AN ABSTRACT OF THE THESIS OF

DONALD DEAN JONES (Name) for the DOCTOR OF PHILOSOPHY (Major)

in GENETICS (Major) presented on August 16, 1971 (Date)

Title: THE INFLUENCE OF CHELATING AGENTS ON CYTOLOGICAL EXCHANGE AND GENETIC RECOMBINATION IN ZEA MAYS L.

Abstract approved: Redacted for privacy

Dr. Ralph Bogart

The present investigation with the chromosomes of Zea mays was conducted to determine: (1) the effects of chelating agents on the process of cytological exchange, and (2) the effects of chelating agents on genetic recombination.

To ascertain the influence of chelating agents on cytological exchange, four known heterozygous paracentric inversion stocks of Zea mays were treated with several concentrations of ethylenediamine tetraacetic acid (EDTA) and dimethyl sulfoxide (DMSO) singly and in combination prior to meiosis. The four chromosomes with known inverted segments were: (1) chromosome two with an inversion involving 19 map units, (2) chromosome three with an inverted segment of 28 map units, (3) chromosome seven with a large inversion in most of the long arm, and (4) chromosome nine with an inverted segment of approximately 33 map units.

Pollen mother cells were utilized for chromosome analysis after fixing in Carnoy's solution and staining with propionic carmine. Cytological exchanges produced by the different treatments were measured by counting the number of dicentric bridges and acentric fragments observed in anaphase I and II.

Depending upon the concentration used, both EDTA and DMSO singly or in combination were found to produce significant increases in cytological exchanges in the four inversion stocks.

Zea mays stocks, heterozygous for four known linkage groups involving seed and seedling characteristics, were treated with EDTA and DMSO and were crossed with untreated homozygous plants by means of hand pollination. The effects of the two chelating agents on genetic recombination were then determined by scoring the progeny from the testcrosses.

Genetic recombination in the testcross stocks was significantly increased by EDTA and DMSO, singly or in combination, in all the testcrosses. In some instances genetic recombination and cytological exchange were both increased significantly in the same chromosome, whereas in other cases no such relationship was observed.

The study may provide valuable clues as to the specific effects that chelating agents exert on genetic recombination and cytological exchange. Furthermore, through the utilization of both cytological and genetic techniques, a better understanding of the entire process of crossing over may be obtained.

Influence of Chelating Agents on Cytological Exchanges and Genetic Recombination in *Zea mays* L.

by

Donald Dean Jones

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1972

APPROVED:

Redacted for privacy

Chairman of Genetics Board
in charge of major

Redacted for privacy

Dean of Graduate School

Date thesis is presented August 16, 1971

Typed by Mary Jo Stratton for Donald Dean Jones

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. Ralph Bogart for his guidance and dedication in helping with my program.

I wish to especially thank Dr. Warren Kronstad for his advice and untiring assistance in this study. The spirit of cooperation between both Dr. Bogart and Dr. Kronstad assisted greatly in completion of this study.

A special note of thanks is extended to Dr. A. L. Haunold, Dr. R. J. Metzger, and Mr. Matt Kolding of the Farm Crops Department.

I wish to thank Mrs. Barbara Fine for her help with the counting and photography work. Also to Miss Thelma Brooks who proofread the manuscript.

The Maize Genetics Cooperative, Urbana, Illinois furnished the seeds for the testcross portion of the study and two of the inversion stocks. Dr. Ellen Dempsey, Indiana University furnished the inversion stocks for chromosomes three and seven.

The Northwest Scientific Association Farm Crops Department and the Genetics Institute provided financial support for this study.

TABLE OF CONTENTS

LIST OF TABLES

INFLUENCE OF CHELATING AGENTS ON CYTOLOGICAL EXCHANGES AND GENETIC RECOMBINATION IN *ZEA MAYS* L.

INTRODUCTION

The effects of chemical mutagens upon chromosome behavior has received considerable attention in recent years primarily due to the specific and predictable action exerted by the chemicals on chromosome behavior during crossing over. The cytological effects of chemical mutagens on the chromosome during crossing over has been studied less than the traditional genetic crossing over. The cytological effects have been shown by use of known inversion strains of *Zea mays* whereas the effects on genetic crossing over have been observed in *Zea mays* by use of testcross stocks.

A group of chemicals which is thought to influence crossing over is the chelating agents, which are postulated to function by complexing divalent cations or by changing the ionic balance in the nucleus. One assumption is that the chelator complexes the divalent cations which are essential for forming molecular bridges between the phosphates of DNA, and this in turn subjects the chromosome to additional breakage and subsequent recombination. The second assumption is that the metallic cations maintain the proper ionic balance in the nucleus and are not a part of the chromosome structure *per se*, consequently when the chelator ties up the cations, abnormal ionic balance occurs producing chromosome breakage.

The exact mechanism to explain cytological exchanges and the resulting new genetic recombination is still unknown, but some investigators believe that cytological exchange is associated with DNA synthesis while others believe the process may occur both at the time of DNA synthesis and at the actual pairing of homologous chromosomes. In each case, crossing over is thought to require some type of breakage and restitution of the chromatids.

The present study was undertaken to answer the following questions:

(1) What are the effects of chelating agents on cytological exchange ?

(2) What are the effects of chelating agents on genetic recombination ?

(3) How do the results of cytological exchange and genetic recombination compare ?

(4) How can chelating agents be utilized by geneticists and plant and animal breeders to increase genetic variability ?

The answers to these four questions may provide additional information concerning the entire process of crossing over which, in spite of extensive research, still remains one of the unsolved problems for geneticists.

LITERATURE REVIEW

Extensive study of the nature of recombination in plants and animals has shown that considerable variation can occur in the process. Both cytological and genetic investigations have been employed in an attempt to explain this variation.

The exact mechanism of crossing over has not been fully explained, even though numerous theories concerning the process have been proposed. It is known that the crossover process depends upon chromosome structure; therefore, information concerning structure and theories concerning crossing over are presented.

Morphology and Chemical Constituents of the Chromosome

The pioneering work on chromosome morphology was conducted by McClintock (1929, 1930). Using acetocarmine stain, she discovered the haploid set of chromosomes for maize. In recent years, the study of chromosome morphology has been greatly increased because of better staining techniques and more elaborate instrumentation. Consequently, researchers are beginning to explore the internal structure of the chromosome.

Two such structures found to be necessary for proper chromosome function are the centromere and the chromonema. The centromere is a small constriction which determines the shape of the

chromosome and is also essential as the attachment site for the spindle fiber (DeRobertis, Novinski and Saez, 1960). The chromonema is a coiled filament running the length of the chromosome (Gall, 1956). Speculation that the chromonema may be double-stranded leads one to believe that it may be a double helix of DNA, but as yet no correlation between the chromonema and the DNA has been observed.

Further studies of chromosome structure have shown that controversy exists between those scientists who believe the chromonema is multistranded and those who support the single stranded view concerning the chromonema. Kaufman and Gay (1960) maintain that the chromosome consists of a bundle of fibrils with each fibril approximately 100A in diameter. Ris (1961) has estimated the number of 100A fibrils in the leptotene chromosome of Tradescantia to be about 32. Each 100A fibril in turn can be subdivided into two units each 40A thick, which represent DNA double helixes surrounded by histone. In contrast, Gall (1963a) suggests that the apparent multistrands seen in electron micrographs are actually the many windings of single strands and that the meiotic chromosome really consists of only two chromatids.

Both the multistranded and single stranded concepts recognize that the basic chemical constituent of the chromosome is a DNA-histone complex. Mirsky and Ris (1951) found that this complex made up 60-90% of the bulk of the chromosome when it was isolated from

the chromosome by treatment with 1M NaCl. Swanson, Merz and Young (1967) suggest that the histone is attached to the DNA phosphate groups, by ionic bonds, and these stabilize the DNA. In addition, acidic protein is believed to run continuously throughout the chromonema, with the DNA segments occurring at right angles to the chromonema and parallel to each other (De, 1964). He also suggests that several of the polypeptide chains of the acidic protein may be bound together by divalent cations.

Mechanism of Crossing Over

One of the first theories to explain crossing over was proposed by Janssen (1909) in which he stated that the chiasmata were points of crossing over. His theory received impetus through the discovery by Stern (1931) and Creighton and McClintock (1931) that genetic crossing over involves the physical exchange of chromatid segments. However, Sax (1932) raised objections to the chiasma type theory and maintained that the chiasma resulted from random coiling of the chromosomes about each other and that crossing over occurred when the chiasma ruptured and the broken ends reunited.

Cooper (1949) observed the presence of chiasmata in the chromosomes of male Drosophila. Since crossing over does not normally occur in the male Drosophila, these observations supported Sax's views. However, the chiasmata observed by Cooper may be only surface associations rather than actual chiasmata (Slizynski, 1964).

Darlington (1935) proposed another theory to explain crossing over. He theorized that the breakage which occurred during crossing over was the result of strains and torsions produced by the opposite directional rotation of the chromatids from that of the chromosomes. Subsequently, the broken ends of one uncoiled chromatid unite with the broken end of the other uncoiled chromatid producing a crossover.

Another explanation of crossing over was presented by Belling (1933). His theory is that chromosome duplication occurs in two stages: (1) the formation of new genes themselves, and (2) the formation of new connections between these genes. The chromatids may exchange segments during the period of new gene formation with the result that the new chain of genes will show crossing over.

Taylor (1958) demonstrated that exchanges between subunits of the chromosome occur during interphase and that the DNA was semi-conservatively replicated. These results present strong objections to the Darlington and Belling theories, which hypothesize that crossing over occurs during prophase. Also contrary to the evidence presented by Taylor is the fact that Belling's model requires the conservative replication of DNA.

The polaron hybrid DNA model (Whitehouse, 1963) is another attempt to explain crossing over. According to this theory, crossing over occurs because the nucleotide chains of opposite polarity break at precise points and the synthesis of new nucleotide chains occur

alongside the old unbroken chains. The newly synthesized chains then separate from the old chains to pair with their new complements from the other chromatid, thereby forming the new crossover.

Uhl (1965) proposed still another theory to explain crossing over. He believes the backbone of the chromosome is a DNA double helix containing "links." Before replication, the DNA strands unwind and the links remain with only one polynucleotide strand. Because these links occur at random, the gaps left by the missing links in the remaining polynucleotide strand are potential sites for the crossing over which may occur when the new links are formed between the new nucleotide chains. At present, the existence of such links is strictly hypothetical; but such links, if they do occur, may represent "punctuation marks" which separate elements of the genetic code in the DNA (Swanson, Merz and Young, 1967). Sherman and Roman (1963) demonstrated that two types of alleleic recombination may occur, one at the time of DNA replication and the other during chromosome pairing in meiosis. Such results tend to bring together the two ideas of crossing over by chromatid exchange during meiosis and crossing over of polynucleotide strands during DNA synthesis.

Environmental Factors Influencing Crossing Over

It was shown by researchers in the early 1900's that many

environmental factors influence crossing over. Plough (1917) observed an increase in crossing over in Drosophila when the temperature was raised or lowered from 25° C. Swanson (1940) observed a decrease in chromosome bridges and fragments of a heterozygous inversion following low temperature treatment but an increase in bridges and fragments at higher temperatures.

Whittinghill (1937) observed crossing over in the male Drosophila following high temperature shock during the larval stage. The majority of the crossovers occurred in males heterozygous for genes on the third chromosome. Peacock (1968) obtained different results by using temperature shock on spermatocytes of the grasshopper Goniaea australasiae. He observed that the frequency of chiasmata in early pachytene decreased greatly following temperature shock treatment and that this frequency was correlated with a reduction in genetic crossing over.

Wolff and Luippold (1955) observed that low temperatures inhibited the repair of radiation-induced chromosome breaks and increased crossing over. Apparently, the low temperature increases the time required for restitution of the breaks which leads to an increase in crossing over. They believe the rejoining of the breaks is dependent upon oxidative metabolism.

The presence of oxygen during irradiation increases chromosomal breakage, according to Thoday and Read (1947). Bean root tips

exposed to oxygen during irradiation showed a significant increase in the number of bridges and fragments at mitotic anaphase as compared with those irradiated in a nitrogen atmosphere.

Bridges (1927), noting the age of the female Drosophila as an influence, observed two minimal periods of crossing over, the first occurring at 11 days and the second at 25 days of age. He further observed that the crossover frequency in chromosome two was less during the first ten-day period.

Influence of Chromosomal Aberrations on Crossing Over

A structural change in the chromosome or the chromatid may occur following breakage after which reunion of the broken ends of the chromosome or chromatid may occur. In some cases rearrangements are produced including deficiencies, duplications, translocations, and inversions. Of these various rearrangements, translocations and inversions have been shown to reduce the frequency of crossing over. Dobzhansky (1931) found that crossing over was reduced in a reciprocal translocation heterozygote, especially near the breakpoints in the chromosome. Rhoades (1968) believes such reduction is due to asynapsis of the non-homologous portions in the translocation. Swanson, Merz and Young (1967), however, observed that homozygous translocations appear to exhibit the same crossing over frequency as that of normal chromosomes.

Zimmering (1955) found that crossing over in the interstitial region of a translocation heterozygote may influence genetic recombination. He observed that the female Drosophila does not produce all possible gametes at the same expected frequency due to the formation of asynaptic dyads, consisting of a long and short chromatid; the recovery of the shorter chromatid occurred more frequently than that of the longer chromatid.

Sturtevant and Beadle (1936) observed a decrease in crossing over within the loop of a heterozygous paracentric inversion in Drosophila melanogaster, but the crossover frequency in homozygous inversions was similar to that of normal chromosomes. However, Sturtevant (1919) showed that decreased crossing over in the pair of chromosomes involved in the inversion actually produced an increase in the crossover frequency between genes of another chromosome pair not involved in the inversion.

Cytological evidence suggests that crossing over in the heterozygous paracentric inversion may not be drastically reduced; rather, the failure of the gametes to survive produces the apparent lack of crossovers (Morgan, 1950). When a single crossover occurs within the loop of a paracentric heterozygous inversion, a dicentric bridge and an acentric fragment occur, causing pollen sterility. However, Sturtevant and Beadle (1936) showed that Drosophila females, heterozygous for paracentric inversions, do not show gamete sterility.

They suggest that the crossover chromatids resulting from a single exchange within the loop are incorporated into the two polar bodies and do not become functional gametes.

Cytological structures found on the chromosome may influence crossing over. The presence of heterchromatic knobs on chromosome ten of maize produces a significant increase in the frequency of crossing over (Rhoades and Dempsey, 1957). In addition, genes located near the centromere on the chromosome will show reduced crossing over (Beadle, 1932a).

Influence of Genes on Crossing Over

Many investigators have shown that crossing over is at least under partial genetic control. Levine and Levine (1955) observed a significant difference in the crossover frequency of two strains of Drosophila, and they suggested that this difference was due to the different genetic constitutions of the two lines. Jessop and Catcheside (1965) found interalleleic recombination at the his-1 locus in Neurospora crassa to be under control of a recessive gene (rec-1). This recessive gene apparently prevents coding for the enzyme necessary to complete the repair process following breakage, and therefore increased the frequency of recombination between alleles by more than ten fold. The lack of sufficient amount of enzyme and consequently the increased time required for repair of the breakage produces a

greater chance for recombination to occur. In addition, the specificity of the rec-1 factor apparently exerts control over the production of a specific regulator gene controlling recombination (Catcheside, 1968).

The genetic control of meiosis and its subsequent influence on crossing over has been observed by Beadle (1932c, 1933). He discovered a gene in maize which caused the chromosomes to stick together and another gene which produced an asynaptic condition. Other abnormal meiotic conditions, including divergent spindle and post meiotic divisions, have been shown to be under genetic control (Beadle, 1933a; Clark, 1940).

Effects of Radiation on Chromosomes and Crossing Over

Radiation effects on the chromosome have been extensively studied and classified as to the kind of aberrations produced. Sax (1957) found that ionizing radiation produced different kinds of aberrations depending upon the stage of nuclear development at the time of irradiation. If the cell is irradiated during interphase, chromosome aberrations occur, but if it is irradiated in prophase, chromatid aberrations including exchanges and dicentric bridges occur.

In addition to the aberrations produced, radiation has been shown to influence crossing over. The extent of the crossing over depends upon the kind of radiation used, the number of exposures, and the stage of cell division at the time of exposure (Stadler, 1928b).

Radiation is thought to produce breakage of both ionic and covalent bonds. Crossing over is caused because the covalent bonds require a source of energy to be reformed. Since these bonds are slow in reforming, crossing over has a longer time to occur (Wolff and Luippold, 1956).

Effects of Chemical Agents on Crossing Over

Chromosome structure and recombination may be influenced by various chemical agents. Such chemicals may exert their influence by inhibiting excision-repair mechanisms, interfering with DNA or RNA synthesis, producing base-pair substitutions, intercalation, and chromosomal and chromatid breakage.

Gigg (1968) observed that caffeine bromo uracil induces chromosome breakage by inhibiting the excision-repair mechanism, thereby allowing time for recombination to occur. In addition, caffeine has been shown to inhibit DNA synthesis and to produce chromosome breakage (Kihlman, 1966).

Actinomycin D has been shown to inhibit the synthesis of DNA or RNA (Suzuki, 1965a). Iyer and Szybalsky (1963) suggest that this chemical produces its effect by crosslinking the complementary strands of DNA. In addition, maleic hydrazide has been shown to interfere with RNA synthesis (Kaufman, Gay and McDonald, 1960).

They believe that this chemical may substitute for the uracil in RNA, thereby producing breakage in the RNA regions of the chromosomes.

Kihlman (1966) reports still another type of effect produced by the acridine dyes--namely intercalation. These dyes insert between two neighboring purine bases of DNA, thereby producing a mutation of the DNA through the addition or deletion of a single nucleotide in the DNA strand during replication.

Still another group of chemicals that influence chromosome and chromatid breakage are the chelating agents. These chemicals are believed to complex the divalent cations that are necessary for chromosome structure (Mazia, 1954). When the cell is treated with a chelator, the divalent cations are chemically complexed and this renders the chromosome more subject to breakage (Davidson, 1958). Kirby (1956) suggests that the metallic cations bond between the carboxyl groups of the protein and phosphate groups of the DNA. However, Zubay (1959) believes the divalent cations may be bound to the nitrogenous bases of DNA and RNA. Experiments conducted by Nilan and Phillips (1957), Frick (1958) and Somers, Cole and Hsu (1963) further support this divalent cation bridge hypothesis.

Another alternative to the molecular bridge hypothesis is presented by Kaufman and McDonald (1957). They believe the chelating agents produce chromosome breakage by modifying the ionic environment of the nucleus. This hypothesis is further supported by the work

of Dornfeld and Owczarzak (1958), who suggest EDTA may interfere with ATP production.

Ihrke (1970) observed that chelating agents would increase the frequency of genetic crossing over. Using the two chelating agents, ethylenediamine tetraacetic acid and dimethyl sulfoxide, his work supports the conclusion that the ionic environment of the nucleus is modified and this modification produces the increase in the frequency of crossing over.

METHODS AND MATERIALS

The investigation was initiated in the spring of 1970 at the East Farm Experimental Site, near Corvallis. The study in which both cytological and genetic experimental techniques were used was conducted to determine the effects of two chelating agents upon cytological exchange and genetic recombination.

Zea mays was selected as the experimental organism for the study because it has well established linkage groups, large chromosomes, a diploid number of 20, and available cytological and genetic stocks. Also the pollen mother cells can be readily removed from the anthers and the chromosomes can be observed clearly in stained preparations.

Four heterozygous paracentric inversion stocks, possessing inverted segments of different lengths and breaks of different distances from the centromere, were used to determine the effects of chelating agents on cytological crossing over. The inverted chromosome two stock contained the smallest inversion, representing 19 map units, with one break at position 30 (gl_2) and the other at position 49 (B) in the long arm. Chromosome three stock contained an inverted segment of 28 map units with breaks at positions 83 (lg_2) and 111 (a_1). Chromosome seven stock possesses the largest inverted segment encompassing most of the long arm with one break near the centromere and the other near the distal end of the chromosome, the exact

locations of the breaks being unknown. The inverted segment on chromosome nine was located in the short arm and consisted of approximately 33 map units; one break was definitely located at position 59 (wx), while the other, not definitely located, was close to position 26 (c_1). The map of the inverted stocks is shown in Table 1.

Four stocks with known genetic markers were used to determine the effects of chelating agents on genetic recombination. On chromosome two, three gene markers are located at positions 11 (lg_1), 30 (gl_2), and 83 (v_4), with the 30 to 83 region spanning the centromere. Chromosome three contains two genetic markers near the distal end of the long arm at positions 111 (a_1), and 122 (et). In addition, chromosome three is thicker than is any other long chromosome in Zea mays and generally has a heterochromatic knob situated between the a_1-et region and the centromere. Chromosome five has the genetic markers located at positions 15 (a_2) on the short arm and 22 (b_t) adjacent to the centromere on the long arm. Chromosome nine, the second shortest chromosome in maize, contains two genetic markers located in the short arm at positions 26 (c_1) and 59 (wx).

These four genetic stocks not only showed defined traits clearly but also expressed these traits in the seed or seedling stage of development. Consequently, they could easily be scored as crossovers or non-crossovers without waiting for the progeny to mature.

The linkage map for the four testcross stocks is shown in Table 2.

Table 1. Inversion map of four Zea mays L. chromosomes showing position of breaks and length of inversions.

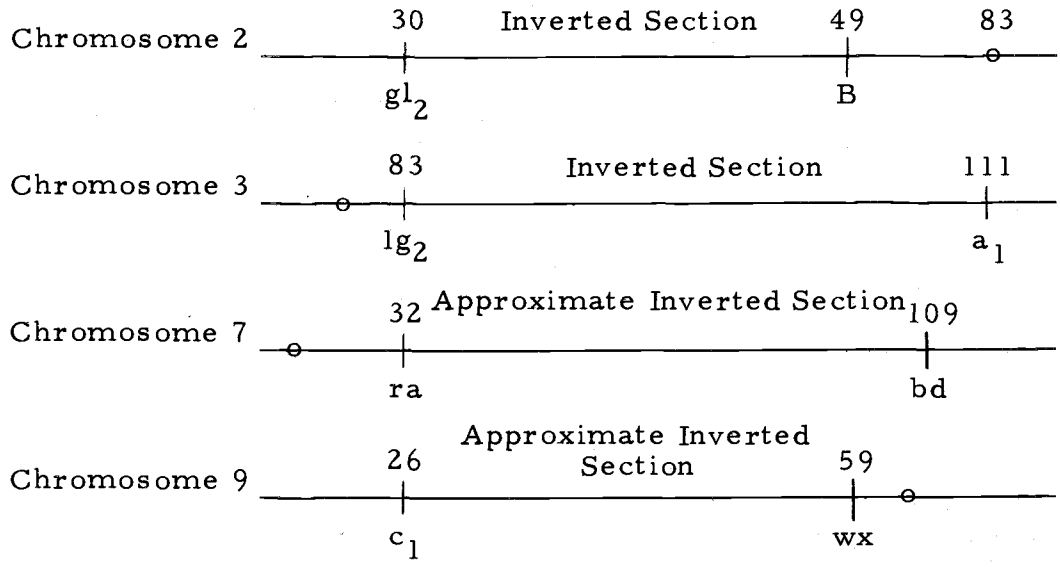
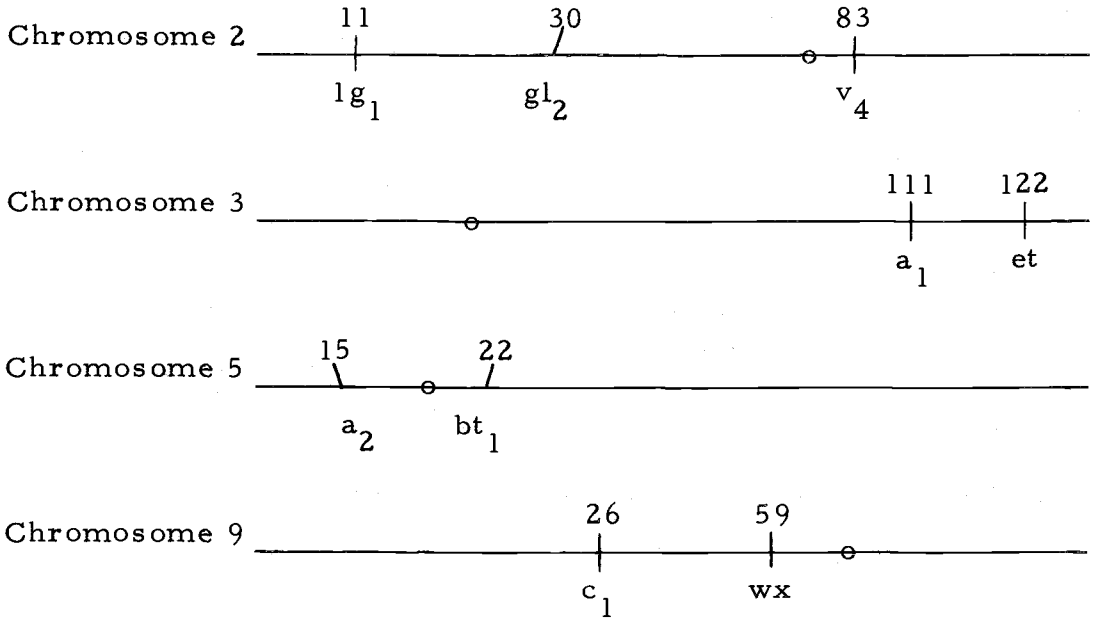


Table 2. Genetic map of four Zea mays L. chromosomes showing position of genes in linkage groups.



Chemical Treatments

The two chelating agents used in the study were ethylenediamine tetraacetic acid (EDTA) and dimethyl sulfoxide (DMSO). In addition, a wetting agent, Tween-20, in a concentration of 1/3 ml/100 ml of solution was applied with each treatment. DMSO, a leftover by-product of lignin following extraction of cellulose, is a clear miscible liquid recently approved by the Federal Drug Administration as a prescription drug for certain skin conditions. EDTA, also known as versene or sequestrene, is a strong chelating agent for heavy metal ions because of its six unshared pairs of electrons--the two nitrogen atoms and the four carboxyl ions. These unshared pairs of electrons form stable five-membered chelate rings with the metal ions.

Utilizing both EDTA, in concentrations of 0.001M and .01M, and DMSO in 2% and 8%, alone and in combination, along with distilled water as a control, allowed for a total of nine treatments. In nine petri dishes containing the different concentrations, five seeds of each of the inversion and genetic marker stocks selected at random were soaked for 24 hours. Ninety planting trays filled with sandy-loam soil were individually marked with letters A-J to indicate the specific treatments, and the seeds which had been soaked in the chemical solutions were planted in the trays. The seedlings grew in the greenhouse for approximately three weeks and were then transplanted in the

field. Untreated seedlings of the inversion and genetic stocks previously grown in the greenhouse also were transplanted in the planting site in order to establish the exact stage of meiotic division for all the plants.

The planting site consisted of river bottom sandy loam soil which had been previously prepared and fertilized. A table of random numbers was used to determine where the seedlings representing each of the treatments would be transplanted in the rows. Five inversion seedlings were grown for each of the nine different treatments making a total of five replications per treatment. Although this same procedure was used for the testcross seedlings, some of the plants failed to produce seed. However, with few exceptions, at least two replications per treatment for the testcross stock were finally realized.

The treated females of the testcross stocks and all the inversion stocks were planted in rows in individually marked hills. The homozygous untreated males for each of the testcross stocks were planted alongside the treated females to facilitate hand pollination.

After the plants had grown to maturity and prior to meiosis, the nine treatments were again applied as a foliar spray; each plant received approximately 2 ml of the chemical spray at five different times with a 48-hour time delay between sprayings. Plastic atomizers were used to apply the spray, and cardboard shields were used to prevent accidental drifting of the spray material.

Following each series of treatments, a plant was sacrificed and the pollen mother cells fixed and stained to ascertain the approximate stage of meiosis in the remaining plants. Once the PMC's were consistently in anaphase II or beyond, the treatments were discontinued.

The treatments utilized in the study are outlined in Table 3.

Method of Determining and Analyzing Cytological and Genetic Data

The tassels from the four inversion stocks containing the pollen mother cells were collected and fixed in Carnoy's solution, consisting of six parts 95% ethyl alcohol, three parts chloroform, and one part glacial acetic acid. The pollen mother cells, teased from the anthers onto a slide, were stained with propionic carmine, consisting of 45 ml propionic acid, 55 ml distilled water, and 0.5 g carmine. The first 50 anaphase I and II figures of the pollen mother cells from five different slides representing the five different plants per treatment were counted and in some cases photographed. The mean number of dicentric bridges and acentric fragments for anaphase I and II for each treatment was determined, and .05 or .01 confidence intervals for each treatment mean were established as outlined by Peterson (1967).

The effects of chelating agents on genetic recombination were investigated using four testcrosses, with the females in each testcross

Table 3. Chelating agents and concentrations utilized.

Treatment	Concentration of solution	Composition
A	Distilled water and wetting agent	Tween-20 @ 1/3 ml/100 ml H_2O
B	EDTA 0.001M	0.0372 g/100 ml H_2O
C	EDTA 0.01M	0.372 g/100 ml H_2O
D	DMSO 2%	2 ml DMSO in 98 ml H_2O
E	DMSO 8%	8 ml DMSO in 92 ml H_2O
F	DMSO 2%/ EDTA 0.001	2 ml DMSO in 98 ml EDTA 0.001M solution
G	DMSO 8%/ EDTA 0.001	8 ml DMSO in 92 ml EDTA 0.001M solution
H	DMSO 2%/ EDTA 0.01	2 ml DMSO in 98 ml EDTA 0.01M solution
J	DMSO 8%/ EDTA 0.01	8 ml DMSO in 92 ml EDTA 0.01M solution

All solutions A through J contain Tween-20 at 1/3 ml/100 ml solution.

being heterozygous for the traits involved. These heterozygous females were treated with the chelating agents in the same manner as described for the inversion lines. Then following maturation of the pollen, the females were crossed with the untreated homozygous males and the ears covered with bags before and after hand pollination to prevent contamination by foreign pollen. (The testcrosses conducted are summarized in Table 4.)

The various traits involved in the testcrosses exhibit simple qualitative inheritance and complete dominance (Weijer, 1952); consequently, the traits could be scored as crossover or non-crossover types; furthermore, such traits observed either in the seed or seedling stages could be scored without the progeny growing to maturity. The characteristics expressed in the seed stage were found on chromosomes three, five and nine. Chromosome three had anthocyaninless aleurone and etched endosperm; chromosome five had anthocyaninless aleurone and brittle endosperm; and chromosome nine had colorless aleurone and waxy endosperm. The phenotypic expression of the various traits is shown in Table 5.

The genetic markers on chromosome two controlled liguleless, glossy and virescent seedling traits. The seedlings were grown in the greenhouse for approximately two weeks with no use of artificial light but with the greenhouse temperature at 70° F during the day and 65° F at night. After the seedlings had reached a height of

Table 4. Testcross combination involving four experimental lines of Zea mays L.

Chromosome line	Type of cross	Sex of treated parent	Female parent	Male parent
2	Test cross	F	$\frac{Lg_1\ Gl_2\ V_4}{lg_1\ gl_2\ v_4}$	$\frac{lg_1\ gl_2\ v_4}{lg_1\ gl_2\ v_4}$
3	Test cross	F	$\frac{A_1\ \ Et}{a_1\ \ et}$	$\frac{a_1\ \ et}{a_1\ \ et}$
5	Test cross	F	$\frac{A_2\ \ Bt_1}{a_2\ \ bt_1}$	$\frac{a_2\ \ bt_1}{a_2\ \ bt_1}$
9	Test cross	F	$\frac{C_1\ \ Wx}{c_1\ \ wx}$	$\frac{c_1\ \ wx}{c_1\ \ wx}$

Table 5. Characterization of marker genes on four chromosomes of Zea mays L.

Symbol	Descriptive title	Stage of classification	Location in chromosome	Position	Characteristics
lg_1	Liguleless	Seedling	2	11	Ligule and auricle missing; leaves upright, envelope stalk
gl_2	Glossy	Seedling	2	30	Leaf surface bright green, water sprayed in fine mist adheres in small drops
v_4	Virescent	Seedling	2	83	Seedling light yellow green; turns green slowly
a_1	Anthocyaninless	Seed	3	111	Absence of anthocyanin pigment produces colorless aleurone; dominant allele has purple aleurone
et	Etched endosperm	Seed	3	112	Kernel has scarred pitted appearance; dominant allele produces smooth kernel
a_2	Anthocyaninless	Seed	5	15	Same as a_1 above
bt_1	Brittle endosperm	Seed	5	22	Mature kernel collapsed, often translucent and brittle
c_1	Aleurone color	Seed	9	26	Colorless aleurone, dominant allele produces purple aleurone
wx	Waxy endosperm	Seed	9	59	Starch in endosperm is amylopectin stains red with iodine, dominant allele endosperm stains blue with iodine

Characteristic descriptions after Neuffer, Jones and Zuber (1968).

approximately three inches, they were scored as crossover or non-crossover types. The harvested kernels from chromosome three, five and nine stocks were dried to a moisture content of approximately 12%, hand-shelled, and classified as parentals or recombinants. Mean crossover values for all the testcross stocks were calculated by converting the percentages of crossing over obtained to Angle=Arcsin $\sqrt{\text{proportion}}$ values. Confidence intervals were established and significant differences for the mean chemical treatments as compared with the control treatment were calculated as outlined in Snedecor and Cochran (1967).

RESULTS

Cytological Exchange in Inversion Stocks of *Zea mays*

Cytological exchange was increased significantly in paracentric inversion stocks of *Zea mays* when the stocks were treated with chelating agents. To ascertain the influence of such agents, the number of dicentric bridges and acentric fragments observed in anaphase I and II of four heterozygous paracentric inversions were recorded. Dicentric bridges and acentric fragments, visible signs of cytological exchange, provided a simple direct method for measuring the exchange frequency within the inversion loop.

Cytological Exchange in Chromosome Two of Inversion Stock

Chromosome two inversion stock has a very short inverted segment of only 19 map units with the breaks at positions 30 (gl_2) and 49 (B). This short segment produced a mean number of dicentric bridges in anaphase I of 0.4 ± 0.68. However, treatments E, F, G, H, and J produced a significant increase in the number of dicentric bridges for the same segment. Treatment E produced a mean of $1.8 \pm .56$ dicentric bridges at anaphase I. Treatment F produced a mean of 3.4 ± 2.08 and treatment G a mean of 2.2 ± 1.36. Treatment H produced the greatest number of dicentric bridges at anaphase I with a mean of

8.3 ± 5.3, and treatment J produced the second largest number with a mean of 4.8 ± 2.29. All of these treatments consisted of DMSO alone or in combination with EDTA. The results of all the treatments for chromosome two inversion stock are found in Tables 6-9 on the following pages.

Chromosome Three Inversion Stock

Chromosome three inversion stock has an inverted segment of 28 map units. The breaks are located at positions 83 (lg_2) and 111 (a_1) near the distal end of the long arm and at a considerable distance from the centromere situated between 26 (ra_2) and 31 (C_g).

The mean number of dicentric bridges for the control at anaphase I was 18.6 ± 2.57. Treatments C (EDTA 0.01M), D (2% DMSO), E (8% DMSO), F (2% DMSO-EDTA 0.001M), G (8% DMSO-EDTA 0.001M) and J (8% DMSO-EDTA 0.01M) all produced significant increases in the number of dicentric bridges at anaphase I. Treatment C produced a mean of 28.6 ± 3.14 dicentric bridges at anaphase I, treatment D a mean of 28.6 ± 3.24, treatment E a mean of 29.8 ± 3.27, treatment F a mean of 33 ± 8.89, treatment G a mean of 30.2 ± 2.69 and treatment J a mean of 23.6 ± 1.42. The results for chromosome three inversion stock are found in Tables 10-13.

Table 6. Number of dicentric bridges at anaphase I and II resulting from exchanges in an inverted segment in chromosome two.

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
A (distilled water)	1	0
	0	0
	0	0
	1	0
	0	0
B (EDTA 0.001M)	0	0
	0	0
	1	1
	1	0
	0	0
C (EDTA 0.01M)	1	0
	0	0
	0	0
	0	0
	0	0
D (2% DMSO)	0	0
	0	0
	1	1
	1	0
	3	0
E (8% DMSO)	2	0
	2	0
	1	0
	2	0
	2	0
F (2% DMSO-EDTA 0.001M)	6	0
	2	0
	4	1
	2	0
	3	1
G (8% DMSO-EDTA 0.001M)	2	0
	1	0
	4	0
	2	1
	2	1

(Continued on next page)

Table 6. (continued)

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
H (2% DMSO-EDTA 0.01M)	5	1
	6	3
	10	0
	13	2
	7	4
J (8% DMSO-EDTA 0.01M)	7	0
	4	2
	6	2
	5	0
	2	2

Table 7. Mean value for dicentric bridges at anaphase I and II for the inverted segment on chromosome two.

Treatment	Anaphase I	Anaphase II
A (distilled water)	0.4 ± 0.68	0
B (EDTA 0.001M)	0.4 ± 0.68	0.2 ± .56
C (EDTA 0.01M)	0.2 ± 0.56	0
D (2% DMSO)	1 ± 1.36	0.2 ± .56
E (8% DMSO)	1.8 ± .56*	0
F (2% DMSO-EDTA 0.001M)	3.4 ± 2.08*	0.4 ± 0.68
G (8% DMSO-EDTA 0.001M)	2.2 ± 1.36*	0.4 ± 0.68
H (2% DMSO-EDTA 0.01M)	8.2 ± 5.3*	2 ± 1.96*
J (8% DMSO-EDTA 0.01M)	4.8 ± 2.29*	1.2 ± 3.86

*Significantly different from the control - $P \leq 0.05$.

Table 8. Number of fragments at anaphase I and II resulting from exchanges in an inverted segment in chromosome two.

Treatment	Fragments (I)	Fragments (II)
A (distilled water)	0	0
	0	0
	0	0
	0	0
	0	0
B (EDTA 0.001M)	0	1
	1	1
	0	0
	0	1
	0	0
C (EDTA 0.01M)	0	0
	1	0
	0	1
	0	0
	0	0
D (2% DMSO)	1	1
	0	0
	2	1
	0	0
	0	0
E (8% DMSO)	0	0
	0	1
	0	0
	0	0
	1	1
F (2% DMSO-EDTA 0.001M)	6	3
	2	2
	4	1
	2	1
	3	2

(Continued on next page)

Table 8. (Continued)

Treatment	Fragments (I)	Fragments (II)
G (8% DMSO-EDTA 0.001M)	2	1
	2	0
	3	1
	0	0
	1	0
H (2% DMSO-EDTA 0.01M)	1	7
	0	11
	3	5
	3	13
	4	8
J (8% DMSO-EDTA 0.01M)	2	5
	4	1
	5	0
	4	4
	3	3

Table 9. Mean value for number of fragments at anaphase I and II for the inverted segment on chromosome two.

Treatment	Anaphase I	Anaphase II
A (distilled water)	0 ±	0 ±
B (EDTA 0.001M)	0.2 ± 0.56	0.6 ± 0.68
C (EDTA 0.01M)	0.2 ± 0.56	0.2 ± 0.56
D (2% DMSO)	0.6 ± 1.11	0.4 ± 0.68
E (8% DMSO)	0.2 ± 0.56	0.4 ± 0.68
F (2% DMSO-EDTA 0.001M)	3.4 ± 2.076*	1.8 ± 0.56*
G (8% DMSO-EDTA 0.001M)	1.6 ± 1.42*	0.4 ± 0.68
H (2% DMSO-EDTA 0.01M)	2.2 ± 1.50*	8.8 ± 3.53*
J (8% DMSO-EDTA 0.01M)	3.6 ± 1.41*	2.6 ± 2.31*

*Significantly different from the control - $P \leq 0.05$.

Table 10. Number of dicentric bridges at anaphase I and II resulting from exchanges in an inverted segment in chromosome three.

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
A (distilled water)	16	0
	20	2
	19	4
	17	1
	21	0
B (EDTA 0.001M)	20	0
	26	0
	22	1
	19	1
	21	0
C (EDTA 0.01M)	30	2
	30	0
	25	1
	27	2
	31	0
D (2% DMSO)	30	0
	28	2
	32	0
	28	0
	25	2
E (8% DMSO)	27	3
	31	3
	27	2
	32	2
	32	2
F (2% DMSO-EDTA 0.001M)	28	2
	30	1
	34	2
	38	0
	35	0

(Continued on next page)

Table 10. (continued)

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
G (8% DMSO-EDTA 0.001M)	32	2
	33	2
	29	1
	28	1
	29	1
H (2% DMSO-EDTA 0.01M)	28	1
	18	0
	21	0
	21	0
	24	2
J (8% DMSO-EDTA 0.01M)	24	0
	25	1
	22	0
	23	2
	24	1

Table 11. Mean value for dicentric bridges at anaphase I and II for the inverted segment on chromosome three.

Treatment	Anaphase I	Anaphase II
A (distilled water)	18.6 ± 2.57	1.4 ± 2.07
B (EDTA 0.001M)	21.6 ± 3.35	0.4 ± 0.68
C (EDTA 0.01M)	28.6 ± 3.14*	1 ± 0.391
D (2% DMSO)	28.6 ± 3.24*	0.80 ± 0.43
E (8% DMSO)	29.8 ± 3.27*	2.4 ± 1.42
F (2% DMSO-EDTA 0.001M)	33 ± 8.89*	1 ± 0.391
G (8% DMSO-EDTA 0.001M)	30.2 ± 2.69*	1.4 ± 0.68
H (2% DMSO-EDTA 0.01M)	22.4 ± 4.69	0.6 ± 0.112
J (8% DMSO-EDTA 0.01M)	23.6 ± 1.42*	0.8 ± 1.04

*Significantly different from the control - $P \leq 0.05$.

Table 12. Number of fragments at anaphase I and II resulting from exchanges in an inverted segment in chromosome three.

Treatment	Fragments (I)	Fragments (II)
A (distilled water)	11	14
	13	9
	7	4
	9	8
	12	6
B (EDTA 0.001M)	16	5
	6	8
	10	6
	8	6
	11	6
C (EDTA 0.01M)	20	10
	20	6
	17	12
	15	13
	19	15
D (2% DMSO)	18	14
	16	16
	16	6
	12	12
	10	10
E (8% DMSO)	15	6
	17	5
	13	5
	20	10
	17	5
F (2% DMSO-EDTA 0.001M)	20	14
	10	6
	10	7
	20	12
	17	8

(Continued on next page)

Table 12. (Continued)

Treatment	Fragments (I)	Fragments (II)
G (8% DMSO-EDTA 0.001M)	21	4
	19	4
	14	4
	17	3
	15	4
H (2% DMSO-EDTA 0.01M)	16	9
	5	11
	11	9
	7	10
	20	10
J (8% DMSO-EDTA 0.01M)	9	9
	9	5
	7	8
	8	5
	2	8

Table 13. Mean value for number of fragments at anaphase I and II for the inverted segment on chromosome three.

Treatment	Anaphase I	Anaphase II
A (distilled water)	10.4 ± 3.0	8.02 ± 7.87
B (EDTA 0.001M)	10.2 ± 7.88	6.2 ± 0.430
C (EDTA 0.01M)	18.2 ± 2.69*	11.2 ± 6.49
D (2% DMSO)	14.4 ± 4.08	11.6 ± 4.77
E (8% DMSO)	16.4 ± 3.25*	6.22 ± 2.97
F (2% DMSO-EDTA 0.001M)	15.4 ± 6.30	9.4 ± 3.76
G (8% DMSO-EDTA 0.001M)	17.2 ± 3.55*	3.8 ± 0.56
H (2% DMSO-EDTA 0.01M)	11.8 ± 21.49	9.8 ± 1.03
J (8% DMSO-EDTA 0.01M)	7 ± 4.71	7 ± 1.94

*Significantly different from the control - $P \leq 0.05$.

Chromosome Seven Inversion Stock

Chromosome seven inversion stock contained the largest inversion used in the study. The inverted segment was found in the long arm with one break near the centromere and the other near the distal end of the chromosome.

The mean number of dicentric bridges at anaphase I for the control was 17.4 $\pm$ 1.42. Treatments B (EDTA 0.001M), C (EDTA 0.01M), F (2% DMSO-EDTA 0.001M), G (8% DMSO-EDTA 0.001M), H (2% DMSO-EDTA 0.01M) and J (8% DMSO-EDTA 0.01M) all produced significant increases in the number of dicentric bridges at anaphase I. Treatment B produced a mean of 30.2 $\pm$ 9.44 dicentric bridges at anaphase I whereas treatment C produced next to the largest number of dicentric bridges with a mean of 33.6 $\pm$ 8.61. Treatment F produced a mean of 23.4 $\pm$ 4.94, whereas treatment G produced the largest number of bridges with a mean of 34.8 $\pm$ 8.72. Treatment H produced a mean of 30.4 $\pm$ 7.77 dicentric bridges at anaphase I and treatment J a mean of 28.4 $\pm$ 7.13 dicentric bridges. The results for all the treatments for chromosome seven inversion stock are found in Tables 14-17.

Chromosome Nine Inversion Stock

Chromosome nine is the second smallest chromosome in maize

Table 14. Number of dicentric bridges at anaphase I and II resulting from exchanges in an inverted segment in chromosome seven.

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
A (distilled water)	16	4
	19	2
	17	4
	18	0
	17	2
B (EDTA 0.001M)	22	3
	31	6
	25	3
	35	1
	38	1
C (EDTA 0.01M)	30	0
	46	0
	31	0
	29	0
	32	0
D (2% DMSO)	20	1
	23	1
	18	0
	19	0
	28	0
E (8% DMSO)	14	2
	23	1
	26	1
	32	0
	21	2
F (2% DMSO-EDTA 0.001M)	18	1
	22	0
	29	0
	24	0
	24	0

(Continued on next page)

Table 14. (Continued)

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
G (8% DMSO-EDTA 0.001M)	34	2
	30	1
	46	0
	28	0
	36	0
H (2% DMSO-EDTA 0.01M)	26	0
	22	1
	35	0
	37	0
	32	0
J)8% DMSO-EDTA 0.01M)	21	2
	25	2
	35	4
	31	1
	30	4

Table 15. Mean value for dicentric bridges at anaphase I and II for the inverted segment on chromosome seven.

Treatment	Anaphase I	Anaphase II
A (distilled water)	17.4 ± 1.42	2.4 ± 2.08
B (EDTA 0.001M)	30.2 ± 9.44*	2.8 ± 2.55
C (EDTA 0.01M)	33.6 ± 8.61*	0
D (2% DMSO)	21.6 ± 5.02	0.4 ± 0.68
E (8% DMSO)	23.2 ± 8.22	1.2 ± 1.03
F (2% DMSO-EDTA 0.001M)	23.4 ± 4.94*	0.2 ± 0.56
G (8% DMSO-EDTA 0.001M)	34.8 ± 8.72*	0.6 ± 0.112
H (2% DMSO-EDTA 0.01M)	30.4 ± 7.77*	0.2 ± 0.56
J (8% DMSO-EDTA 0.01M)	28.4 ± 7.13*	2.6 ± 0.833

*Significantly different from the control - $P \leq 0.05$.

Table 16. Number of fragments at anaphase I and II resulting from exchanges in an inverted segment in chromosome seven.

Treatment	Fragments (I)	Fragments (II)
A (distilled water)	9	10
	15	9
	10	13
	10	15
	9	11
B (EDTA 0.001M)	20	10
	26	13
	23	5
	28	9
	28	6
C (EDTA 0.01M)	15	2
	11	6
	1	5
	5	3
	5	4
D (2% DMSO)	4	5
	9	5
	5	4
	6	7
	12	6
E (8% DMSO)	14	8
	17	7
	11	2
	17	5
	12	8
F (2% DMSO-EDTA 0.001M)	5	10
	5	6
	4	13
	17	9
	17	9

(Continued on next page)

Table 16. (Continued)

Treatment	Fragments (I)	Fragments (II)
G (8% DMSO-EDTA 0.001M)	19	11
	9	10
	10	10
	10	11
	11	11
H (2% DMSO-EDTA 0.01M)	11	3
	14	3
	7	5
	11	5
	12	4
J (8% DMSO-EDTA 0.01M)	22	2
	22	6
	29	3
	26	3
	27	5

Table 17. Mean value for number of fragments at anaphase I and II for the inverted segment on chromosome seven.

Treatment	Anaphase I	Anaphase II
A (distilled water)	10.6 ± 3.52	11.6 ± 2.97
B (EDTA 0.001M)	25 ± 4.3*	8.6 ± 4.0
C (EDTA 0.01M)	7.4 ± 6.88	4 ± 1.96*
D (2% DMSO)	7.2 ± 5.94	5.4 ± 1.42*
E (8% DMSO)	14.2 ± 4.28*	6 ± 3.16
F (2% DMSO-EDTA 0.001M)	9.6 ± 6.3	9.4 ± 3.49
G (8% DMSO-EDTA 0.001M)	11.8 ± 9.27	10.6 ± 0.68
H (2% DMSO-EDTA 0.01M)	11 ± 3.16	4 ± 0.125*
J (8% DMSO-EDTA 0.01M)	25 ± 1.85*	3.8 ± 2.04*

*Significantly different from the control - $P \leq 0.05$.

and has a terminal heterochromatic knob on its short arm. The inverted segment in this stock was approximately 33 map units with one break located at position 59 (wx) and the other near position 26 (C_1).

The mean number of bridges observed at anaphase I in the control was 3.8 ± 0.913. Treatment F (2% DMSO-EDTA 0.001M) produced a highly significant increase in the mean number of dicentric bridges at anaphase I of 12.8 ± 2.57, but no other treatment produced any significant increase. The results of all the treatments for chromosome nine inversion stock are found in Tables 18-21.

Genetic Recombination in Testcross Stocks of *Zea mays*

Chelating agents can increase genetic recombination significantly depending upon the concentration and combination of the chemicals used and the specific chromosome involved. To determine the influence of such agents on genetic recombination, testcrosses were conducted using chromosome stocks of *Zea mays*.

Genetic Recombination in Chromosome Two of *Zea mays*

Chromosome two testcross stock has genetic markers in two different regions. Region I has linkage markers (lg_1 - gl_2) 19 map units apart, and Region II has linkage markers (gl_2 - v_4) 53 map units

Table 18. Number of dicentric bridges at anaphase I and II resulting from exchanges in an inverted segment in chromosome nine.

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
A (distilled water)	3	2
	6	2
	2	2
	3	1
	5	1
B (EDTA 0.001M)	2	0
	3	0
	2	0
	2	0
	1	1
C (EDTA 0.01M)	4	1
	7	0
	2	0
	3	0
	3	0
D (2% DMSO)	7	0
	2	1
	2	0
	3	0
	3	1
E (8% DMSO)	2	0
	3	1
	3	0
	1	0
	3	1
F (2% DMSO-EDTA 0.001M)	14	0
	13	1
	13	2
	14	1
	10	1

(Continued on next page)

Table 18. (Continued)

Treatment	Dicentric bridges (I)	Dicentric bridges (II)
G (8% DMSO-EDTA 0.001M)	1	0
	1	1
	1	0
	6	0
	5	0
H (2% DMSO-EDTA 0.01M)	2	0
	1	0
	3	0
	6	0
	5	1
J (8% DMSO-EDTA 0.01M)	2	0
	5	1
	3	0
	3	0
	0	0

Table 19. Mean value for dicentric bridges at anaphase I and II for the inverted segment on chromosome nine.

Treatment	Anaphase I	Anaphase II
A (distilled water)	3.8 ± 0.913	1.6 ± 0.68
B (EDTA 0.001M)	2 ± 0.86	0.2 ± 0.56
C (EDTA 0.01M)	3.8 ± 2.40	0.2 ± 0.56
D (2% DMSO)	3.4 ± 2.57	0.4 ± 0.68
E (8% DMSO)	2.4 ± 1.11	0.4 ± 0.68
F (2% DMSO-EDTA 0.001M)	12.8 ± 2.57**	1.0 ± 0.877
G (8% DMSO-EDTA 0.001M)	2.8 ± 3.08	0.2 ± 0.56
H (2% DMSO-EDTA 0.01M)	3.4 ± 2.37	0.2 ± 0.56
J (8% DMSO-EDTA 0.01M)	2.6 ± 1.67	0.2 ± 0.56

** Significantly different from the control - $P \leq 0.01$.

Table 20. Number of fragments at anaphase I and II resulting from exchanges in an inverted segment in chromosome nine.

Treatment	Fragments (I)	Fragments (II)
A (distilled water)	1	3
	3	2
	3	4
	1	3
	6	4
B (EDTA 0.001M)	0	1
	0	0
	1	0
	0	0
	0	0
C (EDTA 0.01M)	0	0
	1	1
	1	1
	2	0
	0	1
D (2% DMSO)	1	1
	4	2
	2	1
	6	1
	1	1
E (8% DMSO)	1	1
	0	5
	1	0
	0	1
	0	2
F (2% DMSO-EDTA 0.001M)	3	14
	8	4
	3	6
	4	1
	5	3

(Continued on next page)

Table 20. (Continued)

Treatment	Fragments (I)	Fragments (II)
G (8% DMSO-EDTA 0,001M)	0	0
	1	0
	0	1
	1	1
	0	0
H (2% DMSO-EDTA 0.01M)	1	0
	0	3
	1	0
	0	1
	0	0
J (8% DMSO-EDTA 0.01M)	1	2
	0	2
	1	1
	1	2
	0	2

Table 21. Mean value for number of fragments at anaphase I and II for the inverted segment on chromosome nine.

Treatment	Anaphase I	Anaphase II
A (distilled water)	2.8 ± 2.54	3.2 ± 1.04
B (EDTA 0.001M)	0.2 ± 0.56	0.2 ± 0.56*
C (EDTA 0.01M)	0.8 ± 1.04	0.6 ± 0.68*
D (2% DMSO)	2.8 ± 2.69	1.2 ± 0.56
E (8% DMSO)	0.4 ± 0.68	1.8 ± 2.39
F (2% DMSO-EDTA 0.001M)	4.6 ± 2.57	5.6 ± 5.94
G (8% DMSO-EDTA 0.001M)	0.4 ± 0.68	0.4 ± 0.68*
H (2% DMSO-EDTA 0.01M)	0.4 ± 0.68	0.8 ± 1.62*
J (8% DMSO-EDTA 0.01M)	0.6 ± 0.68	1.8 ± 0.56

*Significantly different from the control - $P \leq 0.05$.

apart. Linkage marker gl_2 is located on the long arm of chromosome two, whereas v_4 is adjacent to the centromere on the short arm.

The mean recombination Angle = Arcsin $\sqrt{\text{proportion}}$ value for the control in Region I was 23.43 ± 15.96. None of the chemical treatments produced a significant increase in recombination for Region I. The mean recombination Angle = Arcsin $\sqrt{\text{proportion}}$ value for the control in Region II was 29.26 ± 9.16. Treatments C (EDTA 0.01M), D (2% DMSO) and F (2% DMSO-EDTA 0.001M) all produced significant increases in genetic recombination for Region II. The results of all the treatments for both Regions in chromosome two are found in Table 22.

Genetic Recombination in Chromosome Three of *Zea mays*

Two linkage markers were utilized in chromosome three. These were a_1 (anthocyaninless seed) at position 111 and et (etched endosperm) at position 122. Both markers are located a long distance from the centromere which is near position 28. In addition this chromosome has a heterochromatic knob situated between the a_1 - et Region and the centromere.

The mean recombination Angle = Arcsin $\sqrt{\text{proportion}}$ value for the control was 20.8 ± 1.07. Treatment B (EDTA 0.001M) with a mean recombination of 29.67 ± 0, treatment C (EDTA 0.01M) with a mean of 29.56 ± 20.58, treatment D (2% DMSO) with a mean of 28.89

Table 22. Genetic recombination data for chromosome two of Zea mays.

	Total number of kernels	Total number of crossovers	Percent crossovers	Angle = Arcsin $\sqrt{\text{proportion}}$	Confidence interval
			Treatment A (distilled water)		
Region I	23	3	13	21.13	23.43 ± 15.96
	26	6	26.1	30.72	$1.62\% \leq 25.33\% \leq 40.25\%$
	20	2	10	18.44	
Region II	23	7	30.4	33.46	29.26 ± 9.16
	26	5	21.7	27.76	$11.81\% \leq 23.89\% \leq 38.61\%$
	20	6	20	26.56	
			Treatment B (EDTA 0.001M)		
Region I	28	4	14.2	22.14	19.995 ± 27.19
	32	3	9.4	17.85	$0\% \leq 11.7\% \leq 53.61\%$
Region II	28	17	61	51.35	46.60 ± 63.02
	32	14	43.8	41.44	$0\% \leq 52.41\% \leq 100\%$
			Treatment C (EDTA 0.01M)		
Region I	22	5	22.7	28.45	27.11 ± 3.06
	26	5	19.23	26.01	$16.61\% \leq 20.78\% \leq 25.20\%$
	44	9	20.45	26.88	
Region II	22	11	50	45.00	41.14 ± 12.00*
	26	12	46.1	42.76	$23.70\% \leq 43.29\% \leq 64.10\%$
	44	15	34.1	35.73	
			Treatment D (2% DMSO)		
Region I	129	39	30.2	33.34	28.02 ± 7.08
	236	77	32.62	34.83	$12.78\% \leq 22.19\% \leq 33.09\%$
	123	19	15.4	23.11	
	145	28	19.31	26.07	
	314	57	14.96	22.75	

(Continued on next page)

Table 22. (Continued)

	Total number of kernels	Total number of crossovers	Percent crossovers	Angle=Arcsin $\sqrt{\text{proportion}}$	Confidence interval
			Treatment D (continued)		
Region II	129	47	36.43	37.12	$37.54 \pm 1.42^{+}$
	236	90	38.14	38.13	$34.71\% \leq 37.11\% \leq 39.52\%$
	123	47	38.2	38.17	
	145	114	36.3	37.05	
			Treatment E (8% DMSO)		
Region I	52	11	21.1	27.35	24.94 ± 19.70
	57	18	31.52	34.15	$0.831\% \leq 17.75\% \leq 49.31\%$
	38	2	5.3	13.31	
Region II	52	27	51.9	46.09	39.88 ± 16.22
	57	17	29.82	33.10	$16.10\% \leq 41.10\% \leq 68.89\%$
	38	16	42.1	40.46	
			Treatment F (2% DMSO-EDTA 0.001M)		
Region I	25	4	16	24.12	24.37 ± 11.58
	17	4	23.5	29.00	$4.90\% \leq 17.12\% \leq 36.12\%$
	34	4	11.7	20.00	
Region II	25	13	52	46.15	43.69 ± 2.28*
	17	8	47.05	43.30	$43.71\% \leq 47.71\% \leq 53.41\%$
	34	15	44.1	41.61	
			Treatment G (8% DMSO-EDTA 0.001M)		
Region I	60	10	16.7	24.12	24.50 ± 9.36
	58	9	15.5	23.19	$4.42\% \leq 17.20\% \leq 30.22\%$
	84	14	16.7	24.12	
	75	15	20	26.56	

(Continued on next page)

Table 22 (Continued)

	Total number of kernels	Total number of crossovers	Percent crossovers	Angle = Arcsin $\sqrt{\text{proportion}}$	Confidence interval
			Treatment G (continued)		
Region II	60	13	21.7	27.76	32.47 ± 9.36
	58	12	20.7	27.06	$15.40\% \leq 28.81\% \leq 44.49\%$
	84	32	38.1	38.17	
	75	27	36	36.87	
			Treatment H (2% DMSO-EDTA 0.01M)		
Region I	No seed set	-	-	-	-
Region II	No seed set	-	-	-	-
			Treatment I (8% DMSO-EDTA 0.01M)		
Region I	28	4	14.3	22.22	21.42 ± 3.15
	49	6	12.2	20.4	$9.83\% \leq 13.31\% \leq 17.29\%$
	74	12	16.2	23.73	
	91	10	10.92	19.28	
Region II	28	9	32.1	34.51	33.97 ± 2.0
	49	14	28.5	32.33	$28.11\% \leq 30.29\% \leq 34.50\%$
	74	25	33.7	35.49	
	91	28	30.6	33.58	

*Significantly different from the control - $P \leq 0.05$

+Significantly different from the control - $p \leq 0.10$

$\pm$ 13.48 and treatment E with a mean of 25.59 $\pm$ 1.49 all produced significant increases in genetic recombination in this region of chromosome three. The recombination values for all the treatments are shown in Table 23.

Genetic Recombination in Chromosome Five of Zea mays

Chromosome five has two genetic markers seven map units apart at locus a_2 (position 15) on the short arm and locus bt_1 (position 22) adjacent to the centromere on the long arm. The control treatment produced a mean crossover Angle = Arcsin $\sqrt{\text{proportion}}$ value of 18.33 $\pm$ 9.73. Treatments C (EDTA 0.01M), F (2% DMSO-EDTA 0.001M), G (8% DMSO-EDTA 0.001M) and J (8% DMSO-EDTA 0.01M) all produced significant increases in genetic recombination for this short segment. The results of all the treatments for chromosome five are found in Table 24.

Genetic Recombination in Chromosome Nine of Zea mays

Chromosome nine has two genetic markers located in the short arm: c_1 (colorless aleurone) at position 26 and wx (waxy endosperm) at position 59. The mean recombination frequency for the control treatment was Angle = Arcsin $\sqrt{\text{proportion}}$ value of 31.38 $\pm$ 3.82. All the chemical treatments produced significant increases in genetic

Table 23. Genetic recombination data for chromosome three of Zea mays.

Treatment	Total number of kernels	Total number of crossovers	Percent crossovers	Angle=Arcsin $\sqrt{\text{proportion}}$	Confidence interval
A (distilled water)	33	4	12.1	20.36	20.36 ± 1.07
	21	3	14.28	20.49	$11.40\% \leq 12.69\% \leq 13.89\%$
	34	5	11.7	20.00	
B (EDTA 0.001M)	45	12	24.5	29.67	29.67 ± 0*
	45	12	24.5	29.67	$24.50\% \pm 0$
C (EDTA 0.01M)	41	9	21.95	27.93	29.56 ± 20.58+
	41	11	26.83	31.19	$2.46\% \leq 24.35\% \leq 58.91\%$
D (2% DMSO)	16	4	25	30.00	28.89 ± 13.48+
	69	15	21.74	27.78	$7.08\% \leq 23.32\% \leq 45.41\%$
E (8% DMSO)	76	12	15.79	23.40	25.59 ± 1.49*
	54	11	20.37	26.81	$16.68\% \leq 18.64\% \leq 19.32\%$
	45	9	20.00	26.56	
F (2% DMSO-EDTA 0.001M)	54	5	9.26	17.70	23.66 ± 70.01
	119	29	24.45	29.63	$0\% \leq 16.10\% \leq 88.30\%$
G (8% DMSO-EDTA 0.001M)	16	5	31.25	33.98	30.50 ± 43.32
	24	5	20.83	27.15	$0\% \leq 25.71\% \leq 92.22\%$
H (2% DMSO-EDTA 0.01M)	72	6	8.33	16.77	29.77 ± 165
	64	30	46.15	52.00	$0\% \leq 24.65\% \leq 100\%$
J (8% DMSO-EDTA 0.01M)	14	6	42.86	40.89	35.66 ± 71.96
	39	10	25.62	30.44	$0\% \leq 33.91\% \leq 100\%$

*Significantly different from the control - $P \leq 0.05$.

+Significantly different from the control - $P \leq 0.10$.

Table 24. Genetic recombination data for chromosome five of Zea mays.

Treatment	Total number of kernels	Total number of crossovers	Percent crossovers	Angle=Arcsin $\sqrt{\text{proportion}}$	Confidence interval
A (distilled water)	28	3	10.71	19.09	18.33 ± 9.73
	33	3	9.07	17.56	$2.25\% \leq 9.89\% \leq 22.12\%$
B (EDTA 0.001M)	20	2	10.0	18.44	18.95 ± 6.48
	27	3	11.11	19.46	$4.64\% \leq 10.54\% \leq 18.42\%$
C (EDTA 0.01M)	32	9	28.1	32.01	32.17 ± 8.76*
	40	8	20	26.56	$15.79\% \leq 28.37\% \leq 42.91\%$
	49	20	40.8	39.70	
	39	25.6	30.40		
D (2% DMSO)	42	9	21.43	27.58	25.09 ± 31.89
	27	4	14.81	22.65	$0\% \leq 17.95\% \leq 70.00\%$
E (8% DMSO)	No seed set				
F (2% DMSO-EDTA 0.001M)	26	5	20.0	25.56	29.29 ± 6.19*
	75	20	26.67	31.09	$15.39\% \leq 23.92\% \leq 33.64\%$
	150	38	25.33	30.22	
G (8% DMSO-EDTA 0.001M)	77	26	33.77	35.55	32.07 ± 9.85*
	65	14	21.69	27.76	$14.30\% \leq 28.19\% \leq 44.61\%$
	241	71	29.45	32.90	
H (2% DMSO-EDTA 0.01M)	47	20	42.5	40.74	37.95 ± 35.06
	51	17	32.24	35.24	$.1\% \leq 37.80\% \leq 91.42\%$
J (8% DMSO-EDTA 0.01M)	43	7	16.28	23.79	28.86 ± 7.89*
	100	34	34	35.67	$12.81\% \leq 23.20\% \leq 35.80\%$
	74	16	21.75	27.80	
	153	34	22.23	28.15	

*Significantly different from the control - $P \leq 0.05$.

recombination except treatments B (EDTA 0.001M) and treatment H (2% DMSO-EDTA 0.01M). The results of the testcrosses for chromosome nine are summarized in Table 25.

Table 25. Genetic recombination data for chromosome nine of Zea mays.

Treatment	Total number of kernels	Total number of crossovers	Percent crossovers	Angle=Arcsin $\sqrt{\text{proportion}}$	Confidence interval
A (distilled water)	189	53	28.05	34.39	31.38 ± 3.82
	98	25	25.5	30.33	$21.40\% \leq 27.10\% \leq 33.21\%$
	185	59	31.9	32.01	
	43	10	23.2	28.79	
B (EDTA 0.001M)	104	33	32.7	34.88	35.27 ± 3.58
	176	63	35.8	36.75	$27.60\% \leq 33.31\% \leq 39.31\%$
	85	26	30.6	33.58	
C (EDTA 0.01M)	48	23	47.9	43.80	42.84 ± 2.18*
	122	56	45.9	42.65	$42.41\% \leq 46.21\% \leq 53.5\%$
	98	44	44.9	42.07	
D (2% DMSO)	No seed set				
E (8% DMSO)	44	16	36.3	37.05	38.38 ± 17.03*
	27	11	40.8	39.70	$13.23\% \leq 38.51\% \leq 50.70\%$
F (2% DMSO-EDTA 0.001M)	108	44	40.7	39.64	39.60 ± 2.66*
	106	47	44.3	41.73	$36.10\% \leq 40.61\% \leq 45.20\%$
	91	34	37.3	37.64	
	124	50	40.3	39.41	
G (8% DMSO-EDTA 0.001M)	61	27	44.2	47.1	42.48 ± 10.63*
	34	16	41.67	43.28	$27.51\% \leq 43.81\% \leq 63.84\%$
H (2% DMSO-EDTA 0.01M)	64	21	32.8	34.94	35.43 ± 7.62
	93	32	34.4	35.91	$21.78\% \leq 33.60\% \leq 46.60\%$
J (8% DMSO-EDTA 0.01M)	91	40	43.9	41.50	43.01 ± 6.80*
	120	52	43	41.21	$34.48\% \leq 46.51\% \leq 58.35\%$
	182	79	43.3	41.15	
	132	61	46.5	52.61	
	203	88	38.4	38.60	

*Significantly different from the control - $P \leq 0.05$.

DISCUSSION

Dicentric bridges and acentric fragments observed in anaphase I and II are cytological evidences of exchanges within the inversion loop. A single dicentric bridge at anaphase I is the result of a single exchange or a three-strand double exchange within the inverted segment, while dicentric bridges at anaphase II generally result either from an interstitual exchange and a single exchange within the loop or from a four-strand double exchange within the inverted segment. Acentric fragments generally do not attach to the meiotic spindle and are visible in the cytoplasm of one of the two cells at anaphase II. Other types of crossovers may produce bridges and fragments as outlined by Burnham (1962).

Correlation Between Dicentric Bridges and Acentric Fragments

McClintock (1938) observed that the percent of dicentric bridges at anaphase I corresponds very closely with the percent of acentric fragments found in the cytoplasm at anaphase II. In addition, the missing or attached fragment at anaphase I can show up on the spindle of one of the anaphase II cells.

In the present study, some of the treatments resulted in significant correlation coefficients between anaphase I bridges and anaphase II fragments. For example, in chromosome two, treatment

B (EDTA 0.001M) produced a mean number of dicentric bridges at anaphase I of 0.4 ± 0.68 and fragments at anaphase II of 0.6 ± 0.68. Treatment C (EDTA 0.01M) produced a mean number of bridges at anaphase I of 0.2 ± 0.56 and of fragments at anaphase II of 0.2 ± 0.56, whereas treatment H (2% DMSO-EDTA 0.01M) produced bridges of 8.2 ± 5.3 and fragments of 8.8 ± 3.53.

In chromosomes three and seven inversion stocks, the number of fragments corresponds closely with the number of dicentric bridges if the fragments observed in both anaphase I and II are totaled. Due to the length of the inversion loops and the distance of the inverted segments from the centromeres, some of the acentric fragments were visible at anaphase I in these two stocks.

Chromosome nine inversion stock has approximately the same number of bridges at anaphase I as it has fragments at anaphase II for treatments A (distilled water) and F (2% DMSO-EDTA 0.001M). Treatment A produced the mean number of dicentric bridges at anaphase I of 3.8 ± 0.913 and the mean number of fragments at anaphase II of 3.4 ± 0.68. Treatment F produced bridges at anaphase I of 12.8 ± 2.57 and fragments of 5.6 ± 5.94.

The results of the study are in agreement with McClintock's conclusion that the number of dicentric bridges and the number of acentric fragments resulting from a crossover in the inversion loop are approximately equal.

Fate of Crossover Products in Paracentric Inversions

The dicentric bridge produced as a result of crossing over within the inverted loop may break at any point in the chromatid between the two centromeres, or it may be cut by the cell plate, leaving the thin strands of the old dicentric bridge near the cell plate. The acentric fragments resulting from cytological exchange generally show up in the cytoplasm of one cell at anaphase II; but if the fragment remains attached to the end of the normal chromatid in anaphase I, it may show up in the spindle of one of the anaphase II cells.

From such crossovers, some sterility in gametes is expected and does indeed occur in the pollen of Zea mays, but sterility in the ovules is no greater than in normal ones--47% pollen abortion but only 4% ovule abortion (Morgan, 1950). Similar results were observed in the eggs of Drosophila (Novitski, 1952). Morgan postulated that when the four meiotic products are formed they are arranged in linear fashion with the two cells containing the dicentric bridge in the center of the old megasporangium and consequently not developing into funtional megaspores. Therefore, the functional megaspore on the end of the linear order becomes a functional megagametophyte. However, no such linear arrangement exists for the microspores, and these do suffer the expected 50% sterility. In the case of

Drosophila, the dicentric bridge is found in the polar bodies and the egg functions normally (Novitski, 1952).

Effects of Chelating Agents on Cytological Exchange and Genetic Recombination

Use of chelating agents produced four specific effects for the four paracentric inversion and testcross stocks of Zea mays: (1) a significant increase in cytological exchanges in the inversion stocks by EDTA, DMSO, or combination of both; (2) a significant decrease in cytological exchanges in the inversion stocks by EDTA, DMSO, or combination of both; (3) a significant increase in genetic recombination in the testcross stocks by EDTA, DMSO, or combination of both; (4) a significant decrease in genetic recombination in testcross stocks by EDTA, DMSO, or combination of both. These results will be discussed in the following sections.

Increase in Cytological Exchanges in the Inversion Stocks

Cytological exchanges in chromosome two inversion stock were significantly increased by DMSO alone or in combination with EDTA. However, the breakage and subsequent recombination was much greater for the two chemicals combined. Chromosome two is next to the longest chromosome in maize, and the inverted segment is between positions 30 (gl_2) and 49 (B). Position 49 is some distance from the

centromere which is between positions 74 and 83; consequently, the centromere probably does not reduce crossing over to any extent.

Chromosome three is influenced differently from chromosome two in that either EDTA or DMSO alone or in combination can increase cytological exchange. Even though the inverted segment is small, it is close to the end of the chromosome; consequently, both breaks are some distance from the centromere. This distance may account for the numerous treatments that produced an increase in cytological exchange. Furthermore, chromosome three may be especially sensitive to the action of the chelators.

Cytological exchanges in chromosome seven inversion line are significantly influenced by treatments B (EDTA 0.001M), C (EDTA 0.01M), G (8% DMSO-EDTA 0.001M), and H (2% DMSO-EDTA 0.01M). EDTA, alone or in combination with DMSO, produces a significant increase in cytological exchange. Since chromosome seven contains a very large inverted segment, it was anticipated that numerous treatments would produce significant increases in cytological exchange. However, only EDTA appears to significantly influence this inverted segment.

Chromosome nine inversion stock is highly influenced by treatment F (2% DMSO-EDTA 0.001M), apparently because these two chemicals combined produce a synergistic effect. One break of the inverted segment is near the centromere at position 59, and this may

in part account for the lack of significant cytological exchanges. No other treatments produced a significant increase in cytological exchange in chromosome nine stock; in fact, these synergistic results for chromosome nine are contrary to those obtained by Ihrke (1970), in which he found that DMSO alone produced a significant increase in genetic recombination. However, he conducted no study on cytological crossing over.

Decrease in Cytological Exchange in the Inversion Stocks

Decrease in cytological exchanges noted in paracentric inversions have been attributed to non-survival of crossover gametes due to formation of dicentric bridges and acentric fragments, but since PMC were used, no reduction in cytological exchange was expected in the Zea mays inversion stocks following chemical treatments. However, in chromosome seven inversion stock, treatment C (EDTA 0.01M) produced a significant reduction in crossing over as shown by the number of dicentric bridges at anaphase II. Treatment C (0.01M EDTA) may actually suppress cytological crossing over when an interstitual crossover plus a crossover in the loop are required. One explanation for this result may be that the chelator is more specific in its effect in one region of the chromosome than in another region. Such action would allow for an increase in interstitual crossing over

but not in crossing over within the loop, or a decrease in interstitual crossing over and an increase in crossing over within the loop. Also the centromere may inhibit cytological exchange when both loop and interstitual exchanges are required.

Increase in Genetic Recombination in the Four Testcross Stocks

Region II of chromosome two testcross stock, including the centromere which in this instance does not appear to suppress crossing over, was influenced significantly by DMSO or EDTA singly or in combination, but the largest increase in genetic recombination results when both chemicals are combined. DMSO has been shown to produce similar synergistic effects when combined with ethyl methanesulfonate (Rana and Mathur, 1969).

EDTA and DMSO significantly influenced recombination in chromosome three, but no synergistic effects are produced by the two chemicals in combination. However, large variances obtained for the combined treatments may actually mask synergistic effects. Furthermore, the two chemicals combined may have produced some sterility as shown by the poor seed set produced in this stock.

The region on chromosome five consists of seven map units and included the centromere. Consequently, the effects of the two chelating agents on a tight linkage group could be observed along with

any influence the centromere might exert on genetic recombination. EDTA, alone or in combination with DMSO, produced significant increases in recombination but DMSO alone had no significant influence on this small region.

Chromosome nine was influenced differently from chromosome five in that both EDTA and DMSO alone or in combination produced significant increases in genetic recombination. However, EDTA in a concentration of 0.01M was very effective in increasing genetic recombination as were treatments F (2% DMSO-EDTA 0.001M), G (8% DMSO-EDTA 0.001M) and J (8% DMSO-EDTA 0.01M).

Correlation Between Cytological Exchange and Genetic Recombination

When both an increase in cytological exchange and genetic recombination occurs following treatment with the chelator, the exchange has not brought about any lethal condition nor in any other manner caused the gamete to be abnormal. Consequently, the increase in cytological exchange is correlated with an increase in genetic recombination as shown by the new phenotypes in the progeny.

However, cytological exchange may be increased whereas genetic recombination may be decreased even though the same chemical treatment is applied. In such case, the chelator may be fragmenting the chromosome to such an extent that intersomatic

selection occurs and the progeny does not survive. But the reverse situation where there is an increase in genetic recombination but not in cytological exchange is difficult to explain. One explanation may be that the chelator has less effect on the inversion loop than on the normal chromosome especially for small inverted segments such as those found in chromosomes two and nine inversion stocks. Too, the chelator may be interfering with restitution thereby allowing sufficient time for genetic recombination to occur.

An increase in both cytological exchange and genetic recombination was observed in chromosome two of both inversion and testcross stocks for treatment F (2% DMSO-EDTA 0.001M), but treatments E (8% DMSO), H (2% DMSO-EDTA 0.01M) and J (8% DMSO-EDTA 0.01M) produced only increases in cytological exchange; some other factor therefore appears to be interfering with genetic recombination.

Treatment E (8% DMSO) was shown to increase both cytological exchange and genetic recombination in chromosome three inversion and testcross stocks, whereas treatments C (EDTA 0.01M), D (2% DMSO), F (2% DMSO-EDTA 0.001M), G (8% DMSO-EDTA 0.001M) and J (8% DMSO-EDTA 0.01M) produced significant increases in cytological exchange but not in genetic recombination. Treatment B (EDTA 0.001M) in turn was found to significantly increase genetic recombination but not cytological exchange.

In chromosome nine, treatment F (2% DMSO-EDTA 0.001M) produced significant increases in both cytological and genetic crossing over, whereas treatments C (EDTA 0.001M and J (8% DMSO-EDTA 0.01M) produced a significant increase in genetic recombination only.

Specific Effects of Metallic Cations in Chromosome Structure

Metallic cations are assumed to be involved in the structure of the chromosome (Mazia, 1954). These cations exhibit varying degrees of attraction to different chelating agents. EDTA, according to Cotton and Francis (1960), has an affinity for calcium and renders the chromosome more subject to breakage than when it is treated with DMSO. Other observations, as those by Selbin, Bull and Holmes (1961), indicate that DMSO has a high affinity for copper, lead and iron but less attraction for manganese and zinc. However, results of the present study indicate that DMSO can produce chromosome breakage as effectively as can EDTA. Therefore,three conclusions may be drawn from the results of this study: either many kinds of metallic cations are associated with the chromosome (Kihlman, 1966); or the chelating agents are merely changing the ionic balance in the nucleus, producing breakage by modification of constituent nucleoprotein gels (Kaufman and McDonald, 1957); or the chelators are interfering with restitution of broken ends of the chromosomes (Wolff and Luipold, 1956).

Although metallic cations may be an integral part of the chromosome (Mazia, 1954) chelating agents are thought to complex these ions, thereby creating a deficiency in the chromosome. Such a deficiency produces breakage and subsequent crossing over in the chromosome as shown by the increase in dicentric bridges in the inversion lines and recombinants in the genetic testcross lines. The divalent cations are believed to link the particulate units of the chromosome together (Mazia, 1954; Steffensen, 1955). Levine (1955) increased chromosome breakage in Tradescantia approximately 17 times more than normal when the plant was grown in calcium deficient medium. The absence of calcium or magnesium ions also causes disintegration of bacterial chromatin and interferes with the multiplication of the bacterial cell (Lauria and Stiner, 1954).

Hyde (1955) postulated that EDTA breaks chromatin into small units and suppresses the movement of chromosomes during anaphase. In addition, marked swelling of metaphase and prophase chromosomes occurred but such swelling did not appear to damage the chromosome permanently.

Chelation of metallic ions may interfere with restitution of broken chromosomes. Wolff and Luipold (1956) suggest that induced chromosome breaks may be at least partially ionic and the chelating agents may be inhibiting rejoining of the broken ends by chemically complexing the metallic cations.

The chelating agents may also affect the energy transfer processes necessary for repair of the breakage (LaChance, 1959). Such an effect would influence the localization of bond breakage and answer in part the question of how chelating agents appear to act on specific regions of chromosomes.

Chelation may also complex cations that are essential for certain enzymes, such as the peroxidases and catalases. If these enzymes occurred in insufficient concentrations, then peroxide and other oxidizing agents may accumulate in large enough concentrations to produce chromosome breakage (Meyer, 1954).

Even though the exact action of the chelating agents is still unknown, the present study does show that such chemicals can be predicted to increase crossing over when the actual concentration of the chelator and the specific chromosome involved are known.

Plant Improvement Considerations

Progress in plant breeding is dependent upon the genetic variability within the plant population. Recombination is one mechanism for insuring that this variability will continue to exist in the species, but close linkage of genes reduces variability and favors conservation of parental types.

Using chelating agents as an artificial means of stimulating recombination between closely linked genes could create additional

variation in the plant species. Some of these rare recombinants could have commercial importance, and, once acquired, they could be maintained in the species because of their close linkage relationship.

There are at least two situations in plant breeding where increased crossing over would be beneficial. One of these involves the linkage of a desirable gene with an undesirable one. For example, in some wheat, Triticum aestivum, varieties the desirable gene for disease resistance is closely linked with an undesirable dwarf gene. An increase in crossover frequency might break up this detrimental relationship and allow both desirable genes to appear in the same individual.

The second situation wherein increased crossing over may be helpful is that in which desirable genes are located in different homologous chromosomes of several varieties of a crop plant. In this case, the plant must be heterozygous for the chromosome pair in order to have both desirable characteristics present. But heterozygosity is difficult to maintain in self-pollinating plants, and is unpredictable in its breeding behavior. Treated with a chelating agent, such a heterozygote might recombine to incorporate both desirable genes on the same chromosome.

Furthermore, chelating agents could be utilized to induce interspecific translocations and thereby the transfer of entire blocks

of favorable genes could be accomplished. Too, once these translocation stocks were formed, they could be used to study the segregation patterns in different translocations.

The chelating agents should be applied to the plant carefully because in some instances the leaf tips developed brown spots following foliar spray application. Such effect can be avoided if the atomizer used to apply the spray produces a mist fine enough to prevent formation of droplets on the leaf.

SUMMARY AND CONCLUSIONS

The objectives of the present study were: (1) to determine the influence of chelating agents on cytological exchange and genetic recombination, (2) to compare the results of cytological exchange and genetic recombination, and (3) to determine if chelating agents can be used as a practical means for increasing genetic variability in a species.

Four paracentric inversion lines and four testcross lines of Zea mays were tested with DMSO and EDTA singly and in combination. The mean crossover frequency was established for each treatment within each line and confidence intervals established.

The conclusions that appear warranted follow:

(1) Chelating agents can increase crossing over significantly depending upon the concentration and combination used;

(2) The chelators are specific in their action in that not all chromosomes are affected in the same way or by the same concentrations of treatment materials;

(3) The chelating agents appear to exert their effect by removing specific cations from the chromosome, or by interfering with the restitution process;

(4) To induce recombination in chromosome two, five and nine of Zea mays, a combination of 2% DMSO-EDTA 0.001 M

could be used; whereas for chromosome three and seven of *Zea mays* a 0.01M concentration of EDTA would be satisfactory.

BIBLIOGRAPHY

Beadle, G. W. 1932. A gene in Zea mays for failure of cytokinesis during meiosis. Cytologia 3:142-155.

_____ 1933a. Polymitotic maize and the precocity hypothesis of chromosome conjugation. Cytologia 5:118-121.

_____ 1933b. Further studies in asynaptic maize. Cytologia 4:269-287.

Belling, J. 1933. Crossing over and gene arrangement in flowering plants. Genetics 18:388-413.

Bridges, C. B. 1915. A linkage variation in Drosophila. Journal of Experimental Zoology 19:1.

_____ 1927. The relation of the age of the female to crossing over in the third chromosome of Drosophila melanogaster. Journal of General Physiology 8:689-700.

Briggs, R. W. and H. H. Smith. 1965. Genetic recombination with ethyl-methane and sulfonate-induced waxy mutants in maize. Nature 207:890-891.

Brink, R. A. and D. C. Cooper. 1935. A proof that crossing over involves an exchange of segments between homologous chromosomes. Genetics 20:22-35.

Brown, W. L. 1949. Numbers and distribution of chromosome knobs in United States maize. Genetics 34:524-536.

Burnham, C. R. 1962. Inversions. In: Discussions in cytogenetics. Minneapolis, Burgess Publishing Company. p. 34-65.

Catcheside, D. G. 1948. Genetic effects of radiation. Advances in Genetics 2:271-358.

_____ 1968. The control of genetic recombination in Neurospora crassa. In: Replication and recombination of genetic material, ed. by W. J. Peacock and R. D. Brock. Canberra, Australian Academy of Science. p. 120-132.

Clark, F. J. 1940. Cytogenetic studies of divergent meiotic spindle formation in Zea mays. American Journal of Botany 27:547-559.

Cooper, K. W. 1949. The cytogenetics of meiosis in Drosophila. Mitotic and meiotic autosomal chiasmata without crossing over in the male. Journal of Morphology 84:81-122.

Cotton, F. A. and R. Francis. 1960. Sulfoxides as ligands. I. A preliminary survey of methyl sulfoxide complexes. American Chemical Society Journal 82:2986-2991.

Creighton, H. B. and B. McClintock. 1931. A correlation of cytological and genetical crossing over in Zea mays. Proceedings of the National Academy of Sciences 17:492-497.

Darlington, C. D. 1935. The time, place and action of crossing over. Journal of Genetics 31:185-212.

Darlington, C. D. and P. C. Koller. 1947. The chemical breakage of chromosomes. Heredity 1:187-221.

Davidson, D. 1958. Effects of chelating agents on cell division. Experimental Cell Research 14:329-332.

De, D. N. 1964. A new chromosome model. Nature 203:343-346.

DeRobertis, E. D. P., W. W. Nowinski and F. A. Saez. 1960. Organization of nucleus and chromosomes. In: General cytology. 3rd ed. Philadelphia, W. B. Saunders. p. 257-271.

Dobzhansky, T. 1931. The decrease in crossing over observed in translocations and its probable explanation. American Naturalist 65:214-232.

Dornfeld, E. J. and A. Owczarzak. 1958. Surface responses in cultured fibroblasts elicited by ethylenediamine-tetraacetic acid. Journal of Biophysical and Biochemical Cytology 4:243-250.

Frick, G. 1958. Interaction between Ca or Mg ions and nucleoprotein from sea urchin sperms. Experimental Cell Research 15:191-199.

Gall, H. 1964. Mutations in plant breeding. Radiation Botany 4: 155-232.

Gall, J. C. 1956. On the submicroscopic structure of chromosomes. Brookhaven Symposium of Biology 8:17-32.

_____ 1963. Kinetics of deoxyribonuclease action on chromosomes. Nature 198:36-38.

Grigg, G. W. 1968. Caffeine-death and a theory of chromosome breakage; an excision-repair suicide model. In: Replication and recombination of genetic material, ed. by W. J. Peacock and R. D. Brock. Canberra, Australian Academy of Science. p. 120-132.

Giles, N. H. 1954. Radiation induced chromosome aberrations in Tradescantia. In: Radiation biology, Vol. 1. ed. by A. Hollaender. New York, McGraw Hill. p. 713-762.

Gulick, A. 1941. The chemistry of chromosomes. Botanical Review 7:433-457.

Hanson, J. C. 1968. The effects of versene on dividing sea urchin eggs. Ph. D. thesis. Corvallis, Oregon State University. 38 numb. leaves.

Herschler, R. J. and S. W. Jacob. 1965. DMSO - a new drug from lignin. The Journal of the Technical Association of the Pulp and Paper Industry 48(6):43A-48A.

Hyde, B. B. 1955. The effect of versene on the structure of plant chromosomes. Biological Bulletin 109:347. (Abstract)

Ihrke, C. A. 1970. Influence of chelating agents on genetic recombination in Zea mays L. Ph. D. thesis. Corvallis, Oregon State University. 78 numb. leaves.

Iyer, V. N. and W. Szybalsky. 1963. A molecular mechanism of mitomycin C action: linking of complementary DNA strands. Proceedings of the National Academy of Sciences 50:355-362.

Janssen, F. A. 1909. LaTheorie de la chiasmatype. LaCellule 25:389.

Jessop, A. P. and D. G. Catcheside. 1965. Interalleleic recombination at the his-1 locus in Neurospora crassa and its genetic control. Heredity 20:237-256.

Kaufmann, B. P., H. Gay and M. J. McElderry. 1957. Effects of ribonuclease on crossing over in Drosophila. Proceedings of the National Academy of Sciences 43:255-261.

Kaufmann, B. P., H. Gay and M. R. McDonald. 1960. Organizational patterns within chromosomes. International Review of Cytology 9:77-127.

Kaufmann, B. P. and M. R. McDonald. 1957. The nature of the changes effected in chromosomal materials by the chelating agent EDTA. Proceedings of the National Academy of Sciences 43: 262-270.

Kihlman, B. A. 1956. Factors affecting the production of chromosome aberrations by chemicals. Journal of Biophysical and Biochemical Cytology 2:543-555.

_____ 1961. Biochemical aspects of chromosomal breakage. Advances in Genetics 10:1-59.

_____ 1966a. Agents affecting DNA and RNA metabolism. In: Actions of chemicals on dividing cells. Englewood Cliffs, Prentice Hall, Inc. p. 88-89.

_____ 1966b. The chromosome breaking chemicals. In: Actions of chemicals on dividing cells. Englewood Cliffs, Prentice Hall, Inc. p. 127-196.

Kirby, K. S. 1956. Evidence for metallic bonds linking deoxyribose nucleic acids to proteins in mammalian tissues. Biochemical Journal 62:31-32.

LaChance, L. E. 1959. The effect of chelating and X-ray on fecundity and induced dominant lethals on Habrobracon. Radiation Research 11:218-228.

Lauria, S. E. and D. C. Steiner. 1954. The role of calcium in the penetration of bacteriophage T5 into its host. Journal of Bacteriology 67:635-639.

Lawrence, M. J. 1958. Genotypic control of crossing-over on the first chromosome of Drosophila melanogaster. Nature 182: 889-890.

Levine, R. P. 1955. Chromosome structure and the mechanism of crossing over. Proceedings of the National Academy of Sciences 41:727-730.

Levine, R. P. and E. E. Levine. 1955. Variable crossing over arising in different strains of Drosophila pseudoobscura. Genetics 40:399-405.

Mather, K. 1936. Segregation and linkage in autotetraploids. Journal of Genetics 32:287-314.

Mazia, D. 1954. The particulate organization of the chromosome. Proceedings of the National Academy of Sciences 40:521-527.

McClintock, B. 1929. Chromosome morphology of Zea mays. Science 69:629-630.

_____ 1938. The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. p. 1-48. (Columbia, Missouri Agriculture Experiment Station Bulletin 290)

_____ 1941. The stability of broken ends of chromosomes in Zea mays. Genetics 26:234-282.

McDonald, M. R. and B. P. Kaufman. 1957. Production of mitotic abnormalities by ethylene-diamine tetraacetic acid. Experimental Cell Research 12:415-417.

McLeish, J. 1954. The consequences of localized chromosome breakage. Heredity 8:385-407.

Meyer, H. U. 1954. Crossing over in the germ line of Drosophila melanogaster males following irradiation of the embryonic pole cells with ultraviolet. Genetics 39:982. (Abstract)

Mirsky, A. E. and H. Ris. 1951. The composition and structure of isolated chromosomes. Journal of General Physiology 34:475-492.

Morgan, D. T. 1950. Cytogenetic study of inversions in Zea mays. Genetics 35:153-174.

Neuffer, M. G., L. Jones and M. S. Zuber. The mutants of maize. Madison, Crop Science Society of America, 1968. 74 p.

Nilan, R. A. and L. L. Phillips. 1957. The irradiation sensitivity of barley seeds low in calcium. Northwest Science 31:139-144.

Novitski, E. 1952. The genetic consequences of anaphase bridge formation in Drosophila. Genetics 37:270-287.

Peacock, W. J. 1968. Chiasmata and crossing over. In: Replication and recombination of genetic material, ed. by W. J. Peacock and R. D. Brock. Canberra, Australian Academy of Science. p. 242-251.

Peterson, R. G. 1967. Introduction to linear estimation and hypothesis testing. Corvallis, Oregon State University, Department of Statistics. 280 p.

Plough, H. H. 1917. The effect of temperature on crossing over in Drosophila. Journal of Experimental Zoology 24:147-209.

Rana, R. S. and H. C. Mathur. 1969. Effect of dimethyl sulfoxide on the mutagenic efficiency of ethyl methanesulfonate in seed treatments. Mutation Research 8:666-667.

Rhoades, M. M. 1933. An experimental and theoretical study of chromatid crossing over. Genetics 18:535-555.

_____ 1936. A cytogenetic study of a chromosome fragment in maize. Genetics 21:491-502.

_____ 1947. Crossover chromosomes in unreduced gametes of asynaptic maize. Genetics 32:101. (Abstract)

_____ 1950. Meiosis in maize. Journal of Heredity 41: 58-67.

_____ 1968. Studies on the cytological basis of crossing over. In: Replication and recombination of genetic material, ed. by W. J. Peacock and R. D. Brock. Canberra, Australian Academy of Science. p. 120-132.

Rhoades, M. M. and E. Dempsey. 1957. Further notes in preferential segregation. Maize Genetics Cooperation News Letter 31:77-80.

Ris, H. 1961. Ultrastructure and molecular organization of genetic systems. Canadian Journal of Genetics and Cytology 3:95-120.

Sax, K. 1932. The cytological mechanism of crossing over. Journal of Arnold Arboretum 13:180-212.

_____ 1941. Types and frequencies of chromosomal aberrations induced by X-rays. Cold Springs Harbor Symposium on Quantitative Biology 9:93-101.

Sax, K. 1957. The effects of ionizing radiation on chromosomes. Quarterly Review of Biology 32:15-26.

Schwartz, D. 1954. Studies on the mechanism of crossing over. Genetics 39:692-700.

Selbin, J., W. E. Bull and L. H. Holmes. 1961. Metallic complexes of dimethylsulphoxide. Journal of Inorganic and Nuclear Chemistry 16:219-224.

Sherman, F. and H. Roman. 1963. Evidence for two types of alleleic recombination in yeast. Genetics 48:255-261.

Slizynski, B. M. 1964. Chiasma in spermatocytes of Drosophila melanogaster. Genetical Research 5:80-84.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical methods. 6th ed. Ames, The Iowa State University Press. 593 p.

Sobels, F. H. and H. van Steenis. 1957. Chemical induction of crossing over in Drosophila males. Nature 179:29-31.

Somers, C. G., A. Cole and T. C. Hsu. 1963. Isolation of chromosomes. Experimental Cell Research Supplement 9:220-234.

Stadler, L. J. 1928. Genetic effects of X-ray in maize. Proceedings of the National Academy of Sciences 14:155-160.

Steffensen, D. 1953. Induction of chromosome breakage at meiosis by a magnesium deficiency in Tradescantia. Proceedings of the National Academy of Sciences 39:613-620.

_____ 1955. Breakage of chromosomes in Tradescantia with a calcium deficiency. Proceedings of the National Academy of Sciences 41:155-160.

_____ 1957a. Effects of various cation imbalances on the frequency of X-ray-induced chromosomal aberrations in Tradescantia. Genetics 42:239-251.

_____ 1957b. Dwarf Drosophila produced by ethylenediamine tetraacetic acid: A relationship to chelation of zinc. Nature 180:390-391.

Stern, C. 1931. Zytologisch-genetische Untersuchungen als Beweise für die Morganische Theorie des Faktorenaustauchs. Biol. Zentralbl. 51:547-587.

Sturtevant, A. H. 1919. Contributions to the genetics of Drosophila melanogaster. III. Inherited linkage variations in the second chromosome. Carnegie Institution Washington Publication 278: 305-341.

Sturtevant, A. H. and G. W. Beadle. 1936. The relations of inversions in the X-chromosome of Drosophila melanogaster to crossing over and disjunction. Genetics 21:554-604.

Suzuki, D. T. 1965. Effects of actinomycin D on crossing over in Drosophila melanogaster. Genetics 51:11-21.

Swanson, C. P. 1940. The distribution of inversions in Tradescantia. Genetics 25:438-465.

Swanson, C. P., T. Merz and W. J. Young. 1967. Cytogenetics. Englewood Cliffs, Prentice-Hall, Inc. 194 p.

Taylor, J. H. 1958. The organization and duplication of genetic material. Proceedings of the 10th International Congress of Genetics 1:63-78.

Thoday, J. M. and T. B. Boam. 1956. A possible effect of the cytoplasm on recombination in Drosophila melanogaster. Journal of Genetics 54:456-461.

Thoday, J. M. and J. M. Read. 1947. Effect of oxygen on the frequency of chromosome aberrations produced by X-rays. Nature 160:608.

Uhl, C. H. 1965. Chromosome structure and crossing over. Genetics 51:191-207.

Weijer, J. 1952. A catalog of genetic maize types together with a maize bibliography. Bibliographia Genetica 14:189-425.

Welcher, F. J. 1957. The analytical uses of ethylenediamine tetra-acetic acid. New York, Van Nostrand. 366 p.

Whitehouse, H. L. K. 1963. A theory of crossing over by means of hybrid deoxyribonucleic acid. Nature 199:1034-1040.

Whittinghill, M. 1937. Induced crossing over in Drosophila males and its probable nature. Genetics 22:114-129.

Wilberg, J. S. and W. F. Neuman. 1957. The binding of bivalent metals by deoxyribose and ribonucleic acids. Archives of Biochemistry and Biophysics 72:66-83.

Williamson, M. B. and A. Gulick. 1944. The calcium and magnesium content of mammalian cell nucleic. Journal of Cellular and Comparative Physiology 23:77-82.

Wolff, S. and H. E. Luipold. 1955. Metabolism and chromosome-break rejoining. Science 122:231-232.

_____ 1956. The production of two chemically different types of chromosomal breaks by ionizing radiations. Proceedings of the National Academy of Sciences 42:510-514.

Zimmering, S. 1955. A genetic study of segregation in a translocation heterozygote in Drosophila. Genetics 40:809-825.

Zubay, G. 1959. The interaction of nucleic acid with Mg-ions. Biochimica et Biophysica Acta 32:233-236.

Zubay, G. and P. Doty. 1958. Nucleic acid interactions with metal ions and amino acids. Biochimica et Biophysica Acta 29:47-58.