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Crossover frequencies for seven selected regions on five chromosomes of maize were measured to ascertain the effect of chelating agents on recombination. Ethylenediamine tetraacetic acid, EDTA, and dimethyl sulfoxide, DMSO, were used in three concentrations singly, and in all combinations. Plants heterozygous for for linked genes governing seed and seedling characteristics were treated with premeiotic foliar spray of EDTA and DMSO for two durations. Appropriate crosses were made and crossover frequencies were calculated from data resulting from test cross and sib cross progeny.

The chelating agents were found to influence the frequency of recombination in each of the regions tested. Crossover reduction was found at high concentrations of the chemicals and at the longer treatment duration. It was concluded that a threshold had been reached causing interference with crossing over or with recovery of

crossover products.

Crossover frequencies were significantly increased in five chromosome regions by several concentrations of the chelating agents. Although the two agents have different cation affinities, both were found to influence crossing over, indicating that the action was by a mechanism other than the removal of a specific cation from the chromosome.

Changes in recombination frequency were found to be of similar magnitude whether the chelating agents were used alone or in combination. It was concluded that the two chemicals did not have a synergistic effect in changing recombination frequency.

A relationship was shown between length of crossover region and effect of the chemical treatment. No such relationship was found between the specific locations in chromosomes and response to chelating agents for the seven regions tested.

Use of chelating agents offers the possibility of increasing genetic variability. Induction of recombination in a short chromosome region may improve a crop variety by breaking an undesirable gene combination, by linking together genes from adjacent regions of homologous chromosomes, or by producing a novel pleiotropic effect.

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

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INTRODUCTION

The nature of the chromosome has become more comprehensible in the years since the elucidation of the role of DNA in heredity. Several aspects of chromosome structure and genetic function, however, are still poorly understood. The basic mechanism controlling genetic recombination and the nature of the crossover process are two relatively unknown phenomena.

Crossing over requires the physical exchange of chromosome segments between the homologous chromosomes. This exchange suggests that a type of physical breakage and restitution must occur in the crossover event. If this is correct, it follows that understanding of the recombination process will be facilitated by an understanding of the chromosome structure. Divalent metal cations comprise one class of compounds found in the nucleus which may have a bearing on chromosome structure and hence genetic recombination.

Organic chemicals having an affinity for positively charged ions may be employed as a means of determining the effects of ion removal from the nucleus. Since the different organic chelating agents vary in ion specificity, characteristics of the cation-chelating agent complexes reflect on the identity of the ion. The determination of whether a particular cation is contained in the chromosome or, conversely, if

the structure of the chromosome is involved with the ionic balance in the nucleus, may be possible by the use of selective sequestering agents.

Involvement of cations in genetic recombination may be one of the evolutionary forces acting upon natural populations. Migration to new territories as well as effects of weather on soil have, over a long period of time, subjected many plant species to changing cation concentrations in soils. These changes may be reflected in the cation concentration of cell nuclei. If variation of cation concentrations in nuclei of reproductive cells affects crossing over, induced cation deficiencies may result in rapid genetic changes having adaptive significance in a plant population.

Recent reports have described changes in calcium concentration in certain tissues and bones of organisms subjected to space flights. It is of more than passing interest to determine whether such changes could affect the chromosomes of plants, animals, and man.

The goals of plant and animal breeding to develop superior agricultural varieties depend upon the variability of the breeding populations. Genetic recombination and mutation are the primary mechanism and source of this variation in sexually reproducing organisms. Hence an agent or process which increases the rate of crossing over, and thus releases phenotopic variability, may be an important tool to the geneticist.

The specific objectives of the present study were: (1) to evaluate the effects of chelating agents on chromosome structure in maize by determining their influence on crossing over at various intervals along the chromosome; and, (2) to explore the possibility of increasing recombination between closely linked genes by use of such chemicals, thereby increasing the phenotypic variability of the species.

LITERATURE REVIEW

Since the second decade of the twentieth century, the nature of chromosomal recombination in both plants and animals has been continuously and extensively studied. Earliest investigations were concerned mainly with mapping genes in chromosomes. It became apparent that a gene's position, as determined by crossover values, was somewhat variable. Efforts have been made over the years to determine the causes of this variability and to bring about changes in crossover frequency. Recombinagenic conditions have been found to be of four major types: those involving the structure of the chromosome, environmental variables, radiation, and chemical factors. A discussion of salient findings on this aspect of chromosome behavior is presented in the following section.

Conditions Affecting Crossing Over

The frequency of crossing over has been shown to vary with changes in chromosome structure. Dobzhansky (1931) has found that translocations reduce crossing over, with the greatest reduction occurring near the breakpoint and effects diminishing more distal to the breakpoint.

Chromosome structure has a pronounced effect on recombination in the case of heterozygous inversions. Sturtevant (1919) observed

that a decrease of crossing over in one pair of chromosome heterozygous for an inversion resulted in a crossover increase between genes in another chromosome pair. Several years later Sturtevant and Beadle (1936) demonstrated a decrease in crossing over within an inversion of Drosophila melanogaster. Rhoades (1955), working with maize, determined that a heteroxygous inversion, while reducing recombination within itself, increased crossing over in distal regions of the chromosome.

The presence of heterochromatic knobs altered the crossover frequency in chromosome ten of maize as shown in a series of studies conducted by Rhoades and Dempsey (1957, 1966). Terminal knobs on chromosome ten led to an increase of crossing over in both chromosome ten and chromosome three.

Of the wide array of environmental factors influencing crossing over, one of the most amply studied is temperature. Plough (1917), working with Drosophila, found that an increase in crossing over resulted when temperatures were either raised or lowered from the normal 25°C. Stern (1926) and Mather (1939) observed similar recombination frequency changes with temperature change in the X chromosome of Drosophila.

In Tradescantia, Swanson (1940) related chromosome bridge and fragment frequencies from an inversion heterozygote to temperatures at which the plants were grown. Bridges and fragments were formed

as the result of crossovers involving the inverted chromosome. Low temperatures were associated with low bridge and fragment frequencies; whereas these frequencies increased with increasing temperatures up to 30°C.

Wilson (1959) reported that increased temperature at meiosis resulted in a gradual decrease in chiasma frequency in Endymion; and that constant temperatures caused a more pronounced effect than did varying temperatures.

In lower organisms, the effects of temperature on Neurospora recombination were found by McNelly-Ingle, Lamb and Frost (1966) to be in agreement with similar studies previously cited for Drosophila. The minimum value of recombination was found to be at 25°C and values consistently increased with higher and lower temperatures over the range of 15°C to 30°C.

Irradiated plant and animal sex cells have been intensively investigated since the work of Muller (1925) revealed an increase in recombination frequency following X-ray treatment of Drosophila. Stadler (1926) was the first to demonstrate the effect of X-ray on crossing over in maize. He found increased chromosome breakage and increased recombination. Powell (1963) induced crossing over in barley by X-ray treatment of the seed as well as by temperature manipulation of the seedling.

Induced recombination as the result of X-ray treatment is thought

to be brought about by two mechanisms; (1) exchanges which produce chromatids equivalent to those produced by spontaneous crossover, termed true crossover; and (2) exchanges occurring between adjacent but not identical loci, termed pseudocrossover (Lucchesi and Suzuki, 1968). However, since most induced crossovers take place in heterochromatic regions largely devoid of known genes, distinction between these two mechanisms has not been proven.

Chemical mutagens, mitotic and metabolic poisons, and other chemical treatments have been employed as recombinagenic agents. Actinomycin-D, an inhibitor of RNA synthesis; and mitomycin-C, a DNA replication inhibitor, caused increased recombination in proximal regions of chromosome three of Drosophila (Hayashi and Suzuki, 1968).

Kaufmann, Gay and McElderry (1957), noting the occurrence of residual RNA in heterochromatic regions of Drosophila chromosomes, studied the effect of ribonuclease on crossing over. They found ribonuclease to be highly effective in increasing recombination of the X chromosome.

Mitotic crossing over in Aspergillus was chemically induced by both N-methyl-N-nitro-N nitrosoguandine (NTG) and 5-fluorodeoxyuridine (FUDR) (Shanfield and Kafer, 1968). Similarly, such cell poisons as formaldehyde, dihydroxydimethyl peroxide and mustard gas have induced crossing over in the normally nonrecombining

Drosophila males (Sobels and van Steenis, 1957). The formation of peroxides by these compounds as well as by X-irradiation has been demonstrated, and could well be involved in the mechanism of crossing over. Meyer (1954) found that crossing over was induced in Drosophila males as a result of exposure to ultra violet light which is known to produce peroxides.

Cation Effects on Chiasma, Crossing Over, and Chromosome Breakage

Chromosome structure and recombination may be influenced by another class of agents, namely the metal cations. Several lines of inquiry have been used to determine the role of these ions. The effects of cations have been studied from the standpoint of environmental and nutritional relationships. Interactions between cation concentrations and recombinagens have been investigated. Divalent metal ions have been shown to be included in the nuclei of many organisms. Experiments have been undertaken to determine the role of cations in the structure of chromosomes. The following sections summarize available information regarding these aspects of the cation-chromosome relationship.

Recently many reports concerning consequences of subjecting chromosomes to varying concentrations of metal ions have been published in the literature. Work by Levine (1955) involved feeding

excess calcium to adult female Drosophila. Progeny were classified for crossovers in three regions of the X chromosome. He found that excess calcium reduced crossing over in eggs hatched six to fourteen days subsequent to treatment. Excess magnesium, however, resulted in no change from the normal crossover frequency for the X chromosome markers.

Conversely, Steffensen (1955) noted a significant increase in breakage of chromosomes of Tradescantia when grown in a nutrient medium deficient in calcium. He determined the breakage frequency to be inversely related to calcium concentration. Steffensen (1953) also demonstrated induction of chromosome breakage by a deficiency of magnesium. Tradescantia grown in low magnesium concentration developed microspores with chromosome breaks. Micronuclei were formed in binucleate pollen in two percent of the cells, double the incidence found in control clones; while chromosome fragments were seven to eight times more frequent at anaphase II in plants grown in magnesium deficient medium than in the controls. No aberrations or stickiness were induced in mitotic cells by the magnesium deficiency.

In a subsequent experiment with maize, Steffensen, Anderson and Kase (1956) were unable to find differences from control cross-over frequencies for several marker gene pairs when the plants were grown in calcium-deficient quartz sand. Shaver (1957) investigated the effect of manganese ions in maize and found an increase in crossing

over. He concluded that the effect of manganese was due to a weakening of chromosome structure by replacement of the calcium and magnesium ions by the manganese in divalent cation bonds associated with chromosomal macromolecules.

Interaction effects of calcium deficiency and other agents have been ascertained in plants. Nilan and Phillips (1957) found that barley chromosomes were more readily broken by X-ray in seeds from plants which were grown under a calcium deficiency. X-rayed Tradescantia microspores which were subjected to low calcium treatment developed more chromosome breaks than those grown with normal calcium concentrations (Steffensen, 1957). Chromosome breakage in Tradescantia induced by calcium deficiency was found to be temperature dependent, with high temperatures during meiosis leading to a higher rate of breakage (Steffensen, 1959).

Hyde and Paliwal (1958) studied the effects of calcium and magnesium on chiasma frequency in Plantago by growing the plants in quartz sand with measured concentrations of the ions. Calcium deficiency resulted in a highly significant increase in chiasma frequency while magnesium deficiency increased chiasma to a lesser degree. Increase of calcium concentration also caused an increase in chiasma. This latter result is at variance with the findings of other workers, and indicates the crossover-calcium relationship may be a matter of environmental balance within the nucleus.

Divalent Metal Ions in Cell Nuclei

The existence of metal ions in cell nuclei has been known since the last decade of the ninteenth century when Frederick Miescher conducted pioneering analyses of cell components. Miescher (as cited by Williamson and Gulick, 1944) was the only one of early cytologists to include definite data on mineral content of cells. He reported that about 0.23% of the dry matter of the nucleus was calcium in water soluble form, and estimated the total nuclear calcium content to be 0.7%.

Microincineration techniques enabled investigators during the 1930's to characterize the mineral components of nuclei and chromosomes more critically. G. H. Scott (1932, 1933) found the nucleus to contain calcium and regarded the bonding between the metal and chromatin to be of considerable strength. Magnesium, calcium, iron, zinc, copper, aluminum, and several other minerals have been found to be associated with the nucleus and chromosomes. Steffensen (1961) reviewed these findings in some detail.

A quantitative determination of calcium and magnesium content in nuclei of mammalian cells was accomplished by Williamson and Gulick (1944). They reported an average nuclear calcium content of 1.35% for the calf lymph gland, calf thymus, and human tonsil tissue. Magnesium content was found to be 0.08% for these tissues. All

tissues were measured on a dry weight basis. These results coincide rather well with Miescher's early estimate of 0.7% calcium in the nucleus.

The Role of Cations in Chromosome Structure

The currently accepted view of the eukaryotic chromosome has derived primarily from work in the field of cytochemistry, electron microscopy, and light microscopy. Since each discipline attacks the chromosome from a unique viewpoint, the composite picture is, at best, slightly out of focus and marred with blank areas. Nevertheless, an abundance of information regarding the chemical organization of chromatin has been gained in recent years, and a generalized understanding of chromosome structure has emerged. Chromosome composition varies among cell types making any characterization of the eukaryotic chromosome an approximation.

The generalized chromosome is composed of 60-90% DNA and histone, the latter making up between 5 and 50% of this complex. Several species of histone have been isolated from chromosomes but all appear to be arginine or lysine rich compounds. Acidic protein of high molecular weight is less intimately associated with the DNA of the chromosome, and remains after the DNA-histone complex is extracted from the nucleus (Mirsky and Ris, 1951). RNA is associated with the chromosome in varying amounts, however its function in

chromosome structure may be trivial.

The role that cations play in the structural integrity of chromosomes has been the subject of considerable research since the proposal of Mazia (1954) that ionic factors bind DNA-histone macromolecules together to form the chromosome. He further postulated the ionic bonds were formed between macromolecules by bridges of divalent cations namely calcium and/or magnesium.

In an attempt to discover the role of calcium in the nucleus, Steffensen (1959) treated male Habrobracon with calcium-45 to produce sperm with labelled nuclei. Following mating to nonradioactive females, he found fertilized eggs which were radioactive. By bringing the label into the cross from the male side he was able to transfer mainly nuclear material, with very little cytoplasmic material transferred. In an experiment with plants, Steffensen and Bergeron (1959) treated Lillium with calcium-45. The radioactive pollen nuclei that were produced remained labelled while growing in the non-radioactive cytoplasm of pollen tubes in styles of nonlabelled flowers. Both of these experiments strongly suggest the binding of the isotope of calcium to the chromosome.

A striking demonstration of calcium-DNA interrelationships comes from the prokaryotic chromosome of T5 bacteriophage. Luria and Steiner (1954) reported that absence of calcium ions resulted in disintegration of the bacterial chromatin and interference with phage

multiplication.

The site of binding of cations to the DNA polyanion may be on the phosphate group of the latter. The nature of the cation, however, is not clearly defined. Moses (1964) cited evidence that indicated arginine-lysine-histidine residues of basic protein may be the cation in this complex. Kirby (1956) found evidence in mammalian tissue to indicate that metallic bonds linked DNA to proteins.

In vitro experiments by Frick (1957) employing extractions in differing ionic solutions, supported the Mazia (1954) hypothesis of divalent cation bridges linking chromosome macromolecules together. Frick found a consistent amount of calcium associated with the DNA-protein complex, and he calculated the relationship to be 11 nucleotides per calcium ion. This did not rule out histones as sharing the cation role with the metal ions nor did it eliminate the possibility of a protein-metal association in the intact chromosome.

Cavalieri (1952) measured the optical density of DNA in the presence of increasing concentrations of magnesium ions up to 1×10^{-4} M, and noted spectral changes. He concluded that magnesium ions were strongly bound to DNA and postulated the site of binding to be at the phosphate and nuclear amino groups, forming a bridge between the phosphate of one nucleotide and the amino group of the next nucleotide. Although this mechanism has been ruled out as the means of building a DNA chain, it may still have validity in the

interconnecting of DNA macromolecules. Jungner (1951) demonstrated the effect of the bivalent ions of magnesium, calcium, and zinc on the aggregate molecular weight of extracted DNA. He found the addition of bivalent ions resulted in greatly increased macromolecular complexes of DNA.

Wilberg and Neuman (1957) also showed a relatively strong binding of calcium and magnesium to DNA but found the ions bound to RNA almost as strongly. They noted, however, that the DNA of their preparation was denatured, hence single stranded, and thus weakened the argument for metal cation bridges as integral components of DNA.

Zubay and Doty (1958) investigated the binding of three classes of cations to undenatured DNA, using sodium, magnesium, and cupric ions as typical members of the alkali metals, alkaline earths, and heavy metals, respectively. They found that sodium ions were not bound to any sites on DNA but magnesium ions were lightly bound to undenatured DNA. Magnesium binding was dramatically increased where the DNA was denatured, reaching a concentration of 0.7 equivalents per nucleotide. Cupric ions were found to be bound more tightly to undenatured DNA than magnesium ions, and they caused an aggregation of DNA molecules which the authors interpreted to be the result of their binding DNA chains together laterally through chelating with phosphate groups on adjacent DNA chains.

The marked increase in binding ability of magnesium ions to

DNA after denaturation of the latter indicates the possibility of magnesium ions chelating to the base groups. Zubay (1959) presented evidence for magnesium-base complexes, and indicated the possibility of magnesium acting as an intermediate complexing agent in the unwinding of the two DNA chains and their subsequent replications.

It must be concluded from the available information, as cited in the preceeding paragraphs, that cations are associated with chromosomes. Further, divalent metallic ions are involved in at least some of the cation-chromosome complexes. The exact nature of this association in living cells remains an open question.

Effects of Chelating Agents on Cells

Investigations into the relationship between chromosomes and cations led to the use of chelating agents on several organisms. Chemicals which complex cations appeared to be an ideal means of removing the calcium, magnesium, and other divalent metal ions from the chromosome, and thus determining the role of cations in chromosome structure and function.

EDTA (ethylenediamine tetraacetic acid under the tradename of Versene) was the chelating agent most often used in these investigations. It has been shown to chelate readily with calcium and magnesium. However, Steffensen (1957) reported EDTA has an attraction for other divalent and trivalent metals in addition to calcium and

magnesium.

Davidson (1958) reported that EDTA acted, not by removal of cations, but rather by tying them in soluble metal complexes. He stated that EDTA exhibited a definite affinity for calcium ions while the chelating agent quinaldinic acid was more reactive with metallic ions such as copper and manganese. Dornfeld and Owczarzak (1958) found a specificity for magnesium and especially for calcium by EDTA in experiments with cultured fibroblasts, and noted reversibility of the EDTA-ion reaction upon removal of the chelating agent.

Dimethyl sulfoxide (DMSO) has been characterized as a chelating agent having a high affinity for copper, lead, and iron and forming complexes of lesser strength with manganese and zinc (Selbin, Bull and Holmes, 1961; Cotton and Francis, 1960). This organic compound has also been shown to be a penetrating agent through both animal and plant membranes (Stoughton and Fritsch, 1964; Kligman, 1965; Herschler and Jacob, 1965).

Information regarding the types of metal ion complexes or chelates that may be found in aqueous solutions for some minerals that have been reported in the cell nucleus is presented in Appendix Table 1.

Results of experiments employing chelating agents fall into two major categories: (1) those which indicate a direct role of calcium and magnesium in maintaining chromosome integrity; and, (2) those

which demonstrate a general interference by the chelating agent with the ionic environment of the cell.

The earliest relevant investigations with chelating agents showed that salivary gland chromosomes of Drosophila and chromosomes of grasshopper testes were broken in vitro when treated with low concentrations of EDTA (Mazia, 1954). The most effective concentration was 0.001 M EDTA, with chromosome dispersing ability decreased as the concentration of the chelating agent increased above 0.001 M. Mazia concluded that the chromosome was held together by divalent cations, calcium, magnesium, or both, which were removed by the EDTA treatment. He suggested that the particles of chromatin, which aggregate in a medium with the ionic strength normally found in the cell, would repel each other and dissolve when the ionic strength was lowered.

In studies of crossing over in Drosophila, Levine (1955) observed that EDTA treatment caused an increase in recombination on the X chromosome. Parallel studies showing a crossover decrease in flies fed excess calcium led him to postulate that calcium ions are necessary for the structural integrity of the chromosome. However, he noted the role of calcium could be one of maintaining the ionic balance of the nuclues.

Eisenstark and Kirchner (1956) found a two fold increase in transduction of a phage marker, and a 750 fold increase over the

controls in crossing over among bacterial strains due to treatment with EDTA. They also found increased recovery of mutants after EDTA treatment. They interpreted these results to be an indication that genetic units in bacteria may be bridged by chelatable elements.

The effects of EDTA on the structure of mitotic plant chromosomes were investigated by Hyde (1955, 1956) who reported chromosome swelling, breakage into smaller units, suppression of anaphase movement, and subsequent cell death. Sublethal treatments slowed division for several days but did not permanently affect chromosome structure. Wolff and Luippold (1956) reported two fold increases in chromosome breakage of Vicia with 0.001 M EDTA treatment. They also found a synergistic effect of EDTA and irradiation. LaChance (1959) found a similar synergistic effect of EDTA and X-ray on chromosome breakage and on deletions in Habrobracon.

Eversole and Tatum (1956), using Chlamydomonas, investigated the effects of EDTA on cation levels by use of crossover data. They found evidence that an EDTA induced increase in crossing over was related to a decrease in magnesium and calcium concentrations. The several regions of the chromosome responded to the treatment in varying degrees, indicating a difference of susceptibility to induced crossing over caused by the cation sequestering agent EDTA. This sensitivity to EDTA was interpreted to be in line with the theory of cation linkage of chromosome macromolecules.

Hyde and Paliwal (1958) concluded that the effect of EDTA on chromosome structure was due to a modification of the relationship between RNA and its associated protein. This chromosomal protein was inferred as being both histone and acidic protein.

Kaufmann and McDonald (1957) analyzed the mode of action of EDTA on mitotic Drosophila and Allium cells. They determined that RNA was involved in the EDTA induced response and nucleo-proteins were degraded sufficiently to affect their staining properties. Summarizing their findings, McDonald and Kaufmann (1957) attributed the effect of EDTA on chromosomes to alterations of the general cell metabolism rather than to direct chelation of divalent cation bridges.

Quinaldinic acid and EDTA treatments both resulted in chromosome swelling, sticky bridges, and mitotic arrest in Allium root tips (Davidson, 1958). The equivalence of effects of these two agents which differ greatly in their cation affinities argues against any mechanism of action involving a particular cation bond.

Hanson (1968) studied mitotic inhibition caused by EDTA in sea urchin eggs and concluded that the chelating agent removed calcium from the cell thereby preventing the formation of gels associated with the achromatic figures.

MATERIALS AND METHODS

Plant Materials

Corn was selected as the experimental organism in this study for the following reasons; its large linkage groups and small number of chromosomes, the well defined cytological characteristics of the chromosomes, the extensive documentation of crossover frequencies, and the availability of stock with linked genes for seed and seedling characters. Genetic marker corn stock was supplied by the Maize Genetics Cooperative, Urbana, Illinois. These inbred lines were homozygous for nine chromosome pairs and either homozygous recessive or heterozygous for marker genes on the remaining chromosome pair. In each case, the marker genes were brought into the cross in the coupling phase, which is to say the dominant alleles were in one member and the recessive alleles were in the other member of the homologous chromosomes.

Each trait employed in this study exhibited simple qualitative inheritance and dominance was complete (Weijer, 1952). Thus, it was possible to classify the progeny on the basis of presence or absence of the trait in question.

Markers on chromsomes two, three, five, seven, and nine were classified for seed and seedling traits. Chromosome three, five, and nine stock were classified for two traits each, while

chromosome three and seven stock were classified for three traits. The location of the pertinent traits on their respective chromosome maps is shown in Table 1.

Chemical Treatments

Ethylenediamine tetraacetic acid (EDTA) in the form of a disodium salt and dimethyl sulfoxide (DMSO) were the two chemicals used as chelating agents. The wetting agent, Tween-20, was applied to all treatments in the experiment, with the exception of one control group for each treatment series, the latter being treated with distilled water alone. Aqueous solution concentrations of EDTA of 0.0 M, 0.001 M, 0.01 M, and 0.1 M; and concentrations of DMSO of 0%, 2%, 8%, and 16% were prepared in all combinations in a four by four factorial arrangement. The composition of each treatment solution is shown in Table 2.

Treatment of the corn plants with the chelating agents was by means of a foliar spray prior to meiosis. Plastic atomizers were utilized for this procedure with an individual atomizer and bottle being used for each of the different treatment solutions.

The marker lines of corn, heterozygous for the genes of interest, were treated with chelating agents at daily intervals before meiosis. One group was treated four times at daily intervals, while a second group was treated daily for eight days. All treatments were applied

Table 1. Genetic map of five maize chromosomes showing position of genes in linkage groups.

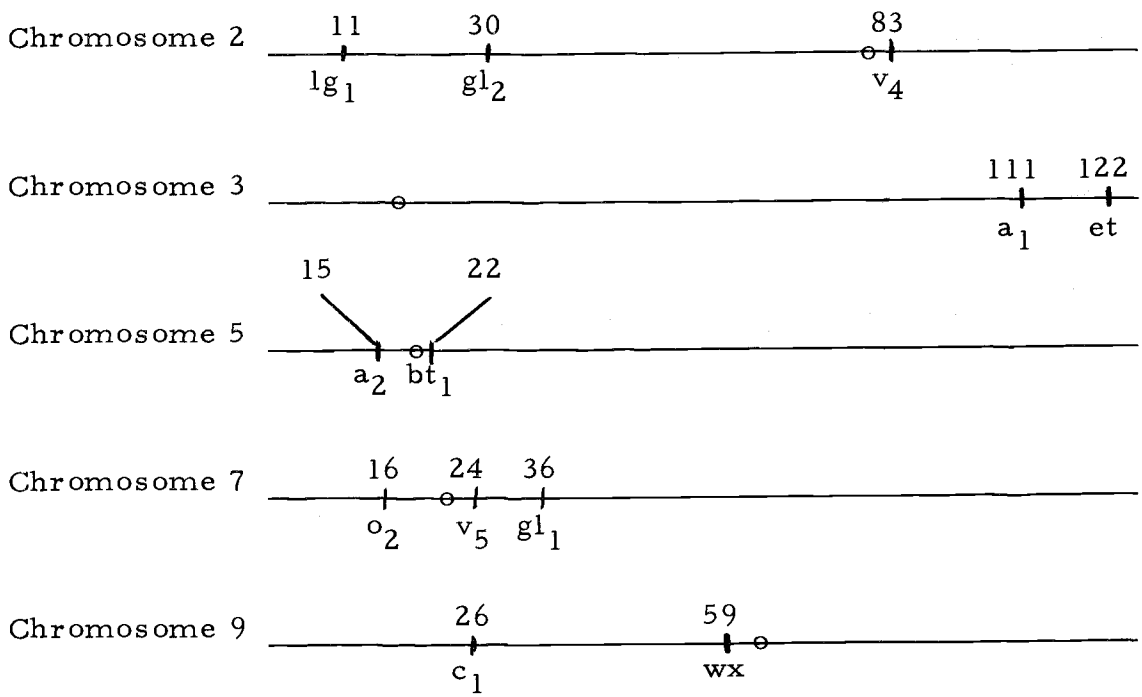

Table 2. Chelating agent solutions.

Treatment	Concentration of solution	Composition
A	Distilled water	
B	Distilled water & wetting agent	Tween-20 @ 1/3 ml/100 ml H_2O
C	EDTA 0.001 M	0.0372 g/100 ml H_2O
D	EDTA 0.01 M	0.372 g/100 ml
E	EDTA 0.1 M	3.72 g/100 ml
F	DMSO 2%	2 ml DMSO in 98 ml H_2O
G	DMSO 8%	8 ml in 92 ml H_2O
H	DMSO 16%	16 ml in 84 ml H_2O
I	DMSO 2%/EDTA 0.001	2 ml DMSO in 98 ml EDTA 0.001 M solution
J	DMSO 8%/EDTA 0.001	8 ml DMSO in 92 ml EDTA 0.001 M solution
K	DMSO 16%/EDTA 0.001	16 ml DMSO in 84 ml EDTA 0.001 solution
L	DMSO 2%/EDTA 0.01	2 ml DMSO in 98 ml EDTA 0.01 M solution
M	DMSO 8%/EDTA 0.01	8 ml DMSO in 92 ml EDTA in 0.01 solution
N	DMSO 16%/EDTA 0.01	16 ml DMSO in 84 ml EDTA 0.01 M solution
P	DMSO 2%/EDTA 0.1	2 ml DMSO in 98 ml EDTA 0.1 M solution
R	DMSO 8%/EDTA 0.1	8 ml DMSO in 92 ml EDTA 0.1 M solution
S	DMSO 16%/EDTA 0.1	16 ml DMSO in 84 ml EDTA 0.1 M solution

All solutions B through S contain Tween-20 at 1/3 ml/100 ml solution.

during the period just prior to meiosis. Each plant was tagged for identification and received chemical applications individually. Plants were spaced widely in the rows to insure against chemical spray accidentally contacting an adjacent plant.

Methods of Determining Recombinant Types

Crossing of the treated plant with an untreated plant was accomplished by hand pollination techniques. The treated plants heterozygous for markers on chromosomes three, five and nine were all test crossed to untreated homozygous recessive plants. Sib crosses to untreated heterozygous plants were accomplished for the chelate-treated heterozygous plants of chromosome two and seven stock. Treated plants were used as female parents in all corn lines. In addition, stock for chromosome three and five were treated and used as male parents in test crosses to untreated homozygous recessive plants. Table 3 shows the crosses that were made.

The spikes formed from the crosses remained bagged until harvest to eliminate the possibility of contamination by foreign pollen. Harvested ears were dried to a moisture content of approximately 12%, hand shelled, and classified for seed characters. Kernels from a single spike were kept separate throughout the process.

Seedling characters were analyzed on greenhouse benches at three to four weeks after germination. The greenhouse temperature

Table 3. Sib and test cross combinations involving five experimental lines of maize.

Chromosome Line	Type of Cross	Sex of Treated Parent	Female Parent	Male Parent
2	Sib cross	F	$\frac{Lg_1 \quad Gl_2 \quad V_4}{lg_1 \quad gl_2 \quad v_4}$	$\frac{Lg_1 \quad Gl_2 \quad V_4}{lg_1 \quad gl_2 \quad v_4}$
3	Test cross	F	$\frac{A_1 \quad Et}{a_1 \quad et}$	$\frac{a_1 \quad et}{a_1 \quad et}$
3	Test cross	M	$\frac{a_1 \quad et}{a_1 \quad et}$	$\frac{A_1 \quad Et}{a_1 \quad et}$
5	Test cross	F	$\frac{A_2 \quad Bt_1}{a_2 \quad bt_1}$	$\frac{a_2 \quad bt_1}{a_2 \quad bt_1}$
5	Test cross	M	$\frac{a_2 \quad bt_1}{a_2 \quad bt_1}$	$\frac{A_2 \quad Bt_1}{a_2 \quad bt_1}$
7	Sib cross	F	$\frac{O_2 \quad V_5 \quad Gl_1}{o_2 \quad v_5 \quad gl_1}$	$\frac{O_2 \quad V_5 \quad Gl_1}{o_2 \quad v_5 \quad gl_1}$
9	Test cross	F	$\frac{C_1 \quad Wx}{c_1 \quad wx}$	$\frac{c_1 \quad wx}{c_1 \quad wx}$

was 70°F during the day and 65°F at night. No artificial light was provided. The expression of the marker genes for both seed and seedling traits are characterized and presented in Table 4.

Statistical Procedure

Crossover percentages between gene pairs were calculated by the conventional backcross method for chromosome three, five, and nine. Crossover frequencies between genes in chromosome two and seven were calculated by the product method of determining linkage from F_2 data. Each crossover percentage derived from seed of a single spike was accepted as a representative value of recombination in the heterozygous parent plant. Analysis of variance within treatment groups, and between treatment groups and controls, was accomplished by the method of least squares. Tests for least significant differences were performed between the two control groups. Where there was no significant difference between control groups (as was the case in all crosses), the control mean was taken to be the pooled mean of borh groups. Tests for least significant differences were then performed between the control mean and all other treatment means. This analysis was done according to the method of Peterson (1967).

Table 4. Characterization of marker genes on five chromosomes of maize.

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	122	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

Table 4. (Continued)

Symbol	Descriptive Title	Stage of Classification	Location in Chromosome	Position	Characteristics
o_2	Opaque	Seed	7	16	Endosperm soft, floury, dull in reflected light, opaque to transmitted light
v_5	Virescent	Seedling	7	24	Same as v_4 above
gl_1	Glossy	Seedling	7	36	Same as gl_2 above
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).

RESULTS

Progeny Numbers and Control Frequencies

Seven regions on five chromosomes of maize were analyzed for crossover frequency. The frequencies were established from F_2 and backcross progenies of plants which were heterozygous for genetic markers flanking the region. Progeny numbers of individual ears, from which the crossover frequency for each region was calculated, are shown in Tables 5 and 6. The treatment groups and the concentrations of chelating agents in each group are displayed in Table 7.

A control value was established for each treatment duration over each region being tested. The two control groups, A and B, were analyzed for differences in crossover frequencies and found not to be significantly different in all 14 experiments, indicating the wetting agent Tween-20 did not influence crossing over. Therefore, the two control groups were pooled and the control frequency taken to be that crossover percentage observed for each region from plants treated with water and water plus wetting agent, noted as treatments A and B respectively. In the experiment involving the a_2-bt of chromosome five, the control group crossover frequencies were pooled for both the four and eight day treatments. The control groups involved did not differ significantly from each other in crossover frequency. This pooling resulted in a larger control population and

Table 5. Progeny numbers of individual ears from which recombination values were calculated taken from plants treated for a duration of eight days.

EDTA Concentration:		0.0 M				0.001 M				0.01 M				0.1 M			
DMSO Concentration:		0%	2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%
Chromosome	Region																
2	lg_1 - gl_2	309	254	422	294	529	465	328	323	387	27	477	392	364	187	320	79
		417		422	330	290	133		159	279	129	278	300	551	136		307
		361							53		389			60			
2	gl_2 - v_4	309	14	422	294	529	465	328	323	387	27	477	392	364	187	320	79
		417	254	442	330	290	133		159	279	129	278	300	551	136	15	307
		361							53		389			60			
									140								
3	a_1 - et	137	45	72	138	98	214	189	80	303	61	251	154	63	75	40	191
		84	189	179		72	258	79	30	209	97	179	284	127			66
		279	69	134		16	333	33		272	196		107				147
		94									63						
5	a_2 - bt_1	190	283	280	279	144	95	14	58	125	66	71	218		147		148
		170		188	104	33	120	111	205	163	111	51	108		300		413
		190		164					178	309		256	156				
		144								109							
7	v_5 - gl_1	75	166	24	104	460	367	38	201	47	188	83	266	96		531	400
		412	139	77	412	31	37	141	247			241		96		163	
		148	412						30			104					
												323					

Table 5. (Continued).

EDTA Concentration:		0.0 M				0.001 M				0.01 M				0.1 M			
DMSO Concentration:		0%		2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%
Chromosome	Region																	
7	$o_2 - v_5$	75		166	24	104	460	367	38	201	47	188	83	266	96		531	400
		412		139	77	412	31	37	141	247			241		96		163	
		148		412									104					
													323					
9	c_1 - wx	87	362	361	530	362	85	260	389	271	345	353	66	364	400	132	343	515
		501	412	87	58	368	184		20	213	321	499		417			313	220
		415	239		131	208												
		28																

Table 6. Progeny numbers of individual ears from which recombination values were calculated taken from plants treated for a duration of four days.

EDTA Concentration:		0.0 M				0.001 M				0.01 M				0.1 M			
DMSO Concentration:		0%	2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%
Chromosome	Region																
2	lg_1 - gl_2	449	208	367	89	329	468	332	431	257	344	491	114	236	273	249	178	54
		309	469	395	77		260		376	261	80	105	571	100	433	570	81	211
		395	264						327	500			262	143		64	119	565
									76				222	276		375	325	204
																283		
2	gl_2 - v_4	449	208	367	89	329	468	332	431	257	344	491	114	236	273	249	178	211
		309	469	395	77		260		376	261	80	105	571	100	433	570	81	54
		395	264		80				327	500			262	143		64	119	565
									76				222	276		375	325	204
																283		
3	a_1 - et	164	247	258	64	33	191	93	113	94	22		91	31	40	126	113	258
		47	82	56	142	576	20	155	159	66	41		199	221	19	240	139	122
		60	67						23		127		130			121		401
		136									100		159					162
											53		34					
5	a_2 - bt_1	190		65	334	134	248	95	209	70	65	138		246	263	92	363	36
		170		98	186	148		23	139			140			157			38
		144		170	39	253						100						
		190		373	373	29												
				196	193													
7	v_5 - gl_1	90	397	310	33	387	110	97	457	362		217	460	587	181		327	251
		54	328			68	177	80	322	203			248	345	322		303	174
		76				377	589			373								

Table 6. (Continued).

EDTA Concentration:		0.0 M				0.001 M				0.01 M				0.1 M			
DMSO Concentration:		0%	2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%	0%	2%	8%	16%
Chromosome	Region																
7	o_2 - v_5	90	397	310	65	387	110	97	457	362		217	460	587	184		327	251
		54	328		33	68	177		332	203			248	30	322		303	174
		76				377	589			24				345				
										373								
9	c_1 - wx	300		23	273	82	462	304	361	598	37	141	409	116	476	470	260	377
		21		390		544	62			81	317	346	285	518	415	391		272
		424		158		502				292	75	234			12	279		
				65														

Table 7. Concentration of chelating agent solutions.

EDTA Concentrations in Rows	DMSO Concentrations in Columns 0%	2%	8%	16%
0 M	A EDTA 0 M DMSO 0%			
0 M	B EDTA 0 M DMSO 0%	F EDTA 0 M DMSO 2%	G EDTA 0 M DMSO 8%	H EDTA 0 M DMSO 16%
0.001 M	C EDTA 0.001 M DMSO 0%	I EDTA 0.001 M DMSO 2%	L EDTA 0.001 M DMSO 8%	P EDTA 0.001 M DMSO 16%
0.01 M	D EDTA 0.01 M DMSO 0%	J EDTA 0.01 M DMSO 2%	M EDTA 0.01 M DMSO 8%	R EDTA 0.01 M DMSO 16%
0.1 M	E EDTA 0.1 M DMSO 0%	K EDTA 0.1 M DMSO 2%	N EDTA 0.1 M DMSO 8%	S EDTA 0.1 M DMSO 16%

All groups except A contain Tween-20 wetting agent (1/3 ml Tween-20 per 100 ml solution). Composition of formula for each treatment is described in Table 2. Groups A and B are combined as a control mean in the tables to follow.

somewhat more precise measure of deviation from the control frequency.

Crossing Over in Chromosome Two

Two regions of chromosome two were tested for changes in crossover frequency due to EDTA and DMSO applications. The lg_1-gl_2 region is on the long arm of the chromosome, distal to a prominent knob. The liguleless gene, lg_1, if mapped at position 11 and the glossy gene, gl_2, at position 30. The second interval analyzed on this chromosome was gl_2-v_4, the latter being located at position 83 on the linkage map. This virescent gene is adjacent to the centromere on the short arm of chromosome two, thus the interval tested spans the centromere.

The lg_1-gl_2 region of chromosome two was analyzed for all treatment concentrations over a duration of four days prior to meiosis as shown in Table 8a. Recombination for the control was found to be 10.28%. Those treatments which differed significantly from this frequency, as displayed in Table 8b, were treatments J (0.01 M EDTA, 2% DMSO), M (0.01 M EDTA, 8% DMSO), and N (0.1 M EDTA, 8% DMSO). All showed increases of recombination which were significant at the 5% level.

Results of all treatments of the lg_1-gl_2 interval over a duration of eight daily applications are shown in Table 9a. The control

Table 8a. Recombination values for the lg_1 - gl_2 region of chromosome two of maize for all treatments over a duration of four days.

DMSO / EDTA	0%	2%	8%	16%
0 M	10.28	9.52	6.28	13.68
0.001 M	12.67	6.27	9.59	11.85
0.01 M	14.03	16.47	14.69	10.30
0.1 M	14.69	11.11	15.14	9.03

Table 8b. Recombination values of treatments involving the lg_1 - gl_2 region of chromosome two which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 10.28			
0.001 M				
0.01 M		16.47*	14.69*	
0.1 M			15.14*	

', *, ** Denote significant differences from the control at the .10, .05, and .01 levels respectively.

These levels of significance will be indicated in this manner for all succeeding tables.

Table 9a. Recombination values for the lg_1 - gl_2 region of chromosome two of maize for all treatments over a duration of eight days.

DMSO / EDTA	0%	2%	8%	16%
0 M	11.11	10.90	15.14	14.68
0.001 M	7.59	10.04	8.91	12.35
0.01 M	14.32	15.79	11.98	13.17
0.1 M	10.58	14.15	15.25	10.97

Table 9b. Recombination values of treatments involving the lg_1 - gl_2 region of chromosome two which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 11.11		15.14"	
0.001 M				
0.01 M		15.79*		
0.1 M				

", * Denote significant differences from the control at the .10 and .05 levels respectively.

frequency was found to be 11.11%. Two treatments were found to differ significantly from the control, as shown in Table 9b. Treatment G (8% DMSO) differed at the 10% level of significance, while J (0.01 M EDTA, 2% DMSO) differed at the 5% level. Both groups, G and J, showed increased crossing over due to the treatments.

The gl_2-v_4 region of chromosome two was found to have a crossover frequency of 35.14% in the control plants. The recombination values for all treatments over four days are shown in Table 10a. Only one group, as shown in Table 10b, differed significantly at the 10% level from the frequency of the controls. Treatment S (0.1 M EDTA, 16% DMSO) had an increased crossover percentage at the 10% level of significance.

Treatment by the chelating agents for eight days prior to meiosis resulted in the recombination frequencies shown in Table 11a for the gl_2-v_4 region. The control frequency was found to be 43.43% in this series, and was reduced in three treatment groups: I (0.001 M EDTA, 2% DMSO), E (0.1 M EDTA), and K (0.1 M EDTA, 2% DMSO) as presented in Table 11b. Reductions in crossover frequencies induced by treatment with the chelating agents were significant at the 10% level for treatments E and I, and at the 1% level for treatment K.

Table 10a. Recombination values for the gl_2 - v_4 region of chromosome two of maize for all treatments over a duration of four days.

DMSO / EDTA	0%	2%	8%	16%
0 M	35.14	39.07	37.21	32.63
0.001 M	36.14	30.17	39.47	37.85
0.01 M	32.63	36.30	40.98	39.18
0.1 M	37.70	36.58	40.83	41.80

Table 10b. Recombination values of treatments involving the gl_2 - v_4 region of chromosome two which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 35.14			
0.001 M				
0.01 M				
0.1 M				41.80"

" Denotes significant difference from the control at the .10 level.

Table 11a. Recombination values for the gl_2 - v_4 region of chromosome two of maize for all treatments over a duration of eight days.

DMSO / EDTA	0%	2%	8%	16%
0 M	43.43	36.42	41.90	37.84
0.001 M	38.11	33.19	35.66	38.42
0.01 M	42.36	36.96	35.10	42.05
0.1 M	35.23	26.21	37.10	39.35

Table 11b. Recombination values of treatments involving the gl_2 - v_4 region of chromosome two which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 43.43			
0.001 M		33.19"		
0.01 M				
0.1 M	35.23"	26.21**		

", ** Denote significant differences from the control at the .10 and .01 levels respectively.

Chromosome Three Recombination

Near the distal end of the long arm of chromosome three is the a_1-et region. Anthocyaninless seed, a_1, is located at the map position of 111, and etched endosperm, et, is at position 122, while the centromere is near linkage position 28. Chromosome three is thicker and heavier than any other long chromosome in maize and has a heterochromatic knob situated between the a_1-et region and the centromere.

Recombination values for all treatments over a duration of four days prior to meiosis for this region are depicted in Table 12a. The control value was found to be 12.35%. The treatments which differ significantly from the control are shown in Table 12b. Treatment E, significant at the 5% level, shows a decrease in recombination frequency; while treatments H and K, significant at the 5% and 10% levels respectively, show increases. Treatment E was 0.1 M EDTA; H was 16% DMSO; and K was 0.1 M EDTA, 2% DMSO.

Treatment of the a_1-et region over eight days resulted in crossover values shown in Table 13a. The control value for this series was 15.69%. Five treatments were found to be significantly different from the control and each had a lower crossover frequency than the control. Four of these groups, as displayed in Table 13b, involved treatments with 16% DMSO. These were: H (16% DMSO), P (0.001 M EDTA, 16% DMSO), R (0.01 M EDTA, 16% DMSO), and

Table 12a. Recombination values for the a_1 - et region of chromosome three of maize for all treatments over a duration of four days.

DMSO / EDTA	0%	2%	8%	16%
0 M	12.35	11.36	12.07	18.74
0.001 M	11.80	11.07	14.40	15.78
0.01 M	14.73		10.84	9.36
0.1 M	7.63	16.20	12.69	13.12

Table 12b. Recombination values of treatments involving the a_1 - et region of chromosome three which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 12.35			18.74*
0.001 M				
0.01 M				
0.1 M	7.63*	16.20"		

", * Denote significant differences from the control at the .10 and .05 levels respectively.

Table 13a. Recombination values for the a_1 - et region of chromosome three of maize for all treatments over a duration of eight days.

DMSO / EDTA	0%	2%	8%	16%
0 M	15.69	11.93	17.40	5.79
0.001 M	11.44	8.46	12.14	8.45
0.01 M	14.98	12.70	12.67	10.32
0.1 M	12.65	21.33	17.50	10.39

Table 13b. Recombination values of treatments involving the a_1 - et region of chromosome three which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 15.69			5.79*
0.001 M		8.46*		8.45*
0.01 M				10.32''
0.1 M				10.39''

'', * Denote significant differences from the control at the .10 and .05 levels respectively.

S (0.1 M EDTA, 16% DMSO). Group I (0.001 M EDTA, 2% DMSO) along with H and P were significantly different from the control at the 5% level while groups R and S differed at the 10% level of significance.

Chromosome Five Recombination

The a_2-bt_1 region of chromosome five spans the centromere. Anthocyaninless, a_2, is located at position 15 on the short arm while brittle endosperm, bt_1, is mapped at position 22, adjacent to the centromere on the long arm of the chromosome.

The recombination for the controls in this short region was found to be 4.18%. Treatments for four days duration resulted in the recombination values shown in Table 14a. Increased crossing over was observed in three groups: E (0.1 M EDTA), J (0.01 M EDTA, 2% DMSO), and S (0.1 M EDTA, 16% DMSO) as shown in Table 14b. These increases were found to be significant at the 10%, 5%, and 5% levels respectively.

Crossover frequencies for the eight day treatments in the a_2-bt_1 region are shown in Table 15a. Group S (0.1 M EDTA, 16% DMSO) was the only treatment to differ from the controls in crossover frequency at the 10% level of significance. The level of increased crossing over of treatment S over the control is given in Table 15b.

Table 14a. Recombination values for the a_2 - bt_1 region of chromosome five of maize for all treatments over a duration of four days.

DMSO / EDTA	0%	2%	8%	16%
0 M	4.18	5.41	5.27	6.33
0.001 M	4.43	6.91	5.73	4.28
0.01 M	1.53	10.05		8.94
0.1 M	8.63	5.43	3.85	9.43

Table 14b. Recombination values of treatments involving the a_2 - bt_1 region of chromosome five which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 4.18			
0.001 M				
0.01 M		10.05*		
0.1 M	8.63"			9.43*

", * Denote significant differences from the control at the .10 and .05 levels respectively.

Table 15a. Recombination values for the a_2 - bt_1 region of chromosome five of maize for all treatments over a duration of eight days.

DMSO / EDTA	0%	2%	8%	16%
0 M	4.18	8.83	5.68	6.83
0.001 M	3.59	5.63	7.17	7.21
0.01 M	4.11	5.42	5.84	7.44
0.1 M		3.71		8.62

Table 15b. Recombination values of treatments involving the a_2 - bt_1 region of chromosome five which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 4.18			
0.001 M				
0.01 M				
0.1 M				8.62"

"Denotes significant difference from the control at the .10 level.

Crossing Over in Chromosome Seven

Two regions of chromosome seven were evaluated for recombination frequency. The centromere is located near position 28 on the linkage map. One region tested, o_2-v_5, is on the short arm while the other region, v_5-gl_1, spans the centromere. Opaque seed, o_2, is located at position 16, virescent seedling, v_5, is at position 24, and glossy seedling, gl_1, is at position 36.

The crossover values for all treatment levels over a duration of four days for the v_5-gl_1 interval are given in Table 16a. The control frequency was found to be 7.58%. As Table 16b indicates, crossover frequency was greatly increased in ten of the treatment groups. At the 1% level of significance increases were found in treatments: E (0.1 M EDTA), F (2% DMSO), I (0.001 M EDTA, 2% DMSO), M (0.01 M EDTA, 8% DMSO), N (0.1 M EDTA, 8% DMSO), and R (0.01 M EDTA, 16% DMSO). Three additional groups showed crossover increases at the 5% significance level: C (0.001 M EDTA), L (0.001 M EDTA, 8% DMSO), and H (16% DMSO). One group, S (0.1 M EDTA, 16% DMSO) was increased in recombination frequency at the 10% level of significance.

For this same region with the eight day treatment duration, the control frequency was found to be 6.81%. Recombination values for all groups treated are given in Table 17a. Two cells had crossover values increased by the chelating agent treatment. Group G (8% DMSO)

Table 16a. Recombination values for the v_5 - gl_1 region of chromosome seven of maize for all treatments over a duration of four days.

DMSO / EDTA	0%	2%	8%	16%
0 M	7.58	20.81	9.39	10.49
0.001 M	13.19	23.60	10.85	9.63
0.01 M		9.16	18.13	22.31
0.1 M	13.66		15.36	10.10

Table 16b. Recombination values of treatments involving the v_5 - gl_1 region of chromosome seven which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 7.58	20.81**		10.49*
0.001 M	13.19*	23.60**	10.85*	
0.01 M			18.13**	22.31**
0.1 M	13.66**		15.26**	10.10"

", *, ** Denote significant differences from the control at the .10, .05, and .01 levels respectively.

Table 17a. Recombination values for the v_5 - gl_1 region of chromosome seven of maize for all treatments over a duration of eight days.

DMSO / EDTA	0%	2%	8%	16%
0 M	6.81	8.29	32.89	11.24
0.001 M	17.46	12.11	11.30	14.78
0.01 M	4.60	12.43	9.41	14.48
0.1 M	25.88		9.51	14.88

Table 17b. Recombination values of treatments involving the v_5 - gl_1 region of chromosome seven which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 6.81		32.89**	
0.001 M				
0.01 M				
0.1 M	25.88*			

*, ** Denote significant differences from the control at the .05 and .01 levels respectively.

and group E (0.1 M EDTA) had increased crossover frequencies significant at the 1% and 5% levels respectively (Table 17b).

The o_2-v_5 region of chromosome seven had a control crossover frequency of 7.76% in the four day treatment series. The crossover frequencies for all treatment cells are shown in Table 18a. Recombination values were increased at the 1% level of significance in treatment F (2% DMSO) and R (0.1 M EDTA, 16% DMSO); increased at the 5% level of significance in treatment M (0.01 M EDTA, 8% DMSO); and increased at the 10% level in treatment N (0.1 M EDTA, 8% DMSO) as shown in Table 18b.

A control value of 11.38% was found in the o_2-v_5 region in the eight day treatments. Values for all treatment groups are displayed in Table 19a. A single treatment, C (0.001 M EDTA), was increased at the 5% level of significance, as shown in Table 19b.

Crossing Over in Chromosome Nine

The short arm of chromosome nine contains the c_1-wx region with colorless aleurone, c_1, located at position 26 and waxy endosperm, wx, located at position 59. Chromosome nine is the second shortest of all maize chromosomes, and has a large terminal heterochromatic knob on its short arm.

Treatment with the chelating agents over a four day duration produced the crossover values in the c_1-wx region shown in Table 20a.

Table 18a. Recombination values for the o_2 - v_5 region of chromosome seven of maize for all treatments over a duration of four days.

DMSO / EDTA	0%	2%	8%	16%
0 M	7.76	25.13	13.80	8.54
0.001 M	10.16	8.67	8.21	11.13
0.01 M		10.97	16.42	19.20
0.1 M	12.85		14.37	7.62

Table 18b. Recombination values of treatments involving the o_2 - v_5 region of chromosome seven which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 7.76	25.13**		
0.001 M				
0.01 M			16.42*	19.20**
0.1 M			14.37''	

'', *, ** Denote significant differences from the control at the .10, .05, and .01 levels respectively.

Table 19a. Recombination values for the o_2 - v_5 region of chromosome seven of maize for all treatments over a duration of eight days.

DMSO / EDTA	0%	2%	8%	16%
0 M	11.38	7.16	12.37	12.89
0.001 M	20.87	12.59	11.18	8.41
0.01 M	8.75	12.38	12.79	14.00
0.1 M	14.04		9.58	8.15

Table 19b. Recombination values of treatments involving the o_2 - v_5 region of chromosome seven which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 11.38			
0.001 M	20.87*			
0.01 M				
0.1 M				

*Denotes significant difference from the control at the .05 level.

Table 20a. Recombination values for the c_1 - wx region of chromosome nine of maize for all treatments over a duration of four days.

DMSO / EDTA	0%	2%	8%	16%
0 M	17.86	16.60	19.41	19.41
0.001 M	16.02	15.13	19.39	20.79
0.01 M	23.50	15.61	17.76	17.42
0.1 M	22.42	18.87	14.23	20.78

Table 20b. Recombination values of treatments involving the c_1 - wx region of chromosome nine which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 17.86			
0.001 M				
0.01 M	23.50*			
0.1 M				

*Denotes significant difference from the control at the .05 level.

The control frequency was found to be 17.86%. One treatment differed significantly at the 5% level from the control. Group D (0.01 M EDTA), as shown in Table 20b, was increased in crossover frequency.

Eight day treatment duration prior to meiosis resulted in the recombination values displayed in Table 21a. The control value for this series was 15.15%. Six groups were found to differ significantly from the control (Table 21b). Three of the treatments received DMSO only: F (2% DMSO), G (8% DMSO), and H (16% DMSO). They each had a higher recombination value than the control; F and H at the 5% level and G at the 10% level of significance. Other groups showing increased crossing over due to treatment with chelating agents were: L (0.001 M EDTA, 8% DMSO), and P (0.001 M EDTA, 16% DMSO), significant at the 10% level; and K (0.1 M EDTA, 2% DMSO), significant at the 5% level.

Table 21a. Recombination values for the c_1 - wx region of chromosome nine of maize for all treatments over a duration of eight days.

DMSO / EDTA	0%	2%	8%	16%
0 M	15.15	22.25	20.81	21.79
0.001 M	20.60	17.30	21.52	21.06
0.01 M	16.10	18.17	18.18	19.17
0.1 M	21.00	25.75	18.66	20.03

Table 21b. Recombination values of treatments involving the c_1 - wx region of chromosome nine which differ significantly from the control frequency.

DMSO / EDTA	0%	2%	8%	16%
0 M	Control 15.15	22.25*	20.81"	21.79*
0.001 M			21.52"	21.06"
0.01 M				
0.1 M		25.75*		

", * Denote significant differences from the control at the .10 and .05 levels respectively.

DISCUSSION

The specific effects of chelating agents in seven selected chromosome regions may be classified into four groups: crossover increases caused primarily by EDTA, crossover increases caused by DMSO, crossover increases caused by both chelating agents, and crossover reduction. The following three sections discuss these relationships.

Crossover Increases in Chromosome Five and Nine

Crossing over in the short region of chromosome five was increased by treatments with EDTA irrespective of the DMSO dosage. Three of the four increases resulted from treatment with high concentrations of EDTA, and the fourth from treatment with medium EDTA concentration. Therefore, it may be concluded that DMSO treatment does not affect recombination in the a_2-bt_1 region. The eight day treatments tended to show less variation from the control mean than did the four day treatments. These results indicated that artificially induced recombination near the centromere of chromosome five would be best accomplished by treatment with high concentrations of EDTA for a short duration prior to meiosis.

Crossing over in the c_1-wx region of chromosome nine differed sharply with the aforementioned results of crossing over in chromosome five. Influence on crossing over was found for all concentrations

of DMSO over the longer treatment duration. Six groups showed significant crossover increases at all three levels of DMSO. Three of these groups were treated with DMSO alone. It may be concluded that DMSO causes an increase in crossing over in this region when treated over an eight day period, and that EDTA acting with DMSO does not alter the results.

Combining the results of both durations, the four highest concentration groups, M, N, R, and S resulted in no significant change of crossover frequency. Four of the seven increases noted for this region came from groups treated with a single chemical. The remaining three groups were treated with a low concentration of one of the agents, and medium or high concentration of the other agent. Taken together, these observations argue against a synergistic action of the two chelating agents. Synergism, however, has been reported for EDTA and other recombination inducing agents (Steffensen, 1961).

Parallel Crossover Increases in Three Regions

Three regions with crossover frequencies ranging from 6.81 to 11.38% show marked similarities in their response to chelating agents. The three regions are lg_1-gl_2 in chromosome two, o_2-v_5 in chromosome seven, and v_5-gl_1 in chromosome seven. All were found to have recombination increased by chelating treatments, and this increase followed similar patterns in each of the experiments.

The salient feature of eight day exposure was that the chemicals acted to increase crossing over when applied alone. Four of the five groups which differed significantly from the control values for these regions were treated with a single agent. In all five cases higher concentrations of DMSO were not effective. It seems valid to conclude that the high concentrations of the chemicals at this longer duration may act by preventing recovery of crossover products.

With treatment over four days, a somewhat different pattern of recombination-treatment relationship emerges. The three regions were increased in their crossover frequency by the heaviest chemical treatments. Groups M and N showed increases in all three regions, group R in two regions and group S in one region. Additionally, treatments by a single chelating agent caused increased crossing over in both regions of chromosome seven.

The v_5-gl_1 span was especially labile, as evidenced by crossover increases due to eleven of the fifteen chemical combinations with the four day treatment duration.

Treatment with high DMSO concentration was effective in four instances over the two chromosome seven spans, indicating that at the shorter duration of treatment this concentration is effective.

It is apparent that both chelating agents increase crossing over in these three regions whether used alone or in combination. Although high and medium concentrations of both chemicals increase crossing

over when used in combination, it is doubtful that there is any synergistic effect. Crossover values for these latter treatment groups are not higher than the values for other groups which also differ significantly from the control frequencies for each of the three regions.

Crossover Reduction

A decrease in crossover frequency was detected for nine treatments among all the chromosome regions tested. Seven of these nine significant decreases involved treatments of either 16% DMSO or 0.1 M EDTA. These, being the strongest treatment concentrations, suggest that the decrease may be due to meiotic irregularities leading to death of the crossover gametes (Hyde, 1955).

Six instances of decreased crossing over due to treatment involve the a_1-et region of chromosome three. All groups involving 16% DMSO were decreased when treated over eight days, with the effect most striking in group H (16% DMSO alone). A diminished effect was found as the amount of EDTA in the treatments increased. This relationship suggests that EDTA may act to increase crossing over in this region while DMSO decreases either the amount or recovery of crossovers.

A decrease in recombination frequency was found in groups treated with 0.1 M EDTA for eight days in the gl_2-v_4 region of

chromosome two, as well as for the chromosome three region cited above. The same treatment for a four day duration did not significantly affect recombination. These results, along with those of chromosome three, suggest a threshold effect, wherein crossing over may be suppressed or, as indicated by Swanson (1940), crossover products screened out.

The gl_2-v_4 region exhibited considerable variation regardless of the treatment applied. Being a long span, with a high recombination value, crossover decreases were much more easily detected than increases would be, owing to the numerical proximity to independent assortment (Sturtevant, 1919). These two considerations detract from the importance of the experimental results for this region.

A survey of the literature revealed no record, in maize or other organisms, of crossover reduction induced by chelating agents. Many instances of crossover increase, however, have been recorded (Steffensen, 1961). Interchromosomal effects have been shown to operate in maize, such that a crossover increase in one region may result in concomitant decreases in crossing over on other chromosomes (Lucchesi and Suzuki, 1968). Since only a single chromosome pair was heterozygous in each of the marker lines of the current investigation, it cannot be determined if increased recombination occurred in other non homologous chromosomes. Nevertheless, an interchromosomal control of crossing over must remain as a possible

explanation for the observed results of these two regions.

Relationship of Chelating Agent Effects to Position and Size of Chromosome Region

Three of the regions investigated spanned the centromere, two were proximal to the centromere on the short arms of their respective chromosomes, and two were distal to the centromere on the long arms of the chromosomes. In the first category were gl_2-v_4 in chromosome two, a_2-bt_1 in chromosome five, and o_2-v_5 in chromosome seven. Little similarity can be detected in response to treatment with chelating agents among these three regions.

The crossover values were increased in the two proximal spans, v_5-gl_1 in chromosome seven, and c_1-wx in chromosome nine by chelating agents. The former, however, was affected to a greater degree and by several more treatment combinations than the latter. The two distal regions, lg_1-gl_2 in chromosome two, and a_1-et in chromosome three, showed opposite responses to the different chemical combinations. It is evident that little relationship exists between the response to chelating agents and the chromosomal location of the seven regions tested.

Examination of the seven crossover intervals in terms of their relative length reveals parallels in chelating agent effect. Two regions were of an intermediate length, and could be compared for response to chelating agents, as could three other regions of relatively short length.

The c_1-wx region of chromosome nine and the a_1-et region of chromosome three were 15.1 ± 2.8 units long. Although the direction of crossover change differed between these regions, the magnitude of change was remarkably similar. Moreover, both regions reacted primarily to DMSO and both were more affected by the long treatment duration.

The three short regions, with a length of 9.1 ± 2.3, are lg_1-gl_2 in chromosome two, and o_2-v_5 and v_5-gl_1 both in chromosome seven. The equivalence of response of these three regions, as discussed earlier, demonstrates a relationship between length of region and effect of chelating agent treatment. Relationship of chelating agents to position or to size of chromosome region have not been discussed in any of the literature cited in the present investigation.

The Role of Metal Cations in Chromosome Structure

The literature reflects a dichotomy of thinking on the specific role of cations involved with chromosome structure and function. Does the cation become incorporated into the physical makeup of the chromosome or does it, instead, influence that structure by its role in the ionic makeup of the nucleus? Results of the present study bear upon the question.

If cations are directly involved in the structure of the chromosome as suggested by Mazia (1954), the likelihood is that one or, at

most, two species of ion would occur in nature. That this is a reasonable assumption may be inferred from the precision and simplicity of those constituents of the chromosome which have thus far been elucidated.

Chelating agents have been shown to have specificity of action with varying affinities for different cations (Davidson, 1958). Of the two agents used in this study, EDTA exhibits greater affinity for calcium and magnesium than does DMSO, while the latter is more reactive with ions of higher molecular weight (Cotton and Francis, 1960). Since calcium and magnesium have been shown in several investigations to be associated with chromosomes (Steffensen and Bergeron, 1959), it is logical to presume that if they are integrally bound to the chromosome, EDTA would have greater chelating ability on the chromosome than would DMSO. Alternatively, if an ion of higher molecular weight, such as a cupric ion, was the chromosomal binding metal ion, one would expect DMSO to be more effective in its removal.

The current study shows no preferential effect by either of the two chemicals over the seven regions tested in maize. Rather, both are shown to be effective in influencing crossing over. This evidence tends to support Kaufmann and McDonald (1957) in the hypothesis of ionic balance in the nucleus, to the exclusion of the hypothesis of metal cation linkage of chromosomal sub units into macromulocules.

Possible Mode of Action of Chelating Agents in Influencing Crossover Frequency

The specific mechanism of crossing over is still in doubt, despite a half century of investigation and speculation. Likewise, the regulation of crossing over is largely undefined. A variety of agents and conditions have been found to influence recombination, yet the manner in which they exert their effect is unclear. Four possible modes of action of chelating agents on crossover regulation are in accord with the current state of knowledge.

Chelation may be responsible for the breakage event of the crossover process. It could affect the role of metal ions in energy transfer processes and thus influence the localization of bond breakage (LaChance, 1959). Alternatively, chelation may inhibit metal dependent enzymes such as peroxidase or catalase leading to accumulation of peroxide and oxidizing agents which in turn may be responsible for chromosome breakage (Meyer, 1954). The possibility remains that chelation of cations directly associated with the chromosome could lead to crossing over (Frick, 1957). In each of these possible mechanisms, chelation would lead to increased chromosome breakage.

Chelating agents may act on the restitution process after breakage has occurred. It might lead to an ion deficiency in the cell which could lower the viscosity of the nucleoplasm, and thereby

reduce restitution. Chelation might be more directly involved in restitution if, at the point of chromosome break, metal ions were needed for rejoining the broken ends. Such a break might be ionic in nature (Wolff and Luipold, 1956). If repair enzymes operate in the restitution process, removal of metal ions could well alter their function.

The synaptinemal complex, composed of protein and nucleic acid, has been seen through the electronmicroscope. This complex is peculiar to meiotic chromosomes, existing from premeiotic interphase through early prophase; and is formed along the interface of the synapsed homologous pair of chromosomes. Crossing over requires the presence of the synaptinemal structure, according to evidence summarized by Moses (1968). Inasmuch as metabolic processes are required for formation of the complex, chelation might alter enzymes responsible for some part of the structure and thereby influence the frequency of crossing over.

Finally, irrespective of the molecular mechanism by which crossing over is accomplished, homologous pairing and meiotic synapsis appear to be necessary adjuncts to the process. The duration and extent of this pairing has been shown to correspond closely with crossover frequency. It follows that an agent or condition which changes either the pairing duration or extent may exert a secondary influence on the amount of crossing over within the paired region.

Chelating agents might well act in this manner, altering recombination frequency by enhancing the opportunity for the molecular and biochemical events of crossing over.

Plant Improvement Considerations

Crossing over is one of the prime mechanisms of releasing natural variability in a population. Progress in a breeding program, which is dependent upon the genetic variance of the population, is therefore related to the amount of recombination as a major source of genetic variation. Close association of genetic factors, on the other hand, is valuable as a means of conserving desirable gene combinations once they have been established. The plant geneticist is faced with the dilemma of maintaining desirable linkages while at the same time searching for the rare recombinant type.

An artificial means of stimulating crossing over could aid in the quest for rare recombinants in experimental plants without sacrificing the benefits of linkage in the breeding population. Once a superior type is isolated it may be readily incorporated into the genome of the general population.

There are three aspects of plant breeding in which increased crossing over would be of particular value. Breaking up undesirable linkages is one such problem. Crop varieties are plagued with linkage groups containing a very desirable trait alongside a detrimental

one, such as disease resistance and the undesirable genetic dwarf habit in some wheat varieties. An agent which could increase recombination to a significant degree would be an aid in recovering the desirable crossover type in a population of manageable size.

Conversely, desirable genes are often located in different homologous chromosomes of several varieties of a crop plant. Under these circumstances, a plant must be heterozygous for the chromosome pair in order to have both desirable characteristics. Heterozygosity, however, is difficult to maintain in self pollinating crop species. By bringing a pair of such genes into the same plant and inducing recombination, the geneticist may be able to find the recombinant type, and develop a strain homozygous for both desirable genes. For genes located close together in the chromosome, normal linkage arrangements would tend to keep them in tandem once crossing over has been accomplished. Recombinagenic agents have obvious utility in this situation.

The phenomenon of pleiotropism is the third area of plant breeding in which increased recombination could be helpful. Several instances of apparent pleiotropism have been found to involve closely linked genes coding for similar products. This enzyme product may be required for several seemingly unrelated processes in the organism. Moreover, heterozygotes have been shown to produce hybrid enzymes having qualities of both parental enzymes. In view of these

considerations, a recombinagen which could be effective over a very short span would have the capability of affecting pleiotropic regions and producing unique new genetic blocks.

The chelating agents EDTA and DMSO have been shown to influence crossing over in regions of varying size in several chromosomes of maize. Their crossover inducing effect has been quite large in some of the regions. The distinct possibility exists that these and similar chemicals, applied to plants under field conditions, can be effective recombinagens. Such crossover inducers may be an important tool in releasing genetic variability and developing novel gene combinations.

SUMMARY AND CONCLUSIONS

The objectives of this study were: (1) to determine the influence on crossing over of the chelating agents EDTA and DMSO, (2) to evaluate the effects of these agents on chromosome structure in maize, and (3) to investigate the applicability of chelating agents as recombinagens.

Five marker lines of maize were treated with the chelating agents, and appropriate crosses were made. The agents were applied in three concentrations, both alone and in combination, in a four by four factorial arrangement. Crossover frequencies were measured for seven regions from test cross and sib cross data. Analysis of variance and tests for least significant differences between treated and control plants were used to determine the effects of the chelating agents.

The following conclusions have been drawn from the results of this investigation: Crossover frequencies in the seven regions of maize chromosomes tested in this study were influenced by the chelating agents EDTA and DMSO. Reduction in crossover frequency attributable to chelating agents was found to be primarily due to high concentration of the agents over the longer treatment duration in two chromosome regions.

This suggests that a threshold may have been reached for these two regions, causing interference with crossing over or with recovery

of crossover products.

Removal of a specific ion from the chromosome structure would be reflected in greater influence of the chelating agent having affinity for that ion. Increased crossover frequency in five chromosome regions, however, was caused by several concentrations of both chelating agents. This indicates that the action of the chelating agents is by a mechanism other than removal of a specific cation from the structure of the chromosome.

Although recombination was influenced by combinations of the two agents, the results within each chromosome region were of similar magnitude to those obtained from the agents acting alone. It is concluded that the two chemicals do not have a synergistic effect in changing crossover frequencies.

Similarities were found in crossover changes of several regions which show a relationship between length of span and effect of chelating agent treatment. No such relationship was found between position of the span on the chromosome and response to chelating agents for the seven regions tested.

For a recombinagen to be of practical value in the improvement of plant and animal species, it must increase crossing over between tightly linked genes. Results of this experiment show DMSO and EDTA to be recombinagenic on spans of varying length and on several maize chromosomes. In the absence of contradictory evidence, it

may be inferred that these chelating agents can increase the production of the more rare recombinants within tight linkage groups.

BIBLIOGRAPHY

Cavalieri, L. F. 1952. Studies on the structure of nucleic acids. V. On the mechanism of metal and enzyme interactions. American Chemical Society Journal 74:1242-1247.

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.

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

Dobzhsnsky, 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.

Eisenstark, A. and C. Kirchner. 1956. Genetic effects of chelation on phage-bacterial systems and on bacteria alone. (Abstract) Genetics 41:640-641.

Eversole, R. A. and E. L. Tatum. 1956. Chemical alteration of crossing-over frequency in Chlamydomonas. Proceedings of the National Academy of Sciences 42:68-73.

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

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

Hayashi, S. and D. T. Suzuki. 1968. A comparative study of induced increases in proximal recombination on Drosophila melanogaster females. Canadian Journal of Genetics and Cytology 10:276-282.

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. (Abstract) Biological Bulletin 109:347.

_____ 1956. The effect of versene on the structure of plant chromosomes. (Abstract) Genetics 41:648.

Hyde, B. B. and R. L. Paliwal. 1958. Studies on the role of cations in the structure and behavior of plant chromosomes. American Journal of Botany 45:433-438.

Jungner, G. 1951. The importance of bivalent ions for the aggregate molecular weight of sodium thymonucleate in aqueous solution. Acta Chemica Scandinavica 5:168-174.

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. 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.

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

Kligman, A. M. 1965. Topological pharmacology and toxicology of dimethyl solfoxide. Part I. Journal of the American Medical Association 193(10):140-148.

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

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

Lucchesi, J. C. and D. T. Suzuki. 1968. The interchromosomal control of recombination. Annual Review of Genetics 2:53-86.

Luria, 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.

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

McNelly-Ingle, C. A., B. C. Lamb and L. C. Frost. 1966. The effect of temperature on recombination frequency in Neurospora crassa. Genetical Research 7:169-183.

Mather, K. 1939. Crossing over and heterochromatin in the X-chromosome in Drosophila melanogaster. Genetics 24:413-435.

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

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

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

Moses, M. J. 1964. The nucleus and chromosomes: A cytological perspective. In: Cytology and cell physiology, ed. by G. H. Bourne. New York, Academic Press. p. 424-545.

_____ 1968. Synaptinemal complex. Annual Review of Genetics 2:363-412.

Muller, H. J. 1925. The regionally differential effect of X rays on crossing over in autosomes of Drosophila. Genetics 10:470-507.

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

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

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

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

Powell, J. B. 1963. Factors affecting recombination in barley. Ph. D. thesis. Pullman, Washington, Washington State University. 133 numb. leaves.

Rhoades, M. M. 1955. The cytogenetics of maize. In: Corn and corn improvement, ed. by G. F. Sprague. New York, Academic Press. p. 123-219.

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

_____ 1966. The effect of abnormal chromosome 10 on preferential segregation and crossing over in maize. Genetics 53:989-1020.

Scott, G. H. 1932. Topographic similarities between materials revealed by ultraviolet light photomicrography of living cells and by microincineration. Science 76:148-150.

_____ 1933. The localization of mineral salts in cells in some mammalian tissues by microincineration. American Journal of Anatomy 53:243-279.

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

Shanfield, B. and E. Kafer. 1968. Chemical induction of mitotic crossing-over in Aspergillus. (Abstract) Canadian Journal of Genetics and Cytology 10:768.

Shaver, D. L. 1957. An apparent effect of manganese on rate of crossing over in maize. Maize Genetics Cooperation News Letter 31:91-94.

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

Stadler, L. J. 1926. The variability of crossing over in maize. Genetics 11:1-37.

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.

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

_____ 1957. Dwarf Drosophila produced by ethylene diamine tetraacetic acid: a relationship to chelation of zinc. Nature 180:390-391.

_____ 1959. A comparative view of the chromosome. Brookhaven Symposia in Biology 12:103-124.

_____ 1961. Chromosome structure with special reference to the role of metal ions. International Review of Cytology 12:163-197.

Steffensen, D., L. Anderson and S. Kase. 1956. Crossing over studies with Drosophila and maize. (Abstract) Genetics 41:633.

Steffensen, D. and J. A. Bergeron. 1959. Autoradiographs of pollen tube nuclei with calcium-45. Journal of General Physiology 34:475-492.

Stern, D. 1926. An effect of temperature and age on crossing over in the first chromosome of Drosophila melanogaster. Proceedings of the National Academy of Sciences 12:530-532.

Stoughton, R. B. and W. Fritsch. 1964. Influence of dimethylsulfoxide (DMSO). Archives of Dermatology 90:512-517.

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.

Strutevant, 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.

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

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

Wilberg, J. S. and W. F. Neuman. 1957. The binding of bivalent metals by deoxyribonucleic 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 nuclei. Journal of Cellular and Comparative Physiology 23:77-82.

Wilson, J. Y. 1959. Temperature effect on chiasma frequency in the bluebell Endymion nonscriptus. Chromosoma 10:337-354.

Wolff, S. and H. E. Luippold. 1956. The production of two chemically different types of chromosomal breaks by ionizing radiations. Proceedings of the National Academy of Sciences 42:510-514.

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.

APPENDIX

Appendix Table 1. Formation of metal ion complexes or chelates in aqueous solutions (after Steffensen, 1961).

Ion	Compound or Complex
Calcium	oxygen complexes only, like the oxygen groups of phosphates and carboxyls
Magnesium	oxygen type complexes predominately
Manganous	oxygen type complexes
Ferric	aromatic nitrogen, polyadenate carboxylate complexes
Ferrous	aromatic nitrogen, mixed nitrogen and oxygen complexes, carboxylic acid (oxygen) complexes
Cobaltic	aliphatic and aromatic nitrogen complexes
Cobaltous	aliphatic amines, mixed oxygen and nitrogen groups
Nickel	aliphatic amines, mixed oxygen and nitrogen groups such as amino acids
Cuprous	thiols, mixed nitrogen and sulfur compounds
Cupric	phenols, aliphatic amines, aromatic amines, thiols