A COMPARISON OF X-RAY AND ULTRAVIOLET EFFECTS ON CHROMOSOMES OF ZEA MAYS[1]

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Received March 3, 1955

PREVIOUS workers have found that in maize the genetic analyses have indicated an apparent difference between the action of X-ray and ultraviolet on (1) gene mutation, (2) the frequency of reciprocal translocations, (3) the type and frequency of endosperm deficiencies, (4) the ratio of endosperm deficiencies to embryo abortions, and (5) the types of embryo deficiencies. These contrasts have been fully discussed by STADLER and co-workers in a series of papers from 1936 to 1951 (STADLER and SPRAGUE 1937; STADLER 1939 and 1941; STADLER and ROMAN 1943 and 1948; STADLER and SWANSON 1951). This discussion will include only those points that have a direct bearing on the differences in the chromosomal aberrations induced by the two radiations.

In *Zea mays* the frequency of reciprocal translocations is low following treatment with ultraviolet radiation in contrast to the frequency found following X-ray treatment (STADLER and SPRAGUE 1937; STADLER 1941). STADLER and SPRAGUE found reciprocal translocation figures at diakinesis in 42% of the 100 plants from X-rayed pollen and no translocations in the same number of plants from ultraviolet treated series. In these experiments the doses of X-rays and ultraviolet radiation used were about equally effective in producing endosperm deficiencies.

Not only does it seem that the frequency of reciprocal translocations is different following treatment with ultraviolet radiation, but it has been suspected that the type of translocation is different (STADLER 1941). SINGLETON and CLARK (1940) recognized a type of translocation associated with a genic deficiency, called a deficiency translocation, in the progeny of ultraviolet treated pollen. A limited amount of evidence is available on the comparative frequency of deficiency translocations following X-rays and ultraviolet radiation. DEBOER (1945) found two deficiency translocations among 3513 plants following treatment with ultraviolet radiation but five deficiency translocations in 1670 plants from X-rayed pollen.

When the occurrence of interstitial and terminal chromosomal deficiencies following X-rays and ultraviolet radiation is compared, a difference is found. Following the use of ultraviolet no cytologically proven cases of interstitial deficiencies have been reported. However, several interstitial deficiencies have been observed in plants from X-rayed pollen (MCCLINTOCK 1931; DEBOER 1945). A number of terminal deficiencies have been reported in progeny following treatment with ultraviolet radiation (STAD-

[1] This work was supported by a grant from the Atomic Energy Commission. Contract AT (11-1)-73 project No. 4.

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Missouri.

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LER 1941; SINGLETON 1939; SINGLETON and CLARK 1940; and DEBOER 1945). Only in DEBOER's investigation is it certain that the chromosome loss was terminal because she was the only one to use a terminal marker. The latter is essential since an interstitial deficiency could appear as a terminal deficiency if non-homologous pairing occurred. Terminal deficiencies have been found in cytologically marked stocks from plants treated with X-rays (MCCLINTOCK 1931; and DOLLINGER 1954).

It seemed desirable, therefore, to make a more extensive study of the types of simple and complex chromosomal deficiencies which occur after X-ray and ultraviolet radiation and also to make a comparison of their frequency. It is important to emphasize that chromosomal rearrangements not involving a deficiency, such as reciprocal translocations and inversions, were not recovered in these experiments since the cases for cytological analysis were selected on the basis of a genetic deficiency.

MATERIALS AND METHODS

The cytological analysis was made on chromosomes 9 and 10. Stocks to be irradiated had the end of the short arm of chromosome 9 terminating in a knob (symbol *K*), either a large one, a medium sized one or a small one, all of these being easily distinguishable from a non-knobbed chromosome 9. The knob on the end of the short arm makes it possible to determine whether the irradiation causes terminal deficiencies. In order to select plants which had radiation damage in the vicinity of the knob, stocks homozygous for the dominant allele of the gene bronze (*bz*), which is located 31 units from *K*, were employed. Pollen from *Bz K/Bz K* plants, after appropriate irradiation, was placed on the silks of *bz k/bz k* (*k* denoting either a smaller knob than *K*, or no knob). The F_1 plants which exhibited the bronze phenotype were selected for study. Presumably this chromosome would be deficient for the dominant allele, *Bz*, as a result of the irradiation. In some families, it was possible to examine the seedlings for bronze variegation, but usually only a small fraction of the total population was examinable. These variegated seedlings were saved also for cytological examination.

For the study of the effects of irradiation on chromosome 10 a stock possessing the abnormal knob 10, as described by LONGLEY (1938), was used. Its subterminal position makes it impossible to establish with certainty whether the deficiency is terminal or interstitial. The stock homozygous for the knob was also homozygous for the dominant allele R^r (one unit from *K*) which produces anthocyanin in the plant. Pollen from such a stock $R^r\ K/R^r\ K$ was irradiated and placed on the silks of $r^g\ k/r^g\ k$ plants (green plants). The F_1 seedlings showing the phenotype of the egg parent were selected for cytological study.

The source of ultraviolet radiation used in these experiments was a Westinghouse Sterilamp WL782H-10 without a filter. Approximately 80% of the energy emitted by this lamp is in the region of 2537 Angstroms, which STADLER and UBER (1942) had found was the genetically most effective part of the ultraviolet spectrum. The dose used in this experiment was approximately 24,000 milliwatts per second per square foot of the treated surface obtained by placing the pollen in a single layer on a quartz slide. The pollen was irradiated from above and below for 10, 20, and 30 second exposures.

TABLE 1

The type and frequency of cytological aberrations in chromosome 9 following ultraviolet and X-ray treatment

Treatment	*n*	*bz*	Terminal df.	Interstitial df.	Fragment chr?	Ring chr.	Deficiency transloc.	Asynapsis	Haploid	Normal chr. 9	Died before meiosis	Unexam.
U.V. 10″	1143	2	1									1
20″	2948	7	4		1				2			
30″	2077	4	3							1		
Total	6168	13	8		1				2	1		1
X-ray 400r	1936	11		1	1		6					3
800r	1003	19	2	2	2	1	4	1		3	3	1
1000r	67	1									1	
Total	3006	31	2	3	3	1	10	1		3	4	4
Control	6397	1							1			

The X-radiation used was obtained from a Coolidge tube with a tungsten target producing radiation in the region of 0.605 Angstroms. The dosages were 400r, 800r, and 1000r.

The meiotic chromosomes were prepared for study by the aceto-carmine smear technique following fixation in a 3:1 mixture of ethyl alcohol (95%) and glacial acetic acid.

OBSERVATIONS

Effects of ultraviolet radiation on chromosome 9

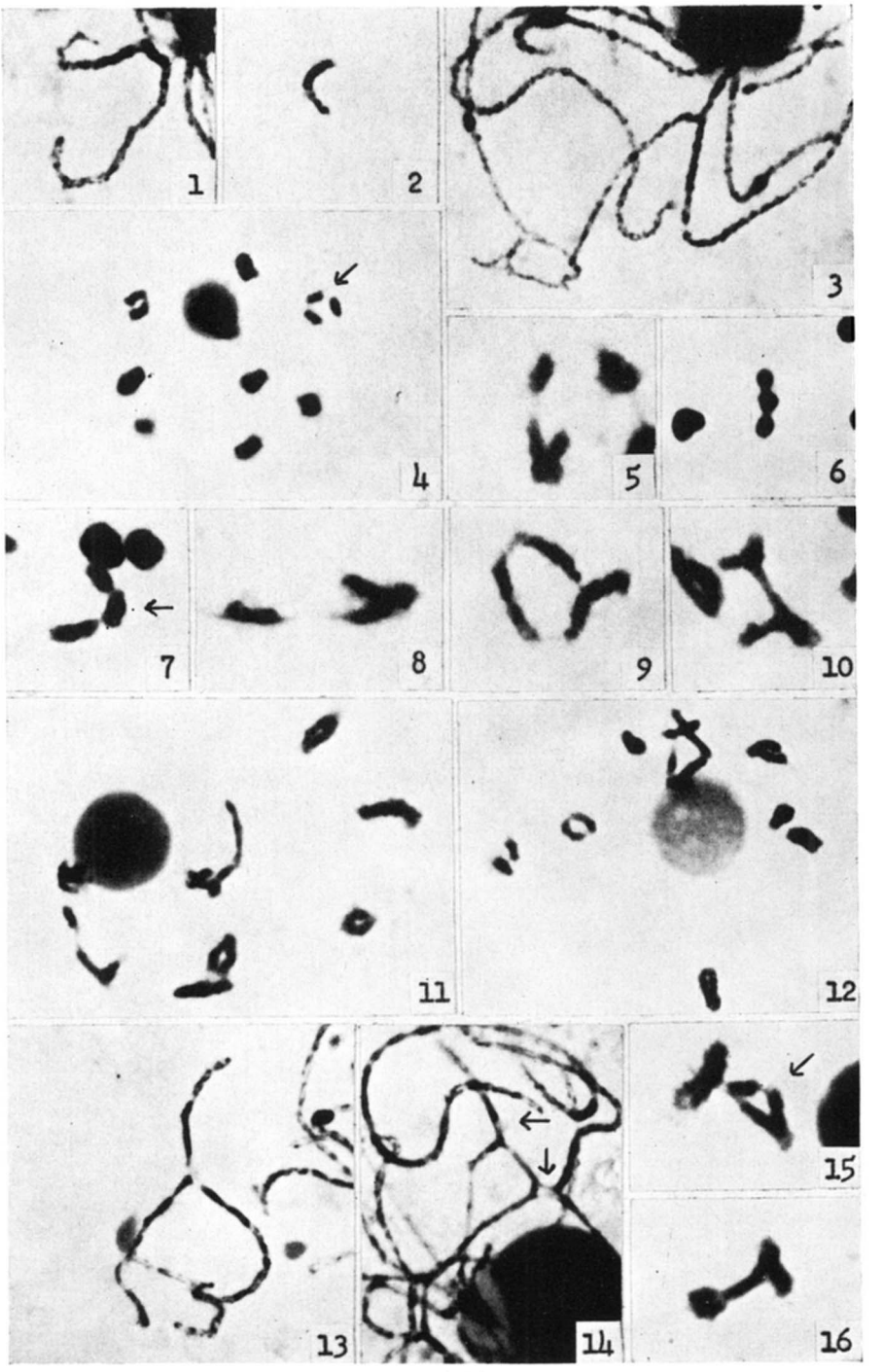
Three F_1 populations totalling 6168 plants were grown from crosses of *bz k/bz k* plants with *Bz K/Bz K* plants, the pollen of which had been irradiated with ultraviolet light for the three periods of time described previously. Thirteen seedlings were bronze indicating that there was either a loss or an inactivation of *Bz*. Twelve were examined cytologically (table 1).

Two of the 12 bronze F_1 plants were haploids, as was the one bronze plant observed in the control population.

Three of the 12 aberrant F_1 plants were found to have lost the whole of the short arm of chromosome 9. Five of the bronze plants had lost the terminal knob and part of the short arm of chromosome 9. One had lost about three quarters of the arm, one about two thirds, another about one half, another about one third, and the last, about one sixth. In all five cases the terminal knob was certainly missing, as can be seen in the one example shown in figure 1.

In one *bz* plant, the treated chromosome 9 was represented in pachytene by a small fragment (fig. 2). In ten pachytene figures, the centromere of this aberrant chromosome was observed in the same position, which would not be true if this were a ring chromosome. In a collapsed ring chromosome, the location of the centromere would vary according to the points at which the ring folded on itself.

One bronze plant from the ultraviolet treated progeny possessed a normal chromo-



FIGS. 1–16

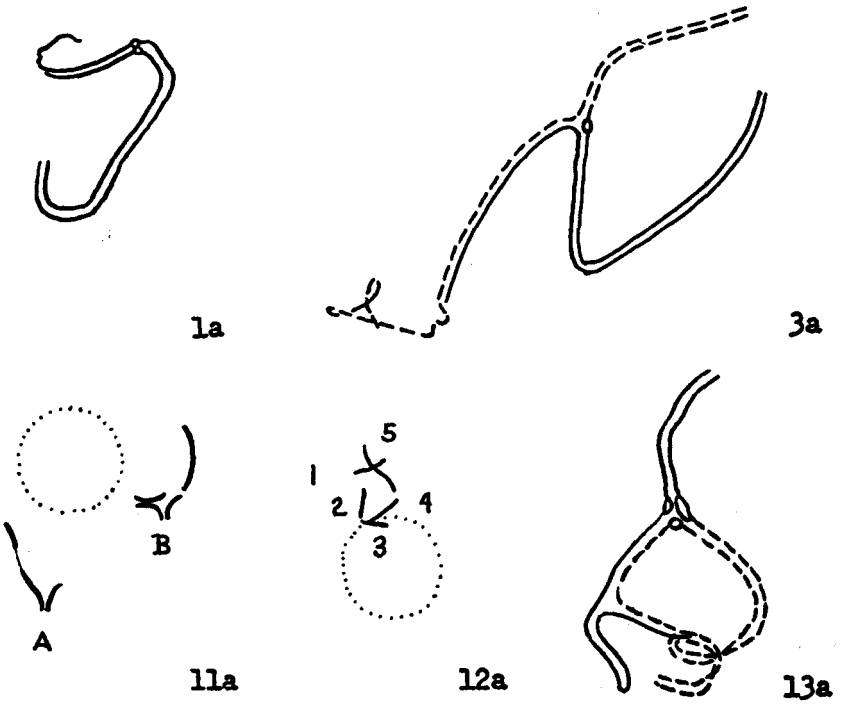


FIGURE 1a.—Diagram of figure 1. The centromeres are represented in the sketch as two circles. FIGURE 3a.—Diagrammatic interpretation of figure 3. The normal chromosome 9 is represented as a solid line; the normal unidentified chromosome is drawn as broken lines; the dicentric chromosome is illustrated by partly broken and solid lines. FIGURE 11a.—Diagram of figure 11. The 3-chromosome translocation (A) consists of three associated chromosomes and the reciprocal translocation (B) of four elements. FIGURE 12a.—Diagram of figure 12 showing the five elements of the translocation. FIGURE 13a.—Diagram of figure 13. The short arm of normal 10 (solid line) is paired homologously with the translocated chromosome (partly solid and broken lines), and the long arm of ten is paired non-homologously with the normal unidentified chromosome. The latter chromosome is also partially synapsed (broken lines) with the translocated chromosome.

FIGURES 1–3.—(× 1300) FIGURE 1.—Terminal deficiency for chromosome 9; see diagram. FIGURE 2.—Fragmented chromosome 9. FIGURE 3.—3-chromosome deficiency translocation involving chromosome 9 and an unidentified chromosome; see diagram. FIGURE 4.—Diakinesis cell with a 3-chromosome deficiency translocation indicated by an arrow (× 1600). FIGURES 5–10.—(× 2600) 3-chromosome deficiency translocation at diakinesis. FIGURE 11.—Diakinesis cell including a 3-chromosome deficiency translocation and a reciprocal translocation; see diagram (× 1600). FIGURE 12.—Type of translocation involving three chromosomes; see diagram (× 1600). FIGURE 13.—3-chromosome deficiency translocation involving chromosome 10 and an unidentified chromosome; see diagram (× 1300). FIGURE 14.—Same translocation in another cell. The two pairs of centromeres (indicated by arrows) are widely separated (× 1300). FIGURE 15.—3-chromosome deficiency translocation at diakinesis (× 2600). FIGURE 16.—3½-chromosome deficiency translocation (× 2600).

some 9 and a normal sized terminal knob. Unfortunately, this plant produced no progeny and thus the analysis could not be extended further.

Effects of X-ray radiation on chromosome 9

Mature pollen from plants homozygous for *Bz K/Bz K* was treated with X-rays (400r, 800r, and 1000r) and pollinated on homozygous recessive plants, *bz k/bz k*. Thirty-one bronze seedlings were found among 3006 seedlings, four of which died before meiosis. Four other plants could not be analyzed because the cytological specimens were poor. These cases were lost since the majority of deficiencies in *Zea mays* are not transmissible in succeeding generations. The results of the study of the 23 remaining plants are presented in table 1.

Two plants had terminal deficiencies of the short arm on chromosome 9 which were analogous to those found in the study of the bronze plants produced from pollen subjected to ultraviolet radiation. In both cases approximately one half of the short arm and the terminal knob were deficient.

Three plants had interstitial deficiencies in the short arm of chromosome 9, the terminal knob being present, unchanged in size. No plants with interstitial deficiencies in this chromosome were observed in the progeny from the ultraviolet irradiated pollen. One of the three interstitial deletions involved approximately one fifth of the short arm of chromosome 9. In the other cases, the deleted segment included about one fourth and two thirds of the short arm.

In three *bz* plants, a fragment of chromosome 9 remained following irradiation. Of course, the possibility of a small ring chromosome is not completely excluded although 25 pachytene figures of the rod-shaped chromosomes were seen in the first case, ten pachytene figures in the second case, and eight in the third case. The constant position of the centromere in these fragments is not a conclusive criterion to distinguish a rod from a ring chromosome since the fragments are so minute that a slight variation in the position of the centromere would not be detected.

One *bz* plant exhibited asynapsis in late pachytene and diakinesis. An examination of an earlier stage of pachytene, when the chromosomes should be paired in asynaptic plants, was not possible since the cytological sample was limited in quantity. This case, therefore, remains undetermined for the alteration associated with chromosome 9. The asynapsis could be due to a coincident loss of another chromosome.

In one *bz* plant a ring chromosome was present. The ring included approximately three fourths of the entire ninth chromosome.

Three bronze plants were found with no visible cytological deficiency. These three cases have not been tested for exclusion of contamination.

Ten plants with the bronze phenotype had a type of chromosomal alteration which was not found among the 12 bronze plants in the ultraviolet series. These plants have a deficiency associated with a translocation. The type of aberration is called a deficiency translocation. In one type, part of one chromosome possessing the centromere is attached to part of another chromosome which possesses its centromere, thus making a dicentric chromosome. The terminal, acentric parts of both chromosomes are lost. In diakinesis, the dicentric chromosome and the two normal chromosomes from the female (nonirradiated) parent make a chain of three chromosomes. For

this reason this type of deficiency translocation will be called a 3-chromosome deficiency translocation. The other type will be called a 3½-chromosome deficiency translocation. This term applies to those translocations in which one acentric fragment is translocated and the other acentric fragment failed to be interchanged, thus leaving one chromosome deficient. In diakinesis a chain of four rather than three chromosomes is associated. The chain of four chromosomes consists of two normal with one translocated chromosome in between, plus a chromosome deficient for some part which being acentric, has been lost. Most of the deficiency translocations involving the *Bz* locus were studied in diakinesis stage only, whereas this type of aberration was analyzed more fully in the pachytene stage in the experiments involving the R^r locus.

Nine 3-chromosome deficiency translocations were identified in the *bz* plants. The 3½ type of deficiency translocation was not found in the chromosome 9 series.

Four of the ten bronze plants with a 3-chromosome deficiency translocation were analyzed in the pachytene and diakinesis stages. In these plants, both breaks probably occurred near the centromere and the centric pieces reunited to form a dicentric chromosome. The acentric fragments, which failed to be translocated, were lost. The distance between the two centromeres of the dicentric chromosome is not always an indication of the original breakage points. This was demonstrated in those deficiency translocations which occurred in variegated plants from the R^r series. In these plants, the breakage point on chromosome 10 was distal to the R^r locus, which is located near the end of the long arm. At pachytene, only the short arm of chromosome 10 and its centromere were present; the long arm was lost, presumably by the process of repeated breakage and fusion. Figure 3 shows the 3-chromosome deficiency translocation at pachytene in one of the plants from the *Bz* series. Three arms are present, two of which are the homologously paired arms, and the third arm represents parts of the two normal chromosomes paired non-homologously. Only one centromere was positively identified in this figure, but two centromeres were seen in other figures. In the diakinesis stage, each of these four plants exhibited 8 bivalents and three associated chromosomes. Three of these four plants are illustrated at diakinesis in figures 4 to 6.

Five of the ten plants with the bronze phenotype had a 3-chromosome deficiency translocation which was analyzed in the diakinesis stage only. In each of these five plants, three associated chromosomes were found (figs. 7–11). In one plant, a coincident reciprocal translocation was present also. Figure 11 shows the three associated chromosomes of a 3-chromosome deficiency translocation and the four associated chromosomes of a reciprocal translocation.

In another bronze plant three chromosomes were involved in a type of translocation. This translocation could be interpreted as either one double 3-chromosome deficiency translocation, which is unlikely, or one deficiency translocation and also a reciprocal translocation. The first should have four associated chromosomes at diakinesis and second type should have five associated chromosomes in the translocation. Among 104 diakinesis cells examined in this plant, 90 cells consisted of either four or five associated chromosomes (fig. 12) and 14 had four associated chromosomes. The uncertainty of the number of chromosomes involved in the translocation origi-

TABLE 2

The type and frequency of cytological alterations in chromosome 10 with the aberrant knob following treatment with X-rays and ultraviolet radiation

Treatment	n	r^g plants	Varieg. plants	Term. df?	Inter-st. df.	Df. transl.	Df. inv?	Uni-valent + frag?	Mono-somic 10	Ring chr.	Hap-loid	Nor-mal chr. 10	Died before meio-sis	Un-ex-am.
U.V. 10″	3614	14	3+2?	4	1	1					4	4	2	3
20″	2526	12	[1]	3		1					3	1	2	2
30″	1283	10	[1]	1		1					3	4		1
Total	7423	36	3+2?	8[2]	1	3[2]					10	9	4	6[3]
X-ray 400r	4272	39	3[4]		1	9[5]	1	1	1	3	10	5	7[6]	4
Control	5310	8[7]		1								7		

[1] No variegation, but only ⅛ were suitable for examination of coleoptile.

[2] Including one variegated plant.

[3] Including three r^g plants, one variegated plant, and two questionable variegated plants.

[4] Approximately three fourths of seedlings were examinable for variegation.

[5] Including two variegated plants.

[6] Including one variegated plant.

[7] Plant losses included two possible unstable mutants and one possible intermediate mutant.

nates from the association of chromosome six with the nucleolus because the centric constriction of the nucleolar organizer chromosome cannot be distinguished with certainty from a terminal chiasma.

Effects of ultraviolet radiation on chromosome 10

In the chromosome 10 series the gene R^r provided the genetic marker and the aberrant knob 10 furnished the cytological marker. Pollen of the $R^r\ K/R^r\ K$ stock was treated with ultraviolet radiation and placed on silks of homozygous recessive plants, $r^g\ k/r^g\ k$. Thirty-six r^g individuals were found among 7423 seedlings, four of which died before maturity. Three plants gave poor cytological specimens and thus were not analyzed. In addition to the 36 r^g seedlings, three were variegated and there were two others which were possibly variegated. The cytological analysis was made on 29 of the 36 r^g seedlings and on two of the three variegated seedlings. The other variegated seedling and the two questionable variegated seedlings included two with sterile tassels and one with a poor cytological specimen. A summary of the types of cytological aberrations observed in the r^g and variegated seedlings is presented in table 2 along with the results from plants irradiated with X-ray radiation and those from control pollinations.

Seven of the 29 r^g plants and one of the two variegated plants exhibited deficiencies for the long arm of chromosome 10 and the knob as did one r^g plant found in the control population. As mentioned previously, the knobbed-10 chromosome may not be used to identify true terminal deficiencies since a segment of euchromatin is located distal to the knob. One plant was deficient for approximately seven eighths of the long arm of chromosome 10, one lacked about two thirds of the long arm, two were deficient for approximately one half of the long arm, and three lacked about one third

of the long arm. The terminal deficiency found in the variegated plant was deficient for three fourths of the long arm. At diakinesis, this plant showed no translocation figure in 25 cells.

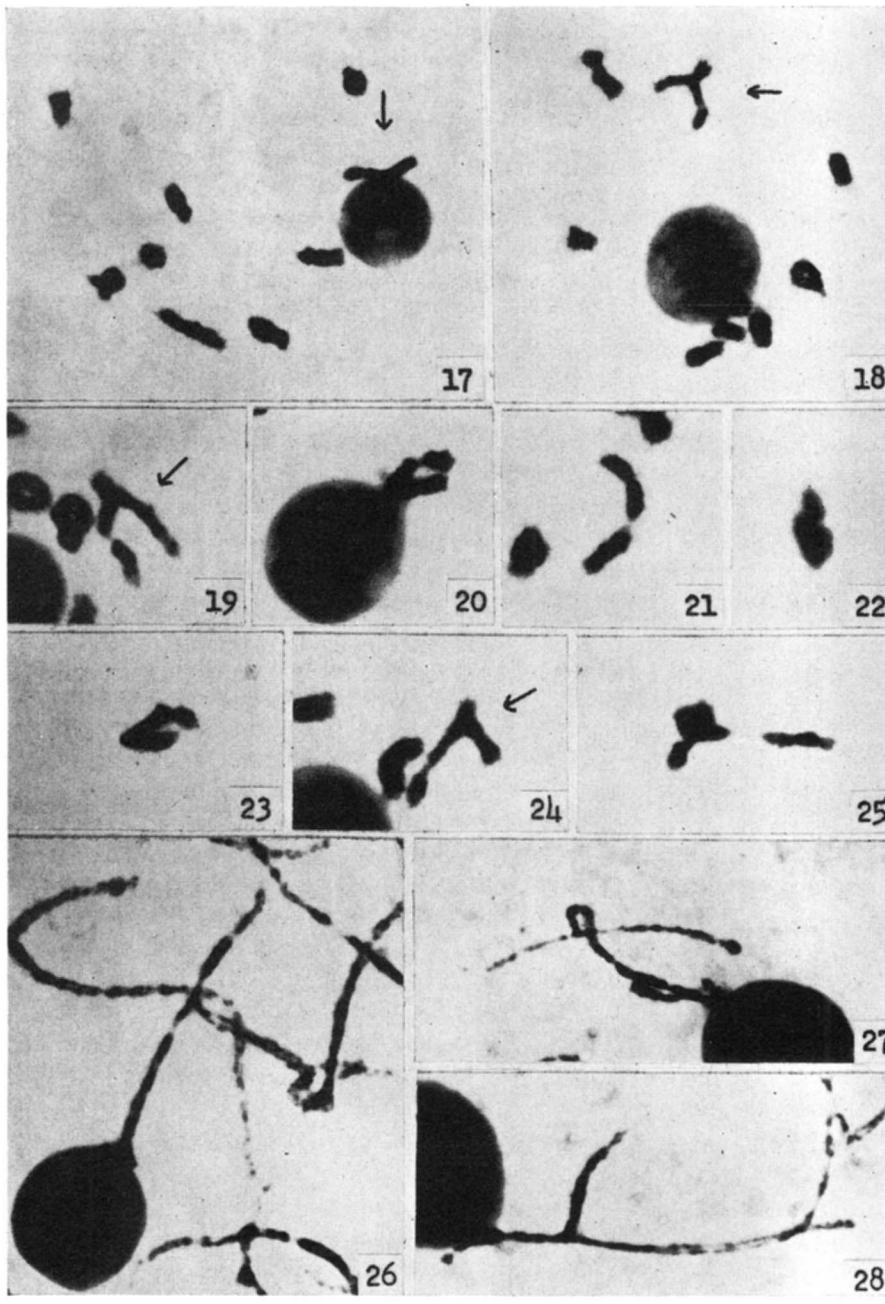
One plant from the ultraviolet treated series possessed a type of chromosomal aberration which was not observed in the 12 bronze plants from the ultraviolet series. This plant exhibited a genetic deficiency for the *R* locus, which was not visible cytologically when heterozygous with a normal chromosome 10. The pollen from this plant was 50% aborted. Its progeny showed no female or male transmission, which would be evidence for a deficiency (r^g) and not a mutation of R^r *K* to R^g *K*. It is important to emphasize the fact that this interstitial deficiency was in compound with a knobless chromosome since a minute interstitial deficiency would not be detected readily in the knobbed chromosome. The aberrant knob on chromosome 10 has a tendency to increase the length of the chromosome by stretching the long arm.

A second type of alteration which did not occur among the bronze plants from the ultraviolet treated series, but did occur in the X-ray treated series of chromosome 9, is the deficiency translocation. Three deficiency translocations were found in the chromosome 10 series in which ultraviolet radiation was used. Two of these translocations were found in r^g plants and one in a variegated plant.

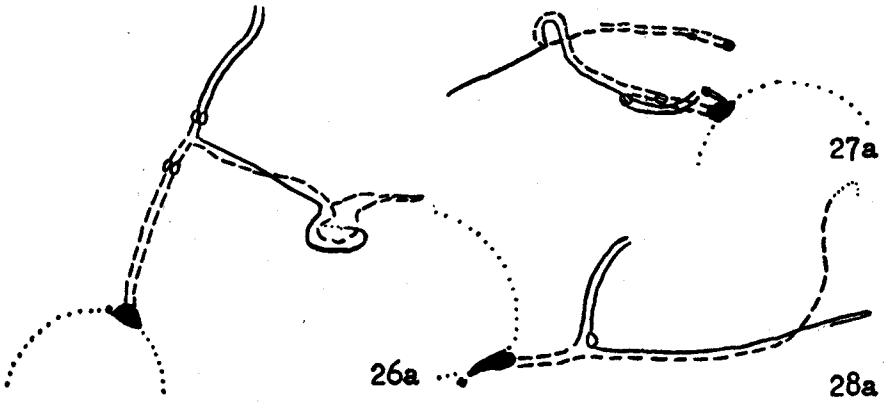
The case from the variegated plant showed a 3-chromosome deficiency translocation at diakinesis and pachytene. One striking difference between this 3-chromosome deficiency translocation and the cases described previously, is the variation in the location of the two centromeres of the dicentric chromosome. The centromere shown in figure 13 is about twice its normal size. This probably represents either two permanently fused centromeres, or two separate centromeres with the orientation of the two chromosomal arms such that the two centromeres had coalesced. Figure 14 shows this same translocation with two widely separated centomeres (indicated by two arrows). The distance between the two centromeres is probably magnified slightly in this figure since one of the centromeres of the dicentic chromosome was adherent to a centromere of another chromosome. This stretched the euchromatin between the two centromeres of the dicentric chromosome. Two additional figures of this translocation were observed at pachytene, one of which shows an enlarged or double centromere and one which shows two separate centromeres; the distance between the two centromeres was similar to the distance shown in figure 14. It would seem likely that these various figures represent the deficiency translocation in different sectors of the tassel.

One of the other two deficiency translocations from r^g plants exhibited three associated chromosomes at diakinesis. Figure 15 illustrates this translocation at diakinesis.

A third type of cytological aberration was found among the r^g plants which was not found in either the ultraviolet or X-ray series of chromosome 9. This plant possessed four associated elements at diakinesis and was designated a 3½-chromosome deficiency translocation. At diakinesis this r^g plant exhibited consistently a chain of four chromosomes and associations with three chiasmata (fig. 16). The following observations were made at pachytene: (1) chromosome 10 and also either chromosome 2 or 4 were involved in some type of translocation (the actual union was not observed); (2) normal chromosome 10 was synapsed with a chromosome 10



FIGURES 17–18.—(× 1600). Diakinesis cells with 3-chromosome deficiency translocations (indicated by arrows) and eight bivalents. FIGURES 19–25.—(× 2600) 3-chromosome deficiency translocations at diakinesis. FIGURES 26–28.—(× 1300) 3-chromosome deficiency translocations between chromosomes 6 and 10 at pachytene; see diagrams.



FIGURES 26a–28a.—Diagrams of figures 26–28. In each diagram the normal chromosome 10 is represented by a solid line; the translocated chromosome is illustrated by partly solid and broken lines; the normal chromosome 6 is represented by broken lines.

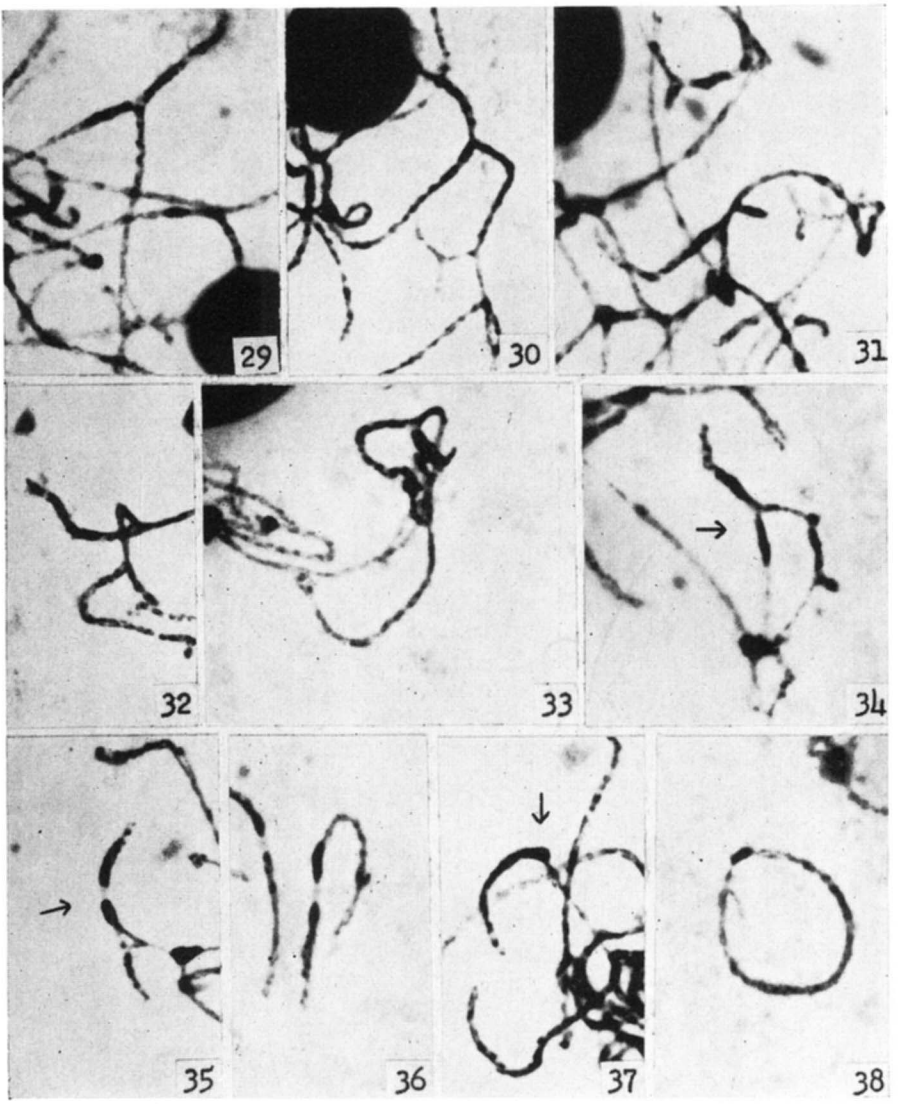
which was a great deal longer than normal; (3) one chromosome 2 or 4 was deficient; (4) normal chromosome 2 or 4 was associated with some chromosome other than the deficient one. Presumably, chromosome 10, and either chromosomes 2 or 4, were part of the same translocation.

The cytological analysis showed that nine of the green plants possessed a normal chromosome ten with the aberrant knob as did five of the plant losses observed in the control pollinations. In addition to these five plant losses in the control, two unstable plant mutants and one possible mutant which was intermediate for plant color were found. Thus the nine plant mutants from the ultraviolet treated series cannot be attributed to the treatment since the frequency of these is not significantly different from that in the control. The self pollinated ears from eight of the plants in the U. V. experiment indicated the mutation of R^r K to R^g K and not to r^g, which would be evidence for a deficiency. The ninth plant gave no progeny. The treated male stock was well marked against contamination. The genotype of the treated male parent was g R^r K/g R^r K, *pr pr*, *Pl Pl*. The cytological examination verified the presence of the knob in each of the nine cases. In eight of the mutants from the ultraviolet treated series, *pr* and *g* were confirmed. The presence of *Pl* has been verified in six of the eight mutants; the remaining two cases are being tested. Each of the eight cases from the control have shown the presence of knob 10 and *pr* in their progeny; the *g* and *Pl* contamination markers need yet to be verified.

Ten of the 29 r^g plants were identified as haploids at diakinesis. These haploids were not analyzed further except for verification of the haploidy at anaphase II where the 10 monads are distributed irregularly to the telophase poles.

Effects of X-ray radiation on chromosome 10

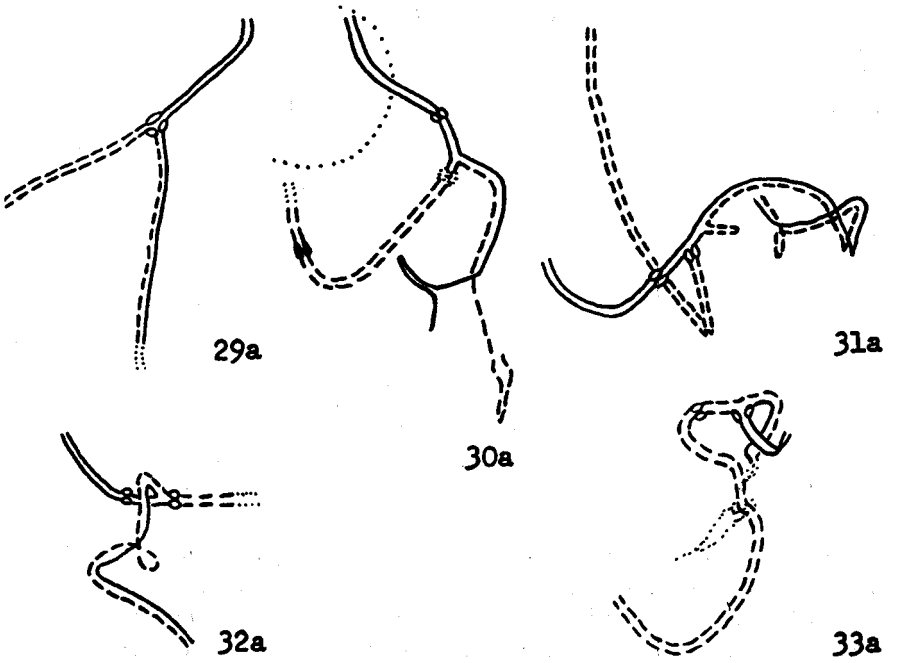
Mature pollen from plants homozygous for the knob and the dominant allele of r^g (R^r K/R^r K) was treated with 400r units of X-radiation and placed on silks of



FIGURES 29–38.—(× 1300). FIGURES 29–33.—3-chromosome deficiency translocations involving chromosome 10 at pachytene; see diagrams. FIGURES 34–37.—Illustrations of a possible deficiency inversion for chromosome 10. FIGURE 38.—A ring chromosome for knobbed-10 chromosome.

homozygous recessive plants, $r^g r^g$. Thirty-nine r^g seedlings were found among 4272 F_1 seedlings, and 29 of these were analyzed cytologically. Six of the r^g plants died in the seedling stage and four gave poor cytological specimens. In addition to the 39 r^g seedlings, three variegated plants were found. Two of these three plants were analyzed. The third case died in the seedling stage. A summary of the cytological results of the 29 r^g plants and the two variegated plants is shown in table 2.

One interstitial deficiency, but no terminal deficiencies, were found among the 29



FIGURES 29a–33a.—Diagrams of figures 29–33. In each diagram the normal chromosome 10 is drawn as a solid line. The translocated chromosome is illustrated by partly broken and solid lines; the other normal chromosome is represented by a broken line.

r^g seedlings. This deficiency involved both the plant and seed color determiners of the R^r locus. This deficiency, which possessed the knob, was invisible cytologically in compound with a knobless chromosome 10. The deficiency showed approximately six percent female transmission. It is now being tested in compound with another knobbed-10 chromosome.

Eight 3-chromosome deficiency translocations were identified in the 29 r^g plants and one in a variegated plant of the X-rayed series. The 3½-chromosome type of deficiency translocation was absent in the X-rayed series of chromosome 10.

In figures 17 to 25, the eight deficiency translocations from the r^g plants and the one from the variegated plant are illustrated in the diakinesis stage. Seven of the eight 3-chromosome deficiency translocations and the translocation in the variegated plant were analyzed in the pachytene stage. In two plants, the 3-chromosome deficiency translocations occurred between chromosome 6, the nucleolar organizer chromosome, and chromosome 10. The dicentric chromosome in both translocations was formed by the fusion of at least the short arm of chromosome 6 and its centromere with the short arm of chromosome 10 and its centromere. In both cases a strand of euchromatin was located between the two centromeres of the dicentric chromosome. It is difficult to determine the origin of this euchromatin since only a limited number of pachytene figures was observed. In figure 26 the long arms of chromosomes 6 and 10 are paired with the thread of euchromatin between the two cen-

tromeres. A pachytene figure from the other plant is shown in figures 27 and 28. In figure 27 the dicentric chromosome is paired homologously with the short arms of chromosomes 10 and 6. The normal long arm of chromosome 6 is synapsed in part with the euchromatin between the two centromeres of the dicentric chromosome. The remainder of the long arms of chromosomes 10 and 6 is paired non-homologously.

In two other plants, one of which was the variegated plant, chromosome 10 and 1 were involved in a 3-chromosome deficiency translocation. The translocation in the r^g plant is shown at pachytene in figure 29. In this particular figure only one enlarged centromere is present. Three additional figures of this translocation were found at pachytene in which two exhibited two separate centromeres and one showed an enlarged centromere. One figure from the variegated plant showed one enlarged centromere.

One r^g plant exhibited a 3-chromosome deficiency translocation of chromosome 10 and 4. In this translocation the short arm of chromosome 10 with its centromere and the long arm of chromosome 4 with its centromere had united to form a dicentric chromosome. A thread of euchromatin was found between the two centromeres.

Figure 30 illustrates a 3-chromosome deficiency translocation between chromosome 10 and either chromosomes 2 or 4. The dicentric chromosome consisted of the short arm of chromosome 10 plus its centromere and long arm of chromosome 2 or 4 plus its centromere. In figure 30, the long arm of normal 10 and the short arm of chromosome 2 or 4 are paired with the euchromatin between the two centromeres; however, this could be non-homologous pairing. The two remaining deficiency translocations are illustrated at pachytene in figures 31 to 33. Figures 31 and 32 are two photos from the same plant. In both of these figures, only the long arm of chromosome 10 is paired with the euchromatin between the two centromeres of the dicentric chromosome. Figure 33 illustrates a 3-chromosome deficiency translocation between chromosome 10 and an unidentified chromosome. In this figure, which clearly shows the two centromeres, only the unidentified chromosome is paired with this region between the two centromeres.

One of the 29 r^g plants possessed an alteration that could be interpreted as a deficiency inversion. Three types of chromosome 10's were observed. One type is represented by figures 34 and 35 in which a knob 10-like substance is now located adjacent to the centromere; normally knob 10 is located near the end of the long arm. These figures show the knob-like substance and the two unpaired long arms of chromosome 10. In the second type, both of the long arms are paired (fig. 36); however, the typical inverted type of pairing was not observed. The third type of chromosome 10 which was observed is illustrated in figure 37. In this type the entire strand of euchromatin distal to the knob is deficient. The pollen from this plant showed 50% abortion.

Ten of the 29 r^g plants were identified as haploid plants from diakinesis and anaphase cells. In each haploid plant, 10 univalents were present.

In one plant the entire irradiated chromosome 10 was deficient. Although no one figure was observed in which each of the 10 chromosomes was isolated, a sufficient number of pachytene stages was examined to verify the absence of even a minute fragment. The examination of the metaphase stage showed also the absence of even

a minute fragment. The diakinesis phase is not a critical stage to determine the absence of minute fragments since small fragments become quite achromatic at this phase.

One of the 29 r^g plants possessed a small fragment or ring chromosome at pachytene. This fragment included about one eighth of the original chromosome 10.

Two ring chromosomes were found in two r^g plants, and one ring chromosome, not deficient for the R^r locus, was included in a variegated plant. The two ring chromosomes from the r^g plants included approximately one fourth and two thirds of the original chromosome 10. The ring from the variegated plant included nearly the entire knobbed-10 chromosome (fig. 38). The original breakage points must have occurred near the end of the short arm of chromosome 10 and in the knob itself.

Five of the 29 plant losses possessed a normal chromosome 10. Four of these cases represented conclusively a plant color mutation of R^r K to R^g K. The presence of *Pl*, *pr*, and *g* contamination markers has been verified in the four plants. In the fifth plant, *pr* and *Pl* have been identified, but the presence of *g* needs yet to be verified.

DISCUSSION

It is difficult to make a quantitative analysis of the effects of ultraviolet radiation and X-rays on chromosomal aberrations, since there is no direct method of measuring comparable doses. A comparison could be made in terms of some genic or chromosomal equivalent. STADLER (1941) determined the relative effectiveness of these two types of radiation on gene mutation in which he was employing doses equal in their effect on specific gene markers in the endosperm. In another comparative mutation experiment (STADLER 1941), the number of plants with segregating pollen was used as an equivalent for determining comparable doses of X-ray and ultraviolet radiation. If a specific equivalent is selected as the basis for the determination of comparable doses, a major difficulty is encountered immediately. The doses of ultraviolet and X-rays assumed to be comparable in their effects on one type of genic or chromosomal standard, may not be equivalent if another standard is selected. In view of this difficulty, only general inferences can be drawn from the cytological results reported here.

Previously, it has been suggested (STADLER 1941) that there may be a qualitative difference in the type of chromosomal aberration which has been produced in plants following treatment with X-ray and ultraviolet radiation. The writer's data show no gross qualitative difference between effects of the two types of radiation. If each kind of aberration in plants from the ultraviolet treated series is compared with those found in the X-ray treated series, it is found ring chromosomes and a questionable deficiency inversion are the only types of aberrations absent in plants following ultraviolet treatment. Both X-ray and ultraviolet treated series included terminal deficiencies, interstitial deficiencies, fragmented chromosomes and deficiency translocations.

There is indication, however, that a quantitative difference exists between the two types of radiation in the frequency distribution of chromosomal aberrations produced. It is reasonable to assume that if the over-all effect of X-rays is greater than that of ultraviolet radiation in the frequency of production of chromosomal

aberrations at the dosage levels used, ultraviolet rays should be less effective than X-rays in the production of any one specific type of chromosomal alteration. In the paragraphs to follow, it will be shown that a low dose of X-rays (400r) is much more effective than the two highest doses of ultraviolet radiation (U.V. 20″ and 30″) on the frequency of total number of chromosomal aberrations. But the total frequency of terminal deficiencies following ultraviolet treatment (U.V. 20″ and 30″) is greater than that produced by X-rays (400r).

The Chi square method of measuring whether the observed ratio of chromosomal aberration fits with the expected ratio has been used to determine the relative frequency of production of chromosomal alterations following a low dose of X-rays (400r) as compared with the two highest doses of ultraviolet rays (U.V. 20″ and 30″). The following data include the simple and complex deficiencies from both of the chromosomes 9 and 10 series:

	Total population	Total no. cases	Total cases expected
U.V. (20″ and 30″)	8834	14	21.7
X-ray (400r)	6208	23	15.3
Total	15,042	37	37.0

Mean value 37/15,042 = 0.002460

Total (sum of d^2/e) = 6.60 = χ^2

P = 0.01–0.02

The results show a larger number of aberrations following X-ray treatment of 400r than following treatment with ultraviolet radiation for 20 and 30 seconds. This conclusion, however, is based entirely on the assumption of random distribution of chromosomal alterations in the two populations.

The following data show that ultraviolet radiation, though less effective on the over-all frequency of chromosomal aberrations, has a greater effect than X-rays on the frequency of terminal deficiencies. This conclusion is based on results from chromosome 9 series only since terminal deficiencies are questionable in the chromosome 10 series.

	Total pop.	Total df. obs.	% Total df.	Term df. obs.	Term. df. exp.	Complex df. obs.	Complex df. exp.
U.V. (all)	6168	9	32.14	8	3.21	1	5.79
X-ray (all)	3006	19	67.86	2	6.79	17	12.21
Total	9174	28	100.00	10	10.00	18	18.00

Tests of significance of these ratios have been estimated from a statistical table prepared by STEVENS (1942). This table is used for the determination of the limits of expectation where small numbers are involved. The following probabilities have been found from the above ratios:

	Exp. (=n)	Obs.	$p = n/N$	Probability
Complex df.—U.V.	5.79	1	0	.005 × 2 = 0.010
Terminal df.—U.V.	3.21	8	0	.025 × 2 = 0.05

The data show a lower frequency of complex deficiencies in the ultraviolet series than in the X-rayed series. The results indicate also a probably significantly larger number of terminal deficiencies following ultraviolet treatments than in the X-ray treated series.

The low frequency of deficiencies in plants following treatment with ultraviolet radiation could be ascribed to the restitution of the fractured chromosomes at the point of original breakage. SCHULTZ (1951), studying the effects of ultraviolet radiation on a ring chromosome in *Zea mays*, attributed the high frequency of ring loss following treatment with ultraviolet to restitution. He believed that sister strands reunite to form interlocked or double rings which are either lost or included both in one daughter cell.

It could always be argued that the doses of X-ray and ultraviolet radiation were not strictly comparable in the present experiment. STADLER (1938) showed that the low rate of deficiencies in plants treated with ultraviolet was not a function of the low dosage. He compared the number of plants showing pollen abortion in the X-ray treated series with those in the ultraviolet treated series. The doses of X-rays used included 150r, 600r, 2400r in one series and 1450r in another series. The dose of ultraviolet used was stated as being near the tolerance limit for the wave length. He found consistently a higher frequency of plants with aborted pollen in the 600r, 1450r, and 2400r X-rayed series than in plants which were treated with a heavy dose of ultraviolet radiation. STADLER concluded that a qualitative difference exists between the two types of radiation.

The low frequency of complex aberrations in the ultraviolet series could be due to a low rate of coincident breaks. An alternative hypothesis to this would be that ultraviolet radiation produces as many breaks per nucleus as X-rays but the broken chromosomal ends do not reattach. If this were the case, there should be numerous broken chromosomes in single cells. These coincident losses were not analyzed in the cytological material examined in the present investigation.

SUMMARY

A cytological analysis was made of the effects of ultraviolet radiation and X-rays on chromosomes 9 and 10 of *Zea mays*. A stock homozygous recessive for the gene *bz* in the short arm of chromosome 9, and a stock with the gene r^g in the long arm of chromosome 10 were pollinated by irradiated pollen from stocks homozygous dominant for the two marker genes and the knob. The F_1 plants which exhibited the recessive phenotype for the specific gene markers on chromosomes 9 and 10 were saved for cytological analysis.

Analysis of the chromosomal aberrations showed no gross qualitative difference between the effects of ultraviolet and X-ray radiation. However, a quantitative difference was found between the two types of radiation in the frequency distribution of chromosomal alterations. A lower frequency of complex deficiencies and higher frequency of terminal deficiencies occurred in chromosome 9 following treatment with ultraviolet radiation (U.V. 10, 20, and 30 seconds) than in chromosome 9 following X-ray treatments (400r, 800r, and 1000r). It could not be determined whether this was due to (1) a low rate of coincident breaks, or (2) the same frequency of

breaks in ultraviolet and X-ray series but failure of fusion of broken chromosomal ends in the ultraviolet series.

ACKNOWLEDGMENTS

To the late DR. L. J. STADLER I wish to acknowledge gratefully the stimulating guidance, encouragement, and advice given by him throughout the course of this investigation. I wish to thank DRS. J. R. SINGLETON, HARRIET B. CREIGHTON, E. R. SEARS, E. NOVITSKI, and J. R. LAUGHNAN for their suggestions and helpful criticisms during the preparation of this manuscript.

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