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Cytological and Genetic Studies of Sterility in Inbred and Hybrid Maize

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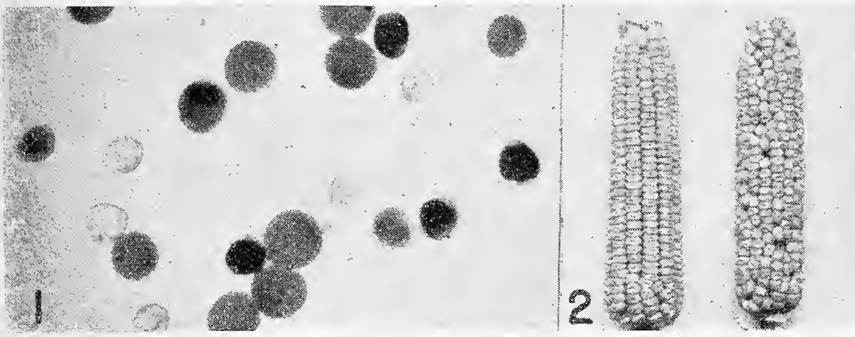
Cytological and Genetic Studies of Sterility in Inbred and Hybrid Maize[1]

Frances J. Clark

A discussion of sterility in maize has applications to both practical and theoretical problems. One aspect is related to the increased growing of hybrid corn in recent years entailing more extensive breeding programs for the production of inbreds and hybrids. It is of practical value to determine and eliminate causes of sterility in inbred lines in order that uniform production may be assured in hybrid corn.

Partial or complete sterility may be manifested in corn by poorly filled ears, aborted pollen, and defective seeds, or a combination of these effects (Figures 1 and 2). Hereditary and non-hereditary failure of seed formation have been described by Mangelsdorf (22). Certain environmental conditions, such as relative growth rates of tassels



Figures 1 and 2.

Figure 1. Photomicrograph of pollen, stained with a weak solution of iodine, showing two types of aborted pollen grains. Smaller grains are darker in the photograph, and empty grains have no included starch. The large grains are normal.

Figure 2. Semi-sterile ear (right) and normal ear of maize. The semi-sterile ear has about 50 percent aborted ovules.

and ears, insect injury, diseases and growing conditions, may also change the development of ears and tassels. Usually such factors may be separated from sterility caused by aberrant chromosome behavior or genetic factors for lethal effects on gametes. However, if the silks receive insufficient pollen, the resulting ear often cannot be distinguished from a partially sterile one. Sterility which is inherent in the genetic or chromosomal constitution of the plant will be transmitted to succeeding generations through the pollen or egg cells

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or through both, and usually results in relatively consistent percentages of aborted ovules or pollen in the progeny.

The results of inbreeding and of crossing inbred lines have been discussed by Jones (14, 15, 16), Jones and Mangelsdorf (18), Jenkins (13), Sprague (37) and others. The problem of hybrid vigor and aspects of heterosis have been reviewed by East (10), Singleton (35) and Jones (17). A decrease in size and vigor of a plant generally accompanies inbreeding and the approach to homozygosity. Certain lines of maize become difficult to maintain because of such weakening, and are particularly susceptible to unfavorable growing conditions. Hybrids from such inbreds may, however, be quite vigorous. This fact has been given theoretical consideration by Jones (17). Causes of poorly filled ears of corn and particularly weak plants have been determined for some stocks by detailed genetic and chromosomal analyses, but such methods have not been generally applied in corn breeding programs.

Problems of theoretical interest are also associated with a cytological study of sterility in maize. Singleton (35) and Dobzhansky and Rhoades (9) have suggested cytological and genetic techniques for determining and locating factors responsible for heterosis. Burnham and Cartledge (7) have outlined a method for locating factors involved in resistance to disease. Theoretical significance can also be given to the variations found among a large number of plants, as in a breeding program. Types of plants that are aberrant in leaf growth, chlorophyll patterns, plant form and fertility of gametes are often observed. The frequency of occurrence of such changes constitutes a special problem to be attacked only in experiments designed for the purpose. However, when large numbers of plants are grown it is possible to observe the kinds of changes which occur and to investigate the causes.

The following report considers cytological and genetic studies of some types of sterility encountered in inbreds and hybrids. Most of the report is based on progenies from semi-sterile ears discovered in a field corn yield test in 1938. Open-pollinated ears were harvested from varieties and hybrids grown in the test plot for calculations of yield. A total of 14,916 ears were harvested from 311 varieties and hybrids included in this test. Of this number, 26 including 22 different hybrids were classified as semi-sterile on the basis of the general appearance of the ears. Twenty-four of these were grown in the next generation. Of this number it was found that the sterility was transmitted to succeeding generations in 12 of the progenies; nine of the progenies had no transmission of the sterility; three of the progenies had possible transmission of the sterility, and two have not as yet been grown. These results give a maximum occurrence of changes causing sterility of 17 in 14,916, or about 1 per 1,000. This cannot be used as a measure of the frequency of mutations, the term being used in a broad sense, since the data were obtained from so many different lines, but is an approximation of the frequency. As such, it may be compared in a general way only with values found in experiments designed to determine the frequency of naturally occurring mutational changes. The cytological and genetic analyses of the progenies from the semi-sterile ears were made in order to determine the kinds of naturally occurring changes which may be detected more particularly with cytological methods.

Another series of semi-sterile ears used in this investigation was obtained from various hybrids grown in other test plots. Six of these were from hybrids with the inbred Connecticut 243; five were from other field corn hybrids, and one was from a sweet corn hybrid. The sterility was found to be transmitted to six of the progenies grown. Five of the progenies showed no transmission of the sterility, and one a possible transmission of the sterility. The progeny from the semi-sterile ear having questionable transmission in this group of hybrid ears is difficult to classify both genetically and cytologically since this hybrid, one with Connecticut 243, segregates a striped plant which is partially ear-sterile, does not always mature and is not inherited as a simple recessive.

The transmissible sterilities identified in the two groups of semi-sterile ears were found to be due to three general causes: (a) translocation between non-homologous chromosomes, (b) inversions of regions of chromosomes and (c) genetic factors for pollen or ovule semi-lethals in which no cytological change could be found. A summary of the results obtained in these series of semi-sterile ears is given in Table 1. Six different translocations have been found, one inversion and three definite pollen or ovule semi-lethals, with the addition of three possible semi-lethals. The progenies in which transmission of the sterility was definitely established showed semi-sterility in approximately 50 percent of the plants, whereas in progenies with doubtful transmission of the sterility about 25 percent of the plants were classified as segregating defective gametes.

The translocations and the inversion that have been identified are different from any that have been previously reported. This indicates that the changes are of a spontaneous nature and not due to any contamination from genetic stocks grown at the same time.

Five inbred lines of dent corn have also been examined. Hybrids with one of the inbreds were found to be semi-sterile, and two of the inbreds were characterized by some pollen abortion. A cytological examination was made to determine the causes of the sterility. Two inbreds were found to have variable amounts of asynapsis; one was found to have a small inversion; one was found to be homozygous for a translocation, and one was found to be mosaic for a chromosomal change. The last inbred is also characterized by degenerative changes in ear development, but any relation between such development and the cytological changes has not been established. One of the inbreds having asynapsis has also been found to segregate a plant with a striped chlorophyll pattern, but the segregation is not that of a Mendelian recessive.

TABLE 1. SUMMARY OF RESULTS FROM THE PROGENIES OF 36 SEMI-STERILE EARS IN FIELD CORN AND SWEET CORN HYBRIDS.

Analysis of the progenies	Field corn test 1938			Other hybrids		
	Semi-sterility transmitted	Doubtful transmission	No transmission	Semi-sterility transmitted	Doubtful transmission	No transmission
Number of ears tested	12[1]	3	9	6[1]	1	5
Number of plants examined (pollen and ears)	481	70	103	74	40	47
Average percent of plants having semi-sterility	45.8	29.5	0	51.1	22.5	0
Translocations	4	0	0	2	0	0
Inversions	4	0	0	0	0	0
Pollen or ovule lethals	2	3?	0	1	1[2]	0
Number of plants examined cytologically	38	0	10	5	3	0

[1] All of the progenies have not as yet been examined cytologically.

[2] This hybrid with C243 segregated a striped plant that is partially ear-sterile, does not always mature and is not inherited as a simple recessive.

The results of the cytological and genetic study of the inbreds and hybrids will be discussed under the following divisions: asynapsis, inversions, translocations, pollen and ovule semi-lethals, and degenerative changes. Unless otherwise noted, all preparations made were aceto-carmine smears from anthers fixed in a fixing solution composed of 3 parts 95 percent ethyl alcohol and 1 part glacial acetic acid, according to the method of McClintock (24).

ASYNAPSIS

The failure of the chromsomes to maintain close synapsis in the meiotic divisions has been found in a number of plants (3, 11, 12, 19, 20, 28, 31). Beadle (1, 2) found such a condition in maize to be inherited as a simple recessive gene (*as*). Variable numbers of gametes are aborted as a result of the action of this gene since one or more chromosomes fail to be included in the nuclei of part of the developing gametes. An examination of the pollen in inbred C243 revealed that about 50 percent of the pollen was of aborted grains—small or without any included starch grains. A cytological examination of meiosis indicated that the cause of this partial pollen sterility was in the lack of complete synapsis of the chromosomes. It is doubtful whether this condition is the same as that reported by Beadle, referred to above, since the asynapsis is not as complete, and the ears show very little if any sterility. Counts of microsporocytes at the diakinesis stage of meiosis are given in Tables 2 and 3.

TABLE 2. COUNTS OF THE NUMBERS OF CELLS HAVING UNIVALENTS AT DIAKINESIS AND METAPHASE. FROM TWO PLANTS OF INBRED C243.

Plant	Number of cells with 10 bivalents	Number of cells with univalents	Total cells	Percent of cells with asynapsis
38-392-1				
Diakinesis	107	234	341	68.6
Metaphase	25	138	163	84.7
42G 17-3				
Diakinesis	26	34	60	56.7

TABLE 3. THE 406 CELLS FROM COLUMN TWO OF TABLE 2 CLASSIFIED ACCORDING TO THE NUMBER OF UNIVALENTS.

Plant	Number of univalents 2	4	6	8	10	12	20
38-392-1	178	130	48	13	1	1	1
42G 17-3	20	10	3	1	0	0	0

It is evident that most of the cells had only one or two chromosomes which did not maintain their synapsis up to the time of diakinesis and metaphase. In many cells in which complete separation of some homologues was observed there were also one or more of the bivalents associated only at the ends. The number of chromosomes with partial asynapsis had no relation to the number of univalents in the cell. When univalents are present in the meiotic divisions, they are often not included in either of the two daughter nuclei due to their lagging on the spindle or being left off the spindle figure. Small micronuclei which become pycnotic develop from univalents excluded from developing telophase nuclei. This is similar to the behavior of fragment chromosomes (26). Quartets that were counted with reference to the presence of micronuclei are tabulated in Table 4.

TABLE 4. NUMBERS OF QUARTETS WITH MICRONUCLEI AND THE NUMBER OF MICRONUCLEI IN THE MICROSPORES.

Plant	38-392-1	42G 17-1	42G 17-2
Quartets with micronuclei	30	113	110
Quartets with no micronuclei	20	25	10
Microspores with:			
0 micronuclei	119	249	164
1 "	61	227	210
2 "	12	60	82
3 "	6	11	21
4 "	1	5	3
5 "	1	0	0
Percent of microspores with micronuclei	40.5	55.0	65.8

The number of micronuclei in a quartet is indicative of the amount of pollen abortion to expect, although a chromosome left out in a previous division is distributed at random to one of four microspores and may be included in a developing nucleus. Two pollen counts were made of two plants of C243, one having 76 percent small and empty grains, the other having 47 percent small and empty grains. The pollen abortion resulting from spores which failed to receive a complete chromosome complement may be variable if the amount of asynapsis is dependent upon environmental conditions. Some indication of this was found in comparing plants grown in the field and in the greenhouse. Powers and Dahl (29) also found evidence that the amount of asynapsis in maize may depend on environmental conditions.

In addition to the plants of C243 which were found to have asynapsis as described above, inbred C14, three plants of which were examined, was found to have some asynapsis. Four plants of the hybrid C243 × C14 were found to have complete pairing of the chromosomes, and likewise complete synapsis was found in one plant each of C14 × C677, Ldg × C243 and C243 × miniature seed. These results show that the asynapsis of both C243 and C14 disappear upon hybridization. If it is gene-controlled, it must be recessive and the two lines must have different genes for asynapsis.

The transmission of the tendency toward asynapsis was tested by examining pollen of plants of the progeny of backcrosses with C243. The backcross, 243 × (243 × 14) gave 23 plants with normal pollen and 16 plants with aborted pollen. The backcross 243 × [Kr (Osf) × 243] gave 29 plants with normal pollen and eight plants with aborted pollen. A 1:1 ratio would be expected from a simple gene ratio. Transmission is effected through both pollen and eggs but, since these numbers are small, it cannot be stated definitely that the inheritance is that of a simple recessive gene. Partial asynapsis seems to occur frequently in inbred plants and may well have some physiological basis for expression.

INVERSIONS

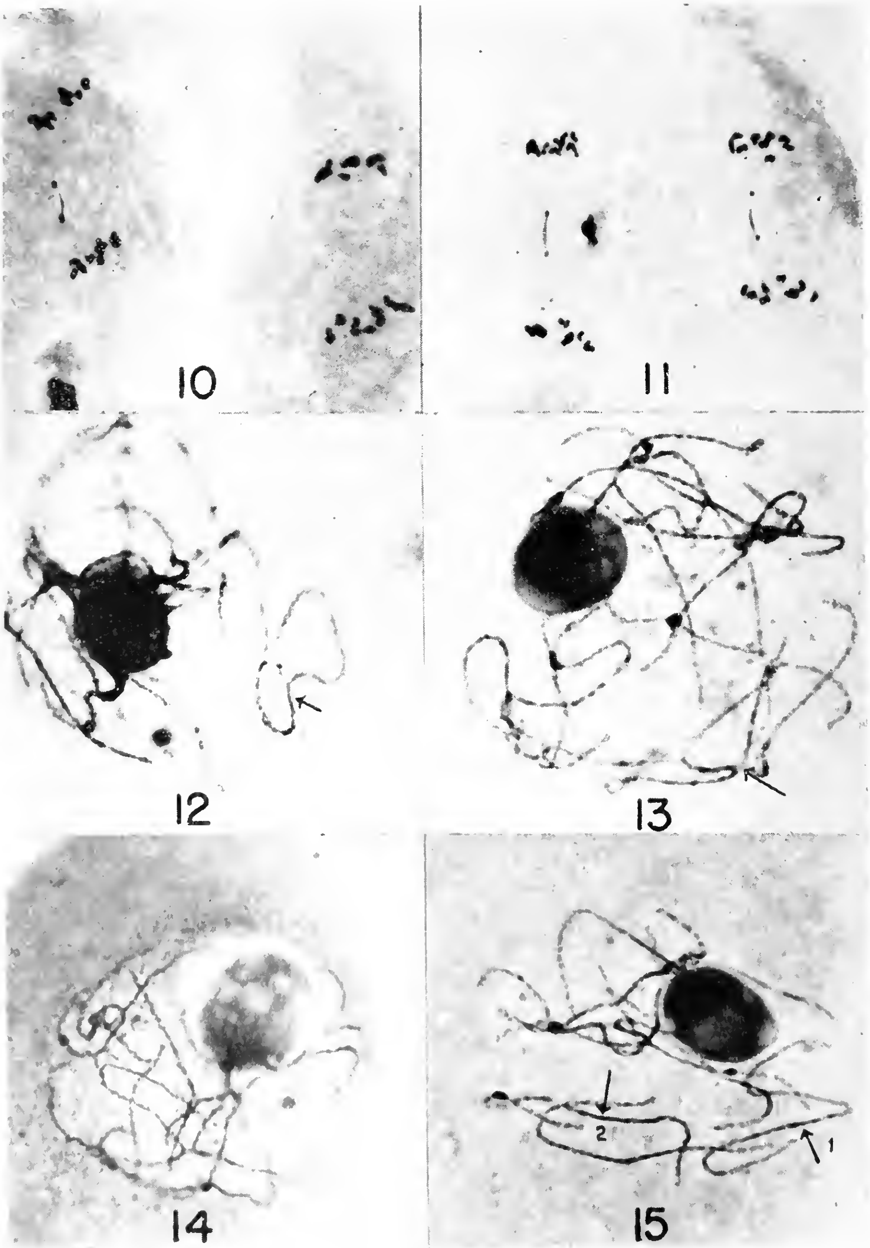
Two inversions were found during the examination of the inbreds and hybrids. One of these included the centromere, as was found by the prophase synapsis of the chromosomes and by the absence of bridges and fragments in the meiotic divisions. The other inversion does not include the centromere, as was shown by the cytological examination. Types of crossovers in inversions, and gametes produced are discussed by McClintock (26), Darlington (8) and Sturtevant and Beadle (39).

The inbred Iowa La has been grown for about 14 years. Cytological examination of plants of this inbred indicated that a small inversion was present although the inbred might have been expected to be free from aberrations after the period of inbreeding. One plant was examined cytologically in the summer of 1938 to determine the morphology of the chromosomes. Bridges and small fragments were found at the meiotic divisions, and it was concluded that the plant was heterozygous for a small inversion. The progeny of this plant was grown and 10 plants of the next generation were also found to be heterozygous for an inversion since bridges and fragments were found. It was concluded that the inversion is a short one since the fragment is so small, but the location on the chromosomes was not observed. There is some evidence that it may be the small terminal inversion, not including the centromere, on the short arm of chromosome 8 (previously reported by McClintock, 25), and the amount of crossing over is similar to that observed for the chromosome 8 inversion. The frequency of crossing over in this inversion was determined by counts of the types of bridges and fragments observed at the meiotic divisions (Table 5).

Table 5. Counts of Bridges and Fragments at the First Meiotic Division in Four Plants Having a Small Heterozygous Inversion.

Plant no.	Non-cross-overs	Bridge plus fragment	Bridge, no fragment	Fragment, no bridge	Two fragments	Total	Percent cross-overs
Anaphases							
836-6	46	13	2[1]	4	1 + 1?[2]	67	29.9
-7	34	3	1[1]	2	0	40	15.0
-9	7	2	0	0	0	9	22.2
-11	38	8	1 + 1?	2	0	50	22.0
Telophases							
-7	322	17	1	48	2[3]	390	17.4

[1] Fragment possibly attached to bridge.
[2] No bridge; bridge in one?
[3] One with bridge; one with no bridge.



Figures 10-15.
(Explanation on opposite page.)

A single crossover within the inverted region is represented in these counts by a bridge and a fragment. If the fragment is included in one of the developing telophase nuclei, only a bridge is observed; if the bridge should break early, only a fragment would be observed. A double crossover (4-strand) is represented by a bridge and two fragments at the first meiotic division. The occurrence of other types of crossovers cannot be demonstrated from the division I configurations, but the presence of bridges in the second meiotic division indicates that they may occur in this small inversion (Figures 10 and 11). Six cells were found (of a total of 140 counted) having a bridge in one of the sister cells resulting from the first meiotic division. A three-strand double crossover, one crossover in the inversion and one cross-over outside the inversion, results in a bridge in one of the sister cells at the second meiotic division. A triple crossover results in a bridge in both sister cells of division II, one sporocyte of this type being observed among the 140 counted. It is evident that numerous cross-overs take place within the limits of this small inversion.

The second inversion was found during the examination of the semi-sterile ears from the field corn test described above. The inversion was found in the following hybrids: 4-8 × R4, 540 × 4-8, 187-2 × 4-8A and Iowa Hybrid 13. Since 4-8 was one parent of the first three hybrids, it may be that this inbred carried the inversion and that it was found in the open pollinated ear of Iowa Hybrid 13 through contamination. Further examinations of the inbreds used in the making of these hybrids will have to be made before it is certain that the inbred 4-8 was heterozygous or homozygous for the inversion, or whether it occurred spontaneously in a generation prior to making the hybrids.

The limits of the inversions are shown diagramatically in Figure 3, and photographs are shown in Figures 12 and 13. Since the inversion includes the centromere, crossovers do not result in bridges and fragments at the meiotic divisions. However, duplicated and deficient gametes are formed as a result of crossing over, and aborted pollen grains and semi-sterile ears are characteristic. Inversions

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FIGURE 10. Chromosome bridge at anaphase of the second meiotic division in one of the sister cells. See text for explanation. Plant 40-836-5 (Pedigree Iowa La.) Magnification × 500.

FIGURE 11. Bridges at anaphase of the second meiotic division in both sister cells. See text for explanation. Plant 40-836-5. (Pedigree Iowa La). × 500.

FIGURES 12 AND 13. Two sporocytes heterozygous for the inversion on chromosome 1. The loop configuration is at the lower part of each photograph. The arrows point to the centromeres. Plant 39-1428-2. (Pedigree 4-8×R4). ×950.

FIGURE 14. Heterozygous translocation between chromosomes 1 and 2. A drawing of this translocation is given in figure 9. Plant 38-382-1. (Pedigree 237 Q2L4A). × 750.

FIGURE 15. Photomicrograph of a sporocyte from the same plant which had the sporocyte shown in figures 9 and 14. In this cell there was no translocation between chromosomes 1 and 2. The numbers 1 and 2 on the photograph are placed by the respective chromosomes. The arrows point to the centromere regions. Plant 38-382-1. (Pedigree 237 Q2L4A). × 750.

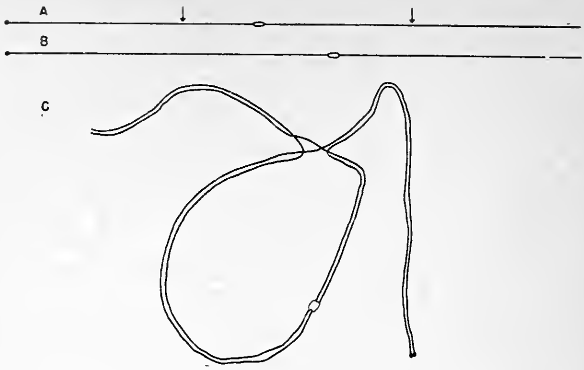

FIGURE 3. A. Normal chromosome 1. The arrows mark the approximate limits of the inversion.

B. Inverted chromosome 1.

C. Outline drawing of synapsis of normal and inverted homologues at mid-prophase of meiosis. Plant 42G 8-1 (Pedigree 540 × 4-8).

The centromere region in all drawings is indicated by the lighter open circle.

including centromeres are rarely reported (Sturtevant and Beadle 39, p. 139). They may occur as frequently as other inversions, but the method of detecting them in maize by the presence of anaphase bridges and fragments does not allow the identification of those inversions including the centromere. The amount of pollen abortion in plants from two of the hybrids is given in Table 6.

TABLE 6. COUNTS OF THE POLLEN PRODUCED BY FOUR PLANTS HAVING A HETEROZYGOUS INVERSION ON CHROMOSOME 1.

Pedigree	Plant	Normal pollen grains	Empty grains	Small grains	Total	Percent aborted
187-2 × 4-8A	40-899-15	691	269	6	966	28.5
		647	252	12	911	29.0
		786	318	11	1115	29.5
	42G 2-3	539	365	38	942	42.8
540 × 4-8	42G 8-1	768	146	—	914	16.0
	8-3	813	227	—	1040	22.0

Since a single crossover within the inverted region results in two normal chromatids and two deficient chromatids from one sporocyte, the amount of crossing over is approximately double the amount of pollen abortion.

If a plant heterozygous for the inversion on chromosome 1 is selfed, the resulting progeny should have one-fourth plants with normal chromosome 1, one-fourth homozygous for the inverted chromosome 1 and one-half heterozygous for the inversion. If a plant heterozygous for the inversion is used as either the male or female parent

TABLE 7. PROGENY OF PLANTS HETEROZYGOUS FOR INVERSION ON CHROMOSOME 1.

Type of cross	No. of plants without inversion	No. of semi-sterile plants	Total	Percent with inversion
Hybrid ear open-pollinated				
4-8 × R4	11	8[1]	19	42.1
540 × 4-8	22	18[2]	40	45.0
187-2 × 4-8A	15	14[1]	29	48.3
Iowa Hybrid 13	22	3	25	12.0
Selfed ears from				
187-2 × 4-8A	2	15[1]	17	88.2
	10	6	16	37.5
	5	7	12	58.3
	3	9	12	75.0
Heterozygous inversion				
× or on normal	11	8	19	42.1
	14	7	21	33.3
	8	11	19	57.9
Totals	123	106	229	46.3

[1] One plant examined cytologically.
[2] Two plants examined cytologically.

in a cross with a normal stock, one-half the resulting progeny should be heterozygous for the inversion and one-half should have normal chromosomes. When any of these three types of crosses is made, there should be one-half the progeny with normal pollen and one-half the progeny with pollen segregating aborted grains, the latter representing plants heterozygous for the inversion. Ears of most plants heterozygous for this inversion are recognizable as semi-sterile. Transmission of the inversion was tested by making the crosses described above. The data are given in Table 7. Classifications were based on

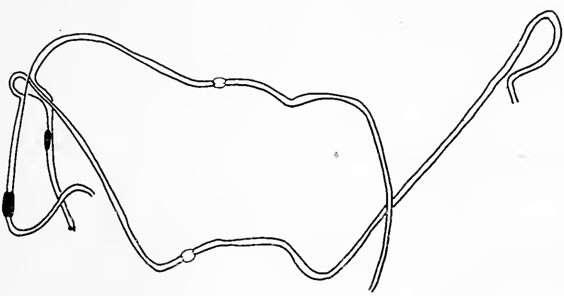

FIGURE 4. Outline drawing of the synapsis at mid-prophase of meiosis in a plant heterozygous for a translocation between chromosomes 1 and 2. An inbred (696-3c) was found to be homozygous for the translocation. Chromosome 1 has prominent chromomeres at the end of the short arm and a heterozygous knob on the short arm. Chromosome 2 has a homozygous knob on the long arm. Plant 38-1210-8 (Pedigree 696-3c × Pamunkey).

pollen or ear examinations and in some instances were verified by cytological examination. Crosses between the inversions from different sources will be made to determine if they are identical. A further proof of this would be in finding the inversion in the inbred 4-8.

TRANSLOCATIONS

Seven different translocations have been identified during the analysis of the semi-sterile ears. One of the translocations is of particular interest since 696-3c, a Lancaster field corn inbred, was found to be homozygous for it.

It was observed that all plants resulting from crosses with this inbred had semi-sterile ears. The hybrid 696-3c × Pamunkey was examined cytologically, and the translocation was found to be present in the heterozygous condition (Figure 4). Approximately 0.4 of the short arm of chromosome 1 was exchanged with about 0.5 of the long arm of chromosome 2. The translocation had occurred in the inbred and became homozygous during inbreeding. Since no apparent growth change occurred in the inbred as a result of the translocation it was not detected until hybrids with it were grown and the translocation became heterozygous. Roberts (32), however, found evidence statistically that some homozygous translocations may affect the development of the maize plant in the rate of maturing and total growth.


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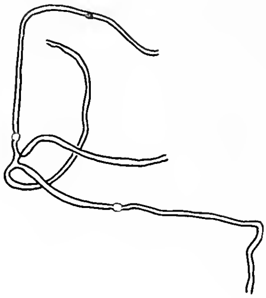
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7

FIGURES 5, 6 AND 7.
Outline drawings of synapsed chromosomes at the mid-prophase of meiosis.

FIGURE 5. Heterozygous translocation between chromosome 1 and 6. The translocation occurred near the ends of each chromosome. Chromosome 6 is attached to the nucleolus and is characterized in this plant by a small and a large knob on the long arm. Plant 40-894-10. (Pedigree 682-8c × 243).

FIGURE 6. Heterozygous translocation between chromosomes 2 and 5. The translocation occurred about mid-way on the long arm of chromosome 2 and very near the centromere on chromosome 5. Chromosome 5 in this plant was heterozygous for a small knob on the long arm. Plant 39-732-4. (Pedigree 609-5 × 2).

FIGURE 7. Heterozygous translocation between chromosomes 6 and 9. The translocation occurred on chromosome 6 beyond the small knob and on chromosome 9 near the end of the short arm. Plant 39-724-6. (Pedigree 474-7 × 474-5).

FIGURE 8. Photograph of "streaked dwarf" type of plant. The upper leaves show the typical streaking and the lower leaves are normal. This plant had not yet lost the upright habit of growth. Plant 38-15-8. (Pedigree 243 $\times$ 14).

In a breeding program small changes would probably not influence the selection within inbred lines.

The six other translocations were found in the progenies of the semi-sterile ears. A sweet corn hybrid was found to have a translocation between chromosomes 6 and 9. Translocations found among the field corn hybrids were between chromosomes 1 and 6, 1 and 7, 2 and 5, 4 and 6, and 6 and 8 (Figures 5, 6 and 7). The presence of chromosome 6 in four out of six of the translocations occurring spontaneously may suggest that the naturally occurring breakage and reattachment of chromosomes is not random. Each of the 10 chromosomes would be expected to occur 1.2 times in six translocations taking place at random between the chromosomes. The presence of chromosome 6 in four of the translocations is significant ($P<.02$), but the number of translocations should be increased before any general conclusion may be drawn.

The disjunction of the chromosomes having translocated regions usually results in approximately 50 percent of the gametes containing duplications and deficiencies, although some low-sterile translocations have been reported (4). The disjunction of translocated chromosomes in wheat may not be random according to the report of Thompson and Hutcheson (40). Their calculations of the effect of crossing over on sterility, however, do not take into account that crossing over occurs in a four-strand stage. A representative count of the pollen produced by plants heterozygous for the translocation between chromosomes 6 and 4 is given in Table 8.

TABLE 8. TYPES OF POLLEN PRODUCED BY A PLANT FROM IOWEALTH HYBRID 129, HETEROZYGOUS FOR A TRANSLOCATION.

Plant	Normal pollen	Small grains	Empty	Total	Percent aborted
42G 11-1	476	248	231	955	50
11-2	664	235	188	1087	39
12-1	470	154	176	800	41

One of the ears harvested in the field corn test and classified superficially as semi-sterile appeared to be a mosaic. Half the ear was well filled with kernels while the other half was only partially filled. Seed from both halves of the ear was planted, and although 11 plants were examined cytologically (at diakinesis) and pollen was examined from 20 plants, there was no evidence of any semi-sterility. Ears from these plants were normal. It was concluded that the apparent semi-sterility of part of the ear was due to environmental or physiological causes.

POLLEN AND OVULE SEMI-LETHALS

Instances of a type of semi-sterility in which certain proportions of the gametes are defective but in which no visible chromosome aberrations can be detected have been reported in maize, (23, 30, 33, 34, 36). These factors may be located on the chromosomes by means of linkage with known genes, and may themselves be used as genetic characters useful in locating other genes. Three types of semi-lethals similar to those reported by others and not associated with detectable chromosome aberrations were found in the inbred and hybrid ears examined.

One of the pollen-segregating types was from the variety Kato. In the first generation from the open-pollinated ear there were 13 plants with normal pollen and one plant that was segregating empty pollen. In succeeding generations there were obtained 11 plants with segregating pollen (eight of these coming from a cross with the pollen parent having the segregating type) and 11 plants with normal pollen. Three of the segregating plants were examined cytologically, and it was found that the chromosomes were all apparently normal with no evidence of any deficiency, inversion or translocation. Any classification for the semi-sterility is based on pollen examinations since plants segregating empty pollen have normally filled ears. The pollen is about 30 percent aborted. Since it was found that the factor causing aborted pollen is transmitted through the pollen, it is suspected that a chromosomal aberration is present that may be small and was not detected in the cytological examinations.

From the hybrid W209-13K × Multiple Leaming 1936, a small-pollen type was isolated. In this instance plants that are segregating the small pollen (smaller in size than normal pollen grains but well filled with starch) also have some ovule abortion and produce ears appearing semi-sterile. From the open-pollinated ear of the hybrid, there were 11 plants with normal pollen and eight with segregating pollen. There were 16 plants with normal pollen and five with segregating pollen in the next generation from a selfed ear. A cross by *su* gave 18 plants with normal pollen and two with segregating pollen. As shown in Table 9, there is no alteration of the expected 1:1 ratio

TABLE 9. CROSSES OF *su* × TWO PLANTS SEGREGATING SMALL POLLEN AND *su*.

Plant	*Su* kernels	*su* kernels	Total
41-559-3	202	177	379
-28	95	92	187
Total	297	269	566
Expected	288	288	

indicating no linkage of the factor for small pollen and *su*. This factor for small pollen is different then from that reported by Singleton and Mangelsdorf, referred to above, as sp_1 reported by them is closely linked with *su*.

There was no evidence of any chromosomal deficiency, inversion or translocation from a cytological examination of a plant segregating the small pollen.

Another type of semi-sterility, similar to the lethal ovule (*lo*) factor reported by Singleton (33) and Singleton and Mangelsdorf

(36) was found in the hybrid 677 × 112-1. These inbred parents are the same as those from which Burnham isolated the *pa* gene (5, 6). The semi-sterility found here was characterized by ears which are semi-sterile in appearance due to the abortion of about 50 percent of the ovules, but the pollen produced by these plants is normal. It was determined that this is not the same *lo* previously reported by making crosses with *su*. There was no disturbance of the *Su* : *su* ratio indicating no linkage with *su* on chromosome 4. The numbers obtained were 227 *Su* and 76 *su* (25.1 percent).

In addition to the three types of pollen and ovule lethals just discussed, there were also three types which have not been grown beyond the first generation from the open-pollinated ears. For this reason they were classified in Table 1 as having questionable transmission of the sterility factors. From the hybrid La (Idt) × Kr (Osf) there were obtained 12 plants with normal pollen and two plants having some anthers with normal pollen and others with empty pollen. Twenty-two normal plants and four plants segregating empty and small grains were obtained from the hybrid 244. From another hybrid, Iowealth Hybrid AQ, 18 normal plants were obtained and nine plants with small or empty pollen grains. Some of the latter plants were very small with narrow leaves, and these may have represented deficiencies of some sort since no mature seed was obtained from them.

DEGENERATIVE CHANGES

In addition to a reduction in size and vigor of maize plants during inbreeding, abnormalities such as small seeds, chlorophyll deficiencies, dwarfed plants, sterile tassels and silkless ears may be found. Some of the variations indicate that mutational changes have occurred since the inheritance is that of simple recessive or dominant genes. When such types of plants occur during inbreeding, they may or may not be eliminated depending upon whether plants carrying the factors in the heterozygous condition are selected for continuing the lines. Other abnormalities occur sporadically, do not behave in inheritance as Mendelian recessives or dominants and may result ultimately in the loss of a particular inbred line.

An example of the latter type of change appeared in line 1-7-1-2, Connecticut 242, (16). A streaking of the leaves occurred, superficially resembling a diseased condition, and the line was ultimately lost. Another type of streaking of the leaves has appeared in the inbred C243, which also has some asynapsis of the chromosomes (Figure 8). The type of streaking, however, does not seem to be confined to this particular inbred. The first five or six leaves of plants of this type are normal. Later leaves have short white streaks, and seem longer and less turgid than normal leaves. When the ear shoot emerges, or after the silks are showing, the plant characteristically bends so that the top of the plant may be parallel to the surface of the ground. As the ear matures, the plant may continue to bend. The production of any mature seed on the ear depends on the degree to which the stripping and bending are manifest. The plants are shorter than their normal sibs.

A preliminary histological examination showed that cell divisions were normal in young leaf sheaths and there was no evidence of any small ring chromosome whose loss might account for the variegation (27). It was observed that cells surrounding the vascular bundles in the leaf may become abnormal in appearance and partially filled with an opaque substance. The subsequent death of these cells could account for the streaking and lack of stiffness of the leaves since in later stages some of the streaking resembles a necrosis.

Five plants which were the characteristic "streaked dwarf" type have been examined cytologically. Three of these were from hybrids with 243, two of which had 243 as the pollen parent (Ldg × 243 and 782-54 × 243) and one with 243 as seed parent (243 × 14). The other two were in the inbred C237 and a related hybrid, 615-11D × 615-9. Pollen from these plants and three additional streaked plants was essentially normal with no segregation type of pollen grain being present. The chromosomes were all apparently normal at the mid-prophase of meiosis, and there was no evidence of any deficiency, inversion or translocation. In addition two normal sib plants were examined cytologically. They were also found to have normally synapsed chromosomes and no chromosome aberration in the heterozygous condition. Pollen from these plants was normal with the exception of a few smaller pollen grains.

The "streaked dwarf" type of plant does not occur in a ratio expected on the basis of a simple recessive gene as shown in Table 10. Seven streaked plants were selfed. In the progeny of these there were 59 normal plants, and none was streaked.

TABLE 10. NUMBERS OF STREAKED AND NORMAL PLANTS RESULTING FROM CROSSES WITH THE "STREAKED DWARF" TYPE OF PLANT.

Plant	F_2		Backcross	
	Normal	Streaked	Normal	Streaked
40-127	4	0	—	—
174	10	0	—	—
814-12	8	1	—	—
815-9	38	1	—	—
814-6 × 2	—	—	14	3
Total	60	2	14	3
Expected	46.5	15.5	8.5	8.5

Another type of degenerative change was found in the line 1-7, C237, (16). At the seventeenth generation of inbreeding two sib lines were separated in this inbred. Ears of one line are more poorly filled than those of the sib line, resulting in a significantly lower yield of grain. Plants with the poor ears are taller than the sib line. Two plants of this poor-ear line were examined cytologically. One of these had sporocytes in which no chromosomal changes could be observed, and the synapsis of homologous chromosomes was normal. The other plant was examined more completely and there were found a few figures of heterozygous translocations. On eight preparations from different anthers, a total of 642 chromosomes could be identified in 137 sporocytes. Of these, five sporocytes had a heterozygous translocation between chromosomes 1 and 2 (Figures 9, 14 and 15), and five sporocytes had heterozygous translocations that were not definitely identified, but were probably between chromosomes 4 and 5 and chromosomes 4 and 10.

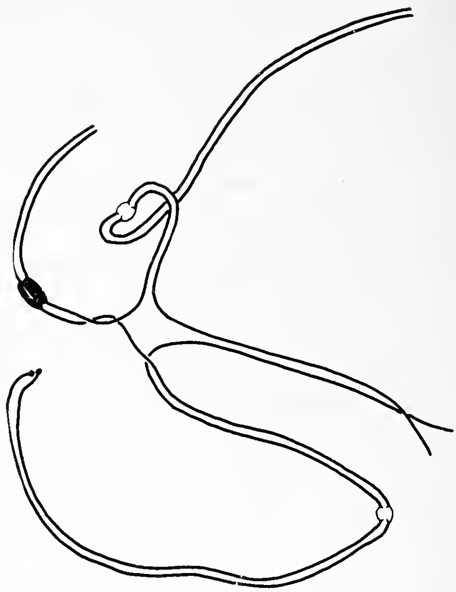

FIGURE 9. Heterozygous translocation between chromosomes 1 and 2 found in a few sporocytes of an anther. A photograph of the sporocyte from which the drawing was made is shown in figure 14. The translocation occurred near the knob on the long arm of chromosome 2 and about mid-way on the long arm of chromosome 1. Plant 38-382-1. (Pedigree 237 (Q21.4A).

Cytological examinations were also made of plants from a cross between the poor ear and normal sib lines. No chromosomal changes were observed in preparations from four plants although one of these had some non-homologous association. One sporocyte from preparations from another plant had a heterozygous translocation that was not completely identified. In preparations from a sixth plant a translocation figure was observed in one sporocyte at diakinesis and one figure of a 1-5 translocation was observed at pachytene. Pollen from a plant in this progeny had 13 percent small and empty grains. This is slightly more than the 3 to 4 percent aborted pollen found in normal plants.

Since a cytological examination is made of relatively few sporocytes, and part of the tassel is left to develop mature pollen, it is difficult to estimate the frequency of occurrence of any chromosomal changes taking place in sporocytes or pre-meiotic cells from an analysis of the chromosomes. However, if any changes are present in the pollen, they should be detected in the next generation. A count was made for any transmission of semi-sterility after using a plant from a cross with the poor-ear line as the pollen parent. From plants of this cross 50 normal ears were obtained and one semi-sterile. Another cross had 30 normal ears, one semi-sterile and one with a portion of the ear appearing semi-sterile. These have not as yet been examined cytologically to determine if any chromosomal change had been transmitted. These numbers are still too small to enable a determination of the frequency of occurrence of any chromosomal changes to be made.

DISCUSSION

In the foregoing account of sterility in inbred and hybrid maize the emphasis has been placed on the kind of change observed rather than on the frequency of occurrence. Any precise determination of the frequency of mutational or chromosomal changes must be made in experiments designed for such a purpose.

In each of the groups of semi-sterile ears used, 50 percent of the ears had no transmission of the sterility or doubtful transmission to succeeding generations (Table 1). This is in agreement with the observation that many poorly filled ears of corn are manifestations of environmental conditions and are not due to any inherent chromosomal changes which affect the fertility of the gametes. That chromosomal alterations do occur was shown by finding translocations and inversions in the succeeding generations from half the progenies grown.

The translocations reported here have arisen without any treatment and are thus considered of naturally occurring origin. They are different from any previously reported translocations. Burnham (5, 6) found a sterility in maize in inbred material, and Lindstrom (21) has emphasized that mutations may be readily isolated from inbred lines of maize. A comprehensive analysis of defective types of endosperm development originating in varieties, inbreds and hybrids has been presented by Mangelsdorf (22).

The frequency of spontaneous chromosomal changes (translocations and inversions) is not known for maize, but Stadler (38) found 0.9 percent of the control plants in an experiment with X-rays and ultra-violet radiation to have segregation for defective pollen. In totalling the ears harvested in the field corn test reported in this discussion, there was found a maximum of 0.11 percent plants with semi-sterile ears, those segregating defective ovules. Since many different lines were totalled in determining this value, it cannot be considered one derived from a "control".

If there is no deficiency associated with a translocation, or no selection against gametes carrying it, lines homozygous for a change are automatically established during inbreeding in the same manner that a gene for a plant character is obtained homozygous in an inbred line. The inbred 696-3c was found to be homozygous for a translocation between chromosomes 1 and 2. Another, a sweet corn inbred, was previously found at the Connecticut Experiment Station to produce all semi-sterile ears when crossed with other inbreds. This was also homozygous for a translocation (identified by Burnham). Inbreds homozygous for translocations are not detected, until crosses are made with them unless there is some effect on the growth of the plants. They are useless in the production of hybrids since all the resulting crossed progeny is semi-sterile.

The type of sterility which is transmitted either through the pollen or through the egg cells is also occasionally found in inbred and hybrid stocks. When the pollen is segregating defective types but the ears are normal, the condition cannot be recognized by field examination. An inbred carrying a factor for pollen abortion would be at no serious disadvantage if it were normal in other respects and produced adequate amounts of pollen in spite of the sterility. When a factor for ovule abortion is present and the pollen is normal, the condition would be easily recognized by the semi-sterile ears produced, and the line would be discarded for commercial production.

Degenerative changes which occur sporadically in inbred material are of interest in theoretical problems but are a hindrance in a practical breeding program. Many of these may have physiological or environmental bases for expression. They are difficult to analyse genetically and there is a possibility that the presence of modifying factors may affect the phenotype, particularly in instances in which the ratios differ significantly from theoretical Mendelian ratios. When sib lines differ after a number of years of inbreeding, as was found to be the case in line C237, the inferences may be drawn that the inbreds may be very susceptible to critical environmental conditions, that homozygosity has not been attained or that the frequency of changes affecting the phenotype of the plant is relatively high. In the line C237 there may also be a condition favoring a rather high frequency of chromosomal changes.

A cytological study of the inbreds and hybrids reported here has shown that many of the changes had occurred without producing any visible effect on the plant growth although the fertility of the gametes was altered. The analysis was undertaken to determine the causes of the sterilities and the types of changes which had occurred.

SUMMARY

An examination of the progenies of 36 semi-sterile ears occurring spontaneously among hybrids grown in test plots showed that 18 of the progenies transmitted the sterility to the next generation, four had

doubtful transmission of the sterility and 14 had no transmission of the sterility.

Six translocations, an inversion and three factors for lethal gametes were found among the progenies in which the sterility was transmitted to succeeding generations.

Five inbred lines of dent corn were analyzed cytologically to determine the causes of sterility in the inbreds and in hybrids with them. Two were characterized by variable amounts of asynapsis. One was found to have a small inversion and one was found to be homozygous for a translocation. The fifth inbred was studied in relation to a degenerative type of ear development and was found to have anthers with the majority of the sporocytes with normal chromosomes. A few sporocytes were found having heterozygous translocations.

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