 13.6 |
| 49:751.2–7 | 71 | 83 | 7 | 12 | 11.0 |
| 49:751.2–9 | 112 | 119 | 24 | 15 | 14.4 |
| 51:11.1–11 | 95 | 127 | 14 | 8 | 9.0 |
| 51:11.2–6 | 71 | 141 | 9 | 23 | 13.1 |
| 51:11.2–22 | 120 | 133 | 12 | 18 | 10.6 |
| 51:12.1–11 | 66 | 136 | 23 | 17 | 16.5 |
| 51:12.1–13 | 86 | 176 | 10 | 16 | 9.0 |
| Total | 846 | 1,198 | 130 | 146 | 11.9 |

The data on crossing over in the *Et-A* region in these tests will be given presently. The data for the two plants marked at all three loci show pronounced reduction of crossing over in the *Et-A* region, but only slight and insignificant reduction in the *A*-$Lg_2$ region.

Additional data for *a-X3*, on crossing over in the *Et-A* region, are given in table 2. The endosperm classification was checked by seedling classification in all cases, and seeds which failed to germinate are omitted. The data are therefore comparable with those for the *Et-A* region in table 1. Data on the *a-X3* effect from table 1 are included in table 2 for comparison.

The results show a pronounced reduction in crossing over in plants hetero-

zygous for *a-X3*, similar to that observed with *a-X2*, and like the latter varyng rather widely in different $F_1$ plants tested.

It should be noted that there are large inequalities in the frequency of corresponding classes in these backcross progenies. This is evident not only in the progenies of *A/a-X* plants, in which inequality is expected from the lowered female transmission of the various *a-X* alterations, but also in the progenies of *A/a* plants, in which this factor would not enter. The marker gene *et* shows distinctly reduced viability in the families used in testing the crossover effect of *a-X2* and *a-X3*, in which the progenies of *A/a* plants used as controls show *Et:et* ratios of 1.00:0.51 and 1.00:0.73 respectively. The reduced frequency of

TABLE 3

*Effect of a-X1, a-X2, and a-X3 on crossing over (ET-A segment). Data from totalled seed counts. (Crossover frequency in female gametes in backcrosses.)*

| $F_1$ GENOTYPE | NUMBER OF CULTURES | NON-CROSSOVERS | | CROSSOVERS | | CROSSOVER percent | *Et:et* RATIO | *A:a(-X)* RATIO |
|---|---|---|---|---|---|---|---|---|
| | | *et A* | *Et a(-X)* | *et a(-X)* | *Et A* | | 1.00: | 1.00: |
| For *a-X1* | | | | | | | | |
| *et A/Et a-X1* | 5 | 598 | 597 | 78 | 90 | 12.3 | 0.98 | 0.98 |
| *et A/Et a* | 6 | 676 | 683 | 114 | 132 | 15.3 | 0.97 | 0.99 |
| For *a-X2* | | | | | | | | |
| *et A/Et a-X2* | 9 | 737 | 892 | 27 | 61 | 5.1 | 0.80 | 1.15 |
| *et A/Et a* | 6 | 484 | 798 | 61 | 108 | 11.7 | 0.60 | 1.45 |
| For *a-X3* | | | | | | | | |
| *et A/Et a-X3* | 7 | 917 | 261 | 8 | 37 | 3.7 | 0.32 | 0.28 |
| *et A/Et a* | 9 | 1,036 | 1,261 | 166 | 153 | 12.2 | 0.85 | 0.83 |

*et* is due in part to lowered female transmission and in part to lowered germination of *et* seeds in some of the cultures. Both tendencies vary widely in different families; note for example that the *Et:et* ratio in progenies of *A/a* plants used as controls for *a-X1* effects is 1.00:0.95.

Since *Et* and *A* are both identified by seed as well as seedling effects, inequalities due to differences in germination may be excluded from data on the *Et-A* region by using the seed counts rather than the seedling counts. All of the data relating to *Et-A* crossing over in tables 1 and 2 were recalculated in terms of the total population of seeds produced. Since the seedling results corrected certain errors of seed classification for *et*, the corrected figures were used, and in the classes affected the same proportionate correction was applied to the seeds which failed to germinate. This recalculation made slight changes in the crossover frequencies observed in individual cultures, but made no material change in the indications of effects on crossing over. In the case of both *a-X2* and *a-X3*, in the data from total seed counts, crossover frequency was lower in every *A/a-X* progeny than in any of the control *A/a* progenies. The effect upon crossover frequency in the totalled data for each group is shown in table 3.

It is evident that marked inequalities of the *Et* and *et* classes remain in the families used for the *a-X2* and *a-X3* comparisons, with a reduction of female transmission of *et* to about 60 percent and 85 percent respectively in the cultures from the *et A/Et a* control plants. In the control cultures this does not distort the crossover frequency, since it affects the crossover and non-crossover classes equally. But in the cultures segregating also for an *a-X* allele of reduced viability, the effect upon these two classes is necessarily unequal. Is this factor, rather than an actual decrease in crossing over, the cause of the reduced frequency of crossover gametes observed in the *A/a-X* plants?

Assume, for example, a crossover frequency of 12 percent in the *et A/Et a-X2* plants, as in their control sibs. In an ear with a potential yield of 200 seeds, we expect 88 gametes of each of the non-crossover types and 12 of each of the crossover types. Assuming 60 percent survival for *et*, the frequency of the *et A* class is reduced from 88 to 53. The frequency of the *Et a-X2* class will be similarly reduced by lower female transmission of *a-X2*, but the extent of this reduction varies in different cultures. We may estimate the reduction in these plants directly by comparing the *a-X* frequency observed with that expected if *a-X* had no effect on viability. With 12 percent crossing over and with full survival of *a-X2*, these cultures would be expected to have a ratio for *A:a-X* of 1.00:1.47, the reduced frequency of *A* being due to the disproportionate elimination of *A* individuals by the action of *et*. Actually the ears had a ratio for *A:a* of 1.00:1.15, indicating survival of *a-X2* at about 78 percent of normal. The expected frequencies of the four classes (with no interaction of viability effects) would thus be

| | *et A* | *Et a-X* | *et a-X* | *Et A* | TOTAL | CROSSOVERS | % OF CROSSOVERS |
|---|---|---|---|---|---|---|---|
| Gamete ratio | 88 | 88 | 12 | 12 | 200 | 24 | 12.0 |
| Survival rate | .60 | .78 | .47 (.60×.78) | 1.00 | | | |
| Expected frequency | 53 | 69 | 6 | 12 | 140 | 18 | 12.9 |

The effect is thus to increase slightly the apparent frequency of crossing over. With lower survival of gametes, such as might occur with *a-X3*, the error would be increased materially, as shown below:

| | *et A* | *Et a-X* | *et a-X* | *Et A* | TOTAL | CROSSOVERS | % OF CROSSOVERS |
|---|---|---|---|---|---|---|---|
| Gametes | 88 | 88 | 12 | 12 | 200 | 24 | 12.0 |
| Survival rate | .60 | .25 | .15 | 1.00 | | | |
| Expected | 53 | 22 | 2 | 12 | 89 | 14 | 15.7 |

It is clear that this error in estimating crossover frequency, when the viability factors are in repulsion-phase, must always be in the plus direction. The apparent increase in crossover frequency is due to an unbalanced reduction in survival of the various classes of gametes, and while both non-crossover classes are reduced in survival, only one of the two crossover classes is so affected.

The illustrations given assume no interaction of the viability factors. For example, in the first illustration, it is assumed that of the 12 gametes of geno-

type *et a-X2* expected, 40 percent would be eliminated by the action of *et*. and of the remainder, 22 percent would be eliminated by the action of *A-X2*, It would not be surprising if the survival of *et a-X2* gametes were less than the expected 47 percent, since the effect of both viability factors in the same individual might be greater than would be anticipated from their effects in different individuals. But even if the cumulative effect were much increased, it could not result in any large decrease in apparent crossovers compared with the control, for it could only affect individuals in the single class which provides only a minority of the observed crossovers. At its extreme, in the example given, with complete elimination of this class, it would give an apparent reduction of crossing over from 12 percent to 8.6 percent. In the second illustration, with lower survival, the maximum decrease possible would not be sufficient to balance the spurious increase in observed crossovers, and the results with complete elimination of the *et a-X* class would still indicate an increase in crossovers over the control. Obviously, the reduction of crossovers to less than half the control frequency, as found with both *a-X2* and *a-X3*, could not be due to these sources of error.

The experimental results (table 3) show further that the *et a-X* class was not eliminated; it provides 27 of the 88 crossovers observed with *a-X2* and 8 of the 45 with *a-X3*. Is this inequality of the crossover classes, and the observed inequality of the non-crossover classes, reasonably accounted for on the basis of viability differences involved?

Consider first the experimental data for *a-X2* (table 3). The survival ratio for *et* in the families used is 0.60, as indicated by the control cultures. With 60 percent survival of *et* in the *et A/Et a-X2* cultures, and with full survival of *a-X2*, these ears should have produced 1228 *Et a-X2* seeds (737/0.60) and 37 *et a-X2* seeds (61×0.60). Actually they produced 892 *Et a-X2* and 27 *et a-X2* seeds, indicating survival of about 73 percent in both classes. In other words, on the assumption of 60 percent survival for *et*, 73 percent survival for *a-X2*, and no interaction of viability effects, a gametic population of 2456 non-crossovers and 122 crossovers would have given the results observed. This represents an actual crossover percentage of 4.7, though among the surviving individuals crossovers would constitute 5.1 percent.

A similar calculation for the *et A/Et a-X3* cultures is shown below:

| | *et A* | *Et a-X3* | *et a-X3* | *Et A* |
|---|---|---|---|---|
| Observed (table 3) | 917 | 261 | 8 | 37 |
| Expected with full survival of *a-X3* | 917 | 1079 | 31 | 37 |
| Indicated survival of *a-X3* | | 24% | 26% | |
| Indicated gametic ratio | 1079 | 1079 | 37 | 37 |

Here also the inequality of the crossover classes is well accounted for by the relative viability of the genotypes concerned, and the crossover frequency indicated, 3.3 percent, is a little lower than that shown by the percentage of crossovers among survivors.

Thus, in spite of the rather unfavorable marker genes necessarily used, the data show pronounced effects of *a-X2* and *a-X3* on the frequency of crossing over. The effect is unexpectedly large, *a-X2* heterozygotes showing a reduction

of about 20 crossover units in the *Et-*$Lg_2$ segment. In *a-X3* heterozygotes, which were adequately tested only in the *Et-A* segment, the reduction in frequency of crossing over in this region was even greater than that shown by *a-X2*.

## CYTOLOGICAL OBSERVATIONS

The pronounced effect of *a-X2* and *a-X3* upon crossing over suggests an effect of these alterations on chromosome pairing in the region of the *A* locus. The additional effect of *a-X3* upon pollen development, and its sharply reduced female transmission, suggests further deficiency at this locus. Cytological examinations were made therefore of plants heterozygous for *a-X3*, in the pachytene stage of the microsporocyte.

The *A* locus is in the terminal one-fifth of the long arm of chromosome 3 (McClintock 1931). This region was carefully examined in two types of material. In the first of these, the normal chromosome 3 carried a distinctive knob, near the midpoint of the long arm, which facilitated identification of the chromosome. Repeated observations failed to show any consistent abnormality in the region concerned. However, the material was not well suited for the detection of small alterations since the knob itself, in heterozygous condition, was responsible for some looseness and the frequent occurrence of buckles, usually in the vicinity of the knob but sometimes also in adjacent regions. More critical material was obtained from plants heterozygous for *a-X3* but lacking the knob. Several pachytene configurations were examined in which chromosome 3 was positively identified. In these there was no suggestion of a deficiency buckle or an inversion loop in the region of the *A* locus; the strands were as closely paired in this region as in other regions of the chromosomes.

The examinations made show that no reciprocal translocation is present, and no deficiency, insertion, or inversion long enough to interfere with visually normal pairing of the affected chromosome with its normal homolog. However, they do not exclude the possibility of a minute alteration that might be detectable only if located in a region most favorable for cytological study. All three *a-X* alterations, if due to deficiency, must be intercalary since the gene *Et*, located distal to *A* and present in the stocks originally treated, was not lost in any of the alterations. It is doubtful that losses as small as the minute terminal deficiences of chromosome 9, recently described by McClintock (1944), would be cytologically detectable if they occupied an intercalary position.

## EVIDENCE FROM THE USE OF AN UNSTABLE DUPLICATION

The unstable duplication used in the experiments now to be described was secured indirectly from a chromosomal aberration found in a progeny from ultraviolet-treated pollen. A stock carrying $A^b$ was irradiated and crossed on an *a* tester stock, in order to secure *A* losses from ultraviolet treatment for study in comparison with those from X-ray treatment. One of the plants of this progeny was a sectorial chimera, in which most of the plant showed the *a* phenotype but a sector of considerable size was of $A^b$ phenotype. Both sectors extended into the tassel, and pollinations made from $A^b$ anthers upon *a* tester

ears showed some transmission of $A^b$ (39 colored seeds+7 colored-colorless mosaics+260 colorless).

The colored seeds yielded colored plants, typical of $A^b$. A few of these were found to show small sectors of *a* tissue. The colored-colorless mosaic seeds regularly gave plants variegated for $A^b$ and *a* tissue, suggestive of the ring-chromosome variegation types described by McCLINTOCK (1938). A typical plant of this sort is shown in fig. 1, A. Cytological examination of these plants showed at pachytene a short supernumerary fragment in addition to the normal complement of ten pairs of chromosomes. This is designated *Dp 3a*, and will be referred to in this paper simply as *Dp*. The fragment was never seen as an open ring though it may have been a collapsed ring. Cytological study of the aberration was undertaken by DR. HELEN V. CROUSE, and will be reported separately.

This unstable duplication covering the *A* locus provides an opportunity for further study of the *a-X* alterations. With the addition of this duplication, the homozygotes and compounds of the *a-X* alterations may be shielded from the zygotic lethal effect, and, if these genotypes are viable in any tissue, the subsequent loss or diminution of the duplication fragment might make it possible to observe their effects.

Crosses were made with all three *a-X* alterations, according to the scheme illustrated below in the case of *a-X1:*

(1) $a\text{-}X1/a^p \times a\ a,\ Dp$.

Most of the seeds produced by this pollination are pale or colorless. There are in addition a considerable number of seeds showing full color due to $A^b$, but all of these are endosperm mosaics. In about half of these mosaics the sector lacking full color is pale; in the remainder it is colorless. The latter class represents the genotype *a-X1/a, Dp.* The plants grown from these seeds show a sectorial phenotype similar to *a a, Dp* (fig. 1, A). A seed showing a typical endosperm mosaic of this class is shown in fig. 3, A.

(2) $a\text{-}X1/a^p \times a\text{-}X1/a,\ Dp$.

This cross similarly yields pale and colorless seeds together with colored seeds showing endosperm mosiacs. Among the latter a new type appears, in which the region lacking color, instead of being healthy endosperm tissue distinguished only by pigmentation of the aleurone layer, is shriveled, degenerate tissue. An example is shown in fig. 3, B.

Seeds of this type are *a-X1/a-X1, Dp*. Plants grown from these seeds are variegated, but are of a type very different from their *a X1/a* (or $a^p$), *Dp* sibs which resemble the plant pictured in fig. 1, A. A typical plant of *a-X1/a-X1, Dp* is pictured in fig. 1, B. The sectors are all relatively small, and in general they are marked by absence of chlorophyll rather than absence of anthocyanin. The plants are distinctly defective in development, but they usually survive to maturity, and produce both tassels and ears. Flowering is delayed, and seed yield is small.

(3) $a\text{-}X1/a^p \times a\text{-}X1/a\text{-}X1,\ Dp$.

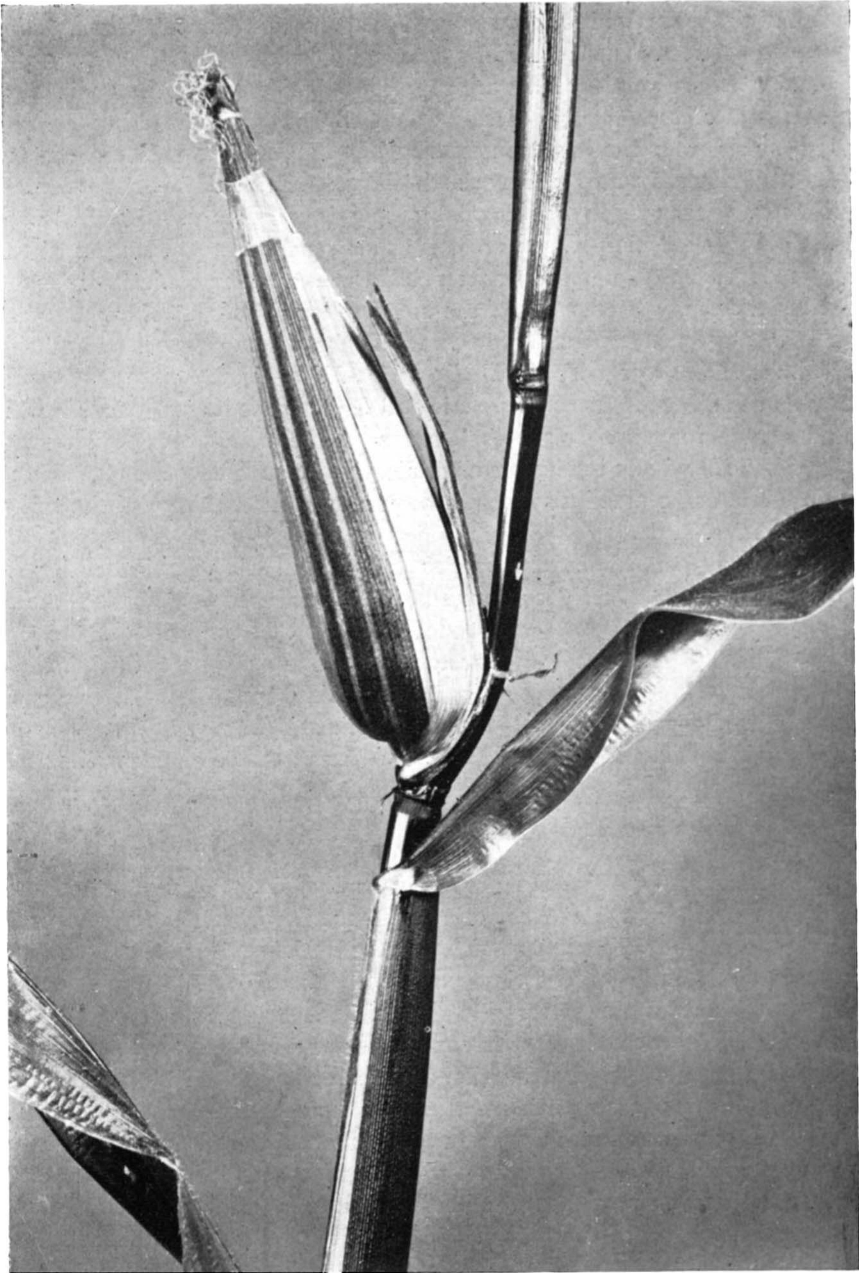
Crosses of this type are used to produce the *a-X/a-X*, *Dp* types in quantity, since all of the seeds produced are colored seed mosaics. The mosaics not showing degenerate sectors are mosaic for pale, not colorless.

Compounds are produced by appropriate substitution of the various *a-X* alterations in crosses (2) and (3). The chief difficulty in extracting the types desired comes from the low transmission of *a-X3*, and to a lesser extent of *a-X2*, in the heterozygotes lacking the duplication. Both alterations are readily transmitted through pollen from plants carrying the duplication. Successful extraction of *a-X2* or *a-X3* in cross (2) thus makes the production of their compounds with *a-X1* a simple matter, but the extraction of the homozygotes requires further use in cross (3) of the non-duplication stocks of low transmission. It was ultimately possible to extract all three homozygotes and all three compounds. The types *a-X3/a-X3*, *Dp* and *a-X2/a-X3*, *Dp* were first secured in crosses using *Dp* stocks as both male and female parents, a procedure which is undesirable for some purposes because of the possible inclusion in the progeny of individuals inheriting a duplication from both parents. The two types were later obtained also from crosses analogous to those of the scheme outlined above.

*Dp*-bearing plants homozygous for *a-X1*, and those representing the *a-X1/a-X2* and *a-X1/a-X3* compounds, were produced by crossing the appropriate types on *a-X1/*$a^p$ (cross-type (3) above). Thus in each of the three crosses, the *a-X/a-X* types desired were produced in a progeny which included sib plants of *a-X/*$a^p$ for comparison. In each case, the *a-X/a-X* plants were distinctly smaller and in general later than their *a-X/*$a^p$ sibs, and were characterized invariably by the occurrence of the characteristic chlorophyll-lacking sectors described above and pictured in fig. 1, B. The variegation type observed showed no consistent differences in the three *a-X/a-X* genotypes included.

These sectors, marked by absence of chlorophyll, in general gave no indication of loss of *A*, for the anthocyanin pigmentation within the sector was in almost all cases unaffected. Examination for anthocyanin effects cannot be made effectively in leaf blade tissue, since little anthocyanin is produced in this tissue. Many of the sectors identified in the blade extend into the leaf sheath, in which, with the proper complementary factors, anthocyanin pigmentation is strong. For pigmentation of the sheaths above the base, the complementary factor *B* is essential, and unless the complementary factor *Pl* is also present, pigmentation is absent in the portion of the sheath shielded from light by overlying tissue. Critical examination for anthocyanin effects in the sectors can therefore be made only in *B Pl* plants, and in some of the sectors of *B pl* plants. Most of the sectorial plants carried *B* and a substantial proportion had *Pl* also.

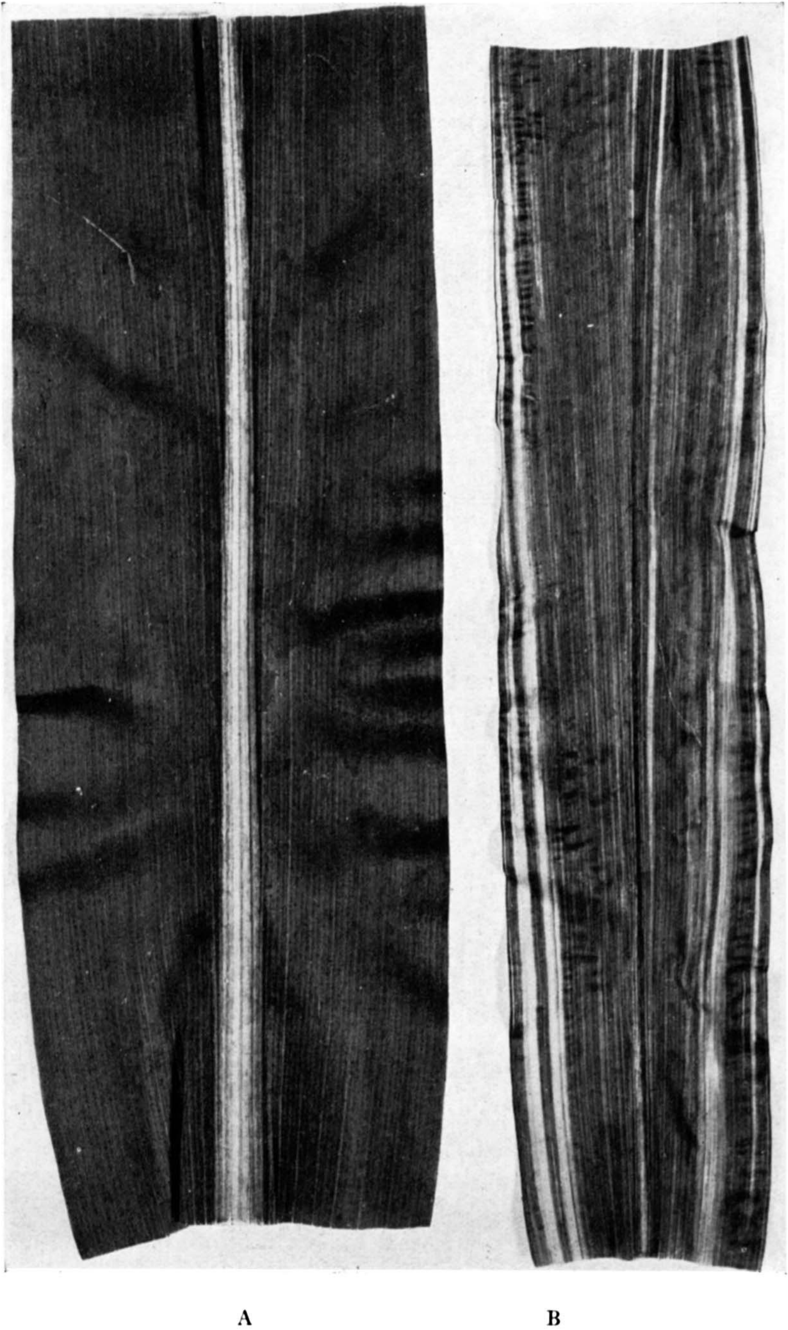
Among these, close examination was made for sectors lacking anthocyanin, with or without associated lack of chlorophyll. In plants heavily pigmented with anthocyanin in the sheath, small sectors lacking chlorophyll cannot always be detected in sheath tissue. The broader sectors found in the leaf blade, clearly marked by absence of chlorophyll, may usually be traced back



A

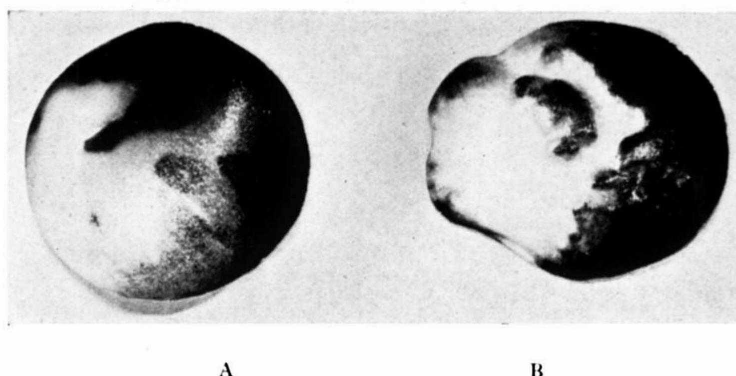
B

FIGURE 1. Characteristic variegated plant types. A. *a/a, Dp*. Note broad to narrow sectors lacking anthocyanin. There are no sectors lacking chlorophyll. B. *a-X1/a-X1, Dp*. Note narrowed sectors lacking chlorophyll. Very few of these sectors lack anthocyanin.



A B

FIGURE 2.—Leaves of plants shown in Fig. 1. A. *a/a, Dp*. No chlorophyll sectors. B. *a-X1/a-X1, Dp*. Numerous chlorophyll sectors.



A B

FIGURE 3. Characteristic endosperm mosaics. A. *a/a, Dp*. The mosaic sector is characterized only by the absence of anthocyanin pigmentation. B. *a-X1/a-X1, Dp*. The mosaic sector is characterized by degenerate endosperm tissue.

to the sheath, where even in the presence of strong anthocyanin the sector is recognizable by its lighter color resulting from the absence of chlorophyll. Such sectors are reasonably numerous, a single leaf sometimes providing a dozen or more. The counts were always made from only one leaf of any plant, to avoid including sectors of common origin in different counts.

It was found that in a large majority of the sectors lacking chlorophyll, anthocyanin pigmentation was present and apparently wholly normal, in comparison with adjoining tissue outside the sector. Much less frequently

A B

FIGURE 4. Sib plants in $F_1$ of $a\text{-}X3/a^p \times a\text{-}X1/a\text{-}X3$, *Dp*. A. *a-X1/a-X3, Dp*. B. *a-X3/a-X3, Dp*.

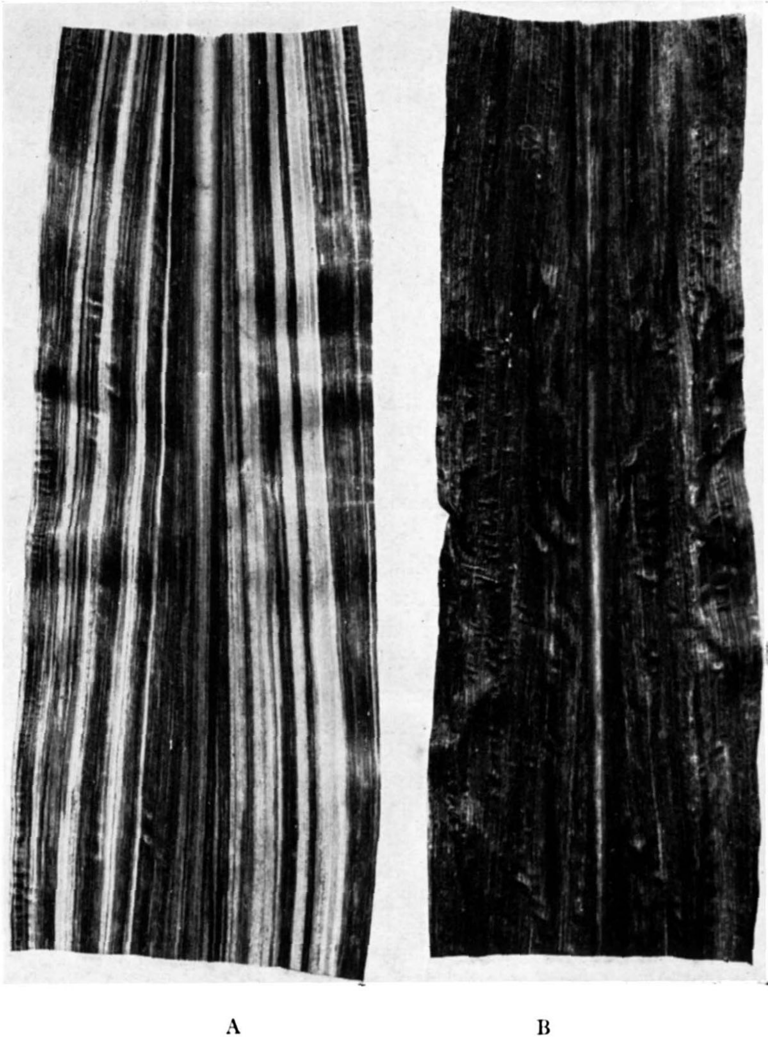



FIGURE 5. Leaf blades of plants shown in Fig. 4. A. *a-X1/a-X3, Dp*. B. *a-X3/a-X3, Dp*.

sectors lacking anthocyanin as well as chlorophyll were found. Usually such sectors were adjoined on one or on both sides by sectors lacking chlorophyll only, as if the sector characterized by loss of both anthocyanin and chlorophyll occurred within a larger sector initiated by loss of chlorophyll only. There were, however, other cases in which sectors showing loss of both anthocyanin and chlorophyll occurred without adjoining sectors showing loss of chlorophyll only.

In rare instances sectors lacking anthocyanin and not lacking chlorophyll occurred. The clearest case of this kind, observed in an *a-X1/a-X3, Dp* plant, was a sector about four mm in width, devoid of anthocyanin and clearly normal in chlorophyll. It was adjoined on one side by normal tissue with deep antho-

cyanin pigmentation, with a sharp line of demarcation between the pigmented and non-pigmented tissues. On the other side it was adjoined by a sector about six mm wide, in which chlorophyll was lacking. Within the latter sector there were several streaks of anthocyanin-bearing tissue, but otherwise anthocyanin was lacking in this sector also. Among sectors lacking anthocyanin, both in cases with and without chlorophyll, there is sometimes clear brown pigmentation similar to that found in a *a B Pl* plants.

These observations suggest that the sectors observed in the *a-X/a-X* plants result not from the loss of the duplication as a whole, but from losses of a portion only. If the *a-X* alterations represent deficiencies which are effectively cell-lethal, this is the result to be expected. Losses of the duplication as a whole, occurring in the course of development, would only eliminate the cell progeny of occasional cells of growing meristems. This might result in the reduced growth and distortion observed in the *a-X/a-X* plants as compared to their $a\text{-}X/a^p$ sibs, but could not produce sectors of phenotypically distinguishable tissue. But if the duplication is capable of partial loss in mitosis, such as the diminution in ring chromosomes demonstrated by McCLINTOCK (1938), and if the cell-lethal effect of the *a-X* alterations is due to a locus different from the *A* locus or the chlorophyll-essential locus (here designated *W*), then partial losses could occur which would given viable sectors lacking chlorophyll or anthocyanin or both. The relative frequency of such sectors would depend upon the sequence of the genes concerned, relative to the centromere of the duplication fragment. The relatively low frequency of sectors lacking both chlorophyll and anthocyanin, and the fact that there are usually found adjoining sectors lacking chlorophyll only, suggests that a factor essential for cell-viability may be so located that losses of a segment including both *A* and *W* would ordinarily include the cell-viability factor, but a loss involving one of the two loci might be followed by a loss involving the other, without including the viability factor.

It should be possible, by detailed study of the cytological behavior of the fragment and of the relations of the various genetic effects in the sectors, to locate these factors more definitely, and to distinguish between the various viability effects involved. For our present purpose, the essential point is the demonstration that all three of the *a-X* alterations are characterized by the absence of distinct and separable genetic effects. This is evident from the varying types of sectors observed.

The phenotypic similarity of *a-X1/a-X1*, *Dp* plants to the compounds *a-X1/a-X2*, *Dp* and *a-X1/a-X3*, *Dp* is best tested in the progeny of crosses of the type $a\text{-}X1/a^p \times a\text{-}X1/a\text{-}X2$ (or *a-X3*), *Dp*. Here the seeds with degenerate sectors include two types, *a-X1/a-X1*, *Dp* and *a-X1/a-X2* (or *a-X3*), *Dp*. There was little basis in the appearance of the plants for any attempted classification of the two genotypes. In the case of *a-X1/a-X1*, *Dp* versus *a-X1/a-X3*, *Dp*, attempted classifications may be checked at flowering by pollen examination, for the plants of the latter class show about 40 percent subnormal pollen due to the effect of *a-X3* in the absence of *Dp*. These trials showed no relation of the attempted classifications to the presence or absence of *a-X3*. The three

*Dp*-bearing *a-X*/*a-X* genotypes including *a-X1* thus appear to be phenotypically indistinguishable.

Of the three remaining *Dp*-bearing genotypes, *a-X2*/*a-X2*, *a-X2*/*a-X3*, and *a-X3*/*a-X3*, the first two also are similar to the *a-X1* homozygote and compounds. These types, obtained in various progenies, were always recognizable as defective plants with chlorophyll-lacking sectors, similar to those previously described. The third, *a-X3*/*a-X3*, *Dp*, is distinctly different. This may be illustrated in the progeny of the cross *a-X3*/$a^p$×*a-X1* (or *a-X2*)/*a-X3*, *Dp*. Because of low transmission of *a-X3* by the female parent, this cross yields only a few seeds of the desired types per ear, but by pollinating several ears of *a-X3*/$a^p$ by pollen from a single plant, small but adequate populations were secured. The results are as follows:

The seeds produced are mostly colored-pale mosaics. The remainder include some with clear degenerate sectors and some with no clear sectors, but usually with a slight pitting of the surface. In some cases no pitting is detectable, and the seed appears fully colored.

Plants grown from the seeds with degenerate sectors are typical *a-X*/*a-X* sectorial plants of the type previously described, and are indistinguishable from those previously found with *a-X1* or *a-X2*. These plants show the segregation of subnormal pollen associated with *a-X3*, but in proportions below 50 percent, as in the *a-X3* heterozygotes previously reported. They are thus identified as the *a-X1* (or *a-X2*)/*a-X3*, *Dp* class expected.

Plants grown from the pitted seeds or from the wholly colored seeds are of a new type, regularly defective in comparison with their *a-X*/$a^p$, *Dp* sibs and usually more defective than their *a-X1* (or *a-X2*)/*a-X3*, *Dp* sibs. These plants usually show no sectors except for numerous minute streaks of chlorophyll-lacking tissue. Occasionally a small sector similar to those found in the other *a-X*/*a-X* types occurs, but ordinarily the plant is distinguishable only by its fine-streaked leaf blades and its generally defective development. A considerable proportion of these plants fail to survive to the flowering stage, but the majority survive to maturity and usually produce a few seeds. These plants show subnormal pollen in proportions approximating 80–85 percent, or subnormal and defective pollen approximating this total. They are thus identified as the *a-X3*/*a-X3*, *Dp* class expected. The contrasted types of *a-X*/*a-X*, *Dp* plants are illustrated in figs. 4 and 5.

Thus *a-X3* must differ from *a-X1* and *a-X2* in some viability factor or factors concerned in the development of the sectors produced as a result of loss or diminution of the duplication fragment. If it be assumed that *a-X3* involves deficiency of a viability factor or factors not involved in the other *a-X* alterations, it does not necessarily follow that sector survival would be appreciably affected, for the evidence indicates that the sectors observed with *a-X1* and *a-X2* result only from partial loss of the fragment. Such partial losses would not necessarily "uncover" the viability deficiency peculiar to *a-X3*. The observed types however indicate that this deficiency is so located that the more frequent types of partial loss which are capable of producing sectors in the usual *a-X*/*a-X* combinations do "uncover" it.

The interpretation applied may best be illustrated by considering the various classes of progeny in a cross of the type $a\text{-}X3/a^p \times a\text{-}X1/a\text{-}X3$, *Dp*. The progeny includes four genotypes, which we may designate for convenience as classes I, II, III and IV as follows:

| | GENOTYPE |
|---|---|
| I | $a\text{-}X1/a^p$, *Dp* |
| II | $a\text{-}X3/a^p$, *Dp* |
| III | $a\text{-}X1/a\text{-}X3$, *Dp* |
| IV | $a\text{-}X3/a\text{-}X3$, *Dp* |

All classes of the progeny include the same *Dp* fragment (or, assuming that the fragment is constantly changing, equivalent samples from the mixture of *Dp* types present in the pollen grains of the plant used as male parent). The fragments present in the progeny plants therefore must undergo elimination and partial loss of the same kinds in plants of the four classes. The total effects of such losses are best indicated by classes I and II, for these carry a chromosome 3 free from viability defects (the $a^p$-bearing chromosome). Any loss of the duplication, in whole or in part, therefore will be without effect upon subsequent development of the affected tissue. In these classes, visible sectors result only from losses which include the *A* locus.

The frequent occurrence of large sectors lacking $A^b$ in classes I and II shows that the *Dp*-fragment is undergoing frequent loss in development, including losses at early stages of development. These losses may be entire or partial, so long as they include the *A* locus. Presumably they include chiefly cases of loss of the entire fragment.

The nature of the sectors observed in class III plants shows that few, if any, such losses, when they occur in plants of this genotype, result in the production of visible sectors. Sectors lacking $A^b$ are extremely rare. Since there is no reason to assume that these losses do not occur, we must postulate a genotype for the *a-X* alterations involved which prevents the development of these sectors. A factor (or factors) essential for viability, present in the normal chromosome 3 in the region covered by *Dp 3a*, must therefore be lacking in both *a-X1* and *a-X3*. Since *a-X2* compounds show the same behavior, it must be lacking also in *a-X2*. The location of this factor is such as to make possible its preservation when *W* and *A* are eliminated in successive losses, though it is commonly lost when *W* and *A* are lost simultaneously. The sectors involving loss of *W* and not of *A*, which make up the great majority of the sectors observed in plants of class III, result from a type of loss not detectable in classes I and II, since these classes are not genetically marked at the *W* locus.

An alternative considered in the interpretation of the variegation observed in the plants of class III was the possibility that the sectors observed are in fact deficient for $A^b$, in spite of the fact that they show anthocyanin. It might be supposed that the boundary of a sector deficient for *A* would not be sharply marked by absence of anthocyanin, since cells at the margin of the sector might include the pigment by reason of diffusion of anthocyanin or precursor substances from the adjoining non-deficient tissue. If the sectors were narrow

enough this might result in apparently uniform anthocyanin pigmentation throughout the sector. On this basis the sectors found in class III plants could be considered the result of the same losses as those found in classes I and II, and the loss responsible in both cases could be loss of the entire fragment. The reduction in size of sector might be considered to be the result of reduced growth in the *a-X/a-X* tissue, and the difference in phenotype the result of an effect upon chlorophyll development involved in the *a-X* alterations. It would still be necessary to postulate effects of the *a-X* alterations upon anthocyanin, chlorophyll, and growth, but no separation of these effects would be demonstrated by the variegation patterns observed.

This possibility is not supported by histological examination of the tissues within and adjoining the sectors. It requires the assumption that a very broad margin must be subject to pigmentation by diffusion, for some of the chlorophyll deficient sectors are eight to ten mm wide, and are uniformly colored by anthocyanin throughout. It is contradicted by the sharpness of the margins of the exceptional types of sector in which anthocyanin, or both anthocyanin and chlorophyll, are deficient, and especially by the fact that this margin is sharp on a side adjoining normal tissue as well as on a side adjoining a chlorophyll-deficient sector. We are forced to conclude that the sectors involving loss of chlorophyll alone represent a second type of loss, distinct from that involving loss of anthocyanin in classes I and II, and occurring frequently enough to produce a large number of sectors on each leaf.

The disappearance, or extreme reduction in size, of these sectors in plants of class IV, similarly shows an additional viability effect in plants of genotype *a-X3/a-X3*. Alterations in the *Dp* fragment of the "second type" mentioned in the preceding paragraph, when they occur in plants of *a-X3/a-X3*, *Dp* genotype, fail to result in sectors comparable to those found in other *a-X/a-X*, *Dp* plants. The chlorophyll-essential factor *W* must be deficient in *a-X3* as well as in *a-X1* and *a-X2*; otherwise the *a-X3* compounds *a-X1/a-X3* and *a-X2/a-X3* could not show the chlorophyll-deficient sectors. The absence of these sectors in the *a-X3/a-X3*, *Dp* plants must therefore be due to their failure to develop, through the action of some viability-affecting factor, or factors, distinguishing *a-X3* from *a-X1* and *a-X2*. The factor must be so located that its normal homolog tends to be lost from the duplication when *W* is lost.

The evidence secured through the use of the unstable duplication thus shows that the *a-X* alterations studied involve the loss of several genetically separable effects observable in somatic development, aside from any additional factors which may be involved in their various effects upon gametophytic survival and pollen development. All three of the alterations involve the loss of effects essential for anthocyanin production, chlorophyll production, and somatic viability. The results of these losses may be suppressed in different combinations by the different derivatives of the *Dp* fragment which are produced in the course of development of a *Dp*-bearing plant. All three effects are suppressed by the "intact" *Dp* fragment, which is maintained in *a-X/a-X*, *Dp* stocks and thus shielded against losses which would make it useless for cover-

ing the *a-X* alterations. The viability and anthocyanin effects are suppressed without suppression of the chlorophyll effect by one type of variant; the viability and chlorophyll effects are suppressed without suppression of the anthocyanin effect by another; and the viability effect is suppressed without suppression of the anthocyanin or chlorophyll effect by a third. Each of these effects may thus be separated from the other two. In addition, *a-X3*, by similar evidence, is shown to involve loss of an additional factor or factors essential for somatic development. This demonstration of the loss of several genetically separable factors in each of the induced alterations constitutes genetic proof of deficiency.

## DISCUSSION

This study of the losses of *A* action induced by X-ray treatment gives no evidence of an effect of the treatment upon gene mutation. It shows that the segmental deficiencies induced in maize by X-ray treatment range to lengths so short as to be distinguishable from gene mutations only in exceptionally favorable material. Among a large sample of "*A* losses" the two selected for detailed study as most nearly approximating the expected effect of gene mutation proved to be deficiencies, distinguished only by the relatively minute length of the segment involved.

The background for the interpretation of these results may be briefly outlined as follows: X-ray treatment, as shown by earlier experiments concerned with the general mutation rate, results in a substantial increase in the frequency of point mutations. These are identified, in experiments with maize, as new Mendelizing characters observed in the $F_2$ of crosses in which one or both of the parents were X-rayed. The X-ray-induced point mutations found in maize prove to be wholly recessive in all cases. They include cases in which the mutant segregates in the expected 3:1 ratio, and also cases in which the ratio is reduced to varying degrees, usually by reduced transmission through male germ cells. The same X-ray treatments produce in large numbers chromosomal derangements of various sorts. There is no appreciable tendency of the induced mutations to occur at points of chromosome interchange, a feature in contrast with the results of comparable experiments with Drosophila. The primary question is whether the point mutations observed represent in the main the result of gene mutations or the result of extra-genic alterations. If the results indicate the latter to be true, a second question arises: Do the X-ray-induced mutations include any cases in which gene mutation must be assumed to have occurred?

The presumption that the point mutations in general represent gene mutations is based upon the assumption that deficiencies would ordinarily be lethal to the gametophyte, or at any rate would be so weakened in haplo phase as to fail in transmission through pollen. If this were so their recessive effects could not appear in $F_2$. This assumption becomes more and more questionable as cases are found of demonstrable deficiencies transmitted through the gametophyte generation. Deficiencies now known range in gametophytic effect from total abortion of both male and female gametophyte through successive levels

of development ranging to apparently normal development and normal functioning. Moreover the cases demonstrated as deficiencies range to the limit of cytological or genetic identification. The assumption that deficiencies do not occur below these limits of detectability is wholly unwarranted.

In selecting individual cases for detailed study as possible gene-mutations, it was assumed that the action of *A* is not essential for normal gametophyte development, and therefore that any deleterious effects of an observed *A* loss upon gametophyte development indicate that other loci are also involved. This assumption was based upon the normal gametophyte development found in all known *A* alleles, including several spontaneous mutations observed from the first generation following their occurrence. The assumption is confirmed by the results of this study, showing two deficiencies of *A* which permit normal development of both the male and the female gametophyte.

Among the population of *A* losses observed in $F_1$, then, we may expect that the major deficiencies including the *A* locus will have visible effects upon gametophyte development, though there may also be minor deficiencies with no detectable gametophytic effect. The *A* losses due to alterations with visible gametophytic effects will include most of the deficiencies, and may include losses of *A* effect due to other extra-genic alterations, but will not include any of the *A* losses due wholly to gene mutation. The *A* losses without visible gametophytic effects will include all of the gene mutations (except such as might occur from a single alteration involving both gene mutation and chromosomal derangement), and may include also some of the deficiencies and other extra-genic alterations.

Unfortunately, it is not possible in general to determine from inspection of the mutant individuals observed in $F_1$ whether or not the alteration concerned has gametophytic effects. The *A* loss observed is induced by X-ray treatment, and the coincidental occurrence of an independent chromosomal derangement induced by the same treatment may produce in the same $F_1$ plant a gametophytic effect not due to the alteration involving *A*. It is technically impossible to extract the *A* alteration in each case for individual study, not only because of the labor involved, but because in many cases the single plant concerned is sterile. The possibility remains therefore that among the population of *A* losses observed there were instances of gene mutation of *A*, which by reason of coincident but independent chromosomal alterations were accompanied by gametophytic or developmental defects.

The nature of the deficiencies identified in *a-X1*, *a-X2*, and *a-X3* is of interest in relation to the question of distinguishing between deficiencies and gene mutations among the point mutations at miscellaneous loci, which are induced in maize by X-ray treatment. It is clear that deficiencies including three or more gene loci may be too minute for cytological detection. In spite of the extremely sensitive reaction of crossover frequency to minute deficiency in maize, the results with *a-X1* show that such deficiencies may occur without pronounced effect upon crossover frequency. Genetic detection of the deficiencies in this case was facilitated by the fact that loci affecting chlorophyll development and somatic viability occur very close to the *A* locus, but there

is of course no reason to assume that such convenient linkages would occur with another gene similarly studied. There may be many genes with phenotypic effects, which are so located that deficiencies of comparable length could remove them without removing other detectable genes. Such deficiencies would be indistinguishable from gene mutations.

The somatic viability locus deficient in all three *a-X* alterations studied is apparently close enough to *A* to ensure that point mutations of *A* would be extremely infrequent in experiments of the usual type on X-ray-induced mutation of miscellaneous genes. So far as we know, no X-ray-induced mutations of *A* have previously been found. None of the three *a-X* alterations here described would appear in $F_2$ in such an experiment, since all are zygotically lethal, due to the associated viability factor. Although the loci yielding mutations under X-ray treatment appear to be a random sample, there are of course many known genes for which X-ray induced mutants have not been found. It is not improbable that with significant comparisons different loci would show large differences in susceptibility to X-ray-induced mutation, due to this cause.

If no viability factor were included in the *a-X* deficiency-segments, the alterations would segregate in $F_2$ as mutant alleles of *A* with effects upon both anthocyanin development and chlorophyll development. Such alterations with diverse effects are commonly regarded as gene mutations, particularly if they occur at loci not already identified by a consistent group of alleles. The X-ray induced mutant *et*, described in connection with the crossover experiments in this paper, is perhaps an analogous case. It affects two characters not obviously related, scarring of the endosperm surface and chlorophyll development in the seedling stage. The homozygote is fully viable and fertile, pollen development is normal, and the mutant is transmitted through both male and female gametophytes.

The three deficiencies designated *a-X1*, *a-X2*, and *a-X3* apparently represent segmental losses of different extent in each case. *Df a-X3* must be assumed to include some locus or loci not involved in the others, to account for its visible effect on pollen development and its much more extreme reduction in gametophyte survival. The additional somatic viability factor or factors involved in *a-X3*, as indicated by the studies with the unstable duplication, may or may not represent the same loci. Similarly *a-X2* must differ from *a-X1*, to account for its much more pronounced effect upon crossing over, as well as its consistently lower gametophytic transmission.

Since these three deficiencies represent breaks at different points in the normal gene sequence, it is reasonable to infer that a larger sample would include deficiencies of smaller extent. A deficiency including the *A* locus and not including the viability and chlorophyll-essential loci here involved would be viable in homozygotes and would be identical in phenotypic effect with the standard recessive allele *a*.

## SUMMARY

1. X-ray-induced losses of the phenotypic effect of *A* (here designated "A losses") were identified in $F_1$ populations produced by the use of X-rayed

pollen. Among about 415 *A* losses observed, only two were normal plants free from segregating pollen defects.

2. The induced germinal alterations responsible were extracted for study in these two cases (*a-X1* and *a-X2*). The alteration involved in an *A* loss showing segregation of subnormal but not aborted pollen was also extracted for comparison (*a-X3*).

3. All three alterations are haplo-viable but with reduced transmission thtough the gametophyte generation. *a-X1* is usually transmitted with normal frequency through female gametophytes, and with about 50 percent of normal frequency through male gametophytes. *a-X2* usually shows about 65 percent of normal female transmission and about 25 percent of normal male transmission. *a-X3* usually gives about 20 percent of normal female transmission, and is not at all transmitted through pollen.

4. Homozygotes of *a-X1* and *a-X2*, and the compounds of *a-X1*, *a-X2*, and *a-X3* in all three possible combinations, are zygotically lethal.[1]

5. Plants heterozygous for *a-X2* and *a-X3* show greatly reduced crossing over in the adjacent region of the chromosome, while plants heterozygous for *a-X1* show approximately normal crossing over in the same region.

6. Cytological examination at pachytene in plants heterozygous for *a-X2* and *a-X3* shows no visible deficiency, intercalation, or inversion, and chromosome pairing in the region involved is not visibly abnormal.

7. By the use of an unstable duplication, *Dp 3a*, covering the region concerned, the various *a-X*/*a-X* homozygotes and compounds were produced, and the sectors resulting from elimination or partial loss of the *Dp* fragment were studied for evidence concerning the factors involved in the various *a-X* alterations. This study showed that all three alterations are deficient for factors affecting anthocyanin production, chlorophyll production, and somatic viability, and that *a-X3* is deficient for an additional factor or factors affecting somatic viability.

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