A CYTOGENETIC STUDY OF INVERSIONS IN ZEA MAYS

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ALTHOUGH spontaneous and induced inversions have been reported in many organisms, the genetic behavior of inversions is known exclusively from studies of Drosophila. It is the purpose of this study to investigate inversions in *Zea mays*, at present the only organism other than Drosophila in which a relatively precise genetic and cytological analysis could be made.

MULLER (1940) proposed that inversions including the centromere be called pericentric and inversions limited to a single chromosome arm be designated as paracentric. Heterozygous inversions of both types form loop bivalents at meiotic pachytene or in the salivary gland chromosomes due to the pairing of the inverted and normally arranged homologues. Single crossover chromatids arising from exchanges within the limits of a heterozygous paracentric inversion are dicentric and form a chromatin bridge at meiotic anaphase or are acentric and lost because of their failure to undergo regular mitotic behavior (MCCLINTOCK 1933). The dicentric and acentric crossover chromatids carry genetic duplications and deficiencies. Single crossover chromatids arising from exchanges within heterozygous pericentric inversions carry genetic duplications and deficiencies but such chromatids each have a single centromere and no chromatin bridges are produced. Double crossover chromatids arising from multiple exchanges within either heterozygous pericentric or paracentric inversions do not carry duplications and deficiencies and may be recovered in viable offspring. Duplication-deficiency chromatids ordinarily cause abortion of the gametophyte generation of plants. In animals duplication-deficiency chromatids result in aneuploid gametes which, though functional, give rise to zygotic or embryonic lethals. Consequently, heterozygous inversions have been found to reduce greatly genetic recombination within the inversion segments in *Drosophila melanogaster* (STURTEVANT 1926; 1931; STURTEVANT and BEADLE 1936) and in *D. pseudoobscura* (DOBZHANSKY and EPLING 1948). In the inversion heterozygote, crossing over is also frequently reduced in the regions adjacent to the breakage points (STURTEVANT and BEADLE 1936; DOBZHANSKY and EPLING 1948).

Pericentric inversions are not abundant in natural populations, due apparently to the mortality caused by aneuploid gametes. Two spontaneous pericentric inversions have been reported in *Drosophila robusta* (CARSON and STALKER 1947) and one in *D. algonquin* (MILLER 1939). In contrast to the scarcity of spontaneous pericentric inversions, there is an abundance of para-

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centric inversions in natural populations of Drosophila. Unlike pericentric inversions, heterozygous paracentric inversions result in little or no mortality in Drosophila and Sciara. Dicentric duplication-deficiency crossover chromatids are excluded from functional egg nuclei, resulting in the production of eggs carrying non-crossover chromatids with full complements of genes (STURTEVANT and BEADLE 1936; CARSON 1946). Normal sperm are produced by Drosophila male inversion heterozygotes, since crossing over does not occur in the male. Meiotic anaphase bridges and fragments reported in many species of plants indicate that paracentric inversions are also frequent in plant populations. In general, crossing over occurs in both sexes in plants. Dicentric chromatids are excluded from the terminal megaspores in megasporogenesis (DARLINGTON and LA COUR 1941) but are included in non-functional, aneuploid microspores. Consequently, pollen abortion, but little or no ovule abortion results from heterozygous paracentric inversions in plants. However, plants produce an abundance of pollen, and the presence of a considerable proportion of aborted pollen presumably would have no great selective disadvantage. Therefore paracentric inversions might be expected to occur frequently in plant populations.

Although the cytological behavior of inversions in maize has been determined by MCCLINTOCK (1931, 1933, 1938), the genetic effects of heterozygous inversions have not previously been reported in plants. The present study comprises a cytological and genetic analysis of X-ray induced inversions 4a, 5a and 2b in maize and a cytological analysis of included inversion 2a/2b. For convenience in nomenclature the inversions studied are designated numerically according to the chromosome involved and alphabetically in the chronological order of their discovery.

MATERIALS AND METHODS

The cytological location of the two points of breakage in the inverted chromosome may be determined in the heterozygote from an analysis of changes of homology in the pachytene loop configuration. Microsporocytes were smeared in acetic or propionic carmine after aceto-alcohol fixation and the points of breakage determined by the use of a map measure from camera lucida drawings of pachytene configurations. If the two points of breakage in a pericentric inversion are not equidistant from the centromere, the inverted chromosome will have arm lengths different from its normally arranged homologue. Comparisons were made of pachytene arm lengths in normally arranged and homozygous inverted bivalents for inversions 5a and 2b.

To obtain the genetic location of the breakage points with reference to linked marker genes, backcrosses were made with the female parent heterozygous for the particular inversion and marker genes and employing the appropriate tester as the pollen parent. Inversion heterozygotes in a segregating backcross population in maize may be conveniently classified due to the pollen abortion resulting from crossing over. The two breakage points of an inversion, when cytologically established, may be used as physical landmarks in determining the cytogenetic position of genes situated on the same chromosome. Since sin-

gle crossover chromatids lead to abortion of the male and female gametophytes in inversion heterozygotes, all loci within the limits of the inversion are completely linked except for recombination effected by double crossover chromatids. Consequently, greatly reduced recombination values between two loci indicate that both may be within the inverted region or one within and one adjacent or both adjacent to the points of breakage and separated by the inversion. Recombination values between an inversion and loci outside the limits of the inversion may be determined by regarding the inversion as a dominant gene for pollen abortion and computing the amount of recombination between it and the marker gene.

The percentage of aborted pollen characteristic of the individual inversions was determined by staining and counting all of the pollen grains of each anther examined. Ovule abortion frequencies were determined from open pollinated ears for comparison with pollen abortion frequencies. The minimum genetic length of an inversion may be estimated from pollen abortion frequencies. The two breakage points of an inversion may be regarded as two loci separated by the length of the inversion with the products of recombination measured as aborted pollen. Each single or three strand double exchange within the inverted sector will produce two viable and two aborted pollen grains. Each four-strand double exchange will produce four aborted pollen grains. The percentage of pollen abortion will equal one-half of the single chiasma and three-strand double chiasma frequencies plus the four-strand double chiasma frequency. The percentage of pollen abortion is therefore a measure of the minimum genetic length of the inversion, since it represents a fairly accurate approximation of one-half the total chiasma frequency, with only double crossover chromatids from multiple exchanges remaining undetected, since they, like noncrossover chromatids, give rise to viable male gametophytes. Maize plants free from known chromosomal aberrations have varying amounts of aborted pollen, usually less than five percent. The percentage of aborted pollen in normal sister plants must be subtracted from the percentage of aborted pollen in the inversion heterozygotes in order to determine an approximation of the minimum genetic length of an inversion. Due to suppression of crossing over by asynapsis and non-homologous pairing (McCLINTOCK 1933), the genetic length of a heterozygous inversion would be less than the genetic length of the same sector in the inversion homozygote or in a normally arranged bivalent.

RESULTS AND DISCUSSION

Inversion 4a

X-ray induced paracentric inversion 4a was discovered and studied cytologically by McCLINTOCK (1938). She located the proximal break at a point approximately one-third of the length of the long arm from the centromere; the distal break was observed to be near the end of the long arm. A diagrammatic interpretation of inversion 4a follows (fig. 1).

Plate I, 1 confirms the observations of McCLINTOCK. Typical inversion loop configurations were not abundant at pachytene in heterozygous in-

version 4a plants, and analysis was often difficult due to frequent asynapsis and non-homologous pairing (plate I, 2).

In order to locate the position of inversion 4a on the genetic map of the fourth chromosome, plants heterozygous for the inversion were crossed with pollen from tester stock not carrying the inversion and homozygous for the recessive genes lazy (*la*), sugary (*su*) and glossy$_3$ (*gl*$_3$). ANDERSON and RANDOLPH (1945)

FIGURE 1. Inversion 4a. The centromere is indicated by the circle and the positions of the breakage points in the long arm by arrows. The heteropycnotic knob is located in the long arm.

place the centromere of chromosome 4 to the left of tunicate (*Tu*) in the long arm and nine units or more to the right of sugary in the short arm. A four-point test of *la su Tu gl*$_3$ by JENKINS (EMERSON, BEADLE, and FRASER 1935) placed *la* to the left of *su* and *gl*$_3$ to the right of *Tu*. The order of the marker genes employed in this study with respect to the centromere is thus *la su* centromere *gl*$_3$. A linkage map of chromosome 4 (fig. 2) has been assembled from data contained in EMERSON, BEADLE, and FRASER (1935) and ANDERSON and RANDOLPH (1945).

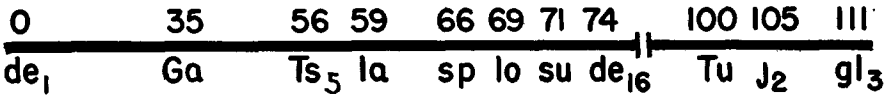

FIGURE 2. Linkage map of chromosome 4. In this and subsequent linkage maps, the approximate genetic position of the centromere is indicated by the cross lines.

F$_1$ plants heterozygous for the inversion and marker genes were backcrossed with pollen from homozygous tester plants with the normal gene order. Sister F$_1$ plants not carrying the inversion were backcrossed with *la su gl*$_3$ to obtain control values for crossing over. Table 1 presents control values for the *la—su* and *su—gl*$_3$ regions. Table 2 presents linkage data for the marker genes and the inversion from a backcross population of 751 individuals. The symbol *I* indicates presence of the inversion and *i* absence of the inversion. Classification for the inversion was effected by examining pollen from each plant. Inversion 4a is known to be located in the long arm of chromosome 4 with the proximal break one-third of the length of the long arm from the centromere. A study of table 2 shows a recombination value of 12.6 for *gl*$_3$ and the inversion. This indicates that the proximal break of the inversion is at least 13 units distal to the *gl*$_3$ locus in the long arm of chromosome 4. Consequently, the physical location of *gl*$_3$ is established within the proximal third of the long arm. The *la—su* interval is 7.5 units long in the inversion heterozygote stocks and 8.1 units long in control stocks. Crossing over in the short arm has not been affected appreciably by the inversion in the long arm. The *su—gl*$_3$ interval has been reduced from 28.0 in the control stock to 22.0 in the inversion heterozygote. The reduction in crossing over for the *su—gl*$_3$ interval in the heterozygous

TABLE 1

Genetic length of the la—su and su—gl₃ intervals.

Data from the cross $\frac{la\ su\ gl_3}{+\ +\ +} \times la\ su\ gl_3$.

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	*la su gl₃*	148
(0)	+ + +	172
(1)	*la* + +	20
(1)	+ *su gl₃*	14
(2)	*la su* +	57
(2)	+ + *gl₃*	75
(1–2)	*la* + *gl₃*	4
(1–2)	+ *su* +	2

Crossover value for *la—su* interval= 8.1% $\left(\frac{40}{492} \times 100\right)$

Crossover value for *su—gl₃* interval=28.0% $\left(\frac{138}{492} \times 100\right)$

TABLE 2

Results from the cross $\frac{la\ su\ gl_3\ i}{+\ +\ +\ I} \times la\ su\ gl_3\ i.$

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	*la su gl₃ i*	210
(0)	+ + + *I*	260
(1)	*la* + + *I*	22
(1)	+ *su gl₃ i*	23
(2)	*la su* + *I*	76
(2)	+ + *gl₃ i*	59
(3)	*la su gl₃ I*	33
(3)	+ + + *i*	36
(1–2)	*la* + *gl₃ i*	3
(1–2)	+ *su* + *I*	3
(1–3)	*la* + + *i*	0
(1–3)	+ *su gl₃ I*	2
(2–3)	*la su* + *i*	5
(2–3)	+ + *gl₃ I*	16
(1–2–3)	*la* + *gl₃ I*	1
(1–2–3)	+ *su* + *i*	2

Crossover value for *la—su* interval= 7.5% $\left(\frac{56}{751} \times 100\right)$

Crossover value for *su—gl₃* interval=22.0% $\left(\frac{165}{751} \times 100\right)$

Crossover value for *gl₃—I* interval=12.6% $\left(\frac{95}{751} \times 100\right)$

inversion stock as compared with the control is statistically significant at the five percent level. The suppression of crossing over is probably the result of reduced chiasma formation in the long arm between the centromere and the locus of gl_3 due to asynapsis and non-homologous pairing, which are often evident in the pachytene configurations of inversion 4a. Since none of the known genes in chromosome 4 lies within the inverted region, it was not possible to demonstrate that crossing over within the inversion is effectively suppressed.

Table 3 gives the pollen sterility of heterozygous inversion 4a plants. In table 4 the pollen sterility of sister plants not carrying the inverison is tabulated. The minimum genetic length of inversion 4a as determined from pollen abortion is 25.3 units (28.2 minus 2.9). Plants heterozygous for inversion 4a

TABLE 3

Single anther pollen counts from plants heterozygous for inversion 4a.

PLANT NO.	NORMAL POLLEN	ABORTED POLLEN	% ABORTED POLLEN
1	1185	470	28.4
2	2186	905	29.3
3	1793	638	26.2
4	1730	695	28.7

$$\text{Average pollen abortion} = 28.2\% \left(\frac{2708}{9602} \times 100\right)$$

TABLE 4

Single anther pollen counts from sister plants not carrying inversion 4a.

PLANT NO.	NORMAL POLLEN	ABORTED POLLEN	% ABORTED POLLEN
1	2303	68	2.9
2	2237	67	2.9

$$\text{Average pollen abortion} = 2.9\% \left(\frac{135}{4675} \times 100\right)$$

produce well-filled ears that are normal in appearance. Examination of two ears from plants heterozygous for inversion 4a disclosed an ovule abortion of 4.0 percent (25 aborted ovules; 595 viable ovules). The striking difference between pollen abortion (25 percent) and ovule abortion (4 percent) indicates either very unequal frequencies of crossing over within the inverted region in male and female flowers or the exclusion of single crossovers from functional egg nuclei. EYSTER (1922) found no significant difference in crossing over in male and female flowers for the *su—Tu* region of chromosome 4. Apparently in maize as in Drosophila dicentric single crossover chromatids carrying duplications and deficiencies are not ordinarily included in functional egg nuclei. A deficient chromatid from a second anaphase bridge due to a chiasma proximal

to the inversion and disparate to a chiasma within the inversion may occasionally enter a terminal megaspore. However, double exchanges of this type also produce two normal chromatids each with a single centromere. McCLINTOCK (1938) found 3.0 percent of the second meiotic anaphase configurations in microsporogenesis arising from such double exchanges in heterozygous inversion 4a plants. Presumably the majority of aborted ovules observed are due to four-strand double crossovers within the inverted region, resulting in duplication and deficiency in all four chromatids. McCLINTOCK (1938) found that 3.1 percent of the first meiotic anaphase configurations in microsporogenesis of inversion 4a heterozygotes had double bridges and double fragments due to four-strand double crossovers within the limits of the inversion. The close agreement between McCLINTOCK's observations and the percentage of aborted ovules indicates that female sterility of paracentric inversion heterozygotes in *Zea mays* largely results from four-strand exchanges within the limits of the inversion.

As shown previously in the linkage map of chromosome 4, the region distal to gl_3 in the long arm does not include any known mutant genes. The location of gl_3 within the proximal third of the long arm of chromosome 4 by the use of inversion 4a reveals that more than two-thirds of the long arm of chromosome 4 is devoid of known mutant genes. Measurement of chromosome 4 at pachytene shows the long arm to be 1.6 times as long as the short arm. Only three of the eleven accurately mapped loci of chromosome 4 are in the long arm, and all three are located within the proximal third of the long arm. Since the distal two-thirds of the long arm is devoid of known mutant genes, there is a disproportionate distribution of mutant genes in the two arms of chromosome 4.

Inversion 5a

Pericentric inversion 5a, discovered by McCLINTOCK (unpublished), involves one-half of the length of the long arm and an extremely short region adjacent to the centromere in the short arm of chromosome 5. The following diagram shows the location of the breakage points in the long and short arms.

FIGURE 3. Inversion 5a. The positions of the breakage points are indicated by arrows.

An analysis of the pachytene bivalent involving the knobless inverted chromosome and its knobbed, normally arranged homologue shows the breakage point in the long arm to be near the large knob. Plate I, 3 shows the pachytene pairing of heterozygous inversion 5a. In figure 3 the two homologues are knobless. The long arm of chromosome 5 contains 52 percent and the short arm 48 percent of the total length of the pachytene chromosome (fig. 4). The ratio of the arms of the homozygous inversion 5a chromosome at pachytene is 3:1 (fig. 5). Due to the location of the breakage points, one-half of the chromatin in the long arm of normal 5 has been transferred to the short arm by the inversion, resulting in a rearranged chromosome with arm lengths strikingly different from the original.

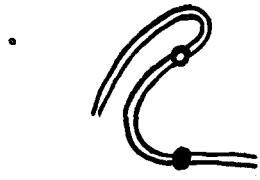

FIGURE 4. Normal chromosome 5 bivalent at pachytene.

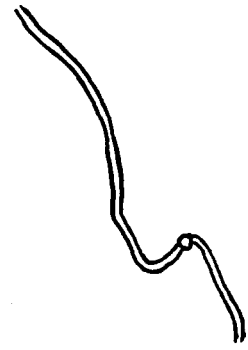

FIGURE 5. Homozygous inversion 5a bivalent at pachytene.

Note the change in arm ratios. The normal chromosome 5 is homozygous for a large knob in the distal half of the long arm. Camera lucida drawings ×900.

The following genetic map of chromosome 5 (fig. 6) was compiled by RHOADES (1940).

FIGURE 6. Linkage map of chromosome 5.

The position of the centromere on the genetic map of chromosome 5 was established by RHOADES (1936). Utilizing a fragment consisting of the centromere and the short arm of chromosome 5, RHOADES located the centromere between *bm* in the short arm and *bt* in the long arm. The *bm*—*bt* interval is only 2 map units long. Consequently, the centromere of chromosome 5 is marked on either side by loci with only 2 percent recombination.

To determine the position of inversion 5a on the genetic map of chromosome 5, plants homozygous for the inversion and *pr* were crossed with a_2 *bm bt Pr* pollen. F_1 plants were used as the female parent and backcrossed with a_2 *bm bt pr* pollen. RHOADES' study of the fragment of chromosome 5 placed the a_2 and *bm* loci in the short arm and the *bt* and *pr* loci in the long arm of chromosome 5. All F_1 plants were heterozygous for the inversion, eliminating the possibility of obtaining control linkage values from sister plants. However, the regions under consideration have previously been carefully studied, and extensive linkage data are available for comparison. Table 5 presents results obtained from crosses of

$$\frac{A_2\ Bm\ Bt\ pr\ I}{a_2\ bm\ bt\ Pr\ i} \times a_2\ bm\ bt\ pr\ i.$$

As may be anticipated from the length and location of the *bm*—*bt* interval and the proximity of the break in the short arm to the centromere, no crossing over

occurred between *bm* and *bt*. The *bm* and *bt* loci were completely linked by the inversion. For convenience the *bm* locus will not be recorded separately from the *bt* locus in subsequent discussions of the genetic behavior of inversion 5a. *Pr* and *pr* cannot be distinguished in homozygous a_2 seeds. In seeds homozygous or heterozygous for A_2, *Pr* produces purple aleurone and *pr* red aleurone. Seeds homozygous for a_2 have colorless aleurone. In computing the crossover value for the *bt*—*pr* region the A_2 class alone was utilized. In assembling the data of table 5, the reasonable assumption was made that class a_2 *bt pr* was numerically equal to its complementary crossover class A_2 *Bt Pr*. This assumption necessitated the removal of two individuals from the a_2 *bt Pr* non-crossover class. These corrections are recorded in parentheses. Since *Pr* and *pr* may not be distinguished in the a_2 classes, the presumed *Pr* and *pr* classifications for the a_2 classes are enclosed in parentheses. This assumption is justifiable since recombination in the *bt*—*pr* region is extremely limited (0.4 per cent) in the A_2 classes. A total of 848 mature plants, obtained from the 1090 seeds of table 5, were classified for the presence or absence of the inverted chromosome by determination of pollen abortion. The presence or absence of the inversion could not be accurately determined in 12 of the plants. In the remaining plants the inversion was found to be completely linked with the *Bt* locus. Recombination of the inversion with *Pr* was observed only in the two A_2 *Bt Pr* crossovers. The *Bt* locus may thus be conveniently and accurately used to follow the segregation of inversion 5a.

TABLE 5

Crossover values for the a_2-bt and bt-pr intervals in heterozygous inversion 5a plants.

$$\frac{A_2\ Bm\ Bt\ pr\ I}{a_2\ bm\ bt\ Pr\ i} \times a_2\ bm\ bt\ pr\ i.$$

GAMETIC TYPES	NO. OBSERVED
A_2 *Bt pr*	499
a_2 *bt* (*Pr*)	540 (538)
A_2 *bt Pr*	20
a_2 *Bt* (*pr*)	29
A_2 *Bt Pr*	2
a_2 *bt* (*pr*)	(2)
A_2 *bt pr*	0
a_2 *Bt* (*Pr*)	0

Crossover value for a_2—*bt* interval = $4.5\% \left(\frac{49}{1090} \times 100\right)$

Crossover value for *bt*—*pr* interval = $0.4\% \left(\frac{2}{521} \times 100\right)$

Table 6 gives additional data on recombination in the a_2—*bt* region in inversion 5a heterozygotes. Combining the data from tables 5 and 6 results in an average crossover value of 5.2 percent for the a_2—*bt* region.

TABLE 6

Data from the cross $\frac{A_2\ Bt\ I}{a_2\ bt\ i} \times a_2\ bt\ i$.

GAMETIC TYPES	NO. OBSERVED
$A_2\ Bt$	373
$a_2\ bt$	366
$A_2\ bt$	18
$a_2\ Bt$	31

Crossover value for the a_2—bt interval = 6.2% $\left(\frac{49}{788} \times 100\right)$

RHOADES (1941) made an intensive study of crossing over in the a_2—bt and bt—pr regions of chromosome 5. A significantly higher amount of crossing over was found in the male flowers as compared with the female. Moreover, high and low rates of crossing over for the a_2—bt region were observed in different lines. Normal and consistent rates of crossing over in male and female flowers were observed for the c—wx region of chromosome 9 in the same lines exhibiting unequal rates of crossing over for the a_2—bt region of chromosome 5. RHOADES concluded that the differences in crossover values observed for chromosome 5 were the result of some peculiarity inherent in that chromosome. Table 7 shows the different crossover values obtained by RHOADES for the $a_2 - bt$ region.

TABLE 7

Crossover values for $\frac{A_2\ Bt}{a_2\ bt} \times a_2\ bt$ *and the reciprocal cross (from* RHOADES *1941).*

	HIGH LINE, %	LOW LINE, %
Male flowers	26.3	13.4
Female flowers	17.3	5.9

A comparison of the crossover value of 5.2 percent for the a_2—bt region in the inversion heterozygotes with RHOADES' values of 17.3 and 5.9 for female flowers in the high and low lines indicates that a_2 is not located within the inversion in the short arm. Due to variation in crossing over in different lines for the a_2—bt region, it is not possible to determine definitely the degree of suppression of crossing over in the a_2—bt region in inversion 5a heterozygotes. RHOADES found a crossover value of 35.4 percent for the bt—pr region in male flowers and 30.3 percent for the same region in female flowers. The crossover value of 0.4 percent for the bt—pr region in the inversion heterozygotes indicates that the pr locus is within the limits of inversion 5a and that in maize as in Drosophila single crossover chromatids are not recovered from exchanges within a heterozygous inversion. Due to the inviability of single exchanges

within the inversion loop, the A_2 *Bt Pr* class necessarily arose from double exchanges in the *bt—pr* and *pr*—inversion break intervals within the inversion loop. The complete linkage of the inversion with *Bt* in the long arm shows the *bt* locus to be included within the inversion, as would be anticipated from RHOADES' study of the chromosome 5 fragment which originally located *bt* in the long arm. The absence of recovered crossovers between *bm* and *bt* in the progeny of the inversion heterozygotes shows that the *bm* locus is either included within the inversion or is closely adjacent to the break in the short arm.

Table 8 presents the results of pollen counts made from single anthers of plants heterozygous for the inversion and tester, and of plants homozygous

TABLE 8

Pollen abortion in inversion 5a heterozygotes and tester plants.

	VIABLE POLLEN	ABORTED POLLEN	% ABORTED POLLEN
Inv. 5a / 5 tester			
Plant 1	1454	877	37.6
Plant 2	1434	783	35.3
Plant 3	2009	1152	36.4
Plant 4	2109	917	30.3
5 tester / 5 tester			
Plant 1	1891	81	4.1
Plant 2	1990	151	7.1
Plant 3	1766	153	8.0

$$\text{Average pollen abortion in heterozygous Inv 5a} = 34.7\% \left(\frac{3,729}{10,735} \times 100\right)$$

$$\text{Average pollen abortion in homozygous 5 tester} = 6.4\% \left(\frac{385}{6032} \times 100\right)$$

for the tester chromosome. The amount of pollen abortion due to inversion 5a equals 34.7 percent minus 6.4 percent or 28.3 percent. The minimum genetic length of inversion 5a is 28 units.

A determination of ovule abortion frequencies revealed considerable disparity between the amount of ovule abortion and the amount of pollen abortion due to heterozygous inversion 5a. Seven ears from open-pollinated plants had a total of 315 aborted and 2192 viable ovules. The average ovule abortion of 12.5 percent was strikingly lower than the average pollen abortion of 28.3 percent. Since inversion 5a is a pericentric inversion, approximately equal amounts of ovule and pollen abortion would be expected. Apparently, in the inversion 5a heterozygote as in the normally arranged bivalent crossing over does not occur with the same frequency in male and female flowers.

Inasmuch as heterozygous inversions cause blocks of genes to be inherited

as a single unit, DOBZHANSKY and RHOADES (1938) suggested their use for a sector by sector analysis of the maize complement for genes of economic value. SPRAGUE (1941) utilized inversion 5a in an attempt to test for the existence of favorable genes located in the inverted segment. Stocks homozygous for inversion 5a and *pr* were crossed with an inbred line homozygous for *Pr*. The F_1 plants were backcrossed to the inversion stock. The resulting seeds were separated into classes homozygous for the inversion, *pr/pr*, and heterozygous for the inversion, *Pr/pr*. No significant differences were observed in the two classes for plant or ear height, number of ears, kernel row number or moisture content. The class heterozygous for the inversion, *Pr/pr*, showed increases, significant at the one percent level, in yield per plot and weight per 500 kernels due to favorable genes contributed by the inbred parent. The 12.5 percent ovule abortion characteristic of pericentric inversion 5a presumably caused a reduction in yield in the *Pr/pr* class. Even greater differences would undoubtedly have been observed if heterozygous inversion 5a had not produced ovule abortion. When possible, it would appear advisable to employ paracentric rather than pericentric inversions in chromosomal analyses for yield factors, since paracentric inversions, unlike pericentric ones, result in very little ovule abortion. However, pericentric inversions may be employed in an analysis for genes controlling plant characters not related to ovule abortion.

Inversion 2b

X-ray induced pericentric inversion 2b was discovered by RHOADES (unpublished). An analysis of pachytene configurations heterozygous and homozygous for the inversion showed that the inversion included the proximal .5 of the short arm and the proximal .15 of the long arm of chromosome 2. A diagrammatic interpretation of inversion 2b follows (Fig. 7).

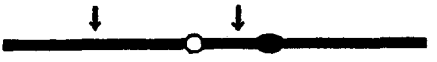

FIGURE 7. Inversion 2b. The positions of the breakage points are indicated by arrows. The short arm is to the left of the centromere, which is indicated by the circle.

The unequal positions of the breaks in the long and short arms with respect to the centromere resulted in a rearranged chromosome with arms of very different lengths (fig. 8). In comparison with normal chromosome 2 (fig. 9) the inverted chromosome has gained chromatin in the long arm and lost chromatin in the short arm.

A study of heterozygous inversion 2b configurations revealed that the long arm of the inverted chromosome possessed a large knob located considerably nearer the centromere than the knob usually encountered in the long arm of chromosome 2. The break in the long arm was near but proximal to the knob (plate I, 4). Frequent non-homologous pairing was evident in bivalents heterozygous for inversion 2b. Many rod-shaped bivalents were observed in which the inverted sector was synapsed non-homologously. Plate I, 5 shows a

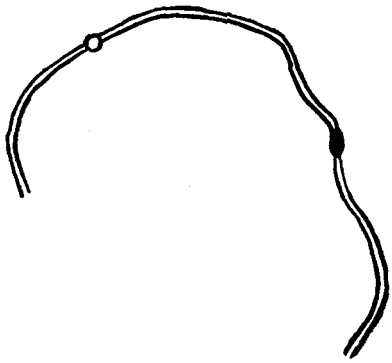

FIGURE 8. Pachytene bivalent of homozygous inversion 2b; knobbed. Camera lucida drawing ×900.

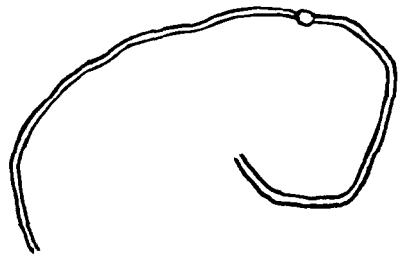

FIGURE 9. Pachytene bivalent of normal chromosome 2; knobless. Camera lucida drawing ×900.

heterozygous bivalent at pachytene exhibiting non-homologous pairing and asynapsis in the inverted and adjacent regions.

The genetic location of inversion 2b was determined by crosses involving the genes *ws*$_3$ *lg*$_1$ *gl*$_2$ *B* and *v*$_4$ in chromosome 2. The positions of these genes and of the centromere are shown on the following linkage map of chromosome 2 assembled from data in EMERSON, BEADLE and FRASER (1935), HAYES and IMMER (1942) and ANDERSON and RANDOLPH (1945). ANDERSON and RANDOLPH

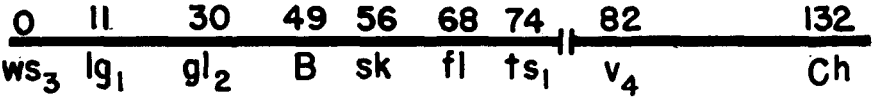

FIGURE 10. Linkage map of chromosome 2.

state that the map distance of the *ts*$_1$—*v*$_4$ interval is uncertain. They estimate the normal value to be approximately 20 units with the centromere five or more units from *ts*$_1$. Table 9 shows the linkage relationships of the inversion with *ws*$_3$*lg*$_1$ and *gl*$_2$. Table 10 presents data from sister plants not carrying the inversion, which were backcrossed with *ws*$_3$ *lg*$_1$ *gl*$_2$ pollen to obtain control values for the *ws*$_3$—*lg*$_1$ and *lg*$_1$—*gl*$_2$ intervals. The data from table 9 show that the break in the short arm of inversion 2b is 38 units proximal to the *gl*$_2$ locus. Thus *gl*$_2$ is physically located in the distal half of the short arm along with *ws*$_3$ and *lg*$_1$. According to the data in tables 9 and 10, crossing over is essentially normal in heterozygous inversion 2b plants for the *ws*$_3$—*lg*$_1$ and *lg*$_1$—*gl*$_2$ intervals. Table 11 shows the amount of recombination between *B* and the inversion break in the short arm. The gene order in the short arm is known to be *ws*$_3$ *lg*$_1$ *gl*$_2$ *B* with *B* located 19 units proximal to *gl*$_2$. The break in the short arm was located 38 units proximal to *gl*$_2$ by the data in table 9. Table 11 shows the break in the short arm to be 17 units proximal to *B*. Consequently *ws*$_3$ *lg*$_1$ *gl*$_2$ and *B* are physically located in the distal half of the short arm of chromosome 2.

According to the linkage map for chromosome 2, the total map length for

TABLE 9

Data from the cross $\frac{+\ +\ +\ I}{ws_3\ lg_1\ gl_2\ i} \times ws_3\ lg_1\ gl_2\ i.$

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	$+\ +\ +\ I$	147
(0)	$ws_3\ lg_1\ gl_2\ i$	139
(1)	$+\ lg_1\ gl_2\ i$	14
(1)	$ws_3\ +\ +\ I$	7
(2)	$+\ +\ gl_2\ i$	35
(2)	$ws_3\ lg_1\ +\ I$	32
(3)	$+\ +\ +\ i$	108
(3)	$ws_3\ lg_1\ gl_2\ I$	83
(1–2)	$+\ lg_1\ +\ I$	5
(1–2)	$ws_3\ +\ gl_2\ i$	0
(1–3)	$+\ lg_1\ gl_2\ I$	15
(1–3)	$ws_3\ +\ +\ i$	6
(2–3)	$+\ +\ gl_2\ I$	11
(2–3)	$ws_3\ lg_1\ +\ i$	8
(1–2–3)	$+\ lg_1\ +\ i$	1
(1–2–3)	$ws_3\ +\ gl_2\ I$	1

Crossover value for the ws_3—lg_1 interval= 8.0% $\left(\frac{49}{612}\times 100\right)$

Crossover value for the lg_1—gl_2 interval=15.2% $\left(\frac{93}{612}\times 100\right)$

Crossover value for the gl_2—I interval=38.1% $\left(\frac{233}{612}\times 100\right)$

TABLE 10

Data from the cross $\frac{+\ +\ +}{ws_3\ lg_1\ gl_2} \times ws_3\ lg_1\ gl_2.$

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	$+\ +\ +$	272
(0)	$ws_3\ lg_1\ gl_2$	210
(1)	$+\ lg_1\ gl_2$	27
(1)	$ws_3\ +\ +$	23
(2)	$+\ +\ gl_2$	46
(2)	$ws_3\ lg_1\ +$	50
(1–2)	$+\ lg_1\ +$	6
(1–2)	$ws_3\ +\ gl_2$	4

Crossover value for ws_3—lg_1 interval= 9.4% $\left(\frac{60}{638}\times 100\right)$

Crossover value for lg_1—gl_2 interval=16.6% $\left(\frac{106}{638}\times 100\right)$

the short arm has a minimum value of 79 crossover units. The 2b inversion break in the short arm was located cytologically in the center of the short arm and genetically 17 units to the right of *B*. Since crossing over may be reduced in regions adjacent to the inversion break, the length of the genetic map distal to the breakage point is 66 units as a minimum estimate. The genetic length of the entire short arm is approximately 79 units. That a disproportionately large amount of crossing over per unit of length occurs in the distal half of the short arm of chromosome 2 is clearly evident from the fact that the map length of the distal half is 66 units while the proximal half of the short arm has a map length of 13 units. McCLINTOCK (1931) located lg_1 within the very short region

TABLE 11

Recombination between the B locus and inversion 2b.

$\frac{B\ i}{b\ I}\times b\ i$		$\frac{b\ i}{B\ I}\times b\ i$	
GAMETIC TYPES	NO. OBSERVED	GAMETIC TYPES	NO. OBSERVED
B i	126	*b i*	49
b I	113	*B I*	48
B I	28	*b I*	9
b i	20	*B i*	13

$$\text{Crossover value for the } B\text{—}I \text{ interval} = 17.2\% \left(\frac{70}{406}\times 100\right)$$

consisting of the terminal four chromomeres of the short arm of chromosome 2. The locus of ws_3 is 11 units distal to lg_1. The interval between the locus of ws_3 and the end of the short arm is genetically unmarked. Consequently, there is a minimum chiasma frequency of 22 percent within the terminal four chromomeres of the short arm of chromosome 2. The surprisingly high chiasma frequency within the short sector composed of the terminal four chromomeres again illustrates the disproportionately high amount of crossing over per unit length in the distal region of the short arm of chromosome 2. Presumably, the lowering of exchange frequencies in the proximal region is controlled in some way by the centromere.

To determine the genetic location of the breakage point in the long arm of chromosome 2, plants heterozygous for the inversion and the marker genes gl_2 and v_4 were crossed with $gl_2\ v_4$ pollen. The data of table 9 disclosed that gl_2 was 38 units distal to the breakage point in the short arm. The locus of v_4 is 52 map units to the right of gl_2. RHOADES (EMERSON, BEADLE, and FRASER 1935) found 37 percent recombination in the gl_2—v_4 interval. ANDERSON and RANDOLPH found a minimum map distance of 7.3 units between the centromere and the locus of v_4 in the long arm. If the locus of v_4 were within the inversion they would show complete linkage except for double crossovers. Since inversion 2b is a comparatively short inversion, double crossovers would be expected to occur infrequently. The data of table 12 disclose 5.5 percent crossing over be-

tween v_4 and the inversion. Therefore, the locus of v_4 is 5.5 units distal to the inversion break in the long arm of chromosome 2. A crossover value of 38.1 per cent for the gl_2—I interval in the short arm was obtained from the data of table 9. The data of table 12 show a crossover value of 34.3 percent for the gl_2—I interval. The average crossover value for the gl_2—I interval computed from the data in tables 9 and 12 is 36.3 percent. The break in the short arm was located 17 units proximal to the B locus, and the break in the long arm 5 units proximal to v_4. Therefore, the region of chromosome 2 located 17 units proximal to B in the short arm and 5 units proximal to v_4 in the long arm is included

TABLE 12

Data from the cross $\frac{gl_2\ i\ v_4}{+\ I\ +} \times gl_2\ i\ v_4$.

REGION	GAMETIC TYPES	NO. OBSERVED
(0)	$gl_2\ i\ v_4$	171
(0)	$+\ I\ +$	157
(1)	$gl_2\ I\ +$	79
(1)	$+\ i\ v_4$	92
(2)	$gl_2\ i\ +$	7
(2)	$+\ I\ v_4$	12
(1–2)	$gl_2\ I\ v_4$	6
(1–2)	$+\ i\ +$	4

Crossover value for the gl_2—I interval = 34.3% $\left(\frac{181}{528} \times 100\right)$

Crossover value for the I—v_4 interval = 5.5% $\left(\frac{29}{528} \times 100\right)$

within inversion 2b. This region corresponds to the proximal 0.5 of the short arm and the proximal 0.15 of the long arm of chromosome 2.

The recombination value for the gl_2—v_4 region in the inversion 2b heterozygotes is 36.0 percent. A comparison of the recombination value for gl_2—v_4 in the inversion 2b heterozygotes with the 37 percent recombination value obtained by RHOADES for gl_2—v_4 in normal stocks indicates no significant reduction of recombination due to inversion 2b. The lack of suppression of recombination in the gl_2—v_4 region is presumably due to the fact that the interval between the locus of gl_2 in the short arm and v_4 in the long arm includes a considerable portion of the length of chromosome 2, and that inversion 2b is a comparatively short inversion including regions adjacent to the centromere where recombination is less than in distal regions.

Table 13 shows the pollen abortion frequencies of heterozygous inversion 2b plants and sister plants not carrying the inverison. The average pollen abortion due to heterozygous inversion 2b was 19.1 percent while the ovule abortion was 20.1 percent.

TABLE 13

Pollen abortion frequencies of inversion 2b heterozygotes and normal sister plants.

	VIABLE POLLEN	ABORTED POLLEN	% ABORTED POLLEN
I/i (heterozygous inversion 2b)			
Plant 1	1456	502	25.6
Plant 2	2192	648	22.8
Plant 3	1739	524	23.2
Plant 4	2080	465	18.3
i/i (homozygous normal chromosome 2)			
Plant 1	2248	62	2.7
Plant 2	2035	57	2.7
Plant 3	2371	101	4.1

Average pollen abortion for $I/i = 22.3\% \left(\frac{2139}{9606} \times 100\right)$

Average pollen abortion for $i/i = 3.2\% \left(\frac{220}{6874} \times 100\right)$

Average pollen abortion due to Inv 2b = 19.1% (22.3% − 3.2%)

Cytological Analysis of an Included Inversion Heterozygote

Complex pairing configurations in the salivary gland chromosomes of Drosophila are found when two inversions involving the same chromosome are present (DOBZHANSKY and STURTEVANT 1938). According to the relative positions of the breakage points of the two inversions, complex inversion types are classified as included, overlapping or independent. Meiotic pairing of complex inversions has not been reported in any organism.

In order to study meiotic pairing of a complex inversion in *Zea mays*, plants heterozygous for two different inversions involving chromosome 2 were crossed. F_1 plants were examined cytologically for the presence of the complex inversion. Pericentric inversion 2b has previously been described in detail. Pericentric inversion 2a, discovered and studied by E. G. ANDERSON, includes 0.7 of the length of the short arm and 0.8 of the length of the long arm of chromosome 2. The location of the breakage points of inversion 2a produced a rearranged chromosome with arms less divergent in length than inversion 2 b (plate I, 4, and 6). The unequal location of the breakage points with respect to the centromere in inversion 2b, as previously described, resulted in a rearranged chromosome with strikingly different arm lengths.

Plate I, 4 and 6 show the pachytene configurations of heterozygous inversions 2a and 2b. Plate I, 7 shows the meiotic pairing of the inversion 2a/2b heterozygote. The abbreviated short arm and the large knob in the long arm of the inversion 2b chromosome enable it to be identified in the complex pachytene configuration. An analysis of figure 7 shows inversion 2b to be included

within inversion 2a. The naturally occurring included inversions analyzed cytologically by DOBZHANSKY and STURTEVANT in *Drosophila pseudoobscura* were of the paracentric type. The inversion 2a/2b configuration is an included pericentric type.

Pollen counts of individual anthers from two plants heterozygous for inversion 2a disclosed an average pollen abortion of 44.6 percent, which is considerably higher than the 19.1 percent abortion typical of inversion 2b. A plant free from any detectable aberration was crossed with pollen from a plant carrying inversion 2a/2b. Pollen samples were examined from 80 F_1 plants and classified into two groups for high and low pollen abortion. Of the F_1 plants 45 had the high pollen abortion characteristic of inversion 2a; 35 had the relatively lower pollen abortion characteristic of inversion 2b, indicating 1:1 segregation of inversion 2a and inversion 2b in microsporogenesis of the 2a/2b plant.

SUMMARY

A cytogenetic study was made of three inversions in *Zea mays*. One of these was a paracentric inversion with both breaks in the long arm of chromosome 4, while the other two involving chromosomes 2 and 5 were pericentric inversions.

Inversion 4a

As previously shown by MCCLINTOCK, the proximal break in the long arm is located at a point approximately one-third the length of the long arm from the centromere; the distal break is near the end of the long arm. Plants heterozygous for inversion 4a have an average pollen abortion of 25.3 percent and an average ovule abortion of 4.0 percent. The striking difference between pollen abortion and ovule abortion frequencies is attributed to exclusion of single crossover chromatids from functional megaspores, while single crossover chromatids are included in the microspores and cause their abortion. The proximal break of inversion 4a is located 12.6 units distal to gl_3, thus establishing the locus of gl_3 within the proximal third of the long arm of chromosome 4. The distal two-thirds of the long arm of chromosome 4 has no known mutant genes. The minimum genetic length of inversion 4a as determined from pollen abortion frequencies is 25.3 units.

Inversion 5a

The break in the short arm is located adjacent to the centromere; the break in the long arm is located at a point one-half the length of the long arm from the centromere. The break in the long arm is near the large knob in the long arm of chromosome 5. Plants heterozygous for inversion 5a have an average ovule abortion of 12.5 percent and an average pollen abortion of 28.3 percent.

The difference between the percentage of pollen and ovule abortion is attributed to higher frequencies of crossing over in male than in female flowers. The locus of a_2 is distal to the break in the short arm. The locus of *bm* is either proximal to the break in the short arm and included within the inversion or distal but very closely adjacent to the break in the short arm. The loci of *bt*

and *pr* are within the inverted region in the long arm. Single crossover chromatids are not recovered from exchanges within inversion 5a. Double crossover chromatids were recovered from exchanges within the *bt—pr* and *pr*—inversion break intervals in 0.4 percent of the eggs of the inversion heterozygotes. The *bt—pr* region is 23 units long in the genetic map of chromosome 5. Recombination within the *bt—pr* region of the inversion heterozygote is effected only by double crossover chromatids.

Inversion 2b

Inversion 2b includes the proximal .5 of the short arm and the proximal .15 of the long arm of chromosome 2. Plants heterozygous for inversion 2b show an average ovule abortion of 20.1 percent and an average pollen abortion of 19.1 percent. The break in the short arm is located 36.3 units proximal to gl_2 and 17.2 units proximal to *B*. The break in the long arm is 5.5 units proximal to the locus of v_4. Crossing over per unit length is considerably higher for the distal half of the short arm of chromosome 2 than for the proximal half.

Cytological Analysis of Included Inversion 2a/2b

The chromosome 2 bivalent of a plant heterozygous for the two pericentric inversions 2a and 2b was analyzed at pachytene. Inversion 2b was found to be included within inversion 2a. The 2a/2b plant showed a 1:1 segregation of inversions 2a and 2b in microsporogenesis.

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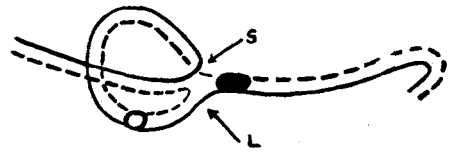
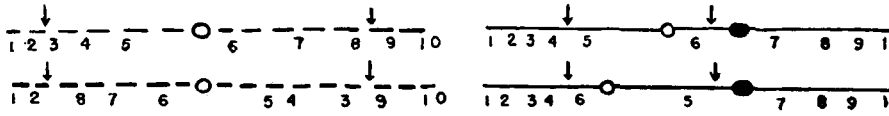
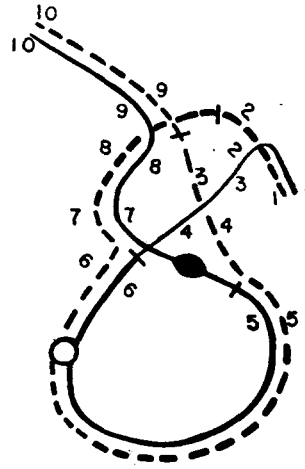


Diagram of plate I, 4. The inverted chromosome 2b is shown by a broken line. S represents the break in the short arm. L the break in the long arm.





Diagrammatic representation of plate I, 7. Inversion 2a is indicated by a broken line. Inversion 2b with the heteropycnotic knob in the long arm is indicated by a solid line. In the rod-shaped diagrams at the top of the figure the two inverted chromosomes are indicated, each below the normal chromosome 2 from which it was derived. The numerical sequence starts at the distal end of the short arm. The breakage points are indicated by arrows and the centromere by a circle.

Interpretation of the 2a/2b bivalent

The changes in homology and the approximate breakage points in 2a and 2b are indicated by the solid cross lines.

Region 1– 2 Normal arrangement distal to the inversion breaks in the short arms of 2a and 2b.

Region 3– 4 Inverted order for 2a; normal order in the short arm of 2b. Asynapsis is probably due to the much abbreviated short arm of inversion 2b.

Region 5– 6 Region inverted in common in 2a and 2b.

Region 7– 8 Inverted order for 2a; normal order in the long arm of 2b.

Region 9–10 Normal arrangement of 2a and 2b distal to the breakage points in the long arms.

Photomicrographs of pachytene configurations (×1800).

1. Heterozygous inversion 4a.

2. Heterozygous inversion 4a showing asynapsis and non-homologous pairing.

3. Heterozygous inversion 5a. One-half of the long arm is involved in the inversion loop. The centromere may be seen adjacent to the pycnotic region at the base of the loop.

4. Heterozygous inversion 2b. (See diagram)

5. Heterozygous inversion 2b showing asynapsis and non-homologous pairing in the inverted and adjacent regions.

6. Heterozygous inversion 2a.

7. The inversion 2a/2b bivalent. (See diagram)

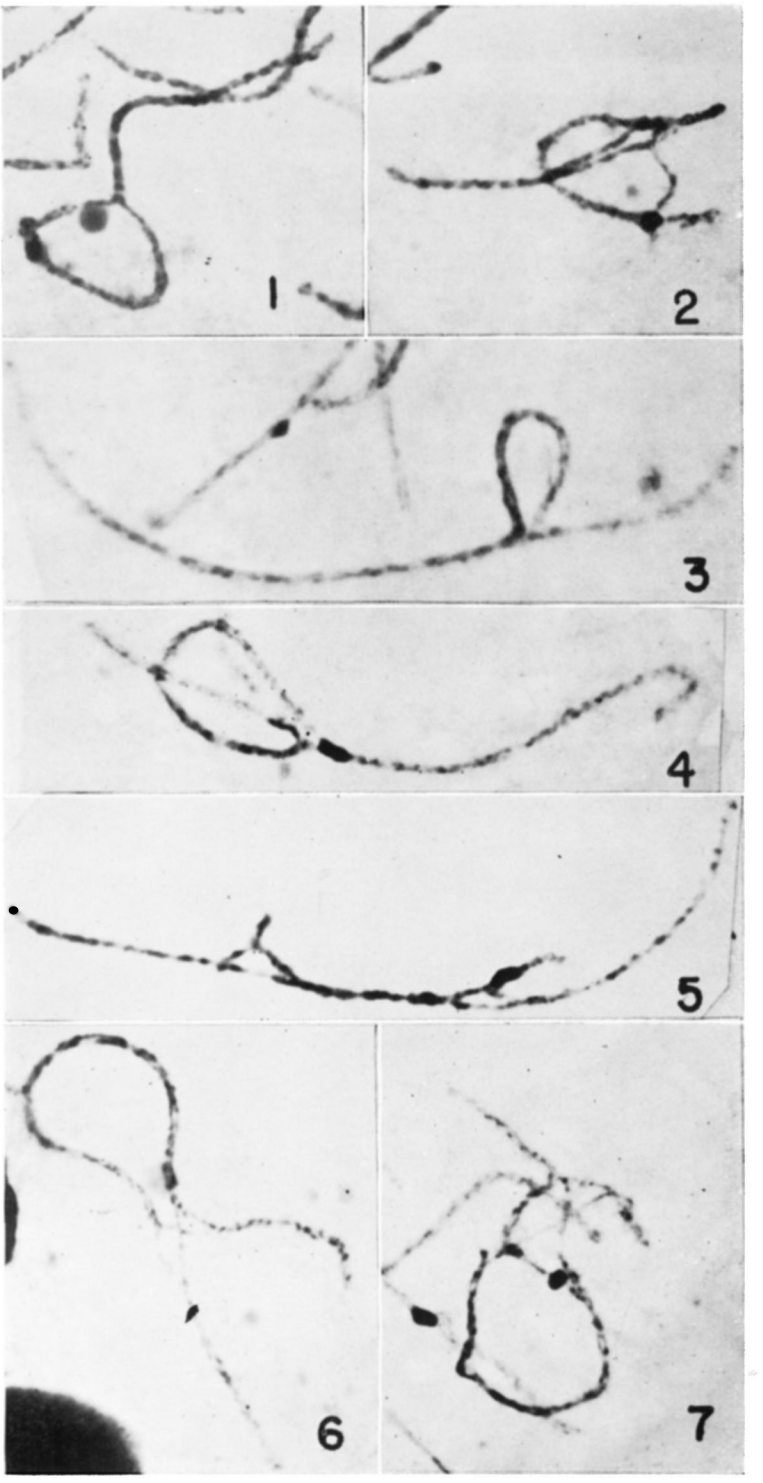


PLATE I

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