# HEREDITARY EFFECTS PRODUCED IN MAIZE BY RADIATIONS FROM THE BIKINI ATOMIC BOMB I. STUDIES ON SEEDLINGS AND POLLEN OF THE EXPOSED GENERATION[1]

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SEVERAL lots of maize seed were exposed to radiations from the first Bikini atomic bomb, the "Able" bomb of July 1, 1946. From these lots cultures were grown at the CALIFORNIA INSTITUTE OF TECHNOLOGY together with controls and a parallel series X-rayed by Dr. L. F. RANDOLPH. Cytological material was collected by DR. RANDOLPH for studies at CORNELL UNIVERSITY on visible chromosomal changes induced by the radiations (RANDOLPH, LONGLEY and LI 1948). Studies on hereditary effects have been carried on at the CALIFORNIA INSTITUTE OF TECHNOLOGY.

This report deals with the immediate generation of plants grown from the exposed seeds, and includes (a) observations on effects shown in the seedling stage and (b) observations and analysis of effects shown in the pollen.

## SEEDLING TESTS

The first planting was made in the greenhouse July 28, 1946. Five lots of the $F_1$ hybrid seed, L289×1205, which had been exposed at Bikini, were included. These five lots had been recommended as probably coming within the range of exposure suitable for our studies. Untreated controls and lots subjected to 5,000, 10,000 15,000, and 20,000 r units X-ray were also included. Germination in all lots was about 98 percent, with the higher dosage lots showing no decrease below the controls or low dosage lots. A second planting of one lot of Bikini (lot A) and 15,000 r unit X-ray was made three days later. Further plantings were made at intervals until August 10.

In the X-ray comparison tests, seed treated with 10,000 r units or more gave a characteristic mottling and streaking of the early leaves of the seedlings. The first seedling leaves were heavily mottled instead of the uniform green of the controls. This is due to deleterious effects on individual cells in the leaf primordia. In the later leaves the spots became progressively more elongated, giving the leaves a finely streaked appearance. After about the fifth leaf, streaks were infrequent and the young plants took on a normal green color,

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with only an occasional plant showing a pale or albino stripe or sector.

In a typical dormant corn embryo, five leaf primordia are visible as lateral outgrowths from the shoot axis, with the lower primordia having a larger number of cells. On any individual cell the radiation may have no detectable effect, or may have effects which prevent chlorophyll development, slow up cell divisions, cause early necrosis, or even prevent cell multiplication. From an already-formed multicellular leaf primordium, the resulting leaf is naturally a mosaic of tissues showing all of these effects. Where more cell divisions and growth must intervene as in the formation of the later leaves from the primary meristem, more rapid growth of the less injured cells and tissues will tend to eliminate cells and tissues which are handicapped by the more serious effects of radiation. Thus, there should be a gradual elimination of abnormal tissue. Abnormal stripes or sectors may survive if the abnormality does not markedly hinder the rate of cell division and development of the tissues.

In the X-ray series, seedlings from the 5,000 r treatment showed a small amount of mottling with little or no reduction in the size of the plants, while those from 10,000 r showed conspicuous mottling and averaged below normal in size. Seedlings from 15,000 r were very conspicuously mottled, and more reduced in size. A small percentage were weaklings which failed to survive. Most of the seedlings from 20,000 r appeared much like those from 15,000 r, but a much larger proportion (8 to 10 percent) were weaklings which did not survive. One lot of the Bikini series showed an intensity of mottling slightly below that of the 15,000 r X-ray treatment, with an absence of weaklings. The effect was much more uniform than in the X-ray series. There were no plants approaching normal and none which could be singled out as excessively affected. The reduction in vigor was more uniform and all plants recovered fairly evenly. The other four Bikini lots showed only slight traces of mottling.

### POLLEN EXAMINATIONS ON THE EXPOSED GENERATION

Pollen examinations were made on plants of the first and second plantings. A portion of the central spike of each plant was collected, the fresh pollen was examined, and the occurrence of sectors of partial sterility was recorded. Sector size varied from a few spikelets to the greater portion of the spike, and in some spikes only partially sterile pollen was found. The limited area of the spike shedding pollen at the time of observation made it impossible to determine the size of sectors in most cases. The data are given only in terms of whether partially sterile pollen was observed on a given spike. No doubt many sectors of defective pollen were missed, so the total frequencies should be considerably higher than the observed frequencies.

For the X-rayed lots the observed frequencies were as follows:

| | *Plants examined* | *With sectors of abnormal pollen* | *Percent* |
|---|---|---|---|
| 5,000 r | 176 | 52 | 29.5 |
| 10,000 r | 170 | 90 | 52.9 |
| 15,000 r | 532 | 335 | 63.0 |
| 20,000 r | 135 | 95 | 70.4 |

In the 15,000 r lot, the first planting gave 72.3 percent and the second only 58.7 percent of spikes showing sectors. The order of planting was such as to place the second planting of 15,000 r at the end of the block. Usually collections and classifications of pollen were made in the order the cultures appeared in the field. It is possible that fatigue on the part of the observer may have contributed to the lower value in the second planting. The average value for 15,000 r should probably be slightly higher than the observed frequency of 63.0 percent.

For the lower dosages the frequency of observed sectors steps up rapidly with the dosage, but above 10,000 r units it levels off toward what may be a maximum of about 75 or 80 percent. Some leveling off is expected due to an increasing occurrence of multiple effects in the same cells. But a great part of the leveling off is no doubt due to the selective elimination of cells and tissues in which the effects are deleterious. The maximum may represent the average value beyond which the plants fail to survive the seedling stage.

The observed frequencies of sectors for the Bikini series follow, the five lots being designated A to E in the order of sector frequency.

| | *Plants examined* | *With sectors of abnormal pollen* | *Percent* |
|---|---|---|---|
| Lot A | 831 | 524 | 63.1 |
| B | 407 | 72 | 17.7 |
| C | 399 | 59 | 14.6 |
| D | 402 | 29 | 7.2 |
| E | 397 | 16 | 4.0 |

The pollen sector counts agree very closely with the seedling observations. Lot A, which seedling observation had placed as equivalent in biological effect to nearly 15,000 r, gave 63.1 percent of observed sectors as compared with 63.0 percent for the 15,000 r X-ray lot. First and second plantings gave nearly the same results, 64.4 and 61.8 percent, respectively. Lots B and C gave about half the frequency of sectors shown by the 5,000 r lot, and lots D and E were much lower. Unfortunately no lower dosages of X-ray were tested. As the dosage-frequency relationship below 5,000 r has not been investigated, we cannot safely extrapolate.

Partial pollen sterility is in nearly all cases due to chromosome alterations or rearrangements which give rise at meiosis to microspores deficient for some portion of a chromosome. Among these are translocations, inversions, and various types of deficiencies. A few pollen abnormalities due to other causes are expected, and likewise a few chromosomal alterations which do not visibly affect the pollen. Both should be low in frequency and should tend to cancel each other. Thus the frequency of partial pollen sterility can be taken as a direct measure of the frequency of chromosome alteration.

## DATA ON SECTOR SIZE AND FREQUENCY

The preceding data give only the frequencies of plants in which sectors of partially sterile pollen occurred within the limited portion of the central spike shedding pollen on the day collected. In order to get an estimate of the size

TABLE 1

*Size and frequency of partially sterile pollen sectors in terms of lateral tassel branches.*

| LATERAL BRANCHES PER TASSEL | PLANTS | NORMAL BRANCHES | PARTIALLY STERILE BRANCHES | ½ | 1 | 1½ | 2 | 2½ | 3 | 3½ | 4 | 4½ | 5 | 5½ | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | TOTAL BRANCHES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 1 | 9 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 9 |
| 10 | 9 | 76½ | 13½ | — | 5 | 1 | 2 | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 90 |
| 11 | 2 | 17½ | 4½ | 1 | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 22 |
| 12 | 10 | 79½ | 40½ | 1 | 3 | 1 | 6 | — | 2 | — | 1 | 1 | — | — | — | — | — | 1 | — | — | — | — | — | 120 |
| 13 | 17 | 145½ | 75½ | 1 | 4 | 2 | 6 | 1 | 4 | 2 | 4 | 1 | — | — | 1 | — | 1 | — | — | — | — | — | — | 221 |
| 14 | 26 | 245 | 119 | 1 | 10 | 1 | 4 | 1 | 5 | — | 2 | 1 | 1 | 1 | 1 | 1 | — | — | 1 | 1 | 1 | 1 | — | 364 |
| 15 | 21 | 198½ | 116½ | 1 | 6 | 2 | 3 | 3 | 3 | 1 | 9 | 2 | 2 | — | 2 | — | — | — | — | — | — | — | 1 | 315 |
| 16 | 15 | 162½ | 77½ | — | 3 | — | 3 | — | 3 | 1 | 2 | — | 3 | — | 3 | 1 | 1 | — | — | — | — | — | — | 240 |
| 17 | 15 | 185½ | 69½ | 2 | 6 | — | 9 | — | 6 | 1 | 1 | — | 1 | — | 1 | — | 1 | — | — | — | — | — | — | 255 |
| 18 | 6 | 80 | 28 | — | 2 | 1 | — | — | — | 1 | 3 | 2 | — | — | — | — | — | — | — | — | — | — | — | 108 |
| 19 | 2 | 21 | 17 | — | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | 38 |
| Total | 124 | 1220½ | 561½ | 7 | 39 | 8 | 37 | 5 | 24 | 6 | 22 | 7 | 7 | 1 | 8 | 2 | 3 | 1 | 1 | 1 | 1 | 2 | 1 | 1782 |

and frequency of sectors, 124 whole tassels from Bikini lot A were examined, taking a sample of pollen from each lateral branch as well as from the central spike. Branches which showed both normal and partially sterile pollen were recorded as one-half of each. The number of lateral branches varied from 9 to 19 with a mean of 14.4. The counts for tassels having the same number of lateral branches have been grouped and the data are presented in table 1.

A total of 1,782 side branches were examined, of which 561½ or 31.5 percent had partially sterile pollen. These partially sterile branches were distributed in 183 sectors. The size of sectors varied from a portion of one branch to nearly the whole tassel, but the small sectors were most abundant. These frequencies may be combined and summarized as follows:

| | | | |
|---|---|---|---|
| ½ to 1 | 46 | 8 | 2 |
| 1½ to 2 | 45 | 9 | 1 |
| 3 | 29 | 10 | 1 |
| 4 | 28 | 11 | 1 |
| 5 | 14 | 12 | 1 |
| 6 | 9 | 13 | 2 |
| 7 | 2 | 14 | 1 |

While the mean size of observed sectors is approximately three branches, the median is two and one-half branches, that is, one half of the sectors are 2.5 branches or less in size, and an equal number 2.5 branches or larger.

To the 183 sectors recorded above, there should be added 10 sectors found only in the central spike. When these are included, the average number of partially sterile sectors becomes about 1.6 per plant. These were distributed on the plants as follows:

| | |
|---|---|
| All normal | 10 |
| One sector | 53 |
| Two sectors | 48 |
| Three sectors | 9 |
| Four sectors | 3 |
| Five sectors | 1[3] |

These figures are based on sectors recognized as distinct in type of pollen. When several sectors of somewhat similar pollen type are present in the same tassel, it becomes virtually impossible to distinguish them. This difficulty is reflected in the abrupt drop in frequency of plants with more than two observed sectors. Except for the total of 31.5 percent of branches visibly abnormal, there is a systematic error running through all these figures. The abnormal tissue must actually be distributed in a larger number of sectors of smaller size than those observed. A reasonable estimate might well be an average frequency of between two and three abnormal sectors per plant, and an average size of sector about two branches.

[3] Only 3 types of pollen were present, with two small sectors of semisterile pollen on opposite sides of the tassel and similarly two small sectors with half of the pollen grains incompletely filled.

Assuming that the effects of the atomic radiations are immediate (not delayed to later cell generations) the above data indicate that, on the average, about seven or eight cells present in the growing tip of the irradiated dormant seed are represented in the reproductive cells of the tassel of the main stalk.

Data were also collected on the abnormal pollen sectors appearing in the central spikes of the same plants. Ten sectors were found which were not present in the lateral branches. Of the 183 sectors observed in the lateral branches, 113, or 61.7 percent, were represented in the central spikes. The other 70, or 38.3 percent, were not so represented. These sectors were distributed in the following size classes.

| | 1 | 2 | 3 | 4 | 5 | 6 | Above 6 |
|---|---|---|---|---|---|---|---|
| + | 17 | 25 | 20 | 22 | 9 | 8 | 12 |
| − | 29 | 20 | 9 | 6 | 5 | 1 | 0 |

Sectors represented in the central spike have a mean of 3.7 lateral branches while those not so represented have a mean of 2.1.

Of the 124 plants observed, the central spikes of 98 showed some abnormal pollen, while 26 showed only normal. At the time of observation, the central spike had shed the entire length, and in some cases the middle portion had ceased shedding. Thus these observations are more inclusive than those made in comparing dosages. The 98 plants showing sectors of partial sterile pollen in the central spike had a total of 162 sectors affecting the lateral branches or an average of 1.7 sectors per plant, with a mean of 3.2 branches per sector. A total of 37 percent of the lateral branches were partially sterile. The 26 plants with only normal pollen in the central spike had only 21 sectors (0.8 sector per plant) with a mean of 1.7 branches per sector. A total of 10.1 percent of the branches were partially sterile. Thus the plants with one or more sectors of abnormal pollen in the central spike had about three and one-half times as many of the lateral branches abnormal as did the plants with wholly normal central spikes.

## DISCUSSION

Any evaluation of the effects of atomic bomb or other radiations on maize seeds must be made upon the complex background of the developing embryo and plant. The impact of radiation is upon a partly grown although dormant plant, in which the growing tip and about five leaf primordia are many-celled structures. Before irradiation, all the cells of the embryonic plant are of the same genetic constitution. On exposure to the atomic bomb, each individual cell is exposed to the radiations, and each one may be expected to respond differently. Following the exposure each seed embryo may be looked upon as a collection of cells of differing genetic composition. Upon germination the seedling develops essentially as a colonial structure, being composed of a mosaic of sectors of which some or all may have been altered from their original constitution.

The best picture of the total effect of the atomic bomb radiations should be the first seedling leaf, which develops from the largest of the leaf primordia and by the fewest cell divisions. The second, third, fourth, and fifth leaves arise from successively smaller and fewer-celled leaf primordia. The percentage of chlorophyll-deficient or otherwise abnormal tissue is greatest in the first leaf, decreasing in each of the succeeding leaves. The later leaves arise from primordia formed from the apical meristem. These later leaves are mostly normal or almost normal in appearance.

It is probable that there is an equal effect of radiation on the undifferentiated cells of the apical meristem and of the already formed leaf primordia; and that the increasingly normal appearance is due to the more rapid growth and division of cells which have not been retarded by deleterious effects of the radiations. The competitive weeding out of handicapped cells should be most pronounced in the parts of the plant which are derived from the apical meristem. Thus the tassel sectors do not reflect the total effects of radiation but rather the composition of the relatively normal tissues which survive in the competition.

In the case of Bikini Lot A, the first seedling leaves were very strongly mottled, with probably more visibly abnormal than normal-appearing tissue. The tassels were nearly all entirely normal green in appearance, indicating the elimination during the growth of the plant of the majority of the apical meristem cells of the embryo. On an average, two or three sectors totaling 31.5 percent of the surviving tissue carried chromosome alterations. Thus in nearly one third of the relatively normal fraction of meristem cells ancestral to the tassel, the atomic radiations had produced chromosomal alterations of types which were not a handicap in the competitive growth which followed.

We can only speculate as to the genetic composition of the cells of the embryonic growing tip which were killed, or which were handicapped in competition. These must make up a large fraction of the meristem cells even at fairly low dosages. At dosages above 15,000 r the percentage becomes so great that frequently there are not enough vigorously growing cells left to enable the growing tip to function. As the dosage of radiation increases, there likewise takes place an increasingly rigorous selection for normality among the cells of the growing tip. Thus the pattern of genetic constitutions among survivors at different dosages should not be interpreted as simply a dosage-frequency relationship of the effect of radiation but also as the end result of selective competition.

## SUMMARY

Plants from five lots of maize seed exposed to atomic bomb radiations were compared with lots exposed to 5,000 r, 10,000 r, 15,000 r and 20,000 r X-rays.

In seedling mottling, Bikini Lot A showed effects a trifle below the 15,000 r X-ray lot. The effects on the Bikini lot were more uniform.

The percentages of central spikes of the tassels showing one or more sectors of partially sterile pollen were

| *X-ray series* | | *Bikini series* | |
|---|---|---|---|
| 5,000 r | 29.5 | Lot A | 63.1 |
| 10,000 r | 52.9 | B | 17.7 |
| 15,000 r | 63.0 | C | 14.6 |
| 20,000 r | 70.4 | D | 7.2 |
| | | E | 4.0 |

Examination of 1,782 lateral branches from 124 tassels of Bikini Lot A showed 31.5 percent with partially sterile pollen. An average of 1.6 partially sterile sectors were recorded per plant. Owing to the difficulty of distinguishing different sectors in one tassel, the actual number was presumably larger. It is estimated that there were between two and three partially sterile sectors per plant with an average size of about two branches.

Data indicate that cell competition in the growth of the apical meristem eliminates the more affected cells, thus modifying the dosage frequency relations.

## LITERATURE CITED

RANDOLPH, L. F., A. E. LONGLEY, and C. H. LI, 1948 Cytogenetic effects in corn exposed to Atomic Bomb ionizing radiation at Bikini. Science 108: 13–15.