GAMETIC LETHALS ON THE FOURTH CHROMOSOME OF MAIZE*

W. RALPH SINGLETON AND P. C. MANGELSDORF

Connecticut Agricultural Experiment Station, New Haven, Connecticut, and Texas Agricultural Experiment Station, College Station, Texas

Received March 8, 1940

PLANTS of maize, heterozygous for *su*, have been observed that produced no *su* seeds when selfed or pollinated by *su* pollen, but whose pollen when applied to *su* ears produced in excess of 96 percent *su* seeds (figure 2, H, I). This anomalous condition is caused by two gametic lethals, small pollen-1, (*sp*), and lethal ovule-1 (*lo*).[1] Preliminary reports on the inheritance of these two factors have been published, (MANGELSDORF 1931, 1932, SINGLETON 1932). Both *sp* and *lo* are closely linked with *su*(*sp su* = 5 percent recombinations; *su lo* = 2.2 percent) and give distorted ratios of *Su*:*su* because of this close linkage and the fact that one gene is lethal to the male, the other to the female gametes. Pollen grains of the composition *sp* are smaller than normal (figure 5, C, D, and E) and in most cases do not function in competition with normal grains. Ovules carrying *lo* usually abort before fertilization and are rarely capable of being fertilized. The ears referred to above were of the composition *sp*++/+*su lo*. The only functional ovules were of the composition *Lo* which were mostly *Su*, in a few cases all *Su*, when the crossing over may have been reduced or due to random sampling an occasional ear was all *Su*. The functional pollen was *Sp* in constitution and was *su* with the exception of recombinations.

ORIGIN OF *sp* AND *lo*

The gametic lethal *sp* was discovered by the junior author while on the staff of the Connecticut Experiment Station in 1924. This factor must have arisen as a mutation in a Leaming strain, Connecticut 112–8, that had been inbred four generations. It is not possible to say in what generation the *sp* factor arose although it is practically certain to have occurred after the first generation, since the original ear selfed once showed no missing kernels, a characteristic of *sp*/+ ears. This condition, when it first appeared, was in the repulsion phase, *sp* linked with *Su*, which accounts for the high percentage of surgary seeds when selfed or backcrossed either way.

The *lo* factor arose in a stock segregating for *sp* and *su*. It must have arisen as a mutation on the homologue of the chromosome carrying *sp*,

* The cost of the accompanying half-tone illustrations has been borne by the Galton and Mendel Memorial Fund.

[1] Factor symbols not accompanied by numerals represent either the first or the only gene with that literal symbol; that is, $sp = sp_1$, etc.

since *lo* was linked with *su* when first observed while *sp* was linked with *Su*. The most probable explanation of the origin of *lo* is that its occurrence in a stock segregating for *sp* was purely a coincidence although there is a possibility of a causal relationship. The first *lo*/+ ear had 11.1 percent of *su* seeds. The two preceding generations had 53 and 64 percents respectively, showing presence of the *sp* factor but absence of the *lo* factor. All studies of the *lo* condition were made by the senior author in Connecticut. The inheritance of this factor was complicated at first because the stock was also segregating *sp* and gave the unusual results described above. However the two factors have been separated and each studied independently and in combination.

INHERITANCE OF *sp* AND *lo*

Disturbance of su ratios

Both the factors *sp* and *lo* were discovered because of their close linkage to *su* and the inheritance of each has been studied largely through its disturbance of the *su* ratio in selfed and backcrossed ears. The *sp* condition was originally termed "high sugary" since it was in the repulsion phase, *sp*+/+*su*, and gave a high percentage of *su* kernels (figure 1).

The other gametic lethal, *lo*, was originally called "low sugary" because the coupling phase, *lo su*/++, the one first observed, gave a very low percentage of *su* kernels. The factor *sp*, produces extremely low sugary ratios when in the coupling phase *sp su*/++, while the original "low sugary," *lo*, produces very high sugary ratios in the repulsion phase *lo*+/+*su* (figure 2).

The disturbance of the *su* ratio in stocks heterozygous for *lo* or *sp* can perhaps best be understood by presenting in tabular form the functional male and female gametes. This is presented in table 1.

TABLE 1

Functional gametes in plants segregating for sp, lo, or sp and lo not counting double-crossovers or survival of sp ♂ gametes or lo ♀ gametes.

GENOTYPE	FUNCTIONAL GAMETES ♀	♂
sp +/+ *su*	*sp* +, + *su* *sp su*, ++	+ *su*, ++
lo su/++	++ + *su*	*lo su* + + *lo* +, + *su*
sp su +/++ *lo*	*sp su* + + *su* + + + +	+ + *lc* + *su* + + + +

Linkage relations of sp with su

Since in preliminary trials it was found that *sp* functions quite rarely (about one percent of the progeny plants were *Sp sp;* MANGELSDORF 1932) in competition with normal pollen, the backcross of *su su* ears by pollen from *sp*/+ plants has been used to give the crossover percent directly. Table 2 gives the total counts of all self pollinations or backcrosses in Connecticut and Texas.

TABLE 2

Percentages of su seeds obtained for selfed ears, and backcrosses to su for stocks segregating for sp, lo, or sp and lo.

GENOTYPE	SELF TOTAL	SELF % *su*	TIMES *su* TOTAL	TIMES *su* % *su*	POLLEN ON *su* TOTAL	POLLEN ON *su* % *su*	EXPECTED SELF RATIO (5×7)	DIFFERENCE (3–8)
1	2	3	4	5	6	7	8	9
sp +/+ *su*	143M	60.8	36M	61.9	158M	93.9	58.1	+2.7
sp su/++	7M	3.2	6M	37.6	41M	9.7	3.6	− .4
lo su/++	31M	1.8	54M	2.4	18M	51.3	1.2	+ .6
lo +/+ *su*	8M	47.1	27M	95.2	34M	48.5	46.2	+ .9
sp ++/+ *su lo*	7M	5.2	1M	8.5	14M	91.6	7.8	−2.6
++ *lo*/*sp su* +	2M	4.8			15M	26.5*		

* Only 28.6 percent of these are recombinations (true C.O. value = 7.6) remainder represent functional *sp* male gametes.

The percent of *Su* seeds, 6.1, in the backcross *su*×*sp*+/+*su* is the maximum average percentage of recombination between *sp* and *Su* in the repulsion phase, and represents the actual crossover value if the amount of functioning of *sp* pollen is negligible.

Functional *sp* pollen

Several cases of supposedly high crossover ratios have been found during the course of this investigation. In 1926 and 1927 the percents were 10.1 and 19.3 based on totals of five and one thousand seeds respectively. Also in 1932 several progenies were found that gave rather high recombination values. These could be explained either by an actual increase in the crossing over or by functioning of the linkage class of *sp Su* pollen grains. To test these two alternatives, some of the *Su* crossover seeds produced by the pollination *su*×*sp*+/+*su* were grown. Pollen was examined to determine whether normal plants or plants segregating *sp* had been obtained. The results of all such examinations are given in table 3. Certain facts are obvious in this table. Of a total of 2294 plants examined, 15.3 percent resulted from functioning *sp* pollen grains. This is considerably greater than the one percent previously reported. It may be argued that the value of 15.3 percent is too high since we were testing, in some cases, progenies

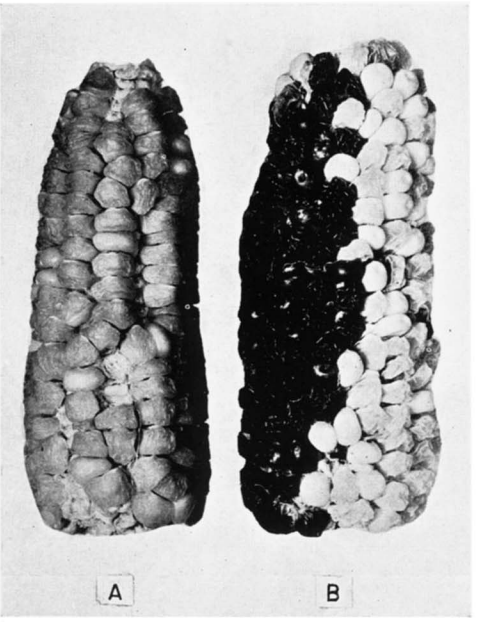

FIGURE 1.—Self pollination and backcross to *su* of *sp*+/+*su* plant. A -*su* times pollen from heterozygous plant; very small percent of *Su* seeds, the crossover class (11.6 percent). B shows a dual pollination, colorless seeds representing self pollinations, the black seeds being pollinated by *ACRPr su*. In making this pollination silks are divided approximately equally and different pollen applied to each half of the silks. Selfed seeds = 58.0 percent *su*, ×*su* = 66.1 percent *su*.

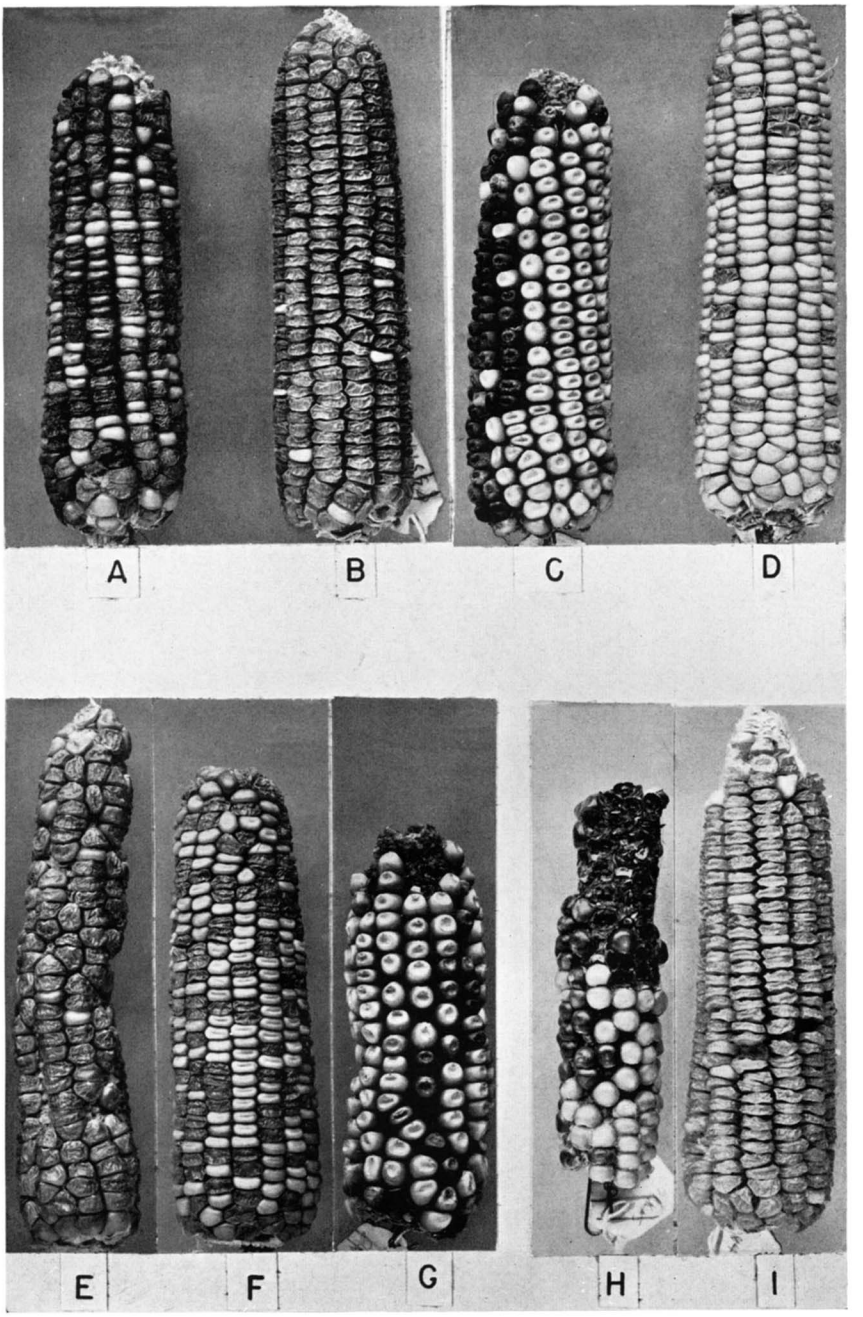

FIGURE 2.—A=ear from *sp*+/+*su* plant selfed (58.4 percent *su*), and crossed by *ACRPr su* (56.5 percent *su*); B=pollen from A put on *su* ear (97.1 percent *su*); C=*sp su*/++ ear selfed (2.2 percent *su*) and crossed by *ACRPr su* (1.6 percent *su*); D=pollen of C on *su* (6.1 percent *su*); E=*lo*+/+*su* ear crossed by *su* (95.2 percent *su*); F=pollen of E on *su* (49.9 percent *su*); G=*lo su*/++ ear selfed (0 *su*, 180 seeds) and crossed by *su* (0 *su*, 20 seeds); pollen when applied to *su* produced 49.8 percent *su*, ear similar to F. H represents *sp*++/+*su lo* ear selfed (0 *su*, 85 seeds), and crossed by *su* (0 *su*, 64 seeds); I=pollen of H on *su* (96.7 percent *su*).

TABLE 3

Percent of sp functioning pollen grains from the cross su×sp +/+ su.

	YEAR SEED COUNTS MADE	% IN C. O. CLASS	YEAR AND PLACE GROWN	TOTAL PLANTS EXAMINED	*sp*/+ NO.	*sp*/+ %	+/+ %	CORRECTED C. O. %
1	1927	9.8	1928 Texas	111	2	1.8	98.2	9.6
2	1926	13.3	1927 Conn.	227	2	6.4	93.6	12.4
3	1929	12.4	1930 Texas	89	0	0	100	12.4
4	1932	10.5	1934 Conn.	116	34	29	71	7.5
5	1932	4.8	1934 Conn.	526	111	21	79	3.8
6	1933	17.5	1935 Conn.	122	67	55	45	7.9
7	1933	8.6	1935 Conn.	1103	135	12	88	7.6
	Total and Average			2294	351	15.3	84.7	
		6.1*	(value for progenies 5 and 7)				84.9	5.2

* Average of all ears counted in Connecticut and Texas.

whose crossover values were obviously high. Two of the populations examined for segregating pollen represented random samples (progenies 5 and 7 in table 3). These two produced a total of 1629 plants of which 246 or 15.1 percent represented functional *sp* pollen instead of recombinations. This left a value of 84.9 percent for recombinations. When this is multiplied by the original crossover value of 6.1, it gives a corrected crossover value of 5.2 percent. This probably represents more nearly the correct value for the recombinations of *su* and *sp* in the repulsion phase.

Our correction factor was based on the number of *sp* pollen grains that not only effected fertilization but *produced mature plants*. Since our experiments have shown a differential viability in favor of *Sp Sp* plants, it is obvious that the number of *sp*/+ plants that survive to the flowering stage is appreciably lower than the seeds of the constitution *sp*+/+*su*. The number of plants lost is not usually known. Any appreciable increase in the number of *sp* functional pollen would further decrease the recombination percentage. In the 1932 ears, the original recombination percent was 4.8. When the crossover seeds were planted in 1935 and the resultant plants were examined for segregating pollen, 21 percent were segregating leaving 79 percent as recombinations. This gives a corrected crossover value of 3.8. This represented several progenies and probably approaches the true crossover value for those progenies.

We are forced to conclude that the original figure of 6.1 percent is slightly too high. Whether there is considerable fluctuation in the recombination percent as well as in the amount of functional *sp* pollen is not possible to determine since we have no accurate way of measuring the *sp*/*Sp* seeds that fail to produce plants. In some cases, at least, we know the value to be as low as four percent and it could be even lower. Since six percent

is the maximum, an average value of five may be taken as the crossover percent. A difference of one percent in the crossing over of these two genes will have little affect on the location of *sp* on the chromosome.

In the coupling phase, the percent of recombinations (plus *sp* pollen survivals) is 9.7 in a total of forty-one thousand. The apparently higher recombination value than for the repulsion phase is probably accounted for largely by *sp* pollen survivals rather than an increased crossover value. This will be discussed more fully under effects upon the male gametophyte.

Linkage relations of lo with su

Like *sp*, the gene *lo* is closely linked with the *su* locus so that its inheritance is studied through its effect on the *Su*:*su* ratios. In table 2 are found the apparent crossover ratios for *lo* and *su* in both the coupling and repulsion phases. Since *lo* ovules are nearly all lethal, the crossover ratio between *lo* and *su* is obtained by backcrossing *lo su*/++ or *lo*+/+ *su* ears by *su* pollen and noting the percentage of "crossover" seeds. The *su* seeds from the cross *lo su*/++ will all be crossovers provided there is no functioning of the *lo su* ovules. This point can be tested in a way similar to that used in testing *sp* survival in the pollen. The "crossover" seeds were grown and the resultant plants examined to determine how many produced *lo*/+ ears and were therefore *lo* ovule survivals instead of recombinations. Table 4 summarizes all "crossover" plants examined.

TABLE 4

Percentage of lo/+ plants produced by "crossover" seeds in both coupling and repulsion phases of lo and su.

GENOTYPE	YEAR	TOTAL PLANTS	*lo*/+ EARS NO.	*lo*/+ EARS %	%++ EARS	ORIGINAL C. O.	CORRECTED C. O.
lo su/++	1932	88	15	17	83	2.3	1.9
lo su/++	1935	37	22	60	40	1.7	0.7
Total and av.		125	37	30	70	2.3	1.6
lo +/+ *su*	1935	318	220	69	31	5.1	1.6
lo su/+ *su*×++	1933	465	9	1.9*			

* =percentage survival of *lo* ovules compared to *Lo* when each given equal opportunity—no selection of seed (all *su*×*Su*).

In both the coupling and repulsion phases some of the apparent crossover seeds were *lo* ovule survivals. The seeds planted represented random samples of the crossover class. The apparent crossover ratio in the repulsion phase was greater than for coupling of *lo* and *su*. However there were more *lo* ovule survivals in the crossover seeds from the repulsion phases. When both crossover ratios from the progenies whose crossover seeds were examined are corrected, they are exactly the same, 1.6 percent in each case. The "crossover value" obtained in the coupling phase for all years studied

was 2.4 as given in table 2. When this is corrected by multiplying by 70 percent (see table 4) it becomes 1.7 percent, the actual crossover ratio for *lo* and *su* for all years grown. Likewise the total figure for the repulsion phase *lo*+/+*su*, 4.8, when corrected becomes 1.5 percent, the actual recombination percentage for *lo* and *su*.

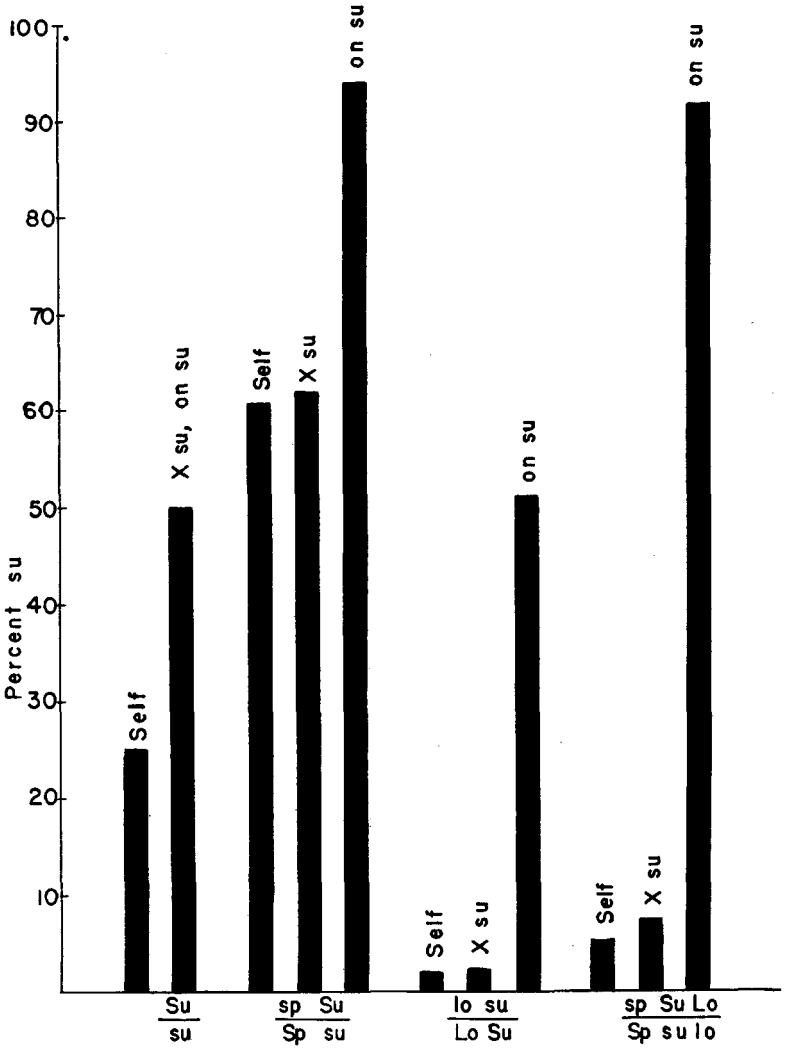

FIGURE 3.—Percent of *su* seeds obtained from self-pollinations and backcrosses to *su* for plants of the compositions *su*/+, *sp*+/+*su*, *lo su*/++ and *sp*++/+*su lo*.

Gametic and zygotic ratios of sp/+ and lo/+ plants

Table 2 gives the gametic ratios (backcrosses to *su*) and zygotic ratios (self-pollinations) produced by plants heterozygous for *sp*, *lo*, and combinations of *sp* and *lo*. This is also shown graphically in figure 3. From the

gametic ratios it is possible to calculate the expected zygotic ratio for comparison with the zygotic ratio found. The expected ratio is given in the eighth column of table 2. By comparing the actual and expected zygotic ratios it is possible to determine whether there is any appreciable differential zygotic mortality between heterozygotes and homozygotes. If there is a regular and appreciable survival of *sp* in the pollen or *lo* in the ovules, there should be some homozygotes produced. If these are eliminated the actual percentage of *su* seeds should be higher than the calculated percentage in the repulsion phase, and lower in the coupling phase. In all cases there is a fairly close agreement between the expected and observed ratios, the differences being noted in the last column of table 2. Some of the differences are negative and some positive. This would indicate no appreciable differential zygotic mortality. To test this point more thoroughly in the case of *sp*+/+*su*, plants from the same progenies were studied in Texas in 1930. Nine progenies were involved which included 255 ears with a total of 57,159 seeds. The total difference for the nine progenies between calculated and observed zygotic ratios was 2.3. In four cases the actual percentage was higher than the calculated and lower in five. All of these data show that differential zygotic mortality is not very influential in disturbing the ratios resulting from selfing heterozygous *sp* or *lo* plants. If there is any differential zygotic mortality it is overshadowed by errors in sampling, differences in crossing over at microsporogenesis and megasporogenesis, and differences in survival of *sp* male gametes.

EFFECTS OF *sp*

Effect on male gametophyte

The effect of *sp* on the male gametophyte was partially discussed in a previous section. The obvious effect is to cause the pollen grains to be reduced in size. In some samples it is possible to count the two classes of pollen grains and in such cases ratios of approximately 1:1 are found (626 normal:643 small in one sample counted). In others, however, there is an overlapping of the two classes. When the distribution of the pollen grains with respect to maximum diameter is plotted as a frequency polygon, the curve for pollen grains from *Sp Sp* plants has only one mode at 96 microns while the curve for *Sp sp* plants is distinctly bimodal with one mode at 84 microns, the other at 99 microns (figure 4).

Of some interest in this connection is the fact that the normal pollen grains from *Sp sp* plants are larger than the grains from normal plants. This is suggested by the frequency distribution in figure 4 and is verified by an actual comparison of all grains with a maximum diameter of 90 microns or more. In *Sp sp* plants these grains average 98.4 microns; in *Sp Sp* plants, 95.3 microns. The difference, 3.1 microns, is probably significant (P

<.03). Evidently the normal pollen grains in heterozygous plants are capable of taking advantage of the reduction in competition resulting from the fact that half of the pollen grains are reduced in size.

Microspores of plants segregating *sp* were examined to see how early the *sp* microspores could be detected. No difference in microspore size or appearance was found at the first microspore division. Just before the second division however, the *sp* grains are slightly smaller and the nucleolus of the tube nucleus is more vacuolated than that of *Sp* pollen grains (figure 5, C and D).

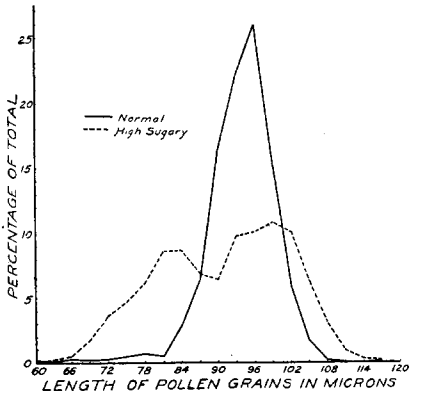

FIGURE 4.—Frequency distribution, with respect to length, of 1350 pollen grains from eleven *sp*+/+*su* (high sugary) plants and 1100 grains from *su*/+ plants.

Although the *sp* grains are smaller than normal they are usually well filled with starch. Preliminary results showed that the *sp* grains rarely effect fertilization in competition with normal grains although they are capable of fertilization when the competition with normal grains is removed by screening (MANGELSDORF 1931, 1932). More recently it has been shown (SINGLETON 1940) that the pistillate parent to which the pollen is applied has considerable influence on the functioning of *sp* pollen. In 1938 the recombination percent of *su* and *sp* was high when pollen of *sp su*/++ was applied to *su*. Crosses on Purdue 39 gave 39.1 percent of *su* seeds, those on Connecticut 81 gave 16.4 percent. Both of these figures suggest that there was considerable functioning of *sp* pollen, more in the case of P39 than for C81. Examination of plants produced by these seeds was made in 1939. The results are given in table 5.

This table shows that the *su* seeds from the P39 cross produced 86.8 percent of *sp*/+ plants while the C81 crosses gave only 55 percent. The corrected crossover ratio for the P39 crosses was 5.2 whereas for the C81 crosses it was 7.4 percent. This difference cannot be a difference in crossing over since pollinations on the two inbreds were made on the same day

TABLE 5

The effect of the pistillate parent upon survival of sp in pollen. Crosses of P39 and C81 by sp su/+ + plants.

PARENTS		NO. SEEDS			PROGENY PLANTS		%	%	CORRECTED
♀	♂	TOTAL	*su*	%*su*	TOTAL	*Sp sp*	*sp*/+	++	C. O.
P39	1174-4	1612	607	37.7	123	111	90.2	9.8	3.7
C81	1174-4	1135	115	10.1	47	32	68.1	31.9	3.2
P39	1175-10	1382	728	52.7	183	165	90.2	9.8	5.2
C81	1175-10	2327	434	18.7	127	58	45.7	54.3	10.2
P39	1176-5	600	156	26.0	114	93	81.6	18.4	4.8
C81	1176-5	1997	350	17.5	91	62	68.1	31.9	5.6
P39	1176-8	864	253	29.3	95	78	82.1	17.9	5.2
C81	1176-8	1365	223	16.3	86	41	47.7	52.3	8.5
Total on P39		4458	1744	39.1	515	447	86.8	13.2	5.2
Total on C81		6824	1122	16.4	351	193	55.0	45.0	7.4

using pollen from the same plants. These results suggest that there might have been a more selective elimination of heterozygous *sp*/+ plants in the case of the C81 crosses. Data are available on this point. These are as follows:

	Seeds planted	Plants obtained and examined	Loss	% loss
P39 crosses	580	515	65	11.2
C81 crosses	512	351	161	31.4
			Difference =	20.2

There was a greater loss from the seed to the mature plant stage for the C81 crosses. If the 20 percent difference in loss is due to elimination of *sp*/+ plants then this amount should be added to the *sp*/+ plants already found for the C81 crosses. Adding this gives 295 *sp*/+ in a total of 453 or 65.1 percent. This leaves 34.9 percent ++ which represent crossovers only. Multiplying this by 16.4, the original "crossover+survival class" gives a corrected crossover value for the C81 crosses of 5.7 which is in agreement with that of the P39 crosses, 5.2 percent. Of course we cannot be sure the additional 20 percent loss for the C81 crosses is all due to elimination of *sp*/+ plants. The fact that *sp*/+ plants tend to be weaker than normal sibs supports the supposition that this is so. At any rate, something has caused the elimination of 31.4 percent of the zygotes between the seed and mature plant stage in the C81 crosses, whereas in the P39 crosses the loss was only 11.2 percent. It seems that the C81 inbred which eliminates more

of the *sp* male gametes than P39 also has a more deleterious effect on zygotes which are heterozygous for *sp*.

In addition to the variable functioning of *sp* pollen grains on different inbreds, and the functioning of *sp* male gametes when screened to remove competition of normal grains, we would expect an increased functioning of *sp* grains if competition were reduced by limiting the pollen so that part of the silks received only one functional grain. We have made no experiments on this point, but data secured from other experiments show that this may occur. Of the 20 ears obtained in 1926 from the backcross *su*×*sp* +/+*su* (89.9 percent sugary) approximately half of the ears were partly filled, some of them undoubtedly so because of insufficient pollen. These ears were arbitrarily divided into two groups, one with less than 200 seeds, the other with more than 200. There were 11 ears in the first group with an average of 127 seeds per ear; nine in the second group with an average of 424 seeds. The percentage of *Su* seeds (crossovers plus *sp* survivals) in the first group was 15.2; in the second, 8.2. The difference is highly significant ($P<.01$). Since the two groups represent random samples of the population in other respects, the differences cannot be attributed to variations in crossing over, and are undoubtedly due to differences in competition between *Sp* and *sp* grains resulting from variations in the amount of pollen available on the styles.

Quite different results were secured in 1930 when a homogeneous population of 55 ears with 94.1 percent of *su* seeds was divided the same way as those in 1926 into two arbitrary classes with more and less than 200 seeds each. In the first group there were 29 ears, in the latter 26. Each group of ears gave an average crossover percent of 5.9. The results of the two tests indicate that competition for limited pollen is undoubtedly a factor in some cases, and not in others.

A third source of variation should be sought in modifying factors which affect the expression of the *sp* gene. Some indication that this occurs is found in the fact that in some samples of pollen from *sp*/+ plants, the small grains can be definitely distinguished from normal grains; in other samples there is an overlapping of the two classes. Yet no correlation between this variation and that in the male gametic ratios has been found, nor is there any correlation between male gametic ratios in two successive generations. The differences are probably due primarily to variations in functioning of *sp* grains rather than to differences in crossing over.

That the variations in "crossing over" are really differences in *sp* pollen survival rather than differences in crossing over is shown by correlating the recombination percent in parent and offspring. If due to a difference in recombination we would expect such differences to be transmitted. The correlation of the crossover percents in parent and offspring is $-.28 \pm .091$

showing no positive correlation and perhaps a slight negative one. The negative correlation is just barely significant.

Effect of sp upon the female gametophyte

The factor *sp* is transmitted largely through the ovules, but there is some lethality of *sp* megaspores. Otherwise, ratios higher than 50 percent *su* could not be obtained for self-pollinations or backcrosses. The figures for these pollinations are 61 and 62 percents based on 143 thousand and 36 thousand kernels respectively. The elimination of *sp* megaspores is also indicated by missing places on heterozygous ears and by the reduced number of kernels compared with *Sp Sp* ears. A comparison of the number of seeds on 61 ++ ears and 237 *sp*/+ ears showed that the *sp*/+ ears had on the average 67.8 percent of the number of kernels on the former ears.

Since there is a semi-lethal action of the *sp* gene upon the megaspores the question arises as to what percent of the *sp* ovules survive and are functional. We can measure directly the percentage of *sp* ovules which accomplish fertilization and produce mature plants. In previously reported three-point tests with *sp su* and *Tu* (EMERSON, BEADLE and FRASER, 1935) 801 plants were grown of which 497 were normal and 304 were heterozygous for *sp*. If *Sp* and *sp* ovules had originally occurred in equal numbers the 304 *Sp sp* plants represent survival for approximately 497 ovules. This is a survival of 61.2 percent.

In the three-point test with *la*, *sp* and *su* reported later in this paper 1898 plants were grown of which 496 were heterozygous for *sp*. Calculated in the same way this represents a survival of 35.4 percent. The weighted average for the two populations is 42.1 percent, which is somewhat less than the 56 percent survival of *sp*[2] reported by RHOADES and RHOADES 1939.

Data already presented indicate that there is sometimes an elimination of heterozygotes between the mature seed stage and the mature plant stage. We can calculate the survival of *sp* through the megaspores to the mature seed stage by the following formula where *s* is percentage of survival, *p* = percentage of non-crossovers and *m* = percentage of functional *su* gametes among the ovules of *sp*+/+*su* plants or the percentage of functional *Su* ovules in *sp su*/++ plants.

$$s = \frac{100(p-m)}{(p+m)-100}$$

If we assume that crossing over between *su* and *sp* is relatively constant at five percent[2] it is found that survival of *sp* through the megaspores to the mature seed stage varies from 0 to 100 percent in different progenies.

[2] Slight differences in crossing over have no appreciable effect upon calculated survival. The results are quite similar if the percentage of crossing over is computed at three percent, the minimum figure, or six percent the maximum.

The average survival in *sp*+/+*su* plants (61.9 percent *su*) is 58.2 percent; in *sp su*/++ plants (37.6 percent *su*) 56.8 percent.

The populations used in the two three-point linkage tests mentioned above permit a direct comparison of survival to the mature seed stage and survival to the mature plant stage. In the three-point test with *Tu* crossing over between *sp* and *su* was 5.8 percent and the percentage of sugary seeds in the backcrossed ears was 61.6 percent. The calculated survival of *sp* to the mature seed stage is 58.4 percent. This is slightly lower than the actual survival to the mature plant stage, 61.2 percent, and indicates that in this particular population there has been no elimination of heterozygotes after the mature seed stage. In the three-point test with *la* the crossing over between *sp* and *su* was 5.0 percent and the percentage of sugary seeds on the backcrossed ears was 36.7 percent. The calculated survival to the mature seed stage is 54.4 percent, which is considerably higher than the actual survival to the mature plant stage, 35.4 percent, and suggests an elimination of heterozygotes. Thus the data not only show extreme variations of survival of *sp* through the megaspores, but support the suggestion already made, that elimination of heterozygotes varies with the stocks used as parents.

The percent of *su* seeds when plants of the constitution *sp*/+ are selfed shows a decided correlation between one generation and the next. The correlation coefficient was $+.40 \pm .04$. This is highly significant. In Texas a similar comparison of six parents and progenies gave a correlation coefficient of $+.67 \pm .23$. Both of these comparisons show that there is a decided tendency for ears that produced a high percentage of *su* seeds one year to give a high percentage the next. Since any percent of *su* seeds in excess of 50 is caused by ovule elimination of *sp* ovules it is evident the degree of ovule elimination is transmitted.

Substitution of megaspores

In maize as well as in many other plants, the embryo sac develops from only one of the four megaspores resulting from the two divisions of the megaspore mother cell. The other three are lost. In several instances where peculiar genetic results have been encountered, it has been suggested that substitution of megaspores may have occurred; that when the megaspore which usually persists, receives a combination of genes with a lethal effect, it may be replaced by a viable sister megaspore. This has been shown by RENNER (1921) to occur in Oenothera and is known as the "Renner effect." Maize is ideal material for investigating this phenomenon because the orderly arrangement of the seeds renders any lethal effect immediately visible.

That the substitution of megaspores in *sp*/+ stocks, if it occurs at all, is not complete, is at once apparent from the fact that ears from hetero-

zygous plants are seldom well-filled and usually exhibit many missing kernels. However, if there is any substitution whatever the number of gaps should be smaller than the number expected to occur on the basis of the calculated survival of *sp* through the megaspores.

In 1926 we compared 29 ears from normal *Sp Sp* plants with 77 ears from *Sp sp* plants. The former had an average of 263 seeds per ear, the latter 179 seeds. Thus, the high-sugary ears had only 68.1 percent as many seeds as normal ears from the same progenies. The average survival of *sp* in 1926 was calculated as 47.4 percent. This means that high-sugary ears in 1926 would be expected to have, on the average, 73.7 percent, $50+(47.4/2)$, as many seeds as normal ears. The difference between this figure and the one actually obtained, 68.1 percent, is easily accounted for as the result of sampling errors. In any case, the difference is in the wrong direction to indicate substitution of megaspores.

In 1930 we compared 160 high-sugary ears with 32 normal ears. The former had an average of 238 seeds per ear, the latter 377. The lower figure is 63.1 percent of the higher. Survival of *sp* in the megaspores in 1930 was computed at 31.0 percent. On this basis ears from *Sp sp* plants would be expected to bear 65.5 percent as many seeds as ears from normal plants. Again the difference is in the wrong direction to suggest substitution of megaspores. The averages for the two years are 69.6 percent calculated and 65.6 percent actually found. That the number of seeds found on ears from *Sp sp* plants is lower in both cases than the number calculated on the basis of survival of *sp* is probably due to the fact that plants heterozygous for *sp* are slightly weaker than normal plants and hence probably bear slightly smaller ears. That the observed and calculated figures agree as closely as they do indicates not only that no substitution of megaspores has occurred, but also that the data on survival of *sp* in the megaspores are reasonably accurate.

Effects of sp upon the sporophyte

Effect upon the heterozygote

That *sp* has a deleterious effect upon the heterozygote has already been suggested by the fact that its survival through the megaspores to the mature seed stage is sometimes greater than survival to the mature plant stage of the succeeding generation. Additional observations support this suggestion.

Plants heterozygous for *sp* are slightly less vigorous and later in blooming than their normal sibs. It is not always possible to demonstrate this fact statistically because of other variables, but it has been observed repeatedly especially in certain progenies. In one season at least (1926), the difference in height of stalk, 2.9 inches, was statistically significant ($P<.03$).

Effect upon the homozygote *sp sp*

Since *sp* survives in part of the megaspores, and *sp* pollen can function, it should be possible to obtain seeds homozygous for *sp*, and if these are viable to determine the effect of *sp/sp* upon the plant. Pollen screening experiments in both Texas and Connecticut have altered the *Su:su* ratio, and have resulted in a considerable functioning of *sp* pollen. So far no homozygous *sp/sp* plant has been found either in ordinary populations or in cases where screened pollen was applied to heterozygous *sp* plants. In one experiment where it was determined that at least 28.7 percent of the functional ovules were *sp* and at least 73.5 percent of the functional pollen grains were *sp* no homozygous plants were obtained in a population of 78 plants derived from 176 seeds. Seedling mortality was high even under ideal conditions which suggests that the homozygous combinations which must have occurred from this pollination were eliminated as seed or seedling lethals.

THE EFFECTS OF *lo*

Effect upon the female gametophyte

The chief effect of *lo* is upon the female gametophyte. The majority of ovules receiving *lo* are lethal, and ears from heterozygous plants are always poorly filled, resembling ears from "semi-sterile" plants in which reciprocal translocations are involved. There is however some survival through the megaspores as was discussed in a previous section. One such test where *lo su/+su* ears were pollinated by *Su* gave 465 ++ ears and nine *lo/+* ears or 1.9 percent of *lo/+* ears. Since this represents an unselected sample it seems safe to say that about two percent of *lo* ovules can take place in fertilizaton.

Effect upon the male gametophyte

There is no visible effect of *lo* upon the male gametophyte, and pollen from *lo/+* plants is quite normal in appearance. Furthermore, there is apparently no elimination of *lo* through the male gametophyte, in fact the contrary seems to be true. In the backcross *su*×*lo+/+su*, the percentage of + seeds was 51.5, while in the backcross *su*×*lo su/++*, the percentage of *su* seeds was 51.3. The total number of seeds in these two populations is 51,606, of which 26,541 or 51.4 percent, represents the class in which *lo* predominates. The deviation from the 50.0 percent expected in this class is $1.4 \pm .148$. The deviation is 9.5 times the probable error, and the odds against its chance occurrence are more than a billion to one.

This situation in which a gene is almost completely lethal to the ovules but advantageous to the male gametophytes receiving it, is unique. Perhaps a hormone-like action which stimulates the male gametophyte and retards the female gametophyte is involved.

Effect upon the sporophyte

We have never noticed any effect of *lo* upon the sporophyte. Plants of the constitution *lo*/+ seem to be as vigorous as those of the constitution +/+ and there is no noticeable difference in time of flowering.

No plants homozygous for *lo* have been obtained. It should be possible to obtain such individuals since about two percent of the functional ovules are *lo* and since the *lo* pollen functions normally. An attempt will be made to obtain homozygous *lo*/*lo* plants.

LINKAGE RELATIONS OF *sp* AND *lo*

Linkage relations of sp

In a previous section we have found the recombination value for *sp* and *su* to be approximately five percent.

TABLE 6

Three-point linkage tests with sp and lo.*

F_1 GENOTYPE	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1 AND 2		TOTAL
Ts5 + + / + *sp su*	738	—	—	50	10	—	—	4	802
			6.2		1.2		.5		
Ts5 sp + / + + *su*	—	367	77	—	—	45	24	—	513
			15.0		8.8		4.7		
Total	1105		127		55		28		1315
			9.7		4.2		2.1		
la + +†/ + *sp su*	781	439	781	14	41	16	2	35	1898
lo + + / + *su gl3*	1748		161		1048		52		3009
			5.4		34.8		1.7		

* For additional tests already published see EMERSON, BEADLE and FRASER 1935.

† Backcross for *su* and *sp* F_2 for *la*.

Various three-point tests with *sp su* and *Tu*, the results of which have already been published (EMERSON, BEADLE and FRASER 1935), show *sp* to be to the left of *su*. Several three-point tests with *Ts5 sp* and *su*, show *sp* to lie to the right of *Ts5* and to the left of *su*. The data are shown in table 6.

The three-point test for *la*, *sp* and *su* given in table 6 proved to be an F_2 for *la* and a backcross for *sp* and *su*. Evidently a +*su*/*la su* plant instead of the double recessive was used for a pollinator. The cross was then *la*++/+*sp su*×*la su*/+*su*. The crossover value for the three genes is as follows: *la* 7.5 *sp* 5.0 *su*. The recombination value for *la* and *su* was 13.2 percent. The crossover values for *la* and *sp*, also for *la* and *su* were calcu-

lated by the product method (IMMER 1930) using the formula ad/bc $=p+p^2/2-3p+p^2$ where p equals the crossover value. The crossover value for *sp* and *su*, 5.0 percent, was calculated directly since this was a backcross for both, and survival of *sp* in the pollinations not involved.

The only data which suggest that *sp* might be to the right of *su* are those showing the crossing over between *sp* and *gl*3 to be 26 percent. Published data (EMERSON, BEADLE and FRASER 1935) show crossing over between *su* and *gl*3 to be 34 percent, and the much lower crossing over between *sp* and *gl*3 would suggest that *sp* lies between *su* and *gl*3. Since all three-point tests show that the contrary is true, this is another example of the variations involved in comparing crossover values from different stocks.

One additional linkage test with *sp* should be mentioned because of the peculiar ratios obtained. The gene *de16*, which causes defective seeds, is located on the fourth chromosome about 3.2 units to the right of *su*. Plants of the composition *sp* ++/+ *su de16* should, when selfed, be high-*su* and high-*de16* seed. In most ears, however, the *su* gene does not express itself in seeds homozygous for *de16*, and since *su* and *de16* are closely linked, there is actually a decided deficiency of visible *su* seeds. In a total population of 2609 seeds from 11 ears, 1430 or 54.8 percent of the seeds were *de16*, while 106 or 4.1 percent of the total were visibly *su*. Yet when pollen from one of these plants was applied to normal *su* plants the percentage of *su* seeds produced was 90.6. A similar situation has been described in which the gametic lethal *lo* is involved.

Among those 11 ears was one in which the + and *su* endosperms in the defective seeds were distinguishable. The four classes of seeds occurred in the following numbers: ++, 117; +*su*, 21; *de16* +, 15; and *de16 su*, 67. Since 40.0 percent of all seeds are *su* and 37.3 percent are *de16*, the results suggest that *sp* has had a greater effect upon *su* than upon *de16* and hence must be closer to *su* than to *de16*. Thus these data, though not critical, are in agreement with other three-point tests which place *sp* to the left of *su*.

Linkage relations of lo

It has already been shown that the percentage of recombinations for *lo* and *su* in the ovules is 1.6 percent. Likewise in published data (EMERSON, BEADLE and FRASER 1935) the recombination percent in the pollen for the repulsion backcross was 1.4 percent. This is very close to the value for ovules. Since the publication of that figure a few additional progenies have been summarized which may alter slightly the recombination percent observed from the pollen. All data are summarized in table 7.

These values are variable especially in the coupling phase. Not much reliance is placed on the figures for this phase since the figures for the most part represent compilations of small progenies.

TABLE 7

Recombination values from backcrosses of lo and su in pollen.

	REPULSION PHASE				TOTAL	RECOMBINATIONS	
	++	+ *lo*	*su* +	*su lo*		NO.	%
1	1	85	80	0	166	1	0.63
2*	1	444	78	2	525	3	0.57
3*	9	190	185	1	385	10	2.6
4	0	92	124	18	234	18	7.7
5	11	265	288	7	571	18	3.2
6	5	160			165	5	3.1
7	23	805	776	24	1628	47	2.9
Total	50	2041	1531	52	3674	102	2.8
			COUPLING PHASE				
8	34	2			36	2	5.6
9	197	19	19	179	412	38	9.2
10	52	15	9	50	126	24	19.0
11	44	0	0	45	89	0	0
Total	327	36	28	274	665	64	9.7

* The totals of 2 and 3 give an average of 1.4 percent, the figure published by EMERSON, BEADLE and FRASER 1935.

In the repulsion phase, the average of all years' trials before 1939 is 2.7 percent. This is higher than the values found from the ovules after correcting for *lo* ovule survival. The values obtained for different progenies vary considerably, from .57 percent to 7.7 percent for one progeny of 234 plants where all the recombinations were of the *su lo* class and there were none of the ++ type. Omission of this progeny from the calculations brings the average figure to 2.4 percent. In four of the progenies, totaling 681 plants, in the repulsion phase the crossover value was less then one percent. It is evident there is considerable variation in the crossover values from the pollen. Part of this might be caused by mistakes in classification, as all *lo*/+ plants were classified entirely by the semi-sterile appearance of the ear. Although on the whole this is a reliable criterion, in the case of ears poorly filled for other reasons there may have been a few mistakes. Also, crossovers of the constitution ++ could have arisen from a contamination in the original pollination. In the 1939 data it is doubtful if this could have been a factor since extreme care was taken to guard against contaminations in the original pollinations. The fact that both crossover classes are numerically so nearly alike leads us to believe that errors in classification were negligible in these progenies.

The conclusions regarding the percentage of recombination between *lo* and *su* are that in the pollen the percent for the repulsion phase varies from one to three with an average of 2.8. The recombination value in the ovules for both coupling and repulsion phases was 1.6. There may be a dif-

ference in the amount of crossing over in microsporocytes and megasporocytes. Since the variation of the crossover values is from 1.6 to 2.8, no serious error will be encountered if any value between those figures is taken as the recombination value for *lo* and *su*. If we take the mid-way point for these two figures we get a value of 2.2 percent (which is the average value published in 1935 by EMERSON, BEADLE and FRASER). The crossover value of 2.2 will be used for *lo* and *su* in calculating linkage with other factors on chromosome 4.

Recombination value of *lo* and *Ts5* is eight percent, and there are two percent of recombinations between *lo* and *de16* (EMERSON, BEADLE and FRASER 1935). Other linkages of *lo* on chromosome 4 are shown in table 8.

TABLE 8

Crossover values of sp and lo with gl3.

GENES X AND Y	LINKAGE PHASE	XY	Xy	TOTAL	RECOMBINATIONS NO.	RECOMBINATIONS %
Lo Gl3	RB	1722	2924	4646	1722	37
Sp Gl3	RB	1210	3530	4740	1210	26

This shows 37 percent of recombinations between *lo* and *gl3*. This would tend to place *lo* to the left of *su*, since *gl3* and *su* show 34 percent of recombinations. However, where two factors are as far apart as 34 units they are not very useful in locating a third gene which is close to one of them. The data with *de16* are more conclusive. This lethal shows 3.2 percent of recombinations with *su* and the value for *lo* and *su* is 2.2 percent. Hence if *lo* were on the left of *su* we should expect about five percent of recombinations between *lo* and *de16*. Actually there was two percent of crossing over between *de16* and *lo* which would be expected if *lo* were to the right of *su*. These data agree with the three-point test between *sp*, *su* and *lo* placing *lo* to the right of *su*. One three-point test for *lo*, *su* and *gl3* was summarized in table 6. The crossing over between *lo* and *su* is 7.1 percent; between *su* and *gl3*, 36.5 percent; between *lo* and *gl3*, 40.2 percent. These data were obtained from crosses in which *lo* survival in the ovules could have been a disturbing element. It is impossible now to correct for that factor although the 7.1 percent of crossovers between *su* and *lo* indicate a survival of *lo* ovules. Even if corrected it would not alter the relative positions of the three factors calculated from these data. This three-point test tends to place *lo* to the left of *su*, in direct conflict to the three-point data of *sp*, *su* and *lo* and emphasizes the variations found in this study.

Combinations of sp, lo and su

The combination of sp ++/+ *su lo* gives the unusual result described in the introduction and shown graphically in figure 3. Actual ratios are

found in table 2. At the time *lo* was discovered it was not known on which side of *su* it was located. If on the same side it would not be improbable that the two were alleles of the same gene. However a simple test rules this out and also gives valuable data on the location of *lo* in relation to *su*. The pollen from a plant, *sp* ++/+ *su lo*, was applied to *su* silks. If *lo* and *sp* were alleles of the same gene then the progenies should either segregate *lo* or *sp*, and no normal ratios should be found. The cross of *su* by *sp* ++/+ *su lo* gave 33 selfed ears. Thirty-one segretated in a normal 3:1 ratio for *su* and two segregated for *sp*. No *lo*/+ ears were found. The occurrence of the 3:1 ratios proves that *sp* and *lo* are not allelic. These ratios also tend to show that *lo* and *sp* are on opposite sides of *su*. If on the same side, the composition would be *sp* ++/+ *lo su* with the crossover values *sp* 2.8 *su* 2.2 *lo* since *sp* is five units from *su*, and *lo* and *su* are 2.2 units apart. Hence we should expect the crossovers at region 2 to be almost equal to those at region 1, 2.8:2.2 percents respectively. The functional male gametes should be +++ (56 percent) and + *lo* + (44 percent). Only *Su* seeds were planted, so *su* gametes need not be considered. A similar comparison of *su* seeds from the cross *su*×*sp su*+/++ *lo* gave similar results. Both sets of data are summarized below.

	Crossovers	
	Region 1	Region 2
su×*sp*++/+*lo su* (*Su* seed)	31	0
su×*sp*+*su*/+*lo*+ (*su* seed)	200	12
Total	231	12
Expected (56%:44%)	136	107

This deviation is highly significant ($P < .01$). The crossovers at region 2 were far too few. If *sp* and *lo* are on opposite sides of *su* then we would expect very few of the second class, double crossovers only. Although not conclusive, these data strongly suggest that *sp* and *lo* are on opposite sides of *su*.

In 1939 a three-point test was conducted for *sp*, *su*, and *lo*. The 212 plants just described were taken from this three-point test. Complete results are presented in table 9. In planting this test it was not possible to grow all the seed available and no effort was made to grow the same relative amounts of *Su* and *su* seed that occurred in the original pollinations. We were more interested in the plants produced by the *su* seeds so relatively larger amounts of the *su* seed were planted. In calculation it was necessary to correct for the *su* seed grown, reducing the *su* seed to the same proportion found in the original crosses. This correction factor is given in table 9.

Also there was some elimination of *sp* male gametes, more in the C81 crosses than in the P39 hybrids. In order not to discriminate against the *sp*

classes it was necessary to increase the individuals in these classes. This was done by dividing the *sp* class found by the percentage of *sp* individuals found, 86.8 percent in the P39 crosses, and 56.4 percent in the C81 crosses.

The use of this correction factor may be questioned but we believe by using it the recombination values are much more accurate than would have been obtained had no correction factor been used. It would be desirable, of course, to have additional data from experiments where no correction factor was necessary.

TABLE 9

Three-point test for sp, su and lo. F_1 genotype=sp su +/++ lo.

	P		R 1		R 2		R 1 and 2		
	sp *su* +	+ + *lo*	*sp* + *lo*	+ *su* +	*sp* *lo*	+ +	*sp* + +	+ *su* *lo*	TOTAL
P39×1174-4, 1176-5, 1176-10	259	272	4	31	7	4	4	4	
P39 corrected for *su* seed planted.*	138	272	4	16.5	3.7	4	4	2.1	
P39×1176-8	65	99	1	11	1	0	0	3	
P39 corrected for *su* seed†	42	99	1	7.1	.6	0	0	1.9	
Total P39 crosses corrected for *su*	180	371	5	23.6	4.3	4	4	4	
Total corrected for *sp*=*sp* class/86.8	207	371	5.8	23.6	5.0	4	4.6	4	
	578		29.4		9.0		8.6		625.0
			4.7%		1.4%		1.4%		
C81 crosses	210	355	7	15.8	4	9	9	5	
C81 corrected for *su*¶	36.1	355	7	27.2	.7	9	9	.9	
C81 corrected for *sp*=*sp* class/56.4	64.0	355	12.4	27.2	1.2	9	16.0	.9	
	419		39.6		10.2		16.9		485.7
			8.2%		2.1%		3.5%		
Total	997		69.0		19.2		25.5		1110.7
			(6.2%)		(1.7%)		(2.3%)		

* Seeds in ratio of 2714 *Su*:1744 *su* planted in ratio of 120:145
Correct number of *su* should have been 120:X::2714:1744=77.1 seeds.
77.1/145=53.2 percent *su* class×53.2 percent=corrected *su* class.

† *Su*:*su* planted=145:145

¶ Seeds in ratio of 7089:1478 planted in ratio of 120:145

A comparison of the recombination values in table 9 shows 6.2 percent for *sp* and *su;* 1.7 percent for *su* and *lo* and 2.3 percent of double crossovers, more than the crossover percent for region 2. This suggests that *sp* and *lo* are on the same side of *su*. Either way the experiment is calculated, the percent of double crossovers is much too high and suggests either faulty classification or that some other factors such as pollen contamination or hetero-fertilization may have tended to increase the individuals in the double crossover class. We have encountered several cases of apparent hetero-fertilization during the course of this investigation. If any of the seeds in the double crossover class were cases of hetero-fertilization they would belong in the parental rather than the double crossover class.

From all the data we would be forced to conclude that it is not possible to tell whether *sp* and *lo* are on the same or opposite sides of *su*. However the most critical part of the data, as well as previous data, strongly suggest opposite sides, as was discussed previously.

CYTOLOGY OF *sp*/+ AND *lo*/+ PLANTS

In regard to the unusual behavior of the *sp* and *lo* genes, it is important to know if any cytological disturbance is present. In a preliminary report (SINGLETON 1932) it was stated there were "no conspicuous irregularities of chromosomes at the reduction division." This referred to examination of the chromosomes in mid-prophase of meiosis. Since that first examination of the pachytene chromosomes, STADLER (1933) has demonstrated a case of small pollen in maize associated with a deficiency in chromosome 10. STADLER'S small pollen behaved genetically similar to *sp*. Since these cases are so much alike, reexamination of chromosome 4 in a number of plants known to be segregating for *sp* was undertaken. A greenhouse progeny in Connecticut was examined cytologically in 1939. Four plants, heterozygous for *sp*, later verified by pollen examinations, were examined at mid-prophase of the first meiotic division. No deficiency was observed in any plant, and pairing of all chromosomes was normal. The factors *sp*, *lo* and *su* are on chromosome 4 which was studied particularly for any evidence of a chromosomal alteration. Pachytene chromosomes have also been repeatedly examined in Texas in plants heterozygous for *sp*, and likewise no deficiency was observed. Plants heterozygous for *sp2* on chromosome 10 also showed no visible deficiency. (RHOADES and RHOADES 1939.)

Two plants, heterozygous for *lo*, were examined in Connecticut in 1939. No deficiencies were observed in any of the chromosomes 4 examined, and all other chromosomes were apparently also normal (figure 5).

DISCUSSION

There are now on record many occurrences of maize plants segregating small pollen, some spontaneous, others induced by X-rays or by ultraviolet radiation. In some instances these may be associated with a visible chromosomal deficiency (STADLER 1933) but in most of the cases so far observed no chromosomal irregularities can be detected. In most segregations for small pollen, the smaller grains are comparatively unable to effect fertilization in competition with the normal larger grains. This condition has been observed so often that it is now regarded as of general occurrence that the small pollen grains are not capable of competing with the larger grains.

In our study of *sp* several observations have shown a considerable proportion of *sp* grains that accomplished fertilization in competition with

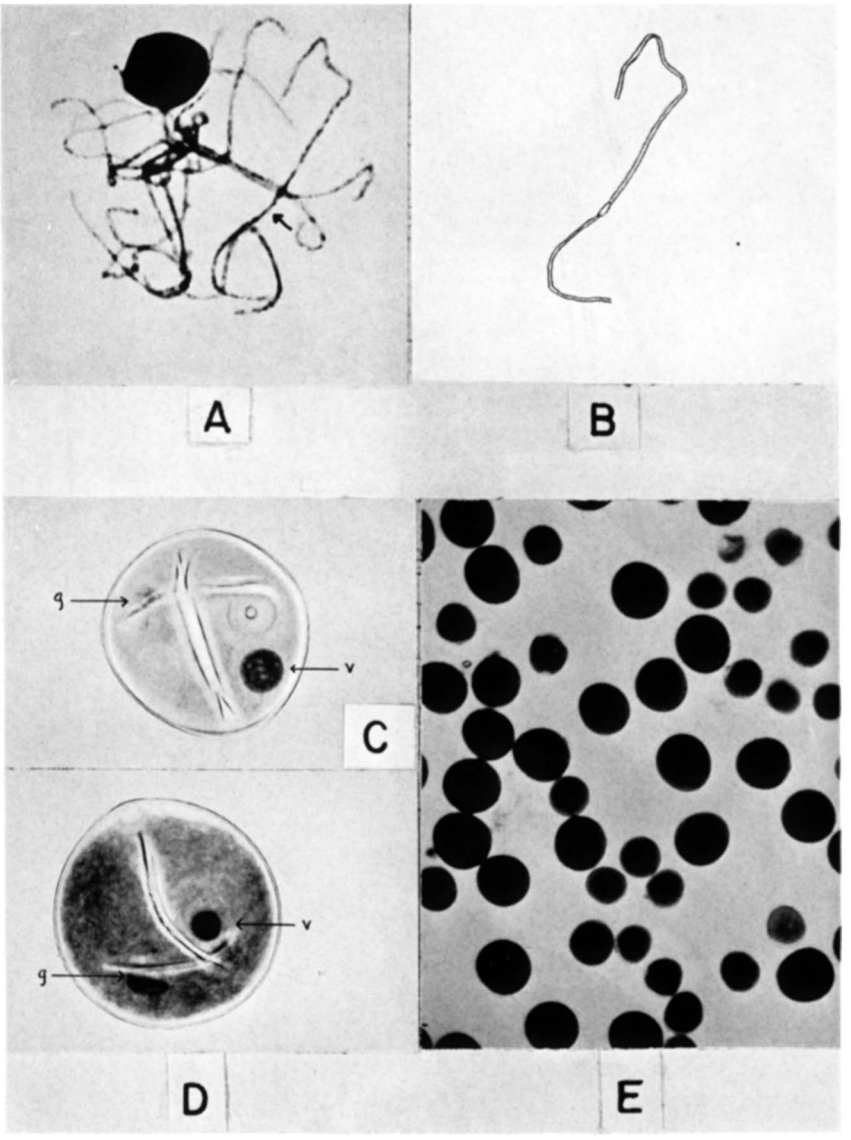

FIGURE 5.—A, pachytene preparation from *sp*+/+*su* plant showing chromosome 4. Arrow points to centromere. No evidence of lack of pairing or heterozygous deficiency. B, drawing of chromosome 4 in A. C, small pollen-1 microspore before division of generative cell. v = nucleolus of vegetative nucleus, g = generative nucleus. Note vacuolated condition of nuceolus of vegetative nucleus. D represents a normal microspore from same plant as C which was segregating *sp*. Note larger size of microspore, denser cytoplasm and smaller but more compact nucleolus of the vegetative nucleus. D is more completely filled with starch at this stage. E shows pollen from *sp*+/+*su* plant stained with iodine. Smaller grains in most cases well filled with starch.

normal; in pollination from one plant in 1938 there was no competitive effect whatever. Also, the stock to which the pollen is applied can make considerable difference in the amount of small pollen functioning.

So far as we are aware, no gene mutations have been found responsible for any gametic lethals in maize. The *sp* reported by MANGELSDORF for the first time in 1931, was the first case of a partial gametic lethal to be observed that was not associated with a visible chromosome abnormality. It is possible that *sp* may be caused by an undetectably short deficiency, but as stated before there is no visible alteration of chromosome 4 in stocks segregating for *sp*. If the abnormality is undetectable, we have no way of knowing whether it has a cytological basis or whether it is in the nature of a gene mutation.

In some ways it behaves like a gene mutation: it is transmissible through the ovules, in some cases with very little or no ovule elimination; in some instances, when pollen from a heterozygous plant is used, the *sp* grains function almost as well as the normal grains. Usually, however, there is almost complete elimination of *sp* grains, the lethal action being complete from a functional standpoint. In linkage relations with characters on chromosome 4, *sp* also behaves like an ordinary gene.

In one important respect the *sp* factor definitely behaves as a deficiency: plants heterozygous for *sp* are quite often smaller than normal sibs. This was especially true when the stock was first grown in 1926. Also one progeny grown in 1927 showed several abnormally small plants. At various other times we have noted that plants heterozygous for *sp* were later in maturity and not as vigorous as sib plants. In this way the factor *sp* behaves more like a chromosomal deficiency than like an ordinary recessive gene. We know of no convincing evidence indicating that a recessive gene in the heterozygous condition causes the plant to be noticeably smaller than the homozygous dominant. All normal recessive genes tested have given just as vigorous plants in the heterozygous condition as do the normal sibs. MANGELSDORF (1928), KARPER (1930), and ROBERTSON (1932) have shown that recessive seed and seedling lethals in maize, sorghum, and barley, respectively, have no measurable effect upon the heterozygote. The *sp* factor seems to be a border line case, cytologically invisible, which acts both like a gene and a deficiency.

This condition is similar to the situation in Drosophila. SLIZYNSKA (1938) analyzed the salivary gland chromosomes of 14 genetic deficiencies in the white-facet region. These are all lethal when homozygous. The longest of the cytological deficiencies included 45 bands, five included one band and one was undetectable cytologically. This last case is like the *sp* condition. This deficiency in Drosophila was a zygotic lethal whereas *sp* is a functional gametic lethal, as well as a zygotic lethal.

SLIZYNSKI (1938) studied 19 spontaneous lethals and 13 lethals induced by X-rays. Of the spontaneous lethals, nine were associated with a cytological deficiency and four of the 13 induced cases were visible cytologically. In more than half of all the cases studied, however, no deficiency in the salivary chromosomes was visible. The reverse condition is shown by DEMEREC and HOOVER (1936). They found a visible cytological deficiency having no genetic effect. DEMEREC (1940) has also made quite an extensive study of X-ray induced and spontaneous Notches in Drosophila. In 27 X-ray induced Notches, three had the full complement of bands, in seven cases one band was missing, in four cases two to five bands were missing and in 13 cases more than six bands were missing. In 10 spontaneous Notches there was one with no bands missing, and nine with one to more than six bands deficient. Thus, lethals and detectable chromosomal deficiencies, although sometimes associated, are not necessarily so.

The *sp* condition in most cases is a functional lethal although the gametes are produced, and the lethality is not necessarily complete. The *lo* factor, however, is a true gametic lethal, the action taking place early in the development of the female gametophyte. Ovule elimination is almost complete for *lo* ovules. In no cases have we found more than a very small percent of the *lo* ovules functioning. This is the only gene of its kind in maize, with its lethal action confined exclusively to the ovules. All other conditions, so far described, that affect the gametes have a more pronounced effect on the male than on the female gamete. Chromosomal disturbances, deficiencies and duplications, have this affect. The *lo* factor however, exercises its lethal affect solely on the ovules. If there is any affect on the male gametes it is one of stimulation since the ratios when pollen from *lo*/+ plants is applied to normal stocks there is an excess functioning of *lo* gametes. Nothing is known of the mechanism whereby *lo* pollen grains are favored over the normal. There seems to be some selective action, making the *lo* gene act as a male influence.

SUMMARY

1. The gene *sp* (small pollen) causes pollen grains to be reduced in size. In plants heterozygous for *sp* two sizes of pollen grains are produced in approximately equal numbers.

2. Pollen grains carrying the *sp* gene usually do not function in competition with normal grains, hence the gene is usually not transmitted through the pollen. When competition between + and *sp* grains is reduced or eliminated as a result of sifting the pollen or because of a sparsity of pollen on the styles, the *sp* pollen grains are capable of accomplishing fertilization. Pollen grains carrying the *sp* gene also regularly function successfully on the styles of certain maize stocks.

3. The *sp* gene is transmitted through part, but not all, of the female gametes; survival of *sp* through the megaspores varies from 0 to 100 percent. The average survival is 42.1 percent to the mature plant stage of the succeeding generation.

4. Plants heterozygous for *sp* are usually slightly weaker than their normal sibs. Heterozygous seeds are slightly smaller and frequently germless.

5. The gene *sp* is a seed or seedling lethal in the homozygous condition; no mature homozygous plants have ever been obtained.

6. The *sp* gene is linked with the *su* locus with approximately five percent of crossing over. In the repulsion phase this results in high-*su* ratios, selfed ears producing 61 percent of *su* seeds and backcrosses by and on *su* producing 62 and 93.9 percent of *su* seeds, respectively. In the coupling phase the ears are low-*su* and the corresponding percentages are 3.2, 38 and 9.7, respectively.

7. Although *sp* has many of the characteristics of a haplo-viable deficiency, cytological studies have failed to disclose such a chromosomal condition.

8. The gene *lo*, which appeared in a stock segregating for *sp*, is a gametic lethal with unique effects. It eliminates practically all the megaspores which receive it but has no effect, or a slightly stimulating effect, upon the male gametophyte.

9. The *lo* gene is also linked with the *su* locus, crossing over being approximately two percent between *lo* and *su*. In the repulsion phase the percents of *su* seeds resulting from selfing and backcrossing by and on *su* are 47.1, 95.2, and 48.5, respectively. In the coupling phase the corresponding percents are 1.8, 2.4, and 51.3, respectively.

10. The *lo* gene is not allelic to *sp*. Plants heterozygous for *sp*, *lo*, and *su* produce 5.2 percent of sugary seeds when selfed, 8.5 percent when backcrossed by *su*, and 91.6 percent when backcrossed on *su*.

11. Various linkage tests with other genes on chromosome 4 show that the order of the genes is probably *Ts5 la sp su lo de16 Tu gl3*.

We are indebted to MISS FRANCES CLARK and DR. R. G. REEVES for preparation and examination of part of the pachytene material. Also, MISS CLARK found the differences observed in the microspores between *sp* and +.

LITERATURE CITED

DEMEREC, M., 1940 A comparison between the X-ray and the spontaneous Notches (Abstract) Genetics **25**: 115–116.

DEMEREC, M., and HOOVER, M. E., 1936 Three related X chromosome deficiencies in *Drosophila melanogaster*. J. Hered. **27**: 206–212.

EMERSON, R. A., BEADLE, G. W., and FRASER, A. C., 1935 A summary of linkage studies in maize. Mem. Cornell Agric. Expt. Sta. **180**: 1–83.

IMMER, F. R., 1930 Formulae and tables for calculating linkage intensities. Genetics **15**: 81–98.

KARPER, R. E., 1930 The effect of a single gene upon development in the heterozygote in sorghum. J. Hered. **21**: 187–192.

MANGELSDORF, P. C., 1928 The effects of a lethal on the heterozygote in maize. J. Hered. **19**: 123–131.

1931 Modification of Mendelian ratios in maize by mechanical separation of gametes. Proc. Nat. Acad. Sci. **17**: 698–700.

1932 Mechanical separation of gametes in maize. J. Hered **23**: 289-295.

RENNER, O., 1921 Heterogamie im weiblichen Geschlecht und Embryosackentwicklung bei den Oenotheren. Z. Bot. **13**: 609–621.

RHOADES, M. M., and RHOADES, V. H., 1939 Genetic studies with factors in the tenth chromosome in maize. Genetics **24**: 302–314.

ROBERTSON, D. W., 1932 The effect of a lethal in the heterozygous condition on barley development. Colorado Agr. Expt. Sta. Tech. Bul. **1**: 1–12.

SINGLETON, W. RALPH, 1932 Complete elimination of certain classes of gametes in Zea. Proc. Sixth Int. Cong. Genet. **2**: 182–184.

1940 Influence of female stock on the functioning of small pollen male gametes. Proc. Nat. Acad. Sci. **26**: 102–104.

SLIZYNSKA, H., 1938 Salivary chromosome analysis of the white-facet region of *Drosophila melanogaster*. Genetics **23**: 291–299.

SLIZYNSKI, B. M., 1938 Salivary chromosome studies of lethals in *Drosophila melanogaster*. Genetics **23**: 283–290.

STADLER, L. J., 1933 On the genetic nature of induced mutations in plants. II. A haplo-viable deficiency in maize. Missouri Agr. Exp. Sta. Research Bul. **204**: 1–29.

1935 Genetic behavior of a haplo-viable internal deficiency in maize. Amer. Nat. **69**: 80–81. (Abst.)