

# ULTRAVIOLET AND X-RAY INDUCED CHROMOSOMAL ABERRATIONS IN WHEAT<sup>1</sup>

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A number of studies have compared the effects of ultraviolet and X-radiation in causing mutations and chromosomal rearrangements. Although superficially similar results are obtained, STADLER (1939) suggested that the action of the two agents might be quite different. He found in maize that rearrangements of chromosomes resulted much more rarely from ultraviolet than from X-radiation. Also, ultraviolet-induced mutants at the locus *A* were recovered which fitted none of the criteria of deficiencies, whereas all of the X-ray-induced mutants obtained were indicated to be deficiencies (STADLER and ROMAN 1943, 1948).

In experiments with *Tradescantia* pollen tubes SWANSON (1940, 1942) reported that ultraviolet radiation produced only simple chromatid deletions, and these were located more medially or distally than those caused by X-rays. It has been suggested (MCCLINTOCK 1941; SWANSON 1942) that broken ends produced by ultraviolet are more stable than those caused by mechanical or X-ray breakage.

EMMERLING (1955), studying induced mutants in maize, could find only quantitative differences in the effects of X- and ultraviolet radiation. Her data showed a lower frequency of complex deficiencies and a higher frequency of terminal deficiencies following ultraviolet treatment. Recently FABERGÉ (1956) found that in maize endosperm no stable broken ends occurred as a result of either ultraviolet or X-ray breakage. His results with the two radiations, though not identical, were so similar that he could combine his data for making most of his analyses.

Since common wheat, *Triticum aestivum* L. emend Thell., is a hexaploid ( $n = 21$ ), it easily tolerates aberrations such as whole chromosome losses which would be lethal in a diploid plant. Although a comparison of X-ray and ultraviolet effects in wheat might therefore yield information of value, such a study has not been possible because the pollen is too short-lived to survive the type of ultraviolet treatment heretofore used. The development of a new method for subjecting pollen to ultraviolet irradiation (FABERGÉ 1957), however, has removed this barrier to the use of wheat in comparative radiation work.

The ultraviolet treatments which yielded the results reported here were made in collaboration with DR. A. C. FABERGÉ, using his apparatus, during the winter of 1955-56. The X-ray data used for comparison were obtained by the junior author in 1948.

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## MATERIAL

Throughout the ultraviolet experiment, wheat plants were used as both male and female parents which were homozygous for the partially dominant gene *Q*, deficiency for which results in the easily detected heterozygous speltoid phenotype. A few pollinations involved parents homozygous also for the recessive gene on chromosome III, virescent, deficiency for which results in normal chlorophyll development. The plants used as female parents were all of the pure variety Chinese Spring, except that the virescent plants had a pair of chromosome III's which had been introduced from another variety. The male parents were mostly pure Chinese also, but included a number of plants obtained from Chinese crossed with an amphiploid of *Triticum dicoccoides* × *Aegilops speltoides*. Four backcrosses to Chinese had largely restored the genotype of Chinese to these lines, but stem-rust resistance and an associated glume character from *Ae. speltoides* had been retained. The amount of ultraviolet energy received by the treated pollen has been calculated by FABERGÉ (1957) as 370,000 to 830,000 ergs per cm<sup>2</sup>.

The X-rayed material, also pure Chinese Spring wheat, received 256r, the pollen being irradiated by the usual method while still in the anthers in the florets.

## RESULTS

*Seed setting and germination*

In the ultraviolet experiment, the spikes pollinated had an average of 23 florets, as determined from a sample of 27 spikes. Hand pollinations showed an average seed set of 31 percent, with a maximum of 17 grains on one spike. The tube-pollinated controls (pollinations through the FABERGÉ apparatus with the ultraviolet sources turned off) averaged six percent set, with a maximum of ten seeds per spike. Treated pollen produced sets averaging 22 percent, with a 13-seed maximum. Thus the radiation did not decrease the seed set. However, some of the seeds from irradiated pollen did not reach maturity. The percentage of aborted seeds was 4.5 from hand pollination, none from tube controls, and 27.2 from treated material.

Of the seeds obtained by hand pollination, 84 percent grew, and all 71 seedlings were normal. These were not studied cytologically. From the 17 tube-control seeds, 13 plants were obtained (76 percent), all of which were studied cytologically. Of the 116 seeds resulting from irradiated pollen, 69 germinated, but only 57 plants were obtained. Thus only 49.1 percent of the seeds from treated pollen produced plants.

In the X-ray experiment, in which ordinary wheat pollinating technique was used, seed setting was strikingly better than following ultraviolet treatment, averaging 87.3 percent. Germination was also much higher, being 99.2 percent.

*Identification of aberrations*

In the ultraviolet experiment all 13 plants obtained following control pollination through the tube had the normal complement of 21 bivalents at metaphase I.

The ultraviolet treatments were of two different durations, one involving exposure through a distance of 8 cm and the other through 18 cm. Data from the two treatments, which yielded 10 and 47 viable seeds, respectively, have been combined,

TABLE 1  
*Chromosome constitution of progeny of ultraviolet-irradiated pollen*

No. of plants	No. of bivalents			No. of trisomes	No. of 3-chrom. translocations	No. of configurations of 4			No. of other multivalents	No. of monosomes	No. of fragments
	Normal	Deficient				Rings	Chains	Defic. chains			
		For <1 arm	For 1 arm <sup>a</sup>								
34	21										
4	20		1								
1	20			1							
1	20		1 <sup>b</sup>								
1	19		2								
2	19				1						
2	19				1						
1	19					1					
1	18	1		1						1	
1	18								chain 5 <sup>c</sup>	1	
1	17		2			1					
1	17	1	1					1			
1	17				1		1				
1	17								chain 6 <sup>d</sup>	1	1
1	16		1			1	1				
1	16	1	1					1		1	
1	16		1				1	1			
1	14		1		1			2			
56		3	14	2	4	4	4	5	2	4	1

<sup>a</sup> As determined at MI. See text for results of root tip studies.

<sup>b</sup> Involved an isochromosome rather than a telocentric.

<sup>c</sup> Including an isochromosome.

<sup>d</sup> Including an apparent telocentric chromosome.

because of the small numbers involved and the lack of any obvious difference in effect. One plant was highly abnormal and died before heading. Two others were speltoid mutants.

Of the 56 F<sub>1</sub> plants studied cytologically, 34 had 21 bivalents. Several whole families were normal. This could have been due to occasional clumping of pollen or to the accidental dropping of an entire anther through the tube, thus preventing effective exposure to the ultraviolet irradiation.

Among the meiotic abnormalities found at MI (table 1), loss of part or all of a chromosome arm was very common. This was observed as a heteromorphic bivalent; or, if the same chromosome also was involved in a reciprocal translocation, it was seen as an apparent telocentric at one end of a chain. There were 23 such deficient chromosomes, 13 of these being apparently telocentric members of heteromorphic bivalents. One was a short isochromosome, probably less than a whole arm, also a member of a heteromorphic bivalent. Three were two-armed members of heteromorphic bivalents. The other six deficient chromosomes were apparent telocentrics involved in multivalent configurations. Studies of a sample of six of the apparent

TABLE 2  
Chromosome constitution of progeny of X-rayed pollen

No. of plants	No. of bivalents			No. of trisomes	No. of 3-chrom. translocations	No. of configurations of 4			No. of other multivalents	No. of monosomes	No. of fragments
	Normal	Deficient				Rings	Chains	Defic. chains			
		For <1 arm	For 1 arm <sup>a</sup>								
28	21										
1	20	1									
7	20		1								
3	20									1	
15	19					1					
12	19						1				
1	19							1			
2	19				1						
5	19		2								
1	19									2	
1	19		1							1	
1	19		1							1	1
1	18	1				1					
2	18					1				1	
3	18		1			1					
1	18						1			1	
2	18	1					1				
4	18		1				1				
3	18								chain 6		
1	18								ring 6		
1	18		1		1						
1	18		2							1	
1	18	1								2	
3	17					1	1				
2	17					2					
1	17				1		1				
1	17								chain 5	1	
2	17		2			1					
1	17		2				1				
1	17	1				1				1	
1	17				1			1			
1	16						1		chain 6		
1	16		1			1	1				
1	16		1			2			chain 8		
1	16	1					2				
1	16		3				1				
1	16	1	2				1				
1	16		1				1	1			
1	15					2					
1	15				1	1				2 <sup>b</sup>	
1	15		2		2						
1	15			2 <sup>c</sup>			1			2	
1	14								chain 6	4	
1	14		3						chain 8		
1	13			1	1				chain 9		
124		8	49	3	9	37	33	3	8	23	1

<sup>a</sup> As determined at MI. See text for results of root tip studies.

<sup>b</sup> Including one isochromosome.

<sup>c</sup> Including one isochromosome each.

telocentrics in root tips of offspring revealed that few or none were actually telocentric. Five of the six had a tiny but clearly discernible second arm.

Nineteen translocations were found, including two that involved more than four chromosomes. Only the four translocations which formed rings of four could with certainty be attributed to reciprocal translocation without any loss. Ten translocations definitely involved loss of chromatin, four of them being deficient for a whole chromosome, and six for part of one chromosome. Four other translocations were either deficient or else involved a piece too short to permit ring formation.

The data for the progeny of X-rayed pollen are given in table 2. Of the 124 plants, 28 had the normal complement of 21 bivalents at metaphase I. Fifty-seven heteromorphic bivalents were found, and 49 of these had one telocentric or apparently telocentric member. Thirty-eight translocations formed rings at metaphase I indicating that no chromatin had been lost. Thirty-eight others involved either a translocated piece that was too short to permit ring formation or a deficiency not large enough to observe at this stage. Fourteen translocations involved definite loss, 11 being deficient for one whole chromosome, the other three for most or all of one chromosome arm. Root tip studies of four apparently telocentric members of heteromorphic bivalents showed that at least three of these were not actually telocentric, but had a short second arm.

Two plants were found in the ultraviolet experiment and two in the X-ray series that had an extra chromosome. Since the two plants from ultraviolet treatment (one with  $20_{II} + 1_{III}$  and the other with  $18_{II} + 1_I + 1$  chain of five including an isochromosome) both came from the same pollen parent of hybrid ancestry, it is quite possible that the extra chromosome was already present before the treatment. The pollen parent, which was not examined cytologically, may have had a trisome. This plant could thus have given rise directly to the trisome in the first plant and, following misdivision of one chromosome and translocation of its homologue, to the chain of five in the second plant.

The extra chromosomes in the X-ray series are less likely to have come from trisomic parents, because pure Chinese Spring plants were used. However, nondisjunction, while uncommon in this variety, is not unknown. One of the two plants with extra chromosomes had  $15_{II} + 1$  chain of four +  $2_I + 2_{III}$  (both trivalents including an isochromosome), and the other had  $13_{II} + 1$  chain of nine +  $2_{III}$ . In the former plant both trivalents were presumably trisomes (secondary), and in the latter plant one of the trivalents must have been a trisome. Since the division to form two generative nuclei had already occurred at the time of irradiation, there was no possibility for the treatment to cause the duplication of a chromosome, except by a delayed effect involving nondisjunction at an early division of the zygote. This would have resulted in a sectorial plant, part of which had a trisome and part a monosome. Such sectoring could scarcely have been detected, since only one spike each of these two plants was examined cytologically. Further assumptions are necessary to explain the origin of the added isochromosomes solely as a result of the irradiation.

In the X-ray series one plant had an isomonosome, in addition to one other monosome, one three-chromosome translocation, a ring of four, and 15 bivalents. The isochromosome can perhaps most easily be explained as the result of failure of a pair of

chromosomes to synapse in the female (untreated) parent, followed by misdivision of one of the univalents. Since the isochromosome was present as a monosome, it is necessary to assume that the homologue from the treated parent was lost, presumably as a result of the irradiation.

*Comparison of the effects of X- and ultraviolet radiation*

From ultraviolet-treated pollen two speltoid mutants were obtained, and three were found in the X-rayed series. One of the former and all three of the latter had cytological aberrations. It is not known whether any of the mutations were dependent on the cytological aberrations.

Tables 1 and 2 show that the cytological abnormalities obtained from the two radiations fell into the same classes. The average number of breaks per cell (calculated minimum) was 2.8 for the effectively treated ultraviolet material and 2.9 for the comparable X-rayed material (i.e., among plants with aberrations). In the X-rayed material there were a few chain configurations longer than any obtained by ultraviolet treatment.

Three of the offspring of X-rayed pollen were simple monosomics with no other abnormality, a class that was not found in the ultraviolet-treated material. This is presumably of no significance, however, since the X-ray experiment included more than four times as many plants with aberrations. The total number of monosomes, including those in plants with other aberrations, was not significantly different, being 21 in X-rayed and four in ultraviolet-treated material.

Heteromorphic bivalents were somewhat less frequent in the X-rayed material, but the difference was not significant. Furthermore, the proportion of heteromorphics having two two-armed members to those having one apparently telocentric chromosome was the same. Only three multivalent configurations in the X-rayed material as against six in the ultraviolet-treated involved apparently telocentric chromosomes. This is a significant difference between the two types of material. These data can be interpreted as evidence that chromosome ends broken by ultraviolet are less likely than those broken by X-rays to reunite with other broken ends, and after failure to reunite are less likely to form chromatid bridges and be lost.\*

DISCUSSION

It is a reasonable assumption that when two chromosomes are broken, translocation will as frequently bring the two centric pieces together (asymmetrical exchange) as it will unite the centrics with the acentrics (symmetrical exchange). The dicentrics formed are either lost or, perhaps following a breakage-fusion-bridge cycle, break apart and heal. In diploid organisms the frequency of loss of these dicentrics cannot easily be ascertained, because the resulting plants, being deficient for two whole chromosomes, do not ordinarily survive. In hexaploid wheat, however, plants deficient for two chromosomes not only are viable, but are reasonably normal unless the

\* KIRBY-SMITH and CRAIG (Genetics 42: 176-187. 1957) showed that in *Tradescantia* pollen the ends of chromosomes broken by ultraviolet rejoin less often than do those produced by ionizing radiation, thus giving rise to fewer translocations relative to deficiencies.

chromosomes concerned happen to be related. It is therefore of interest to try to determine what became of the dicentrics in the present material.

In the X-ray experiment 70 reciprocal translocations (excluding the definitely deficient and the complex types) were recovered, which presumably means that there were about the same number of asymmetrical exchanges, each giving rise to a dicentric. That there were only five instances of two monosomes in a single plant (counting one plant with four monosomes as two instances) indicates that the dicentrics were seldom lost. In the ultraviolet series there were eight reciprocal translocations and no plants with two monosomes. Thus the ultraviolet data agree with those from X-ray in indicating little loss of dicentrics.

Presumably some dicentrics undergo a breakage-fusion-bridge cycle which eventually results in the shortening of the region between the centromeres to the extent that the two centromeres behave as one, as observed by HAIR (1952) and EMMERLING (1955). This makes a three-chromosome translocation. Even if all of the three-chromosome translocations arose in this way, there were only four in the ultraviolet series and nine in the X-ray experiment (or 11, considering that the chains of five and of nine are comparable to chains of three), far short of the number needed to account for all of the assumed dicentrics.

Dicentrics which eventually break and heal will usually give rise to chromosomes with one arm of altered length. Since the bridge formed by the dicentric is deficient for the terminal segment of each of the arms composing it, the derived arms will most often be shorter than the original; but, due to an eccentric break, an arm may occasionally be longer, or very short, or even completely absent. The derived chromosomes with a short or missing second arm will form heteromorphic bivalents. It seems probable that most of the heteromorphic bivalents observed in at least the X-ray experiment arose in this manner, for relatively few of the dicentrics can be accounted for in other ways.

Another possible manner of origin of deficient members of heteromorphic bivalents is from single breaks that heal either directly or following chromatid reunion and a breakage-fusion-bridge cycle. It seems probable that a number of the heteromorphic bivalents in the ultraviolet experiment and some in the X-ray series arose in this way.

Since the deficient member of the large majority of the heteromorphic bivalents had a very short second arm, there is a possibility that the wheat chromosomes are most easily broken proximally, as has been suggested by MAC KEY (1954). However, if most of the deficient chromosomes are formed following breakage-fusion-bridge cycles, of either chromosome or chromatid type, there is no necessity for assuming that the position of the original break is represented by what becomes the end of the shortened arm of the chromosome.

The data reported here show that chromosomal rearrangements are obtained in high frequency with ultraviolet as well as with X-radiation, but indicate that more of the ultraviolet-induced translocations involve loss. In nonpolyploid material, where chromatin losses are less likely to be tolerated, the result could be infrequency or absence of detectable translocations, such as has been reported by STADLER and others (see SWANSON and STADLER 1955, for summary). Only in experiments such as those of

EMMERLING (1955), designed to select the occasional surviving deficient translocations, would frequencies comparable to those from X-rays be expected.

#### SUMMARY

From wheat pollen given dosages of ultraviolet and X-rays roughly comparable in capacity to break chromosomes, 56 and 124 plants, respectively, were obtained, all of which were analyzed cytologically. Reciprocal translocations and part- and whole-chromosome deficiencies were found following both types of treatment, but more of the ultraviolet translocations were deficient for an apparently terminal segment. This is regarded as evidence for less reunion and more healing of broken chromosomes following ultraviolet treatment. The virtual absence of translocations in previous ultraviolet experiments is attributed to the strong tendency of ultraviolet-induced translocations to involve deficiency. In diploid species these deficiencies would frequently cause inviability. The data suggest also that dicentric chromosomes are seldom lost in this material, but instead often break and give rise to chromosomes with terminal deficiencies.

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