

GENETICAL AND CYTOLOGICAL STUDIES OF SEMISTERILITY AND RELATED PHENOMENA IN MAIZE†

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This paper is a preliminary report of studies on two new partially sterile lines of maize derived from a pedigreed strain of genetic material grown at the University of Wisconsin in 1927. The parent line is unrelated to the stock in which semisterile-1, reported by Brink,⁴ arose. In semisterile-1, approximately 50 per cent of the pollen and ovules abort and, when selfed or crossed with normals, the plants throw a ratio of 1 normal : 1 semisterile. In order to account for these results and for the origin of semisterility, Brink⁴ and Brink and Burnham⁶ advanced the hypothesis that a chromosomal change involving non-homologues had occurred. Simple translocation of a portion of one chromosome to a non-homologue would give a plant in which, with random distribution in the two pairs, 50 per cent of the spores either would be deficient or would possess a portion in duplicate. This unbalance was assumed to be sufficient in both cases to cause abortion. From a semi-sterile plant selfed, a new normal type was isolated which, when crossed with standard normals, gives all semisterile plants in F_1 .⁶ The new normal, which should be homozygous for the translocation, was designated x -normal, as contrasted with the standard or o -normal. It has been found also that semisterile-1 shows linkage with factors in two different groups, $P\ br$ and $B\ lg$.⁵

To explain somewhat similar phenomena in *Datura*, Belling and Blakeslee² advanced the hypothesis of segmental interchange between two non-homologous chromosomes. Semisterility in *Stizolobium*, originally explained as due to two factors, has been reinterpreted more recently by Belling¹ on the basis of such interchange. According to this hypothesis, with random distribution, 50 per cent of the spores are deficient in chromatin material and are assumed to abort. A diagram of the assumed chromosomal constitution is given in figure 1. The combinations $A_1\ B_2$ and $A_2\ B_1$ are assumed to abort, while $A_1\ B_1$ and $A_2\ B_2$ form apparently normal spores. A plant with the constitution $A_2\ A_2\ B_2\ B_2$ would correspond to the x -normal type in maize. Both the interchange hypothesis and the simple translocation hypothesis fit the breeding data thus far obtained in maize. Although duplication of chromatin material may cause abortion in certain cases, as in the simple trisomic *Datura* called *Echinus* where about half of the pollen is devoid of starch,³ deficiency seems a more probable cause of abortion. This favors the idea of segmental interchange.

In maize it should be possible to obtain critical genetic evidence as to the type of change in different semisterile lines.

Classification for different degrees of sterility is based on estimates of the relative amounts of normal and aborted pollen. The two classes are easily distinguishable. The aborted grains are either entirely devoid of starch or nearly so, while the normal ones are well filled. Counts for sterility on the ear are made most easily from two to three weeks after pollination. For cytological study of meiosis in microsporocytes, the aceto-carmin smear method was used.

At diakinesis of the first meiotic division in normal plants ten bivalent chromosomes are found regularly. At the corresponding stage in semisterile-1 only eight bivalents occur, plus a group of four chromosomes arranged either in a ring or in a chain. In occasional cells there are ten bivalents. Apparently some chromosomal change has occurred which causes the association of non-homologues in this semisterile race. With the conjugation of homologous ends, ring configuration might be expected

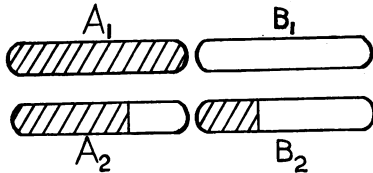


FIGURE 1

Diagrammatic representation of the assumed chromosomal constitution of a semisterile plant on the basis of segmental interchange. One end of chromosome A has interchanged with an end of chromosome B.

aborted spores result. More study is necessary to determine the detailed behavior of the altered chromosomes. The longest chromosome pair appears to be included in the group of four. Since semisterile-1 is linked with the *B lg* and *P br* groups, and since *B lg* is the third from the largest chromosome,¹⁰ the longest one in maize must carry the factors in the *P br* group.

The new partially sterile lines were derived from sibs in a progeny of twenty-six plants from a homozygous waxy ear. Thirty-four plants from the same ear have been grown since, but all were normal. Crosses were obtained with only two of the partially sterile plants.

The first partially sterile line to be considered shows the typical breeding behavior of a semisterile. An average for eight plants showed 56.9 per cent pollen abortion. Although more than 50 per cent sterile, it breeds as a semisterile and will be referred to hereafter as semisterile-2. The cross of semisterile-2 with *x*-normal-1, i.e., the *x*-normal of semisterile-1, gives all partially sterile plants in F_1 . About half of these are semi-

if segmental interchange had occurred; although, with crossing-over in the "four-strand" stage, rings might occur as a result of simple translocation. Referring to figure 1, which represents segmental interchange, the order of the chromosomes in the ring would be $A_1 A_2 B_1 B_2$. Only when alternate chromosomes in the ring go to the same pole would normal spores result. When adjacent non-homologues go to the same pole, i.e., A_2 with B_1 and A_1 with B_2 ,

sterile. The other half are more than 75 per cent sterile, and will be referred to as the 75+ per cent sterile class. Pollen counts on eleven plants in the latter class gave an average of 78.5 per cent sterility. Kernel counts on eight of the same plants gave an average of 83.7 per cent ovule abortion. These results in F_1 indicate that semisteriles-1 and -2 are different. Had they been similar, there would have been a ratio of 1 normal : 1 semisterile. As far as tested, the semisterile F_1 plants are of the semisterile-1 type. Plants in the 75+ per cent sterile class were crossed with standard normals. The results from these crosses, given in the last line of table 1, show a close approximation to a ratio of one normal: one 75+ per cent sterile : two semisterile. These are the results expected if the two semisteriles are entirely independent of each other. The intermediate classes will be considered later.

TABLE 1

SEGREGATION FOR WAXY AND PARTIAL STERILITY IN THE F_1 FROM THE CROSS OF NORMAL WITH (75+ PER CENT STERILE FROM: SEMISTERILE-2 $wx wx$ \times SEMISTERILE-1 $Wx Wx$)

	NUMBER OF PLANTS											
	NORMAL		75+ PER CENT STERILE				SEMISTERILE				INTERMEDIATE	
	$Wx Wx$	$Wx wx$	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$	30 ± PER CENT STERILE	65 ± PER CENT STERILE
Cross A,												
Normal = $Wx Wx$	74	10	18	61	86	80	1	..	1	9		
	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$	$Wx wx$	$wx wx$
Cross B,												
Normal = $wx wx$	8	1	3	6	11	10	1	2	..	1		
Total	82	11	21	67	97	90	2	2	1	10		
Total	93		88		187		4		11			

The above conclusion is supported also by cytological evidence. At diakinesis in microsporocytes of the 75+ per cent sterile plants, there are six bivalent chromosomes plus two separate groups of four chromosomes each. Two separate groups would be expected if semisterile-2 arose as the result of an interchange or a translocation involving two non-homologous chromosomes, neither of which is involved in semisterile-1. In semisterile-2, rings are formed frequently, but chain configurations are more frequent than in semisterile-1. Possibly the size of the piece involved in the change may affect the relative frequency of chains and rings. Chains might be more frequent where the piece was very small in relation to the size of the chromosomes concerned. Two of the smaller chromosome pairs apparently are involved in semisterile-2. The deviation from 50 per cent sterility may be due to a lack of random distribution in the two pairs.

The segregation for waxy in the crosses of 75+ per cent sterile with standard normals is shown also in table 1. Linkage of semisterile-2 with wx should give, in cross A with $Wx Wx$, an excess of the two parental

types: normal $Wx Wx$, and 75+ per cent $Wx wx$. In cross B with $wx wx$, the reverse should be true. A 1:1 ratio is expected in the semisterile class with or without linkage. Although the numbers are small, apparently waxy is linked with semisterile-2, the calculated cross-over value being 17.7 per cent. The amount of recombination in the two classes is very different, but possibly this is not significant.

The plants showing intermediate degrees of sterility (table 1) have not been tested thoroughly. Cytological evidence and abnormal genetic ratios thus far obtained indicate that they are 21-chromosome plants. I am indebted to Dr. McClintock for suggesting the hypothesis that these intermediate types have an extra chromosome of one of the kinds composing the ring. The presence of an extra chromosome in a spore enables certain deficient combinations to survive which otherwise would abort, thus markedly reducing the amount of sterility. The four plants belonging to the class intermediate between normal and 50 per cent sterile showed 27.1, 32.9, 33.1 and 39.8 per cent pollen sterility, respectively. Only two of these were examined cytologically. Both were found to have 21 chromosomes. At diakinesis in the one showing 32.9 per cent aborted pollen there is usually a group of five chromosomes in addition to eight bivalents, making a total of twenty-one. In certain cells, the group forms a chain; in others a single chromosome is attached at its ends to two bivalents, showing that the extra chromosome belongs to one of the groups involved in semi-sterility. If, in partially sterile 20-chromosome plants, three chromosomes of the quadripartite ring go to the same pole, spores with $n + 1$ chromosomes will be formed. Anaphases have been counted in which there were eleven chromosomes at one pole and only nine at the other. The amount of sterility in the resulting 21-chromosome plant depends on which three chromosomes in the ring went to the same pole to form the $n + 1$ spore. Referring to figure 1, the two types of combinations possible in the $n + 1$ spore would be $A_1 A_2$ with B_2 and $A_1 A_2$ with B_1 . The amount of sterility also depends on whether or not there is random pairing in the set containing the extra chromosome. There might be preferential pairing of the two normal chromosomes. The frequency with which the extra chromosome lags at meiosis and is eliminated also will affect the degree of sterility.

From 75+ per cent sterile plants, there should be two classes of intermediates: those falling between semisterile and normal, and those between semisterile and 75+ per cent sterile. The former class is distinct; but in the latter there is probably some overlapping with 20-chromosome semisterile-2 plants which show an average of about 57 per cent abortion. The upper class of intermediates has not been examined cytologically.

Data from two 21-chromosome plants backcrossed to a $c sh wx$ stock are given in table 2. On the basis of interchange as represented in figure 1,

both plants are assumed to have arisen from an $n + 1$ egg of the type $A_1 A_2 B_2$. The extra chromosome in B11-13 belongs probably to the $sh wx$ set. In the crosses with both plants, the ratios for sh and c deviate widely from the normal 1 : 1 ratios obtained from semisterile sibs. Close fits to the observed numbers and also to the degree of sterility may be obtained by assuming the proper amount of preferential pairing and the proper frequency of lagging univalents in the trisomic groups. The assumption that the break in the $sh wx$ chromosome occurred on the side of wx away from sh , placing sh about 38 units from the break, fits the observed data. Further study is necessary to determine the amounts of preferential pairing and lagging, and to determine the frequency of crossing over between sh and semisterile-2.

TABLE 2

SUMMARY OF BACKCROSS DATA FROM TWO EXCEPTIONAL 21-CHROMOSOME PLANTS IN THE PROGENY OF THE CROSS: [75+ PER CENT STERILE FROM ($C Sh wx \times ? Sh Wx$)] \times NORMAL $c sh wx$

CROSS	PER CENT POLLEN ABORTION	C						c			TOTAL
		Wx	Sh wx	Wx	sh wx	Wx	Sh wx	Wx	sh wx	wx	
B11-13 $\times c sh wx$	32.9	94	73	..	2	1	..	9	38	217	
$c sh wx \times$ B11-2	27.1	..	189	..	11	..	1	..	365	566	

From B11-2, the $Sh : sh$ ratio through the pollen is approximately 1 : 2. This might appear to be a normal trisomic ratio, since in maize $n + 1$ pollen functions only occasionally.¹⁰ However, the progeny in which plant B11-2 was found came from the cross: 75+ per cent sterile $Sh Sh \times$ normal $sh sh$. Since the female parent in which the $n + 1$ spore probably arose was $Sh Sh$, Sh seeds would have been in excess in the backcross given in table 2 if the extra chromosome were the one bearing this factor. The extra chromosome is probably the other one involved in semisterile-2. With random pairing in the trisome in a plant of the type assumed for B11-2 and B11-13, it would be possible to get apparent trisomic ratios for factors which were not in the trisome, provided they were closely linked with semisterility. In this case, where sh is assumed not to be closely linked with sterility, the assumption of preferential pairing in the trisome gives a close fit to the observed numbers and also to the degree of sterility.

Plant B11-2 was crossed also on two $Pr pr$ plants, giving 233 Pr :156 pr seeds. The deviation from the expected 3 : 1 ratio is over ten times its probable error, and suggests that the $pr v_2$ chromosome is the other one involved in semisterile-2. This is not certain, however, since ratios for pr often deviate widely from the expected (Fraser, unpublished data from Cornell).

In two other exceptional plants, abnormal ratios for $waxy$ were obtained in the pollen. One plant which showed 37.7 per cent aborted pollen had only 11.9 per cent waxy grains. Another plant showing 9.7 per cent

pollen sterility had 16.3 per cent waxy grains. In semisterile plants heterozygous for waxy, 50 per cent of the pollen is waxy.

The abnormal shrunken and waxy ratios furnish additional evidence that the *sh wx* chromosome is one of those involved in semisterile-2.

A partially sterile sib in the culture in which semisterile-2 was found was crossed with *x*-normal-1. All but one of the forty F_1 plants were partially sterile. The exception was a normal which may have resulted from a stray pollination. About half of the F_1 plants were semisterile, while the other half were somewhat less than 75 per cent sterile (referred to as 75- per cent). Pollen counts on nine plants in the latter class showed an average of 68.7 per cent sterility, while kernel counts on the same plants gave an average of 71.3 per cent abortion. These results indicate that the new sterile is different from semisterile-1. Since the new class from the corresponding cross with semisterile-2 showed 78.5

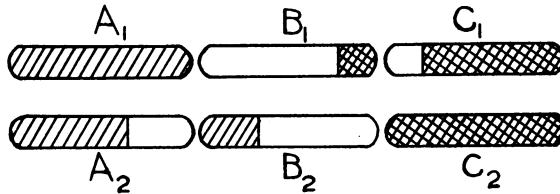


FIGURE 2

Diagram of the assumed chromosomal constitution of a 75 per cent sterile plant from the cross of semisterile-1 with semisterile-3, according to the segmental interchange hypothesis. One member of the B pair has interchanged with an A chromosome while the other member of the B pair has interchanged with a C chromosome. If homologous ends associate, a ring of six chromosomes might be expected at diakinesis.

per cent pollen abortion, the new type is also different, apparently, from semisterile-2. Plants belonging to the 75- per cent sterile class were crossed with standard normals. Out of 97 plants from these crosses, 90 were semisterile, one was normal, one was 81.5 per cent sterile and five belonged to a distinct class intermediate between normal and 50 per cent sterile. Practically every functional spore from the 75- per cent sterile plants must have been a semisterile producer. This suggests that the new type, semisterile-3, also involves interchange or translocation between non-homologous chromosomes, but that one of them is the same as is involved in semisterile-1. A diagram of the assumed chromosomal constitution of a 75- per cent sterile plant, based on segmental interchange is given in figure 2. In the pair common to both steriles, the B_1 member has interchanged with chromosome C_1 , while the B_2 member has interchanged with A_2 . Separation at meiosis of the members of this com-

mon pair would give only two kinds of normal spores, $A_1 B_1 C_1$ and $A_2 B_2 C_2$. In crosses with standard normals, the first would give semisterile-3 and the other would give semisterile-1.

On the above hypothesis, configurations involving six chromosomes should be found at meiosis in 75— per cent sterile plants. Cytological examination of microsporocytes at diakinesis does show a group of six chromosomes plus seven bivalents. The group is either a large closed ring or, occasionally, an open one. On the basis of Belling's interchange hypothesis illustrated in figure 2, the order of the chromosomes in the ring of six would be $A_1 A_2 B_1 C_2 C_1 B_2$. Only the combinations of alternate chromosomes in the group would give normal spores. The deviation from 75 per cent sterility is probably due to lack of independent distribution of the three pairs involved. Semisterile-3 may be considerably less than 50 per cent sterile; or the association of the three pairs in a ring or chain may prevent their independent distribution.

If opposite ends of the two members of the common chromosome pair are involved in semisteriles-1 and -3, as illustrated in figure 2, cross-overs between them might occur. In crosses with standard normals, these would give normal and 75± per cent sterile plants. One normal plant and one with 81.5 per cent sterility were found. They are being tested to determine whether they are the result of crossing-over or stray pollinations.

Two of the three pairs in the ring of six chromosomes must be *B lg* and *P br*, respectively, since these pairs are involved in semisterile-1. Either the *B lg* or the *P br* chromosome, therefore, must be concerned in semisterile-3. Certain configurations at diakinesis indicate that *B lg* is the one involved. Genetic tests are in progress.

The five plants belonging to the class intermediate between normal and 50 per cent sterile have been examined cytologically using either root tip or microsporocyte material. All proved to have 21 chromosomes. Where microsporocyte material was available, groups of five chromosomes were found in addition to eight bivalents, showing that the extra chromosome belongs to the group involved in semisterility. The frequency of these plants is very high, over six per cent having been found following crosses where the 75— per cent sterile plant was the female parent. The 20-chromosome, partially sterile plants thus furnish another source for the isolation of certain simple trisomics in maize.

In *Pisum*, an unexpected linkage of two factors which are ordinarily independent has been found by Hammarlund.⁹ Håkansson⁸ examined plants from this cross cytologically and found a ring of four chromosomes at meiosis. No mention of sterility was made in these papers; but by comparing the number of plants per progeny from the normal line with that from the cross which shows linkage, I find that the latter progenies

are about half as large. In the normal line the average for seven progenies is 115.6. In the cross showing linkage, the average for 28 progenies is only 53.0. This suggests that Hammarlund's double dominant line *K*, which shows linkage of the two factors in its crosses, may be an x -normal type. Since only the parental combinations of chromosomes in the two pairs involved in semisterility are viable, two factors closely linked with sterility but in different chromosome pairs would show apparent linkage with each other. This would be true whether or not either or both were in the interchanged or translocated piece. Unless both were linked with sterility to about the same degree, the recombination classes should not be of equal size. Examination of the data shows that the two classes are approximately the same size. Another case of ring-formation in *Pisum* was reported recently by Miss Richardson¹² in a cross showing 50 per cent sterility.

Ring formation in *Oenothera* has been interpreted by Darlington⁷ on the basis of segmental interchange between non-homologous chromosomes. One of the cases described in maize in the present paper shows how configurations involving more than two pairs of chromosomes may be built up by combining the proper semisterile types.

Müntzing¹¹ has studied partial sterility in crosses of *Galeopsis* species. Although the complete story has not been published, the breeding behavior suggests a situation similar to that in maize and in *Datura*. Cytological examination revealed no abnormalities, but only one diakinesis preparation was examined.

Summary.—1. In semisterile-1 and in two new types, semisteriles-2 and -3, there is an association of non-homologous chromosomes at meiosis. A group of four chromosomes occurs plus eight bivalents.

2. Sufficient evidence is not available to decide definitely whether this is the result of segmental interchange or simple translocation.

3. The combination of semisteriles-1 and -2 gives a new class which is somewhat more than 75 per cent sterile, and in which two separate groups of four chromosomes occur plus six bivalents.

4. Neither of the two pairs involved in semisterile-2 is concerned in semisterile-1. The *sh wx* pair is one of those involved in semisterile-2.

5. The combination of semisteriles-1 and -3 gives a new class which is a little less than 75 per cent sterile, and in which there is a group of six chromosomes plus seven bivalents.

6. One of the pairs involved in semisterile-3 is probably the same as is concerned in semisterile-1, and, therefore, must be either *P br* or *B lg*.

7. Exceptional 21-chromosome plants showing intermediate degrees of sterility have arisen from partially sterile 20-chromosome plants. The extra chromosome belongs to the group involved in semisterility.

† This work was begun in the Department of Genetics at the University of Wisconsin.

The greater part of the analysis was made at Cornell University, certain parts being finished at the Bussey Institution.

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¹ Belling, J., *Zeit. indukt. Abstamm. Vererb.*, **39**, 286-288 (1925).

² Belling, J., and A. F. Blakeslee, *Proc. Nat. Acad. Sci.*, **12**, 7-11 (1926).

³ Blakeslee, A. F., *Ibid.*, **10**, 109-116 (1924).

⁴ Brink, R. A., *J. Hered.*, **18**, 266-270 (1927).

⁵ Brink, R. A., (Abstract) *Anat. Rec.*, **44**, 280 (1929).

⁶ Brink, R. A., and C. R. Burnham, *Amer. Nat.*, **63**, 301-316 (1929).

⁷ Darlington, C. D., *J. Gen.*, **20**, 345-363 (1928-29).

⁸ Håkansson, A., *Hereditas*, **12**, 1-10 (1929).

⁹ Hammarlund, C., *Ibid.*, **4**, 235-238 (1923); **12**, 210-216 (1929).

¹⁰ McClintock, B., and H. E. Hill, (Abstract) *Anat. Rec.*, **44**, 291 (1929).

¹¹ Müntzing, A., *Hereditas*, **12**, 297-319 (1929).

¹² Richardson, Eva, *Nature*, **124**, No. 3128 (1929).

EVIDENCE THAT NATURAL RADIOACTIVITY IS INADEQUATE TO EXPLAIN THE FREQUENCY OF "NATURAL" MUTATIONS

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I. The Problem.—When it was found that irradiation of *Drosophila* by artificial means results in a pronounced increase in the mutation frequency, the question immediately arose (Muller, 1927) whether the mutations that occur in untreated material are caused by radiation of a similar type from radioactive substances naturally occurring. Since this problem was raised, results have been published by Olson and Lewis, Babcock and Collins and others, which have been taken as indicating that such was actually the case.

II. The Inadequacy of the High-Energy Radiation from the Outer Environment.—In order to test the above possibility in our material, we have made calculations of the relation between the artificial and natural radiation effects, for comparison with the artificial and "natural" mutation frequencies. As measures of the intensity of the radiation in both cases, we have taken the ionization per cc. per second in air. In order, then,