CHAPTER 4 INTERCHANGES

In many species of plants and animals, occasional individuals are heterozygous for a change in which end segments of non-homologous chromosomes have exchanged positions. In certain plant species an examination of the mature pollen in such individuals shows that part of the grains are completely or almost completely devoid of stored food material. Seed set is usually reduced by a similar amount. In other plant species or in certain cases other lines, such individuals have essentially normal pollen and seed set. In animals individuals heterozygous for such a change show zygotic sterility, reduced egg hatch or reduced litter size. Partial sterility may have other causes; but wherever it is known to be the result of an exchange of segments of non-homologous chromosomes it is termed an interchange, a reciprocal translocation, or simply a translocation. Transposition would have been a more desirable term, since translocation is used in plants for a very different phenomenon.

The importance of interchanges lies in the fact that they are one of the types of structural change which may result in changes in chromosome morphology and number, and in some cases changes in physiology. Also they may be used as tools to gain basic information in cytology and genetics and as aids in breeding (Anderson 1935). For a review of the literature on interchanges, and a survey of the plant species in which they have been reported, see Burnham (1956).

Semisterility and the interchange hypothesis

Stizolobium

Belling was the first to recognize the phenomenon. Crosses made in a breeding program for the improvement of the Florida velvet bean, Stizolobium deeringianum, Bort., were found by Belling (1914, 1915a) to have about 50% of visibly aborted pollen and 50% of a normal seed set. Belling termed this semisterility. About half the offspring were semisterile and half had normal fertility. The fertile ones bred true in the next generation; but the semisterile ones again segregated in a 1:1 ratio. Microtome sections from one F_1 plant showed 50 normal or complete embryo sacs and 40 aborted, about a 1:1 ratio. He recognized the segregation as being expressed in the gametophyte stage. A two-factor scheme was proposed which would explain the results.

In 1920, Belling joined the group working under Blakeslee at Cold Spring Harbor. Blakeslee had found off-type plants in Datura stramonium, the Jimson weed, which were not breeding in a typical Mendelian manner. Belling found that these plants had an extra chromosome. In one of them, which reoccurred only in crosses with a certain strain ('B' white), a small extra chromosome often was attached to two pairs of large ones. This led to the conclusion that non-homologous chromosomes could exchange segments (Belling and Blakeslee 1924), and to an explanation by Belling (1925) of the breeding behavior of semisterility in Stizlobium based on "segmental interchange between non-homolgoues" (Fig. 17).

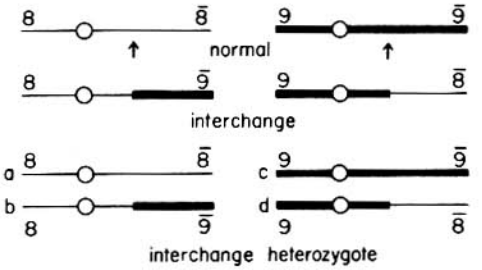


FIG. 17. Diagrams illustrating the interchange hypothesis; from top to bottom:

Normal chromosomes, 8. $\bar{8}$ and 9. $\bar{9}$, break points indicated by arrows.

Interchange chromosomes 8. $\bar{9}$ and 9. $\bar{8}$ resulting from an exchange of terminal segments, $\bar{8}$ and $\bar{9}$. Also designated as 8^{9} and 9^{8}.

Interchange heterozygote in which a and c are the normal, b and d the interchange chromosomes.

The order of the chromosomes and their ends in the ring configuration is:

8. $\bar{8}$ - $\bar{8}$. 9 a - d
( ) or ( )
8. $\bar{9}$ - $\bar{9}$. 9 b - c

In the diagram (Fig. 17) the normal chromosomes are designated as a and c and the interchanged ones b and d. If at meiosis, a separated from b and c from d, four combinations might be expected a plus c, b plus d and a plus d, b plus c. The first two have the entire chomosome complement, the last two have a duplication and a deficiency. This would account for the observed 50% spore abortion (a result of the deficiency), the observed breeding behavior, and for the origin of semisterility as a single event, an exchange, involving two chromosomes. No cytological observations were ever reported on meiosis in the Stizolobium material, but rings were found later in crosses between certain strains of Datura (Blakeslee 1928). Certain of them were associated with partial sterility, others not (including crosses with 'B' white). The reasons for these differences will be discussed later, page 84.

The first translocation in Drosophila to be analyzed cytologically was described as having a piece of the X chromosome attached to one end of the Y chromosome (Stern 1926). The first translocations produced by X-rays in Drosophila were described as having a segment of one chromosome attached at or near the end of another (Painter and Muller 1929, Muller 1930). Later work has indicated they were probably interchanges.

Semisterility in maize

CYTOLOGICAL VERIFICATION. In maize, plants with about 50% pollen abortion were found in a culture being classified for the non-waxy and heterozygous waxy genotypes (Brink 1927). The ears had about 50% of the normal seed set, and further studies (Brink and Burnham 1929) showed a breeding behavior similar to that found by Belling in Stizolobium. The subsequent work on maize is used to illustrate the various aspects of the behavior of chromosomal interchanges in plants. By this time, a ring of four chromosomes had been reported in crosses with the 'B' white strain of Datura (Blakeslee 1928). Brink's semisterile-1 and two new ones, semisteriles-2 and -3 found in a stock used as a standard normal, and crosses between them and semisterile-1 were grown by the writer at Cornell University in 1929. Identification of the maize chromosomes morphologically had been completed by McClintock (1929) and she was also making rapid progress in associating each with its linkage group by means of trisomics (plants with 2n + 1 chromosomes). Cytological examination of the semisterile plants showed a ring of four chromosomes plus 8 pairs (Burnham 1930). At the pachytene stages of plants heterozygous for semisterile-2 there was a 4-armed, cross-shaped configuration (McClintock 1930) which provided convincing evidence of an exchange of terminal segments of non-homologus chromosomes.

The relative arm lengths and centromere positions showed that chromosomes 8 and 9 had exchanged segments of their long arms, represented in the cross-shaped configuration by the two arms of the cross without a centromere. Hence, there had been an exchange of segments, or interchange or reciprocal translocation, and not an attachment of a segment of one chromosome on the end of a non-homologue (simple translocation). The center of the cross should represent the interchange points. The normal chromosomes 9. $\bar{9}$ and 8. $\bar{8}$ are in alternate positions with the interchanged ones 9. $\bar{8}$ and 8. $\bar{9}$. The two interchanged chromosomes may be designated also as 9^{8} and 8^{9}, that is, 9 with a piece of 8 and 8 with

a piece of 9, the segments with the centromeres being the base, and those without a centromere being the superscripts. The segments without the centromeres are considered to be the interchanged pieces.

By measuring the distances from the centromeres to the center of the "cross" and from the ends of the long arms to the center of the cross, the positions of the breaks were determined. It is expressed as the distance from the centromere to the break divided by the total length of the arm. For this 8-9 interchange, the average was 8 L .2, 9 L .4. At diakinesis and metaphase, the chromosomes were usually still associated at their ends to form a ring of four chromosomes (⊙ 4).

The crosses of semisterile-1 with semisteriles-2 and -3 produced some plants with about 75% sterility. These "high sterile" plants, when crossed with a normal line produced progenies segregating for various degrees of sterility (Table 15).

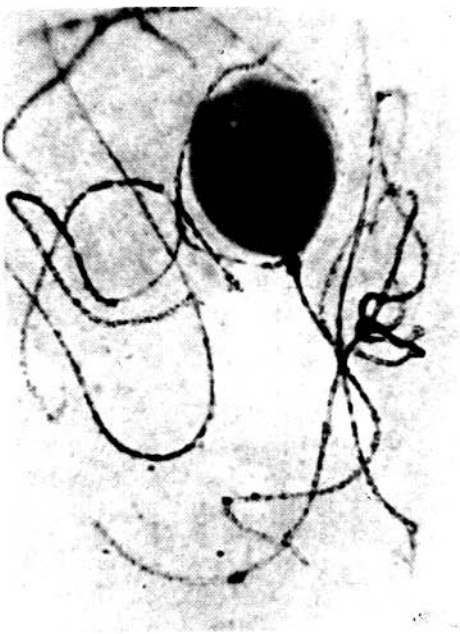
Table 15. Progenies from "high sterile" plants crossed with standard normals, classified for various degrees of sterility. H.S. = high sterile, S.S. = semisterile, F. = fertile (Burnham 1930).

Type of cross	F.	S.S.	H.S.	±30%	± 60%
(H.S. from S.S.-1 x S.S.-2)xF.	93	187	88	4	11
(H.S. from S.S.-1 x S.S.-3)xF.	1	90	1	5	

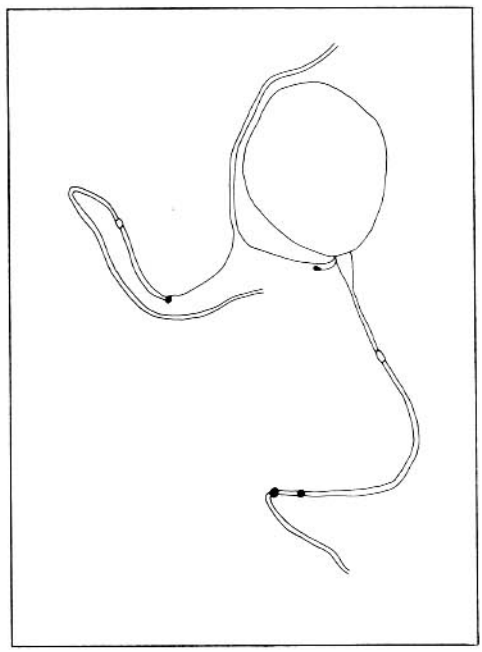
The test involving "high steriles" from the S.S.-1 x S.S.-2 cross segregated in a ratio of 1 fertile (F): 2 semisterile (S.S.): 1 "high sterile" (H.S.) plants and a smaller number with intermediate degrees of sterility (line 1 of Table 15). Cytological examination of the "high sterile" plants showed 2 ⊙ 4. Hence the two pairs of chromosomes involved in semisterile-1 were different from those involved in semisterile-2. This explains the observed 1:2:1 segregation of the three major types. Also, if each ⊙ 4, by itself, accounts for 50% sterility, a plant with both would be expected to have 75% sterility. Plants with intermediate degrees of sterility, about 30 or 60% (Table 15), were checked by root tip and sporocyte examinations and found to have 21 chromosomes, the extra one associated with the four involved in the interchange. The phenotype of several 21 chromosome plants (narrow, stiff leaves) from crosses involving semisterile-3 identified chromosome 7 as one of those in the ring. The behavior of these plants will be described later (pages 146 and 154).

The test involving "high steriles" from S.S.-1 x S.S.-3 showed semisterile plants almost exclusively (line 2 of Table 15). This suggested that one chromosome was involved in both interchanges. Cytological examination of H.S. F_1 plants from the original cross showed a ⊙ 6 and thus verified the prediction.

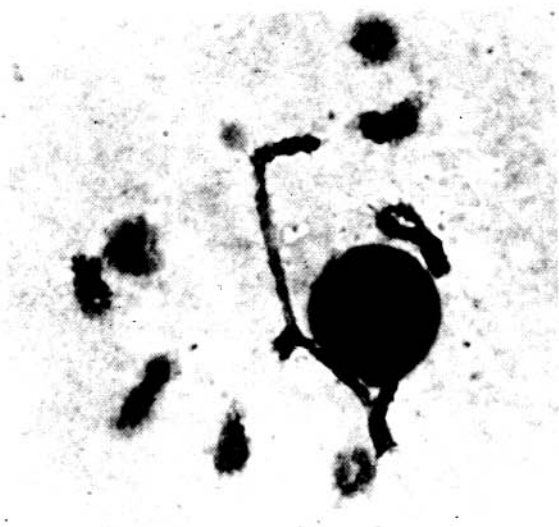
Plants with low sterility that were found in the original culture segregating semisterile-1 were found to have a chain or string of four chromosomes attached to the nucleolus. Pachytene analysis showed that one break was in the satellite, the other in the long arm of chromosome 1. Since the prominent terminal chromomere of the satellite could be recognized in its new position (Fig. 18), this demonstrated that an interchange had taken place, and that translocations interpreted as being simple might well be interchanges in which one interchange segment was very short and had no cytologically recognizable feature (Burnham 1932a). The stock homozygous for this interchange shows a striking change in morphology (Fig. 18). The nucleolus organizer is nearly median in one chromosome pair and there is a short chromosome. The longest chromosome pair and chromosome 6 have been replaced by a short chromosome and one in which the nucleolus organizer is nearly median.



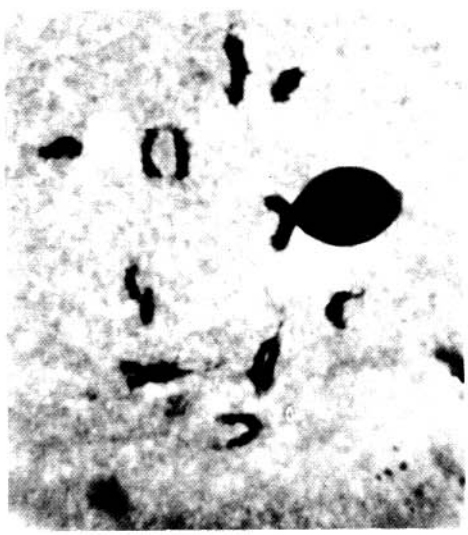
A



B



C



D

FIG. 18. A. Photomicrograph of a T-shaped pachytene configuration in the Tl-6b interchange heterozygote in maize. One break was in the satellite of 6, the other in the long arm of 1.
B. Semidiagrammatic camera lucida drawing of the configuration shown in A.
C. Tl-6b heterozygote at diakinesis showing "chain" configuration.
D. Tl-6b homozygote at diakinesis showing the new morphology of one of the interchange chromosomes, 6^1, with a sub-median attachment to the nucleolus. The normal chromosome 6 has only the few chromomeres of the satellite beyond the point of attachment. (Burnham, unpublished).

Other naturally occurring lines with partial sterility were found, several by R. A. Emerson in the genetic stocks (Burnham unpublished); two in corn breeding programs and several others. Large numbers have been produced by irradiation, see page 93.

GENETIC VERIFICATION. Based on the interchange hypothesis, semisterility should show linkage with factors in two linkage groups. Also factors close to the interchange points should show linkage with each other. In lines homozygous for the interchange, genes in the pieces exchanged should show new linkages and should no longer be linked with the genes in the remainder of the chromosome from which they came.

Linkage between semisterility and several characters in Stizolobium had been reported by Belling (1915b), but it was not known whether those factors belonged to the same or different linkage groups. In maize, linkage had been found between waxy (wx) and partial sterility in the progeny from intercrosses involving semisteriles-1 and -2 (Burnham 1930). Linkage of semisterile-1 with factors in two linkage groups was demonstrated by Brink and Cooper (1931).

The method is typical for such tests in higher plant species that are easily crossed and in species in which plants with a ring are partially sterile. The method can be applied to inversions as well. Plants carrying the interchange were crossed with the genetic marker stock. The semisterile F_1 plants, heterozygous for the interchange and for the marker genes, were backcrossed to a normal stock carrying the recessives. In crosses with normals, semisterility behaves as if it were a dominant character, i.e. plants which carry the interchange are semisterile. By classifying the progeny for sterility as well as for the genetic characters the presence of linkage can be detected. The data Brink and Cooper obtained from the linkage test involving semisterile-1 and brachytic (br) and fine striped (f) in linkage group 1 are in Table 16.

Table 16. Data from semisterile-1 $\frac{+\ +\ T}{br\ f\ +}$ plants backcrossed to br f normal plants (Brink and Cooper, 1931, from Table 11, p. 607, Genetics 16). S.S. = semisterile, and F. = fertile

+ +		+ f		br +		br f		
S.S.	F.	S.S.	F.	S.S.	F.	S.S.	F.	Total
333	19	1	8	17	6	25	273	682

Obviously there is linkage with semisterility; since if independent, a 1:1 ratio under each of the four phenotypes for the genetic markers would be expected. Since semisterility behaves as if it were located at the interchange point in each of the two chromosomes, the order of the genes in relation to this point can be determined. The data in Table 16, summarized in the conventional manner in terms of recombination events: non-recombinants, single recombinants in regions 1, 2 and doubles in 1 and 2 are as follows:

$+\ ^{1}\ +\ ^{2}\ T$	0	1	2	1, 2	Total
br f +	333-273	8-17	19-25	1-6	682

recombination: br-f = 4.7%; f-T = 7.5%; br-T = 10.1%

Data from similar tests of semisterile-1 with liguleless (lg) and virescent -4 (v_4) in linkage group 2 are summarized as follows:

$+\ ^{1}\ +\ ^{2}\ T$	0	1	2	1, 2	Total
lg v_4 +	163-105	124-126	13-25	18-14	588

recombination: lg-v_4 = 48.0%, v_4-T = 11.9, lg-T = 49.0

in the linkage map

The three-point data indicate the position of the interchange breakpoint as well as the linkage groups involved.

Independence was obtained in tests with at least two markers in six of the other eight linkage groups and one in each of the other two. Hence this illustrates the first prediction, that linkage of semisterility is with factors in two and only two linkage groups. Since McClintock had found that Br and f are on the longest, chromosome 1, and lg and v_4 are on the next to the longest, chromosome 2; semisterile-1 was designated as T1-2a, the letter a indicating it was the first one between 1 and 2.

The second prediction, that new linkages and the loss of old ones would be found in lines homozygous for the interchange, could not be tested until homozygous interchange stocks carrying the markers had been established following crossing over. As shown in Table 26, page 85, in a stock homozygous for T5-9a, waxy (wx) and virescent (v_1) were independent (Burnham unpublished). Since in normal stocks these two genes are linked, the break must have been between them. Also pr in chromosome 5 was linked with wx both in plants heterozygous and in plants homozygous for this 5-9a interchange. Such tests in interchange homozygotes serve as an additional check on the location of the interchange point in relation to the positions of genes in a given linkage group. The results also constitute a genetic proof that chromosome 9 in T5-9a had been broken into two segments. For a different type of genetic proof of this in Drosophila, see page 81.

One further point of use in the analysis of interchanges is the clue that such data may furnish as to the location of the centromere (see page 85 and Anderson and Randolph, 1945).

Behavior in pachytene and later stages

At pachytene in an interchange heterozygote, a cross-shaped configuation is formed (Fig. 20). In certain maize interchanges the position of the center of the "cross" varies widely, in others it seems to be less variable (McClintock 1932, Burnham 1932a, 1934a, 1948). For example, for T2-6a with the breaks in the long arm of 2 and in the short arm of 6, the "cross" varied from a position in the short arm of 6 to one well out on the long arm as shown in Figure 19.

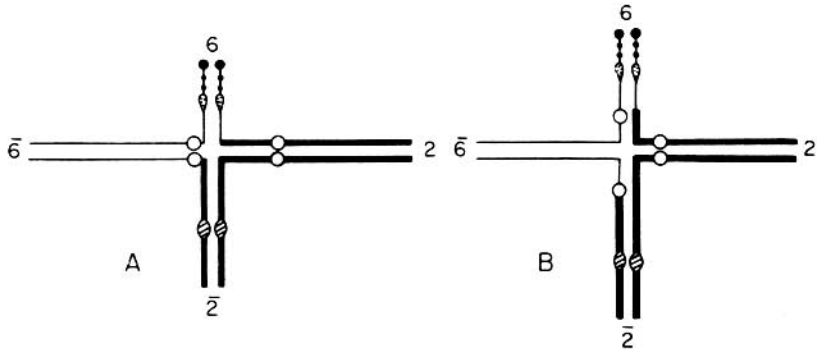


FIG. 19.• Diagrams interpreting the synapsis in two different observed positions of the center of the "cross" in the T2-6a heterozygote in maize. The thick line represents chromosome 2, the other chromosome 6.
A. The center of the "cross" is at the original exchange break points, and homologous parts are associated.
B. The center of the "cross" is not at the exchange points, and there is association of non-homologous segments.

The interpretation in terms of homologies of two of the positions of the "cross" is shown in Fig. 20.

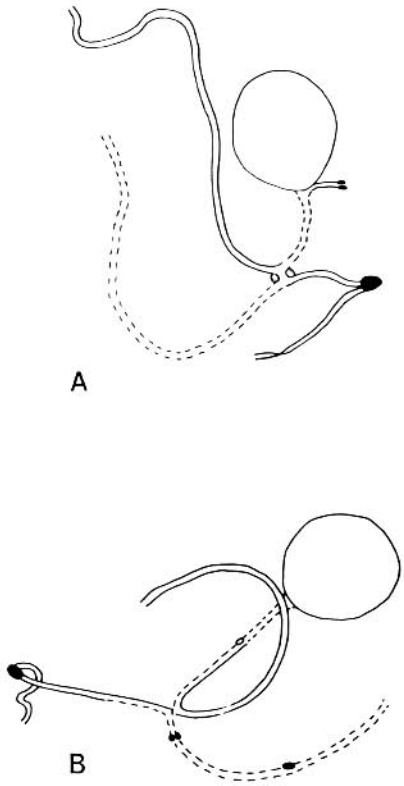


FIG. 20. A camera lucida tracing of pachytene configurations observed in T2-6a heterozygote in maize.
A: "Cross" at the true positions of breakage.
B: "Cross" at a position resulting from non-homologous pairing.
(Burnham, unpublished).

In some figures there was asynapsis near the center of the cross, but in others there was close pairing in all segments even when the cross was far out of its true position and involved some non-homologous pairing as in Fig. 20B. If pairing is zipper-like, pairing in an interchange heterozygote might be expected to go beyond the actual points of interchange if it begins earlier in one arm, or closer to the point of interchange. Also rates of pairing may be variable or may be different for different chromosomes.

Following pachytene, the chromosomes become shorter and at diplotene begin to open out. In smear preparations in maize the chromosomes at this stage stick together and the interchange configurations are difficult to analyze. At the diakinesis stages, the chromosomes in the ring may still be associated in the four arms as a ring of four, sometimes by subterminal chiasmata, sometimes only at the ends, or one arm may fail to show an association and a chain or string of four configuration results. If opposite arms of the original group of four fail to remain associated, the group then forms two "pairs". These failures to remain associated probably occur most often in the interchanged arms of the cross-shaped configurations. An arm probably remains associated at diakinesis only if there had been a cross-

Table 17. Frequencies of diakinesis configurations, relative lengths of interchanged segments and pollen abortion percentages in a few maize interchange heterozygotes.

Interchanges	Chromosome Break positions 1st	2nd	length of pieces interchanged	⊙ 4	Chain of 4	"Pairs"	Pollen Abortion	Authority
T8-9a	L.2	L.4	long, long*	108	7	-	59%	Burnham, 1934
T1-6a	L.2	L.54	long, long	mostly	-	-	50%	Burnham, unpublished
T2-3a	S.9	L.6	short, long	96	194	-	50%	Burnham, unpublished
T1-6b	L.25	sat.	long, very short	-	all	-	25%	Burnham, 1932
T5-9a	L.7	S.[illegible]	med., med.	9	40	88	50%**	Burnham, unpublished
T3-6	S.8	sat.	short, very short	0	.31	.69	19-47%***	Clarke & Anderson 1935

* The lengths of the interchanged pieces are .8 of the long arm of 8, .6 of the long arm of 9.

** 50% of the pollen grains smaller, most of them partly filled with starch.

*** includes small but filled as well as partly filled grains.

over in that arm. The short arm of chromosome 6 may be an exception. What happens to a crossover in the segment between the centromere and the break point will be answered in part a little later. Interchange heterozygotes differ greatly as to the relative frequencies of rings-of-four, chains-of-four and "pairs" at diakinesis (Table 17).

The data in Table 17 show there is a general relationship between the frequencies of the various configurations at diakinesis and the relative lengths of the interchanged segments. The shorter the segment the more likely is it to fail to pair with the homologous segment. For example, when both interchange segments are long most of the configurations are a ⊙ 4 as in T8-9a. If one interchange segment is short, chains of four are frequent, as in T2-3a. In T1-6b, which forms only chains, one interchange piece is part of the satellite, a very short segment which has never been observed to pair with its homologue. If both interchange segments are short, "pairs" are frequent as in T5-9a and T3-6. For T5-9a, the probability of "pairing" failure is about .8. Data of this sort may be used to compare the pairing capabilities of distal segments of different chromosomes.

Interchanges in maize also show differences in degree of pollen abortion and in the appearance of the pollen which also is related in general to the lengths of the interchanged segments (Table 17). For example, the interchange heterozygotes that form mostly rings usually show about 50% of spore abortion, as in T1-6a. The excess in T8-9a (59% observed) has not been explained. This approaches the $\frac{2}{3}$ sterility expected if the four chromosomes in the ring pass at random, two to each pole. Although 3-1 segregations will add to the sterility and in some interchanges may be as high as 10%, their frequency in T8-9a is too low to account for the excess sterility over 50% (Burnham 1948). In T2-3a, the deficiency for the short terminal segment of chromosome 2 resulted in pollen grains without starch. As expected, the effect of the deficiency differs, depending on the segment involved. For example, pollen grains with a deficiency for 0.22 of the long arm of 10 were filled with starch but smaller in size (Stadler 1935). In T5-9a, pollen abortion was about 50%, but the aborted grains were mostly smaller and only partly filled with starch. In T3-6 the visible pollen abortion was variable, and included pollen that was somewhat smaller than normal but with normal starch content as well as some that was partly filled. In T1-6b, only 25% pollen abortion was observed. Here there was no visible effect on the pollen of the deficiency for the short interchange segment of the satellite. This class of spores also carried a duplication which prevented functioning in the pollen. It did function in the ovules. The resulting plant was hyperploid for the distal 0.75 of the long arm of chromosome 1 and had certain distinctive morphological features, and in the original stock seemingly greater susceptibility to smut.

The relative frequencies of the different kinds of segregation would be expected to affect the percentage of pollen abortion.

Orientations at meiosis and the kinds of segregation

Direct Observations

In maize, in the association of four chromosomes the orientation at metaphase I is either "open" or "zigzag" (i.e. adjacent or alternate) when the four centromeres are oriented toward the poles as shown in Fig. 21A, B and C. In barley, Tjio and Hagberg (1951) noted configurations in which two chromosomes opposite each other in the ring were not coorientated with reference to the poles and the equatorial plate (Fig. 21D).