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On the Genetic Nature of Induced Mutations in Plants

II. A Haplo-Viable Deficiency in Maize

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On the Genetic Nature of Induced Mutations in Plants[1]

II. A Haplo-Viable Deficiency in Maize

L. J. Stadler

The germinal variations induced in maize by X-ray treatment include, in addition to types readily classified as chromosomal aberrations and gene mutations, certain anomalous types which may with some reason be assigned to either group. Chromosomal aberrations in plants are distinguished from mutations chiefly by means of the effects of deficiency, since those which do not result directly in the loss of chromosomal substance, usually involve chromosomal rearrangements which result in the production of deficient spores in meiosis. The anomalous types mentioned are intermediate in genetic behavior between typical deficiencies and typical gene mutations.

The typical deficiency is lethal to the haploid gametophyte, and therefore results in "semi-sterility." Numerous instances of induced deficiency have been found in progenies produced by the use of X-rayed germ cells (8, 10). In many deficiencies the heterozygous sporophyte is distinctly defective; in others the effect of the heterozygous deficiency on the development of the affected plant is imperceptible. In either case the microspores and megaspores which bear a deficient chromosome complement are aborted; half of the mature pollen is defective and non-functional, and half of the ovules are sterile. Since reproduction is entirely from the non-deficient gametes, the deficiency is not transmitted to the progeny.

Typical mutations, on the contrary, are transmitted regularly without sterility, and have no appreciable effect on the development of the gametophyte. Many mutations of spontaneous origin have been examined in the course of extensive experiments on the frequency of mutation of various genes for endosperm characters in maize (9). In plants heterozygous or homozygous for the mutant gene, pollen and ovule development are normal, and mutant gametes function in full proportion.

Similarly the typical mutations induced in barley and maize by X-ray treatment appear to be free from deleterious effect on the gametophyte (11). Most of the induced mutations are recessive genes for chlorophyll defects of various types, and the majority do not survive the seedling stage. The recessive mutant types occur in the progeny of plants free from partial sterility or defective pollen,

1. Cooperative investigations, U. S. Department of Agriculture, Division of Cereal Crops and Diseases, and Missouri Agricultural Experiment Station, Department of Field Crops.

and commonly comprise approximately 25% of the selfed progeny, indicating that mutant gametes of both sexes function normally. Such variants are classed as gene mutations, since they meet every test which may be used to distinguish gene changes from grosser chromosomal variations. This classification, however, does not imply that the gene mutations are in all cases the result of intra-genic transformations (11, 12).

The intermediate cases are induced variations which are not wholly eliminated in the gametophyte generation, but which do not permit complete survival or normal functioning of the gametophytes. Varying degrees of defective gametophyte development are found in cases of this class. In some of these transmission of the variant through the female gametophyte is wholly normal, but variant male gametophytes function in low proportion or not at all. In many of such cases the variant pollen is visibly reduced in size, and in some it is visibly defective in structure. In more extreme cases the variant is partially eliminated also in the female gametophyte. In some of these, the seeds developed from defective female gametophytes pollinated by normal pollen are visibly defective.

The total frequency of such cases in maize appears to be considerably higher than that of the typical induced mutations, though much lower than that of the typical deficiencies which are wholly eliminated in the gametophyte generation. An accurate comparison of the frequency of occurrence of the various types of induced variation cannot be made until detailed analysis of a large number of representative cases has been completed.

The classification of these intermediate cases is more or less arbitrary. If it may be assumed that deficiency is always lethal to the haploid gametophyte generation (by analogy with Drosophila, in which all deficiencies are said to be lethal when homozygous or haploid), they must be considered gene mutations, distinguished from the "typical" gene mutations only by their lowered viability in the gametophyte generation. All of the intermediate variants may be described conventionally as mutant genes: one, for example, as "a mutant recessive allelomorph of *A*, of low viability in the male gametophyte;" another as "a dominant gene for defective seeds, lethal to the male gametophyte;" a third as "a pollen tube growth factor reducing the frequency of transmission through the pollen of various linked genes;" etc., etc. Certainly it must be assumed that there are mutant genes adversely affecting the development of the gametophyte, and that in some instances the same mutant gene may have recognizable effects on both sporophyte and gametophyte. Furthermore it is a plausible assumption that many changes within the gene, perhaps the great majority of gene-changes, are highly injurious, and in plants

these may be expected in some instances to be injurious or perhaps lethal to the haploid gametophyte. It is therefore not unreasonable to assume that these variations are due to gene mutations. If they are so classified they comprise the majority of the gene mutations induced by X-ray treatment in maize.

On the other hand the possibility must be considered that deficiency may not always be lethal to the gametophyte, and that the deficiencies identified by their gametophyte-lethal effect may be only the more extreme members of a larger group. On this hypothesis it would be expected that less extreme deficiencies would show varying degrees of viability in the gametophyte generation, and would be transmitted in corresponding proportion to the next sporophyte generation. Deficiencies injurious but not entirely lethal to the gametophyte would be transmitted as variations of the intermediate type. Deficiencies having no injurious effect on the male or female gametophyte, if such occur, would be carried by half of the male and female gametes, and their sporophytic effects would be inherited as typical gene mutations. The supposition that some deficiencies may be viable in the gametophyte generation is supported by the fact that the haplolethal deficiencies previously studied are in general rather "long," and that partially developed pollen is found even in some of these. Since the long deficiencies previously identified vary greatly in length, it is likely that shorter deficiencies also are produced, and these should in general be less injurious in their effects. Burnham (1) has described a reciprocal translocation in maize in which one of the interchanged segments is very short, comprising only a portion of the satellite of chromosome VI. Spores deficient for this segment (and carrying a long segment of chromosome I in duplicate) are not aborted, and in one instance a female gamete of this constitution was functional. Thus the induced variations of the intermediate type, which may be regarded as gene mutations affecting gametophyte development, may with equal plausibility be regarded as deficiencies of sublethal effect. It is possible that the intermediate group includes members of both classes.

Induced variations of this sort therefore have not been included among the mutations in the barley and maize experiments previously reported. The statements made regarding the frequency and nature of the induced mutations apply in all cases to variations of the type described above as "typical mutations."

The argument for considering even the "typical" induced mutations as wholly or largely the result of induced deficiency was stated in the preceding paper (12). The present paper is concerned with the analysis of one of the "intermediate" cases, an induced variation involving the gene R^r. This variation is representative of the more

extreme cases among the intermediates, since it is transmitted in greatly reduced proportion through female gametes and not at all through male gametes. It may be described as a recessive allelomorph of R^r, of low viability in the gametophyte generation. Other cases of less extreme effect will be considered in later papers of this series.

The gene R^r and its allelomorphs affect anthocyan color in the aleurone layer of the endosperm and in various other parts of the plant. In the presence of the dominant genes *A* and *C* the dominant *R* gives colored aleurone, the recessive *r* colorless. In certain stocks the endosperm combination *r r R,* resulting from the crossing of *r* x *R,* is mottled, though the reciprocal endosperm combination *R R r* is fully colored like the homozygous *R R R.* The superscript refers to an associated effect on plant color, R^r and r^r, in the presence of the appropriate complementary factors, producing color in the anthers and certain other parts of the plant which are free from anthocyan color in the presence of R^g and r^g. For a fuller description of the R^r series and its relation to complementary genes, see Emerson (2).

ORIGIN AND GENETIC BEHAVIOR

A cross of a *C* R^g *b pl* x *A C* R^r *B Pl,* in which the ear was irradiated (dose 450 *r*) 16 hours after pollination, yielded among the plants of the following generation a colored plant with green anthers. This is a type which could arise as a result of the loss of the R^r gene or of its replacement by R^g or r^g. The plant was normally vigorous and produced a good ear. Its pollen was slightly abnormal, approximately half of the pollen grains being distinctly smaller than the remainder and less completely filled with starch.

The ear, pollinated by *A c* R^g, produced a good set of seed, clearly better than the semi-sterile seed-set characteristic of typical deficiencies. If the original change affecting the R^r gene involved its loss or its replacement by r^g, some of these seeds should have received the dominant *R* only from the pollen parent, and therefore might be mottled. All of the seeds were rather heavily colored, but a small proportion of them showed an irregularity in color development suggesting the mottled pattern, which is highly variable in different stocks.

It was noted that the possibly mottled seed were with few exceptions a little smaller than the neighboring self-colored seeds. There were also several self-colored seeds which appeared to be significantly smaller than neighboring seeds. The difference in size was too slight to permit classification of the entire F_1 population, but numerous instances of large and small seeds side by side on the ear suggested a genetic difference. Ten pairs of adjacent seeds showing distinct contrast in size were selected and planted in the greenhouse. Eight plants from small seeds reached tasseling, and all but one segregated

large and small pollen like the parent plant. All of the ten plants from large seeds produced normal pollen. A supplementary planting in the field in the following season was made from the somewhat less certainly classifiable seeds remaining on the ear. Of the fourteen plants from small seeds all but three showed the typical pollen-size segregation, while all sixteen of the plants from large seeds produced normal pollen. This planting included also three plants from seeds considered probably mottled though not small. All three of these showed the pollen-size segregation. Thus the induced pollen variation was transmitted through the female gametes of the variant plant in at least twenty-one cases. The seeds heterozygous for the induced variation tend to be small and mottled.

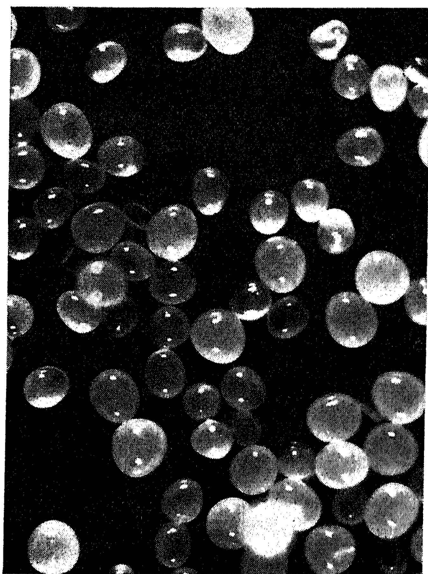


Fig. 1.—Fresh pollen of heterozygous plant. Photomicrograph, X 66. (13-861.1-1.

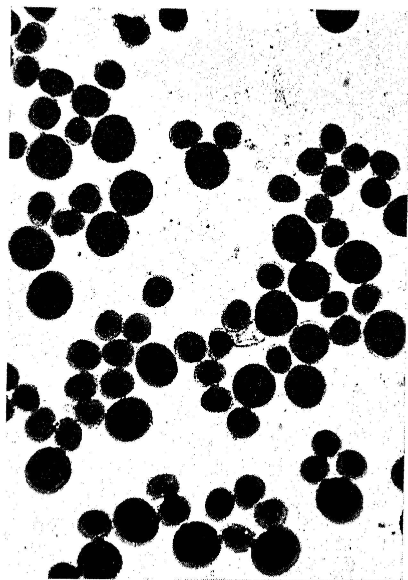


Fig. 2.—Pollen of heterozygous plant stained in iodine-potassium iodide solution. Photomicrograph, X 66. (13-861.1-1)

The appearance of the fresh pollen of heterozygous plants is shown in Figure 1. About half of the pollen grains are considerably smaller than normal pollen, though not otherwise distinctly abnormal. When stained with iodine most of the small pollen grains are seen to be more or less deficient in starch content, though there are many small pollen grains apparently well filled with starch. A sample of pollen stained in iodine—potassium iodide solution—is shown in Figure 2.

In both the greenhouse planting and the field planting there was a slight but quite consistent difference in growth between the plants with segregating pollen and those with wholly normal pollen. The plants were grown together under the same conditions, but without special precautions to insure strict uniformity. The heterozygous plants, though well developed and of normal appearance, were 2 to 4 days later in pollen-shedding and in silking and were 6 to 12 inches shorter at maturity than their normal sibs. The difference was very consistent in the greenhouse, and was distinctly apparent though less strikingly consistent in the field. This difference may be due in part

TABLE 1.—RELATIVE FREQUENCY OF *R* AND NO-*R* SEEDS AND OF GERMLESS SEEDS ON BACKCROSS EARS OF HETEROZYGOUS PLANTS
(*R*/no-*R* x *r*)

Plan	Test Ear*	*R* Seeds		no-*R* Seeds		Ratio (no-*R*)/*R*
		Total	Germless	Total	Germless	
13-843.1-1	(1)x 825.1-3	54	1	7	0	
	a x 1323.2-2	64	0	23	2	
Total		118	1	30	2	0.25
13-843.1-2	(1)x 825.1-3	16	0	0	0	
	(2)x 822.1-13	41	0	10	1	
	a x 1323.2-2	72	0	26	3	
Total		129	0	36	4	0.28
13-843.1-3	(1)x 822.1-13	137	0	9	0	
	a x 1323.2-2	185	0	19	1	
Total		322	0	28	1	0.09
13-843.1-4	(2)x 825.1-1	14	0	0	0	0
13-843.1-7	(1)x 825.1-1	42	0	2	0	0.05
13-843.2-3	a x 1323.2-2	79	0	12	1	0.15
13-843.4-1	(1)x 822.1-8	43	0	8	0	0.19
13-843.4-2	(1)x 826.1-5	155	0	37	0	
	(2)x 822.1-13	168	0	52	5	
Total		323	0	89	5	0.28
13-843.4-4	(1)x 822.1-13	90	0	5	0	
	(2)x 825.1-6	139	0	6	0	
Total		229	0	11	0	0.05
13-843.4-6	(1)x 822.1-13	7	0	4	0	
	(2)x 826.1-5	77	0	17	1	
Total		84	0	21	1	0.25
13-843.4-7	(2)x 825.1-6	101	0	27	0	0.27
TOTAL		1484	1	264	14	

*Ear Designations:
(1)—First ear of main stalk
(2)—Second ear of main stalk
a —Ear of first tiller

Testers:
822—A C r
825—A C r^{Nv}
826—A C r^{St}
1323—A C r

to the smaller size of the seed from which the deficient plants were grown, although the three deficient plants grown from large seeds and the four non-deficient plants from small seeds resembled in growth the other plants of similar genetic constitution rather than the plants from seeds of similar size.

Pollination of the heterozygous plants by *R*-tester stocks ($A\ C\ r$, $A\ C\ r^{St}$, and $A\ C\ r^{Nv}$) showed the absence of *R* in the affected chromosome. Eighteen ears, representing 11 heterozygous plants, were pollinated by *R*-testers. Each of the plants tested yielded some no-*R* seeds, except one plant which produced only 14 seeds on the test ear. The proportion of no-*R* seeds was much below 50% in all cases. The relative frequency of *R* and no-*R* seeds on these ears and the frequency of germless seeds in each group, are shown in Table 1.

The set of seed was incomplete on all of these ears, and the proportion of sterile ovules was high enough to allow of the assumption that the deficiency of no-*R* seeds was caused by infertility or abortion of no-*R* ovules. The proportion of no-*R* ovules setting seed varied widely in different ears, but at best (for example, in ears 843.1-1a, .1-

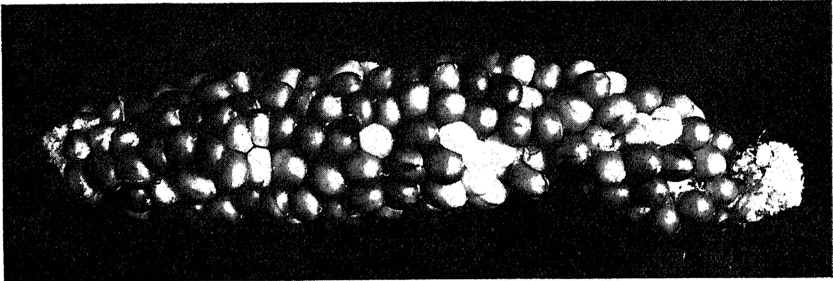


Fig. 3.—Ear of heterozygous plant pollinated by *A C r*, showing typical slight reduction in size of no-*R* seeds. (13-843.4-2 (2) x 822.1-133).

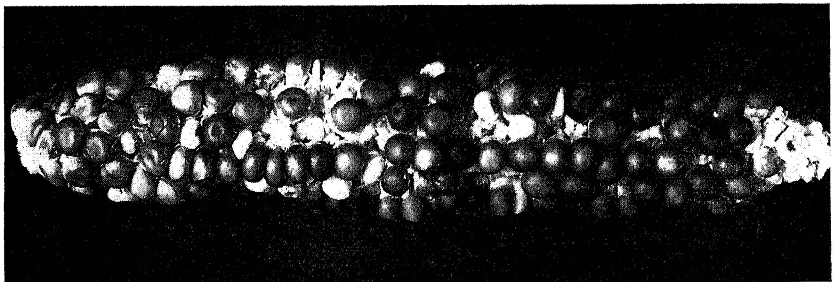


Fig. 4.—Ear of heterozygous plant pollinated by *A C r*, showing extreme reduction in size of no-*R* seeds. (13-843.1-3a x 1323.2-2)

2a, .4-2 (2), and .4-7 (2) the frequency of functional no-*R* ovules was less than one-third of that of functional *R* ovules. The frequency of germless seeds was significantly increased in no-*R* ovules. Only 1 of the 1484 *R* seeds was germless, while more than 5% of the no-*R* seeds were germless.

The no-*R* seeds were more or less reduced in size on all of the heterozygous ears, though the difference was inconspicuous in most cases. A typical ear of a heterozygous plant pollinated by *A C r*, showing the slightly smaller size of the colorless seeds, is shown in Figure 3. The degree of reduction in size of the no-*R* seeds varied considerably in different ears, but was extreme in only one (.1-3a). This ear is shown in Figure 4. (For further data on seed size in this population see page 15.)

Similar test-pollinations of ears of ten plants not showing the pollen defect gave no indication of segregation of ovule sterility, seed size, or no-*R* gametes. Thirteen well-filled test ears were produced, totaling some 2000 seeds.

Pollination of *R*-testers by pollen of the heterozygous plants showed to transmission of no-*R* gametes through the male gametophyte. Six plants were tested, producing eight ears totaling about 900 seeds, all showing the presence of *R* in the male gametes tested.

The difference in pollen size is great enough to permit the separation of large and small pollen by screening. Mangelsdorf (4) has shown that "tiny" pollen, associated with a factor modifying the *su* ratio in maize, may be mechanically separated from normal pollen by screening, and that when freed from the competition of normal pollen the "tiny" pollen may function normally in fertilization.

Pollen from heterozygous plants was screened and applied to the silks of *R*-tester plants. The screen used was a "250-mesh" wire cloth testing sieve, with specified openings of .061 mm. (manufactured by the W. S. Tyler Co., Cleveland, Ohio). The pollen was shaken from the anthers of shedding tassels into a clean glassine bag, taken to a nearby shed for protection from foreign pollen, passed through the sieve, and immediately applied to fresh silks of the tester plants. The time from shedding to pollination was usually about five minutes. Since the pollen used in each case was that obtained from the shedding anthers of a single tassel, the quantity of screened pollen applied to each ear was small. This was carefully distributed over the silks to reduce competition. Samples of the screened pollen used showed fairly effective separation. The proportion of large grains in the screened samples varied from one to three per cent. These large pollen grains were probably shriveled when they passed through the sieve.

Twelve ears were pollinated by screened pollen. Seven of these produced no seed; the other five set a few scattered seeds, none of them colorless. The few colored seeds were probably produced by *R* pollen of small size, since normal plants produce a small proportion of small pollen capable of passing through the screen. They could have come also from large pollen grains which were shriveled when screened, or from pollen contamination. In any case, it is obvious that the small no-*R* pollen is not able to bring about fertilization under the conditions of these trials, even when freed from the competition of normal pollen.

CYTOLOGICAL OBSERVATIONS

The gene *R* is known to be located in the long arm of chromosome X. McClintock and Hill (4) showed that in plants giving trisomic ratios for *R* the smallest of the ten chromosomes is present in triplicate. A plant deficient for r^r, produced by the use of X-rayed pollen, was shown by McClintock (3) to be deficient for the entire long arm of the smallest chromosome.

Chromosome X is readily recognized in the meiotic prophase in microsporocytes by its relatively short total length, its arm-length ratio of approximately 2.6:1, and its characteristic deep staining in the parts of the chromosome adjoining the region of spindle fiber insertion (McClintock 5, and unpublished observations.[1]). This chromosome has no distinctive knobs in the stock studied, but knobs were present in this stock on the other short chromosomes—in all plants on chromosome VII, and in some plants on chromosome VIII and on Chromosome IX. The most favorable stage for examination and measurement of chromosome X is a rather late pachytene, when the chromosomes have shortened somewhat, since this chromosome is often found separated from the mass of other chromosomes at this stage.

Sporocyte material from three non-deficient plants of culture 13-843 showed the normal chromosome-pair X, clearly double through its entire length. These included plants .1-5 and .4-3, two of the plants which, though grown from small seeds, later proved to be non-deficient.

Sporocytes from six deficient plants of the same culture, .1-2, .2-1, .2-2, .2-3, .4-4, and .4-6, showed one of the paired chromosomes to be distinctly shorter than the other. In all cases the terminal region of the long arm of one of the paired chromosomes extended well beyond the end of the other chromosome. Typical configurations of this chromosome pair are shown in Figures 5 and 6. Usually the two chromosomes were closely synapsed for the full length of the shorter

1. I am greatly indebted to Dr. McClintock for unpublished information regarding the morphology of the maize chromosomes.

chromosome, as in Figure 5, but in several instances, they were unsynapsed for a short distance back of the end of the shorter chromosome, as in Figure 6. Other configurations observed are shown in outline sketches in Figure 7. The deficiency appears to be terminal in all figures. If it is internal it extends to a point so near the end of the chromosome that the remaining terminal region shows no ten-

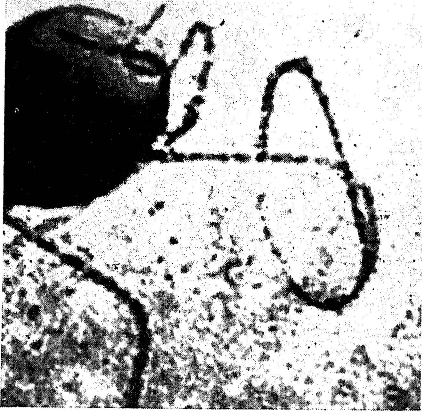
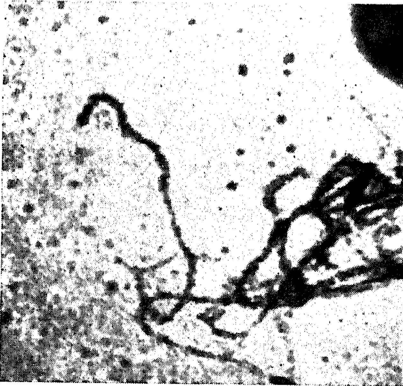


Fig. 5.—Typical pachytene figure of chromosome-pair X. Photomicrograph, X 1500. (13-843.4-6)



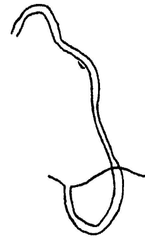


Fig. 6.—Pachytene figure of chromosome-pair X, showing short unpaired region at deficient end. Photomicrograph, X 1500. (13-843.4-6)

dency to pair with the homologous region of the non-deficient chromosome.

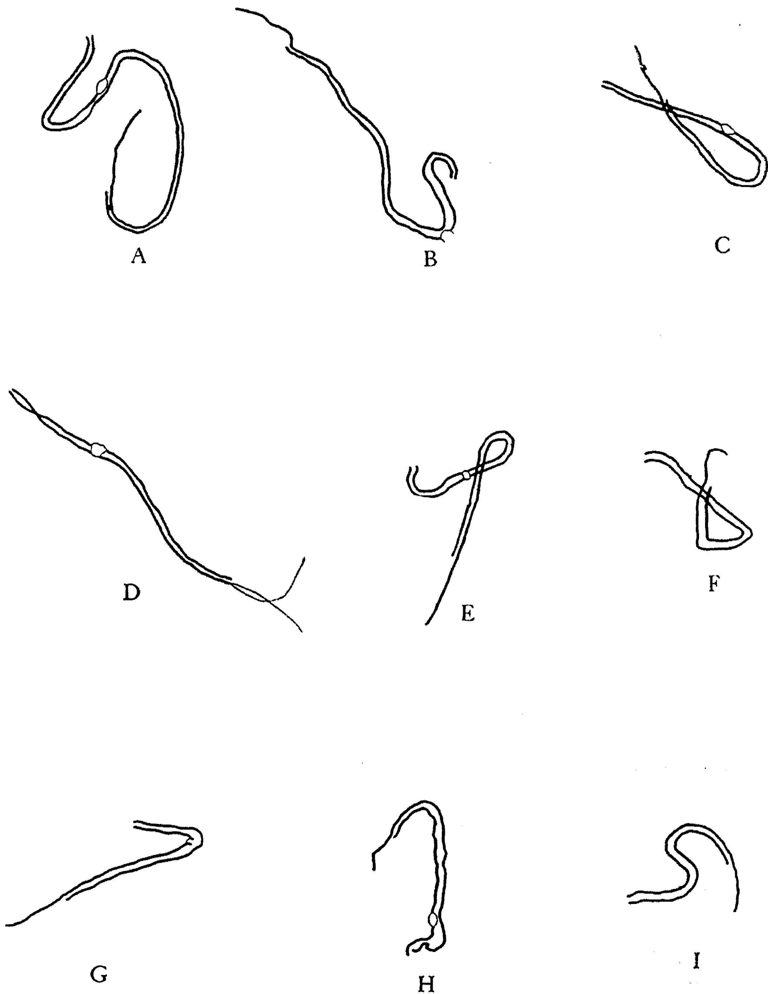


Fig. 7.—Outline camera lucida drawings, showing pairing of deficient and non-deficient chromosome X at various stages of pachytene. The shorter chromosomes represent the later stages. X 1500. (A, B—13-843.4-4; C, F, G, H, I—13-843.1-2; D—13-843.2-3; E—13-843.4-6).

The length of the deficiency was measured in camera lucida drawings of numerous flat figures. In chromosomes at about the stage illustrated in Figures 5 and 6 the deficiency averages approximately .22 of the length of the long arm of the non-deficient chromosome, or .16 of the length of the entire non-deficient chromosome. In later stages, when the chromosomes are shorter (Figure 7, f-i), the unpaired region appears to have shortened less than the paired region, and the deficiency thus appears to involve a slightly larger fraction of the chromosome.

In some figures it was possible to distinguish the two chromatids of the non-deficient chromosome in part of the unsynapsed region. In one case (Figure 7d) the chromatids were distinct throughout the length of this region. The separated chromatids are extremely fine strands and are readily distinguished from the separated chromosomes which sometimes appear in similar configurations at an unsynapsed end of a chromosome-pair.

Viable deficiencies of possible use in further investigations will be numbered in series for each chromosome. The deficiency here described is designated deficiency X-1.

Phenotypic Effects of Deficiency X-1

The somatic effects of this deficiency may be determined only in the heterozygous form, since the failure of transmission through male germ cells makes it impossible to produce the homozygote. The heterozygous deficiency apparently has a slight effect on the development of the plant. The heterozygous plants, as previously stated, are slightly shorter and slightly later in flowering than their non-deficient sibs. The significance of this difference and its possible relation to the size of the seed planted, however, cannot be positively determined without a more strictly controlled comparison.

The heterozygous deficient seeds, produced by pollinating heterozygous plants by normal pollen, are commonly somewhat reduced in size. The difference is inconspicuous on most ears, but it may be clearly demonstrated by comparing the weight of each deficient seed with that of a non-deficient adjacent seed. The size of the seed varies considerably from the base to the tip of the ear, and in a comparison based on the size of bulked groups of seeds, this variation may mask small differences between the groups.

The significance of the difference in seed size between deficient and non-deficient seeds was determined in all backcross ears producing four or more deficient seeds, by determining individually the weights of paired deficient and non-deficient seeds occupying comparable positions on the ear. All deficient seeds were included except a few moldy or otherwise clearly injured seeds. In each case

the deficient seed was paired for comparison with the non-deficient seed adjoining it to the right, or, if this position was not occupied by a non-deficient seed, with the nearest non-deficient seed at the same level. The results are summarized in Table 2.

TABLE 2.—COMPARATIVE WEIGHT OF PAIRED DEFICIENT AND NON-DEFICIENT SEEDS

Pedigree	Number of Pairs	Mean Weight of Paired Seeds no-R	Mean Weight of Paired Seeds R	Ratio *wt. no-R* / wt. R	Mean difference	Significance of mean difference[1]
		mg.	mg.		mg.	P
843.1-1 (1) x 825.1-3	5	191	245	0.78	54	.9950
843.1-1a x 1323.2-2	20	104	148	0.70	44	.9999+
843.1-2 (2) x 822.1-13	9	179	215	0.83	36	.9025
843.1-2a x 1323.2-2	22	128	165	0.78	37	.9999+
843.1-3 (1) x 822.1-13	8	106	167	0.63	61	.9999
843.1-3a x 1323.2-2	18	40	100	0.40	60	.9999+
843.2-3a x 1323.2-2	11	111	180	0.62	69	.9999+
843.4-1 (1) x 822.1-8	8	90	163	0.55	73	.9998
843.4-2 (1) x 826.1-5	37	129	200	0.65	71	.9999+
843.4-2 (2) x 822.1-13	47	120	188	0.64	68	.9999+
843.4-4 (1) x 822.1-13	5	145	230	0.63	85	.9886
843.4-4 (2) x 825.1-6	6	138	205	0.67	67	.9998
843.4-6 (1) x 822.1-13	4	241	325	0.74	84	.9969
843.4-6 (2) x 826.1-5	16	182	249	0.73	67	.9999+
843.4-7 (2) x 825.1-6	27	170	227	0.75	57	.9999+

[1]Values of P from Student's tables (13)

In each of the ears the deficient seeds were lighter in weight than the comparable non-deficient seeds, their average weight ranging from 40% to 83% of that of the non-deficient seeds. The difference in weight was statistically significant according to conventional standards in all but one of the 15 ears.

The deficiency has a marked effect on the development of the male gametophyte, and the mature pollen grains developed from deficient microspores are in general distinctly defective in size and structure. The range in size of pollen grains of a deficient plant in comparison with those of a non-deficient plant is shown in Table 3. The measure-

TABLE 3.—FREQUENCY DISTRIBUTION OF POLLEN-GRAIN DIAMETERS IN NON-DEFICIENT AND HETEROZYGOUS-DEFICIENT PLANTS

Plant	Maximum Diameter in Microns 73-77	78-82	83-87	88-92	93-97	98-102	103-107	108-112	113-117	118-122	123-127	128-132	Total
Non-Deficient						2	37	54	117	71	19		300
Heterozygous-Deficient	1	13	19	46	31	39	14	39	33	53	9	3	300

ments were made from photographs of fresh dry pollen magnified 125 X. Since the fresh pollen grains are not perfectly regular in shape (see Figure 1) the measurement of the maximum diameter does not in all cases accurately represent the size of the pollen grain.

The frequency distribution for size of pollen of the heterozygous plant is clearly bimodal, although the limits of the two size groups cannot be clearly defined. Most of the large pollen grains are in the diameter range 108-122 microns with a mean of 115 microns. This corresponds fairly well to the distribution of pollen grain diameters in pollen from the non-deficient plant. Most of the small pollen grains are in the range 88-102 microns, with a mean of 95 microns, about 18% less than that of the large grains. Although the small pollen group undoubtedly is made up largely of deficient grains it probably includes also some non-deficient grains, for homozygous non-deficient plants usually produce some small pollen.

The course of development of the deficient microspores was traced by examining microspores of heterozygous plants at various stages from meiosis to pollen shedding. The period from meiosis to pollen shedding in this strain of maize normally extends over about two weeks. The maturation process begins in the primary florets of spikelets a little below the tip of the main axis of the tassel, and rapidly extends upward and downward. A day or two later it begins similarly in the uppermost whorl of tassel branches, and later in branches of lower whorls. Within five or six days it has reached the base of the tassel. Meanwhile a second wave of maturation has begun in the secondary florets of the same spikelets, and passes similarly to the base of the tassel. Anthers at various stages may therefore be found in different parts of the same tassel. About five days after the beginning of meiosis microspores in the most advanced region of the tassel are found undergoing the first nuclear division. After another period of about five days the second nuclear division in the microspore takes place. Pollen shedding usually begins four or five days later. The time intervals stated are those usually found during the normal growing season. As the season advances the intervals are shortened somewhat.

The microspores of plants heterozygous for the deficiency are uniform in appearance until some time after the first postmeiotic nuclear division. This division occurs almost simultaneously in all of the microspores from a given region of an anther. For example, in a preparation from one anther about 90% of the microspores will be found in late prophase; in a preparation from an anther of the next more advanced floret, an equal proportion will be found to have completed the division, and to contain two distinct nuclei, each with a nucleolus. Occasionally a single anther will show microspores in various

stages from late prophase to interphase, but such anthers are not more common in the deficient plants than in normal plants. The deficient microspores therefore, are not appreciably delayed in their development up to the time of the first nuclear division. Microspores of a heterozygous deficient plant at the time of the first nuclear division are shown in Figure 8.

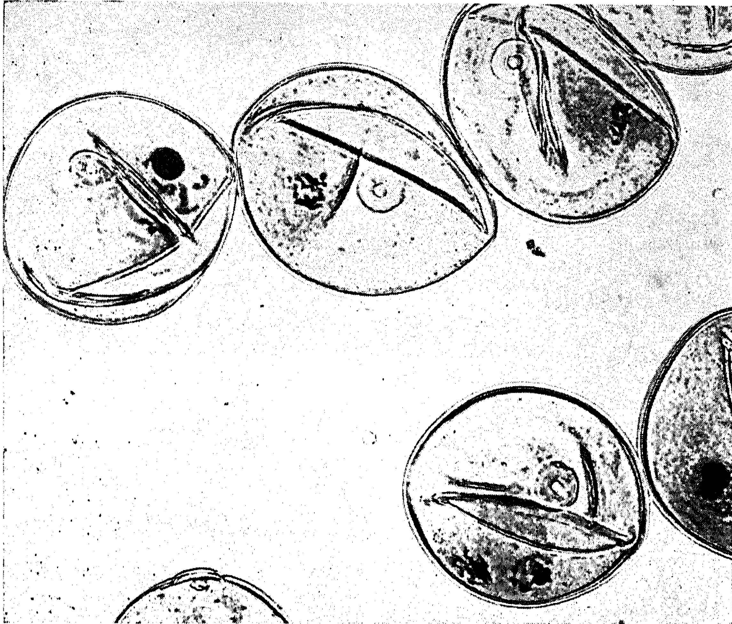


Fig. 8.—Microspores of heterozygous deficient plant at the time of the first nuclear division. The deficient and nondeficient spores are indistinguishable at this stage. Photomicrograph, X 425.

Shorty before the second division a difference in size and content of the microspores appears. The sequence of stages may be indicated by comparing the development of the microspores in successive florets of a single branch. The first indication of heterogeneity appears in florets about four spikelets below that in which the first metaphases are found. Here about half of the microspores appear to be slightly larger and more densely stained than the remainder. In the next floret the difference is increased, and is distinct enough to permit the classification of almost all microspores as large or small. The prophase begins slightly earlier in the large microspores. Preparations showing the large microspores in metaphase show most of the smaller microspores in early prophase. Three or four spikelets higher on the same branch the smaller microspores are found in meta-

phase. Here the larger microspores contain three distinct nuclei and a considerable quantity of starch. The comparative development of large and small microspores at the time of the division of the generative nucleus in the smaller microspores is shown in Figure 9. The division of the generative nucleus is completed normally in the smaller microspores, and the accumulation of starch proceeds as in the larger microspores. However, at the time of pollen shedding, as we have seen, the starch content of the smaller pollen grains is much less, even in proportion to their size, than that of the larger pollen grains. In anthers about to shed pollen, the small pollen grains are smaller and less well-filled with starch than are the large pollen grains found in the immature anthers of the secondary florets.

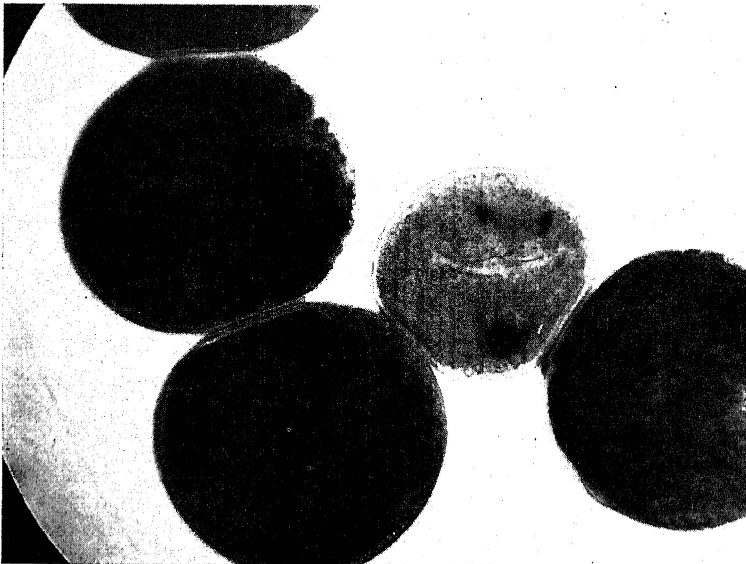


Fig. 9.—Division of the generative nucleus in a deficient microspore. Note the greater size and more advanced development of the non-deficient microspores. Photomicrograph, X 425.

This incomplete filling suggests that the deficient pollen grains may shrivel more quickly after shedding than the normal pollen grains. This might account for the failure of the small pollen to accomplish fertilization. Normal maize pollen shrivels within a short time after shedding, unless protected from exposure to drying conditions. The rapidity of shriveling varies widely with temperature, humidity, and the extent of exposure of the pollen grain surface. The effect of shriveling on pollen germination was determined in normal pollen by

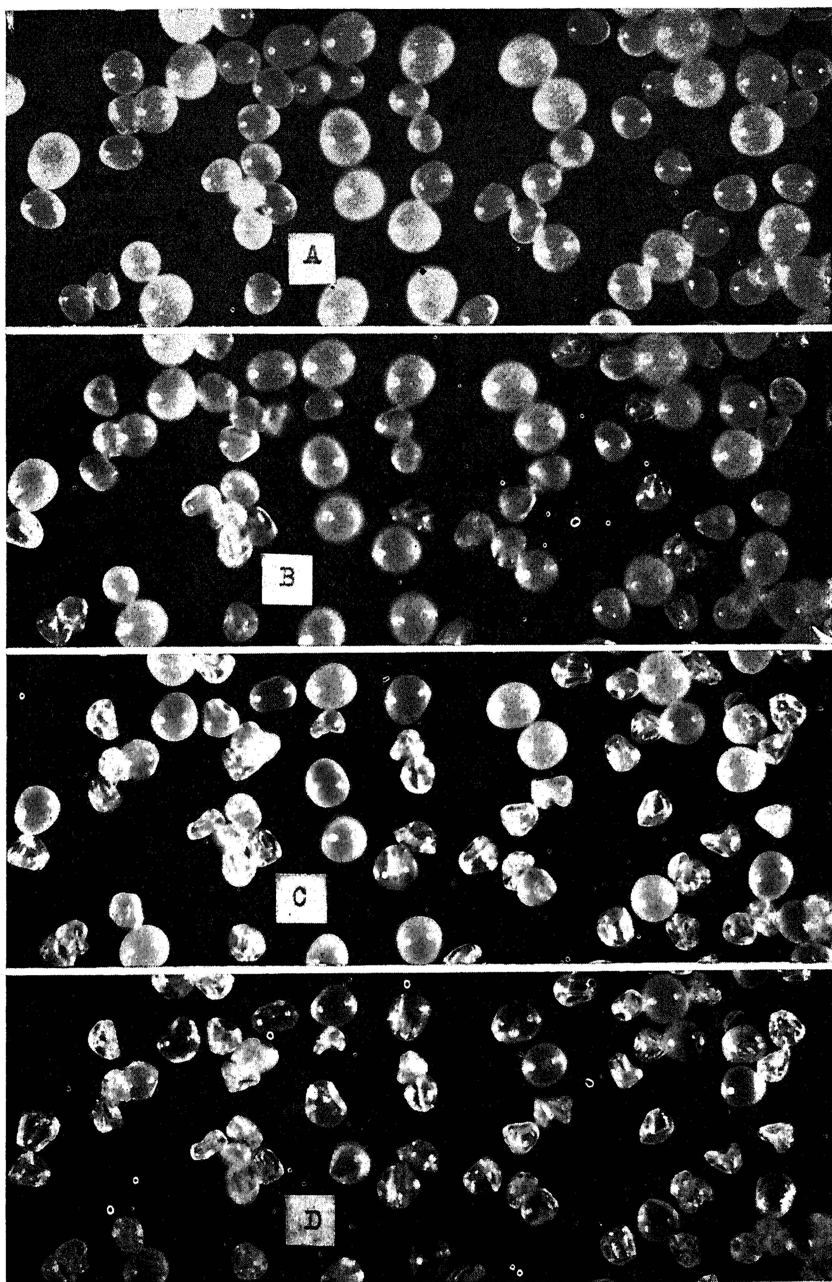


Fig. 10.—Stages in the shriveling of pollen of a heterozygous deficient plant. (A—30 seconds after shedding; B—3½ minutes; C—6½ minutes; D—10½ minutes). Photomicrographs, X 66.

germination methods similar to those described on page 21. It was found that some of the pollen grains which have begun to shrivel before being placed upon the silks regain their spheroidal shape and later germinate, but that pollen grains which have passed the first stages of shriveling, when placed under conditions optimum for germination, remain shriveled and show no activity.

The rate of shriveling in large and small pollen was compared by photographing a sample of pollen from a heterozygous deficient plant at brief intervals from time of shedding until all of the pollen had shriveled. The pollen was shed directly from a single anther to a glass slide. The absolute time intervals determined are not applicable to field conditions, since the pollen was exposed in a single layer and was observed at room temperature and humidity, modified by the heating effects of the lamps used in illumination. Under open pollination conditions shriveling would probably be more rapid because of the exposure of the entire surface of the pollen grains and the usual higher temperature and lower humidity. Under controlled pollination conditions shriveling might be slower because of protection of the pollen from exposure. However, the relative rate of shriveling of the large and small pollen grains is of interest.

Four stages in the shriveling of a specimen of pollen are shown in Figure 10, which shows the appearance of the same sample at periods of ½, 3½, 6½, and 10½ minutes after shedding. Some shriv-

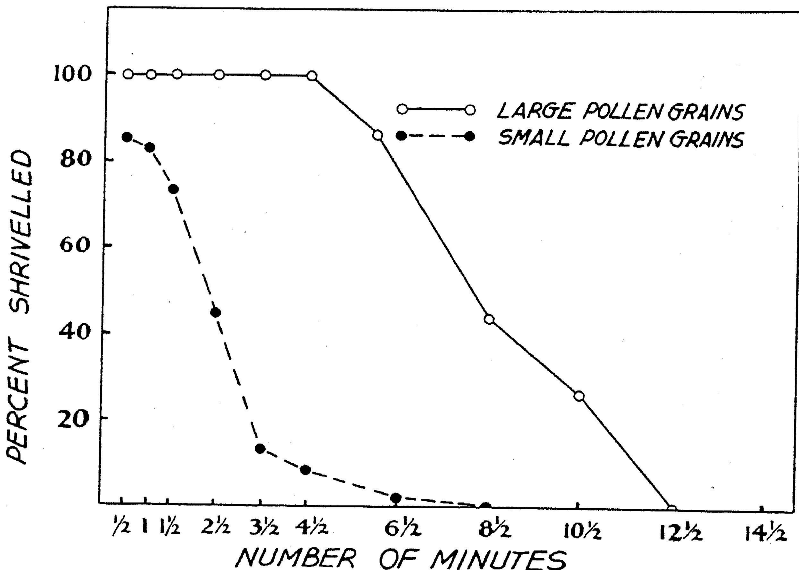


Fig. 11.—Course of shriveling of large and small pollen of heterozygous plant. (13-861.1-1)

eling of the small pollen grains had already occured when the first photograph was taken, 30 seconds after shedding. After 3½ minutes almost all of the small grains had shriveled, while none of the large grains had shriveled. A few of the large grains had shriveled after 6½ minutes, and most of them had shriveled after 10½ minutes. All of the pollen grains had shriveled when the next photograph was taken; 12½ minutes after shedding. The course of shriveling in both classes of pollen is shown graphically in Figure 11.

In order to determine whether the deficient pollen grains might germinate if placed promptly upon the silks, fresh pollen from a heterozygous deficient plant was germinated on silks under observation. Sprague (7) has recently reported observations of the germination of maize pollen on silks mounted for study in a Van Tieghem cell.[1] A similar method was used in these observations. Fragments of fresh silks were placed on a cover slip and fastened at each end by means of vaseline. Pollen was shed from a fresh anther directly upon the silks, and the cover slip was inverted and sealed in place in the Van Tieghem cell with vaseline. Best results were obtained when no water was added to the cell. Observations were made immediately and repeated at various intervals to determine the behavior of the large and small pollen grains. Since Sprague has shown that *Wx* and *wx* pollen grains differ materially in the time required for establishment of the pollen tube, only *Wx Wx* plants were used.

Only the pollen grains in contact with silk hairs were included in the observations. The pollen grains observed were classified in four size groups on the basis of micrometer measurements, to permit the separate consideration of grains of the intermediate size classes. The location of each pollen grain was indicated on a sketch of the silks, to facilitate the repeated observation of the individual grains. Pollen was recorded as "germinated" only if the emerging pollen tube could be seen or if the characteristic streaming movements of the tube contents could be seen within the silk hair. In many of the pollen grains which did not extrude a pollen tube, active protoplasmic movements and progressive diminution of the solid contents were observed. This disappearance of the solid contents is presumably the result of digestion of food reserves. Among the pollen grains not recorded as germinating, those showing clear streaming movements were recorded as "active," others as "inactive."

Several germination trials were made with similar results. The results of a single representative trial are here summarized. In this

1. Dr. Sprague kindly gave me in advance of publication a description of his technic.

trial 8 sections of fresh maize silk were mounted in parallel in a single Van Tieghem cell, and the locations of 181 pollen grains were recorded. A quick survey of the preparation was made before beginning the individual records, to identify the most quickly germinating pollen grains. This examination began 15 minutes after pollination, and was completed in the next 15 minutes. It showed 46 germinating pollen grains, all of which were of the "large" or "probably large" class. The first examination of the individual pollen grains, which occupied the period from ½ hour to 4 hours after pollination, gave the following results:

SIZE CLASS (WITH DIAMETER IN MICROMETER UNITS)

	Large (30+)	Probably Large (28-30)	Probably Small (25-27)	Small (25—)
Germinating	100	5	1	8
Not Germinating				
Active	1	0	0	54
Inactive	4	0	0	8

Of the large pollen grains observed in this trial almost all germinated within the first observation period. Very few of the small pollen grains germinated, though almost all of them showed active streaming movements and many showed some apparent diminution of solid contents. During this examination activity within the pollen grain appeared to be as great in the small pollen as in the large.

The same pollen grains were individually re-examined during a second period from 4 to 7 hours after pollination, and a third period from 24 to 27 hours after pollination. In the pollen grains which had germinated, internal movements and progressive diminution of solid contents continued in most cases, and many were empty and collapsed at the third examination period. The pollen grains recorded as "active" though not germinated at the first period showed continued activity, but none of them germinated. Of the 54 small pollen grains of this class, 49 were still active in the second period of observation, and 22 in the third. The remainder had shriveled, or in a few instances had fused with other pollen grains or had burst. Active protoplasmic streaming continues in some of these pollen grains for surprisingly long periods—one was still in active movement 108 hours after pollination. The pollen grains recorded as inactive at the first examination remained inactive, with the exception of two pollen grains which showed slight activity in the second examination. All members of this group were shriveled or fused at the time of the third examination.

It is clear that the great majority of the deficient pollen grains are incapable of germination. This may be true of all of the deficient pollen, for it is possible that the few small pollen grains which germinate may be non-deficient pollen of small size. However, the proportion of germinating pollen grains which are distinctly small in size, in this and in other trials, appears to be too large to be entirely accounted for in this way, and it seems probable that a small proportion of the deficient pollen grains are capable of germination.

The failure of small pollen grains to germinate obviously is not due to any lethal effect of the deficiency. The deficient microspores pass through the two nuclear divisions, and are able when placed upon receptive silks to accomplish the first steps of germination. Their failure to complete germination by the extrusion of a pollen tube, as well as their tendency to shrivel prematurely on exposure to drying conditions, may be due merely to their incomplete development when shed. The development of the deficient microspore lags behind that of the non-deficient microspore in the later stages. When the non-deficient pollen grains are mature the anther dehisces, and the pollen, deficient and non-deficient, is shed. If immaturity only is the cause of the failure to germinate, it may be possible to increase the proportion of deficient pollen germinating by treatments which delay the shedding of the pollen.

In the female gametophyte, as in the male, the deficiency has a distinctly injurious but not a lethal effect. A small proportion of the megaspores (varying on different ears from 0 to about 30 per cent) develop sufficiently well to permit seed production when pollinated by normal pollen. Although the seeds produced are in general somewhat smaller than the non-deficient seeds, the difference, as we have seen, is sometimes hardly appreciable.

In order to compare the development of deficient and non-deficient megaspores at the time of pollination, ovules of heterozygous deficient plants were examined microscopically. The specimens for examination were taken when the ears were well silked, three or four days after the emergence of the first silks. At this stage silks from the entire length of the ear have emerged, and normal ears pollinated at this time commonly produce a full set of seed.

In normal plants at this stage the ovule contains a large mature embryo sac. In the development of the female gametophyte the megaspore goes through 3 nuclear divisions, producing an embryo sac containing 8 nuclei. A growth period follows, during which the embryo sac becomes greatly enlarged and the nuclei within it are differentiated as egg, polars, synergids, and antipodals. The polar nuclei migrate

to the middle region, and the antipodal cells begin a series of cell divisions. In the mature embryo sac the group of antipodals usually includes 24 or more cells. These later degenerate, and apparently play no part in the development of the seed.

In the ears of heterozygous deficient plants at this stage the ovules and silks are uniform and normal in external appearance. Ovules were sectioned in blocks of 8 (4 x 2) to permit the comparison of embryo sacs of neighboring ovules. These blocks were cut radially, in sections 16 microns in thickness. Approximately half of the ovules, distributed at random over the ear, were found to contain large mature embryo sacs similar to those of comparable non-deficient plants. The remainder contained embryo sacs of distinctly smaller size. In all cases examined the smaller embryo sacs appeared to have passed through all three nuclear divisions, and in many instances they

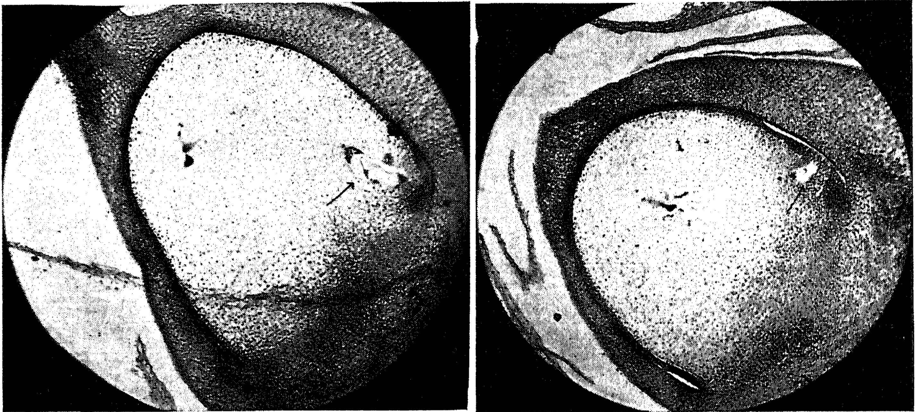


Fig. 12.—Ovules from heterozygous deficient plant (13-863.1-1), showing typical large and small megaspores. Photomicrograph, X 40.

contained a small group of antipodal cells, showing that some of the divisions in this region had occurred. The small embryo sacs differed somewhat in size and in degree of development, and it is possible that some of the deficient embryo sacs were not distinguishable from the normal type. A typical small embryo sac and a comparable large embryo sac from the same region of the ear are shown in Figure 12. The distribution of large and small embryo sacs in the blocks of ovules examined is indicated in Figure 13.

These observations show that the deficient megaspore, like the deficient microspore, completes the essential cell divisions, but lags

somewhat behind the non-deficient individual in development. Only a small proportion of the deficient megaspores are sufficiently well developed to function normally in fertilization and seed development. The observed increase in the frequency of germless seeds may be due to incomplete development of the egg at the time of fertilization or to some other consequence of the imperfect development of the female gametophyte. It is possible that the proportion of functional megaspores might be increased somewhat by delayed pollination.

A		B		C		D		E		F		G	
S	L	S	L	L	L	S	L	L	L	S	L	S	—
S	L	S	S	S	S	S?	L	S	S	L	L	L	S
L	L	—	S	L	L	L	S	S	L	L	S	S	L
S	—	—	S	—	S	L	—	S	L?	L	L	L	S

Fig. 13.--Distribution of large and small megaspores in blocks of ovules examined. (L—large megaspore; S—small megaspore; ?—classification doubtful; — —megaspore lost in preparation.

DISCUSSION

It is clear that deficiency is not necessarily lethal to the haploid gametophyte in maize. The deficiency described in this paper involves the loss of approximately one-sixth of chromosome X. In spite of this large loss, a considerable proportion of the female gametophytes function normally and transmit the deficiency to the next sporophyte generation. The deficiency is not transmitted through male gametophytes, but this is not due to any directly lethal effect. It results primarily from failure of the deficient pollen to germinate, a failure which may be due merely to the incomplete development of the deficient pollen grains at the time of pollen shedding. Similarly, the failure of the majority of female gametophytes to function normally is associated with incomplete development at the time of fertilization. It may be possible to secure transmission of the deficiency through male gametes, and to improve the transmission through female gametes, by treatment which permits the more complete maturing of microspores and megaspores before fertilization.

It is possible that the deficient region may include other known genes of the *R* linkage group. According to the standard linkage map of maize recently published by Emerson (3) only 3 loci are definitely placed in chromosome *X*, *R* at *O*, g_1 at 15, and *nl* at 33. The deficiency

includes the locus of R and not that of g_1. There are however several other genes known to be linked with R but not definitely placed with reference to g_1 and *nl*. Of these only one, v_{18}, has been tested for inclusion in the deficient region. It is not included.

I have elsewhere (12) called attention to the possibility that maize may be a polyploid species, and have pointed out that this may make possible the survival of deficient gametophytes because of gene reduplication. In the related genera, Coix and Sorghum, species with 5 pairs of chromosomes are known. Possibly the survival of haploid tissue with this large deficiency is due to the presence in some other part of the chromosome complement of genes identical or homologous with some of the genes lost. The occurrence of transmissible deficiencies and duplications may provide material for the experimental investigation of this possibility.

On the other hand, the survival of deficient gametophytes may not be rare in seed plants, even in species in which polyploid origin is not a factor. The life of the gametophyte is brief and simple. There is no necessity for the assumption that every gene is indispensable during this period. However, if the genes which are indispensable are sufficiently numerous, any deficiency long enough to be detected cytologically is likely to include one or more of such genes. The observations here reported show that in maize no gene essential to gametophyte survival is located in the rather long chromosome-segment lost in deficiency X-1. Other haplo-viable deficiencies not yet reported show that this condition is not rare in maize; various chromosome segments long enough for cytological detection in the pachytene chromosome may be lost without lethal effect. Whether this is true also in species of plants not suspected of polyploid origin is as yet unknown.

The related assumption, that every gene, at least in single dose, is essential to the diploid sporophyte (that is, that every deficiency is lethal when homozygous), cannot be tested in plants except in the case of deficiencies which are transmissible through both the male and the female gametophyte. In the case of the deficiency under discussion transmission through the male gametophyte has not yet been accomplished, and the homozygous effect of the deficiency is therefore unknown.

On the assumption that deficiencies are necessarily haplo-lethal, this induced variation would be interpreted as a gene mutation of R^r to r^g. The mutant "gene" however is not identical in effect with the previous known gene r^g, for in addition to the characteristic r^g effect on aleurone and plant color, it has certain effects on development which are not found in the r^g gene previously known. It would therefore be regarded as a new allelomorph at the R locus, resembling r^g in its typical recessive effects on aleurone and plant color, but with lowered

viability in the gametophyte generation. Its specific effects on seed size and plant growth also are characteristic effects of the new allelomorph, and in these effects it is dominant to the older allelomorphs.

Thus interpreted, the occurrence of such induced variations might be considered evidence of the ability of X-rays to bring about intragenic transformations, that is, to produce new genes from old. In the case of *R* it can be shown that the assumed gene mutation induced by the treatment is not identical with the mutations spontaneously occurring in the same gene. The gene *R* is one of 8 genes of maize in which mutation has been studied extensively in untreated material (9, 12). Recessive mutation of *R* is fairly frequent. In all cases in which the allelomorph R^r is involved, the recessive mutation affecting aleurone color is found to produce no change in the effect of the gene on plant color. In view of its mutation behavior therefore, the gene R^r may be regarded as two genes completely linked, or as a gene composed of two more or less independent parts. The type of mutation which occurs spontaneously affects one of these genes or parts of the gene, without affecting the other. Deficiency could hardly fail to affect them together.

X-ray treatment has no appreciable effect on the frequency of this type of mutation of *R*, as reported in 1930 (9) and confirmed repeatedly in similar experiments in later seasons. But X-ray treatment does induce such variations as the case reported in this paper, which may be interpreted as a mutation of R^r to a less viable r^g. In this instance it may be demonstrated cytologically that the "mutant allelomorph" of *R* is not a gene but the absence of a rather long chromosome segment. Its cytological identification however is made possible by the unusual advantages of the pachytene technic; in species in which this is not applicable a deficiency of equal length probably could not be cytologically identified. The interpretation of the induced variation as mutation or deficiency would then be wholly arbitrary. Ordinarily such a variation is regarded as a gene mutation unless there is genetic or cytological evidence indicating deficiency.

Moreover, even in the pachytene chromosomes, deficiencies of much shorter length or less favorable location probably would be undetectable. Stretching and illegitimate pairing tend to mask slight inequalities of the synapsed chromosomes, particularly in non-terminal deficiencies. It is probable therefore that the shortest induced deficiencies may present a normal appearance in the pachytene chromosome. These also are the cases which would be least likely to be identified as deficiencies by genetic evidence.

The fact that so large a loss as that involved in deficiency X-1 does not more radically disturb development in the haploid gameto-

phyte generation in maize strengthens the suspicion that shorter deficiencies (perhaps not cytologically detectable) may be transmitted without loss through both male and female gametophytes. The phenotypic effects of such a deficiency would be inherited as if due to a change within the gene.

The experiments reported were supported in part by a grant of funds and equipment by the National Council, Committee on Effects of Radiation on Living Organisms.

I am indebted to Mr. Luther Smith for much valuable assistance in this investigation.

SUMMARY

Many X-ray induced variations in maize are eliminated in part in the gametophyte generation. These are intermediate in genetic behavior between typical deficiencies, which are wholly eliminated in the gametophyte generation, and typical mutations, which are transmitted without loss by both male and female gametophytes. Such variations are commonly considered mutations of reduced viability.

A case of this kind involving the gene *R* is found to be due to the loss of a terminal segment of chromosome X, about 1/6 of the length of the entire chromosome. The deficiency is transmitted through the female gametophyte, with somewhat lowered viability, but is not transmitted through the male gametophyte. This deficiency is designated deficiency X-1.

The effects of deficiency X-1 may be summarized as follows:

(1) The deficient male gametophyte develops apparently normally until some time after the first nuclear division. The second nuclear division is delayed somewhat in deficient microspores but apparently proceeds normally.

(2) At the time of pollen-shedding, the deficient pollen grains are small and still incompletely filled. They shrivel much more quickly after shedding than the mature non-deficient pollen grains. If deficient pollen grains are placed immediately on receptive silks, streaming movements and digestion of reserves proceed as in the non-deficient grains, but emergence of the pollen tube (except possibly in rare instances) does not occur.

(3) The deficient female gametophyte, like the non-deficient, undergoes 3 nuclear divisions, producing an embryo sac of 8 cells. At the time of pollination approximately half of the ovules of the heterozygous plant contain embryo sacs of reduced size and sub-normal development.

(4) Deficient female gametophytes are functional, though with much reduced fertility. The ears of plants heterozygous for the deficiency produce less than 1/5 as many deficient as non-deficient seeds.

(5) The proportion of germless seeds produced is higher among deficient female gametophytes than among non-deficient gametophytes of the same ears.

(6) Seeds heterozygous for the deficiency are slightly reduced in size.

(7) Plants heterozygous for the deficiency are probably reduced slightly in size and delayed slightly in flowering.

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