1973

Somatic Association Of Chromosomes And An Organized Nucleus In Zea Mays (I)

John David Horn

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SOMATIC ASSOCIATION OF CHROMOSOMES
AND AN ORGANIZED NUCLEUS
IN Zea mays L.

by

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Department of Plant Sciences

Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada
March 1973

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ABSTRACT

A cytogenetic and statistical study of somatic association was undertaken in maize root tip nuclei. Cells were blocked at metaphase using a cold shock (5°C for 24 hr.) unless otherwise noted. The stocks studied included: 1. a genetically normal stock; 2. a stock segregating for seeds disomic or trisomic for chromosome two; 3. Seneca-60, a commercial single-cross hybrid sweet corn; 4. Seneca-60 (prometaphase); 5. Seneca-60 treated with 8-hydroxyquinoline; 6. Seneca-60 treated with monobromonapthalene; 7. a chromosome 3 tester stock; 8. a stock heteromorphic for abnormal chromosome 10; 9. a stock heteromorphic for abnormal chromosome 10 treated with 8-hydroxyquinoline; 10 a stock homomorphic for abnormal chromosome 10; 11. a chromosome 3 tester stock with 2 B chromosomes; 12. a stock carrying the translocation heterozygote T2-88376; 13. a stock carrying the translocation homozygote T9-10b.

Standardized distance measurements were taken from photographs (X5000 final magnification) from 50 cell samples for each stock and treatment. For each of the
twenty chromosomes per nucleus, measurements were taken among centromeres, between long telomeres and between short telomeres for each chromosome of each stock and treatment. Analysis of variance and the Kolmogorov-Smirnov test for goodness of fit (a non-parametric test that approximates Chi-squared) were used to analyze the data. The use of these statistics allowed analysis of the differences in the shape and means of the distributions of distances between chromosomes.

Specific chromosomes were found more associated with their homologues than with another non-homologous chromosome at somatic metaphase. Those chromosomes which showed a non-random association with their homologues at metaphase, consistently demonstrated this association from one normal stock to the next. I referred to this as a pattern of association. The type of association pattern found was modified by the addition of an extra chromosome, the addition of heterochromatin or alterations in chromosome arm lengths.

The use of chemical antimitotic agents such as 8-hydroxyquinoline or monobromonaphthalene for the disruption of the spindle prior to fixation prevented the detection of somatic association at metaphase.

I have proposed a generalized model of the organization of the maize interphase nucleus based upon the normal and abnormal association patterns viewed at metaphase. Cytological evidence supporting the model system
was presented. The phenomenon of somatic association of homologous chromosomes at metaphase was concluded to be one of several predictable spatial orientations that chromosomes may have relative to one another in an ordered nucleus. The type of chromosome organization in the nucleus is dependent to some extent on the stage of the nuclear cycle considered.
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I thank the Maize Cooperative (University of Illinois) for the provision of some of my experimental material.

I would like to thank my parents for their generous assistance during this period.

I am most grateful to my wife Patsy for her continual encouragement and moral support. I would also like to thank Patsy for her assistance in measuring chromosomes, recording (x,y) coordinates, in the preparation of the figures and tables and in her typing and correcting of the revised manuscript. I am also grateful to my son John who contributed of his time and patience.

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CHAPTER 1

INTRODUCTION

Cytological observations of chromosome morphology and behavior during mitosis were reported almost one hundred years ago. Since that time our knowledge about the structure and behavior of the somatic chromosome has increased substantially through the research efforts of investigators such as Muller, Bridges, Sturtevant, Bovari, Belar, Taylor, DuPraw, Gall and others.

Research efforts of investigators such as White (1937), Carlson (1956) and DuPraw (1966) demonstrated the attachment of chromosomes to the nuclear membrane in a variety of higher plants and animals. The chromosomes attached to the nuclear membrane at anaphase and remained attached through-out interphase to the next prophase. The attachment of chromosomes to the nuclear membrane could explain the earlier observations of Boveri (1909), Belar (1925, 1926, 1929) and Manton (1935) which showed that the chromosomes were spatially fixed and immobile in the interphase nucleus, from telophase to prophase. It is tempting to suggest that the organization of the interphase nucleus must be fixed by this chromosome attachment.
to the nuclear membrane within any one cell.

Recently, proposals describing how the chromosomes are organized in the interphase nucleus were reviewed by Coming (1968).

The repeated organization of the chromosomes into a predetermined and spatially fixed orientation would have major implications for the pairing of chromosomes during meiosis. An ordering of the chromosomes could enhance the recognition of homologues during chromosome pairing in meiosis. A further reduction in the degree of randomness associated with homologue recognition prior to meiotic pairing could be accomplished by organizing the chromosomes in the interphase nucleus so that a chromosome is spatially closer to or more associated with its homologous partner(s) than with any other (non-homologous) chromosomes in the nucleus (read Brown and Stack, 1968).

The study reported in this thesis includes an examination of the relative positions of each chromosome with respect to all other chromosomes at somatic metaphase. It was assumed that the repeated non-random somatic association of a specific chromosome with any other specific chromosome, from stock to stock and cell to cell, was a reflection of a non-random association of these chromosomes during the interphase that immediately preceded the mitosis scored.

Our inability to directly observe the position
of each chromosome as an individual unit during inter-
phase required the use of indirect methods of examination
in order to determine how the chromosomes are arranged
with respect to one another within the nucleus.

Our investigation examined some of the variables
that can affect the organization of the nucleus. For
example, the effects of three different antimitotic
agents were examined. The effects of the addition of
heterochromatin and an extra chromosome were studied.
The significance of nuclear order for the recognition of
homologous chromosomes was examined indirectly by changes
in the somatic association patterns observed in trisomic
and translocation stocks.

Information from these studies led to the pro-
posal of a model for the arrangement of the chromosomes
within the maize nucleus throughout the cell cycle. A
model of mitosis was also proposed to show how an ordered
nucleus can be divided at mitosis and still maintain
nuclear order.

Maize afforded an ideal organism for a study of
this nature as all the chromosomes are readily identifi-
able at somatic metaphase. Several hundred genetic
stocks and aberrations were available for experimental
use. Moreover, the mechanisms of behavior of many of
these mutants have been well established allowing the
results of this study to be examined in the additional
perspectives of the information available about these mutants.

A perusal of the literature indicated that several questions about the phenomenon of somatic association remained to be answered. Among those questions that warranted further investigation were:

1. Are all chromosomes of a complement significantly more associated with their homologous partner than with non-homologues?

2. Is somatic association a reflection of the organization of the interphase nucleus? If so, can some representation of the relative positions of the chromosomes within the interphase nucleus be derived?

3. What is the role of the nucleolus organizer in somatic association and how is this association affected by the addition of heterochromatin?

4. What are some of the effects on somatic association of different antimitotic agents used prior to fixing the cells for study?

We decided to approach these and other questions about somatic association by using a series of normal and aberrant stocks from the maize genetics arsenal. Information obtained from a mitotic analysis of these stocks was subjected to extensive statistical analysis.
CHAPTER 2

LITERATURE REVIEW

2.11 Somatic association:

Somatic association is defined as the tendency for a specific, identifiable chromosome to lie closer to any other specific chromosome in the nucleus than might be expected on the basis of a random distribution of the chromosomes. This definition implies a pattern of association; that is a specific chromosome will have some predictable, non-random, spacial relationship to some other specific chromosome or chromosomes.

Any study of somatic association is based on the ability of the investigator to assign a consistent and reliable identity to the chromosomes under consideration. In most organisms, including corn, this identity assignment, with a few exceptions, can only be made at metaphase.

Several criteria for the measurement of somatic association of any pair of chromosomes have been used. These methods are reviewed in detail in section 2.15.

In general, the methods of measuring the degree of association between specific somatic chromosomes can
be divided into 2 categories. The first method involves the use of some subjective criteria to measure somatic association, such as both chromosomes lying within a 'chromatid-width' or one arm length of one another (Ferguson-Smith and Handmaker (1961), Bishun (1966), Cohen and Shaw (1967)). The second involves the statistical comparison of the distribution of distances found between any specific chromosomes over a number of cells to an expected distribution. The methods of deriving an expected distribution were varied and are reviewed in section 2.15.

The most common points of reference between which the measurements are made are the centromeres of the chromosomes in question. The centromere is a repeatably defined reference point. However the centromere is most clearly visible in metaphase, again restricting an examination of somatic association to one stage of mitosis.

Historically, interest in non-random chromosome associations at metaphase arose from a desire to provide a mechanism to explain two observed cytogenetic phenomena. These two events were firstly: the non-random distribution of breaks between chromosomes during a breakage-fusion event and secondly; the mechanism by which recognition of the
homology of chromosomes occurs during chromosome pairing in meiosis.

Somatic association was also proposed as a mechanism to account for the high frequency of recovered anomalies associated with the chromosomes assigned to the D and G groups in the human karyotype. Shaw (1961), Ferguson-Smith and Handmaker (1961) and Cohen and Shaw (1967) were among the human cytogeneticists who noted a high frequency of somatic association of the satellited acrocentric human D and G group chromosomes in metaphase preparations. These satellited chromosomes also participated in the organization of a nucleolus.(Ferguson-Smith 1964).

Some tendency for nucleoli to fuse in prophase cells has been observed (Darlington 1965). A high frequency of nucleolar fusions was observed in human cells in culture (Nankin 1970). Shaw (1961) and Ferguson-Smith and Handmaker (1961) suggested that the fusion of several smaller nucleoli to form a single larger nucleolus could cause association of these nucleolar organizing chromosomes during interphase. A higher than expected frequency of recovered acrocentric-acrocentric interchange events within the D and G group chromosomes could be explained by their spatial proximity during interphase as a consequence of nucleolar fusion.

The essential point of somatic association as a
mechanism for homologue recognition prior to pairing in meiosis is that the homologous chromosomes must lie closer to each other than to any other (non-homologous) chromosomes. If this is not the case, then some other factor which allows homologue recognition and pairing must be operating and somatic association of chromosomes would not be a necessary adjunct to pairing in meiosis.

Studies involving a comparison of the distances found between homologous and non-homologous chromosomes over a sample of cells are detailed in section 2.13. An evaluation of the statistics used in these studies is given in section 2.15.

The obvious limitation of any study of somatic association is that the observations of association are made at metaphase and the results are extrapolated to predict the spacial arrangement of the chromosomes at interphase.

A study of the mechanics of mitosis (2.17) gave no indication of any factor operating during mitosis which would cause a randomization or repositioning of the chromosomes relative to one another. Recently Heddle (1973), (personal communication) examined the association of the acentric and dicentric fragments from an X-ray induced exchange between 2 chromosomes in cultured human lymphocytes. He found that the acentric and dicentric which were derived from 2 chromosomes close together dur-
ing interphase, were also associated at metaphase.

It seems unlikely that the positions of the chromosomes in the nucleus, relative to one another can change from interphase to metaphase. We will demonstrate in this study that there are exceptions to this that result from the mechanics of dividing an organized nucleus. Those chromosomes which demonstrate a close and consistent association at metaphase, are however likely to be associated during somatic interphase.

2.12 Somatic association of satellited chromosomes:

Shaw (1961) reported association of the 10 short acrocentric D and G group human chromosomes at somatic metaphase. She also reported that these satellited chromosomes were orientated toward the centromere region of chromosome 1. Edwards (1961) revealed the association of the 10 acrocentrics of the D and G groups with chromosomes 1, 2 and 3. These observations were based on a small number of observations with no finite measure to definite association. Cohen and Shaw (1967) later demonstrated association of the D and G group chromosomes but could not find association of the D's and G's with chromosomes 1, 2 or 3.

Ferguson-Smith and Handmaker (1961, 1961a, 1963, 1964) established that all 10 human acrocentric chromosomes which fell into the D and G groups of the human
karyotype could be satellited. A maximum of 6 of the 10 acrocentrics participated in a nucleolus organizer function at one time. Ohno (1961) had reported that while all the D and G group chromosomes could be satellited, of the 10 D and G group acrocentrics, only 6 were satellited at any one time during somatic metaphase. Reitalu (1964) showed that a maximum of 6 of the 10 D and G group chromosomes were associated at somatic metaphase. The author suggests that the number of satellited acrocentrics in the human be recounted using chromosome banding techniques now available for more accurate numerical classification of the chromosomes in the human karyotype.

Ferguson-Smith and Handmaker (1961, 1961a, 1963) and Prokafieva-Belgovskaya et al (1966) suggested that the association of the acrocentric chromosomes of the D and G groups was a direct result of their earlier participation in a nucleolus organizing role.

Nankin (1970) studied the frequency of association of the satellited D and G group chromosomes in cultured human lymphocytes as a function of the length of culture time of the cells. He found that the number of associated acrocentrics decreased from 33.7% at 48 hours to 17.7% after 92 hours of culture time.

Similarly, Nankin noted that at 24 hrs after culturing the cells, 89% of the nucleoli visible at prophase were fused to form a single nucleolus. After 18 hrs of
culture time, only 26.8% of the prophase cells had a nucleolus. After 92 hrs in culture, only 13.1% of the prophase cells had a nucleolus. Nankin concluded that the decrease in the frequency of acrocentric associations observed at prophase as culture time increased was a function of the decreased number of nucleoli found that persisted into prophase. The fusion of nucleoli to form one nucleolus is common in prophase of many organisms (Darlington 1965).

Gillies (1972) has shown, in serial sections through the meiocytes of Neurospora crassa, that all the bivalents (7) save one are attached by both ends to the nuclear membrane. The single exception was the end or telomere of 7th bivalent, the nucleolus organizer (NO) carrying arm of the NO chromosome. Gray (1927) has reported that the nucleoli of the eggs of the echinoderm Echinus are free to move about in the nucleus as the eggs were changed in position. The non-attachment of the telomere of the NO carrying arm may permit nucleolar fusions at a high frequency in some organisms.

Heitz (1931) (using Vicia faba) and Darvey and Driscoll (1972) (using wheat) demonstrated that the nucleolar position or the pattern of arrangement of the nucleoli during interphase remained constant from cell to cell. This phenomenon was particularly evident in daughter nuclei from a recent division.

Morgan (1971) (using Zea) and Darvey and Driscoll
(1972) (using wheat) have reported on the frequency of nucleolar fusions in these 2 organisms. Morgan reported approximately 2 fusions per thousand interphase cells in *Zea*. Darvey and Driscoll (1972) reported 57% nucleolar fusion in interphase nuclei of wheat. There was no preferential fusion observed between homologous or non-homologous nucleoli in polyploid wheat with 4 NO chromosomes. Nucleolar fusions are a common phenomenon in meiotic prophase in many organisms (Lin 1955).

Miles (1968) and Darvey and Driscoll (1972a) examined the somatic association of the satellited NO carrying chromosomes in *Zea mays* and *Triticum aestivium* respectively. In both organisms the satellited (NO) chromosomes were randomly associated at metaphase. Both studies utilized the statistical approach proposed by Feldman (1966a). (See section 2.15 for a review of Feldman's statistical methods.) Palmer (1972) in a study of the last premeiotic mitosis in *Zea mays* did not find any association of the 2 satellited chromosome 6's.

The fusion of nucleoli is a mechanism to account for the association of the satellited D and G group chromosomes in man. The fact that no other chromosome associations were found in the same cells, the fact that colchicine was used (see section 2.16) in these studies (Merrington and Penrose, 1964; Cohen and Shaw, 1967; and Galperin, 1968), and the fact that somatic association between satellited chromosomes is a random event in
other organisms like *Zea* and *Triticum* lead one to conclude that the observed somatic association of the D and G group chromosomes may be a phenomenon unique to man.

The observed mobility and fusion capabilities of individual nucleoli (Gray (1927), Darlington (1965 p.40), Nankin (1970)) indicate that the telomeres of the NO chromosome arms are free moving in some organisms. The observations of Heitz (1931), Miles (1968), Palmer (1972), Darvey and Driscoll (1972), and Gillies (1972) all suggest that the nucleolus organizer chromosomes have some fixed spatial relationship to one another.

2.13 **Somatic association between homologous chromosomes:**

Metz (1916) working with *Diptera* and Boss (1954) with newt reported the close co-alignment of homologous chromosomes during somatic mitosis.

The suggestion of non-random chromosome associations at somatic metaphase in human cells was first proposed independently by Shaw (1961), Edwards (1961) and Ferguson-Smith and Handmaker (1961). Studies which involved the identification of individual chromosomes of the human complement prior to 1969 were limited to the assignment of chromosomes to a group. In rare cases, a few pairs of chromosomes such as 1, 2, 3 or 21 could be assigned individual, numerical identities.

The use of chromosome banding techniques developed
by Casperson et al (1969), Drets and Shaw (1970) and Wang and Federoff (1972) have allowed a more consistent identity assignment to human metaphase chromosomes. There are no reports to date of somatic association which have taken advantage of the increased 'resolving power' provided by these techniques.

Schneiderman and Smith (1962) and Barton and David (1962) showed that those human chromosomes which could be identified showed somatic association with their homologues at metaphase. Merrington and Penrose (1964), using the same statistical approach as Barton and David (1962), found only the satellited acrocentrics of the D and G chromosomes were associated. In contradiction to the results of Barton and David (1962) and Schneiderman and Smith (1962), Merrington and Penrose (1964) found no association between any other chromosomes in the human metaphase.

Feldman et al (1966) examined the distribution and means of the standardized distances between homologous and non-homologous telocentric chromosomes in somatic metaphase figures of *Triticum aestivum* L. The telocentric chromosomes served as unique markers. The telocentrics were derived from homologous arms of a chromosome (complete homology), non-homologous arms of the same chromosome (homologous centromeres) and from non-homologous arms of different chromosomes (no homology).
Feldman et al used a cold treatment (0°C for 24 hrs) as the antimitotic agent rather than colchicine or colcemide as most investigators had used. Feldman et al found that homologous telocentrics, whether of the same or opposite arm derivation (common centromere) were significantly more associated at somatic metaphase than the non-homologous telocentrics. From these results, Feldman concluded that homologous chromosomes were more associated than non-homologous chromosomes. Feldman et al (1966) also concluded that the centromere was the associating agent as the centromere was the only region of homology that 'opposite arm' telocentrics possessed.

Avivi et al (1969) used the same telocentric system in wheat as Feldman et al (1966a). Cold arrest (0°C for 24 hrs) and colchicine (0.02% for 3 hrs) were used as the antimitotic agent in 2 parallel studies. Avivi found that homologous telocentrics were significantly (p < 0.05) more associated than non-homologous telocentrics when cold (0°C for 24 hrs) was used to block mitosis at metaphase. When colchicine was used as the antimitotic agent neither homologous nor non-homologous telocentrics were associated.

Dubuc and McGinnis (1970) examined in hexaploid oats, the relative positions at metaphase of a pair of telocentric chromosomes and the shortest pair of chromo-
somes (#21) after cold arrest (0°C 24 hrs). Fedack and Helgason (1970) examined the association of homologous and non-homologous telocentric chromosomes in cold arrested somatic metaphase figures of barley. It was concluded from both these studies that homologous chromosomes were more associated than were the non-homologous chromosomes.

Darvey and Driscoll (1972) found that both homologous and non-homologous telocentrics were significantly associated at somatic metaphase in normal wheat (*Triticum aestivum*). Darvey and Driscoll questioned the results of Feldman et al (1966a) as, unlike Feldman, they found some instances where non-homologous telocentrics were non-randomly associated as were the homologous telocentrics.

It may be that the telocentrics which were originally derived from either homologous or non-homologous autosomes have a tendency to be associated because of their similar and unique morphology. Every telocentric chromosome would not show association because of some limited genetic control of the ordering of the interphase nucleus. As a consequence of the clearly abnormal nature of induced telocentric chromosomes, some caution should be exercised in extending these results to predict the behavior of 'normal' biarmed chromosomes. For example, it will be shown below that not all the chromosomes of maize are associated with their homologous
partner at somatic metaphase. This conclusion could not have been arrived at either by the examination of a sample of some of the chromosomes or by data generalized solely from the observations of a pair of telocentric chromosomes.

Sadasivaih et al (1969) examined somatic association for 4 pairs of homologues of *Avena*. Homologues were more associated than non-homologous. Distances were measured between homologous telomeres of the long and short arms as well as homologous and non-homologous centromeres. There was no difference in association observed between homologous telomeres or homologous centromeres. This lack of difference is not entirely surprising considering the degree of coiling which chromosomes undergo prior to metaphase.

Maguire (1967) examined various sized chromosome knobs at interphase in premeiotic and somatic root-tip cells of the KYS strain of maize. In both cells, root tip and premeiotic, these heterochromatic knobs were closely aligned to their homologous partner knobs. Chauhan and Abel (1968) showed premeiotic pairing of homologous regions of centric heterochromatin during interphase in *Impatiens* and *Salvia*. The observations of Chauhan and Abel were less than convincing, however because of the very low frequency with which the centric regions were closely associated into a pair by pair re-
relationship.

Palmer (1971) in a study of the last premeiotic mitosis in normal and meiotic maize found no evidence for premeiotic pairing in the behavior of the satellited No chromosome 6's in maize. The spatial relationship of the 6's at metaphase in both normal and ameiotic maize was similar, that is, not paired prior to meiosis. Moens (1969) demonstrated that closely coaligned homologous chromosomes moved closer together and then paired at meiosis in the primary spermatocytes of Locusta migratoria.

In summary, there is some evidence that homologous chromosomes are associated at somatic metaphase and non-homologous chromosomes are not. The data of Maguire (1967) would suggest that this co-alignment is common for all homologous at interphase. The data of Palmer (1971) and Moens (1969) indicated that final chromosome pairing is reserved for meiosis prior to the formation of the synaptonemal complex during meiotic prophase.

2.14 An Organized Somatic Metaphase Plate:

Miller et al (1963, 1963a) examined the relative positions of the metaphase chromosomes in cultured human leukocytes. All identifiable chromosomes were ranked on the basis of the distance that each chromosome lay from the center of the metaphase spread. Miller found that
the Y chromosome and chromosome 13 lay more toward the periphery of the cell than might be expected by chance. The longest chromosome(s), and the G group chromosomes, had a tendency to lie more centrally.

Ockey (1969) utilized the same ranking techniques as Miller et al. (1963) to rank the chromosomes of labelled (tritiated thymidine) human fibroblast cells. He found that whenever the chromosomes could be identified by morphology, the more peripheral the chromosome's position on the metaphase plate, the more frequently that chromosome was late labelling in the S period preceding mitosis. The time of uptake of tritiated thymidine is often used as a temporal marker and a late labelling chromosome is simply one which was replicating later in the cell cycle than an earlier replicating chromosome. Ockey found that the D and G group chromosomes which were centrally located in the metaphase plate, were also early replicating.

Barton et al. (1964, 1965) drew 2 circles through the two X chromosomes in metaphase figures of the human female. The center of the metaphase was the center of these circles. Barton et al. calculated the number of chromosomes that could be expected to fall inside and outside each circle if the chromosomes were randomly distributed with respect to the X chromosomes. The two X chromosomes were found to be peripheral in the metaphase figure as more chromosomes lay inside each circle than
could be expected by chance.

Miller et al (1963, 1963a), Barton et al (1964, 1965), Ockey (1969), concluded that in cultured human cells, there is a tendency for chromosomes to occupy specific regions of the metaphase plate. This tendency is consistent over a population of cells. It may reflect in some fashion the distribution of the chromosomes relative to each other during interphase.

The tendency for the D and G group chromosomes to occupy the more central regions of the metaphase plate may be responsible for their observed somatic association in many studies. The central location of the nucleolus may be responsible for this association.

2.15 Statistical Approaches to the Measurement of Somatic Association:

There have been many reports of the non-random association of chromosomes at somatic metaphase. The methods by which somatic associations have been measured vary from subjective and intuitive observation to well founded statistical approaches. Some of the more common methods of measuring chromosome associations are given consideration here.

Shaw (1961) and Edwards (1961) defined association of the D and G group chromosomes on the basis of a cytogeneticist's intuitive feelings. This was not perhaps an
entirely invalid approach. Ferguson-Smith and Handmaker (1961) and Zellweger et al (1966) defined any 2 chromosomes as associated if they were connected by a chromatic strand or if they lay within one chromatid width of each other.

Van Brink et al (1962), Ferguson-Smith and Handmaker (1963), Frøland and Mikkelsen (1964) and Bishun (1966) defined association as 2 metaphase chromosomes that were either joined by a chromatin fiber or that lay within 2 mm. of one another at 4000 X magnification. Somewhat similar criteria for association were used by Cohen and Shaw (1967).

By means of these somewhat subjective measurements of somatic association, these investigators all reported non-random associations of the D and G group human chromosomes in somatic metaphase cells. An advantage of defining association as existing between all and any chromosomes that lay within a predetermined distance of a specific, reference chromosome was that any number of chromosomes could have been accommodated with a simple change in the expected.

An additional source of prejudice which could affect a study of somatic association beside the means of evaluating the degree of association is the timing of chromosome identification. For example, if one is looking for somatic association between chromosome 3's, one would
find the 1st chromosome 3. Then the closest chromosome to the chromosome called 3 that looked like a chromosome 3 would be called the second chromosome 3.

This prejudice can be reduced by identifying all the chromosomes of all the metaphase figures. It can be eliminated by the use of cells in which the chromosomes had been previously identified for some study other than somatic association. The study of Cohen and Shaw (1967) used cells in which the identification of chromosomes was accomplished blindly with respect to the study of chromosome associations at metaphase.

The remaining studies that will be reviewed here involved statistical manipulations of distances measured between chromosomes at metaphase. The majority of these studies approached the statistical analysis of chromosome distribution in metaphase as a deviation from an expected distribution. The expected was often derived as a function of the frequency of chromosome associations expected if the chromosomes were spread homogeneously through the metaphase spread.

The measurements of the distances between chromosomes at metaphase were usually made by marking the X and Y coordinates of the centromeres of the chromosomes on a grid axis. The centromere was a consistent and readily identifiable point from which distance relationships between the chromosomes can be measured. The use of a com-
puter allowed a rapid calculation of all possible distances between any points (chromosomes) that were consistently identifiable.

Schneiderman and Smith (1962) and Barton and David (1962, 1963) measured the distribution of distances between pairs of homologous and non-homologous chromosomes in the human and compared the variances and means of the distributions. They concluded that homologues were more associated than non-homologues. In addition to the problems of chromosome identification in karyotyping the human cell karyotype, Schneiderman and Smith used a t-statistic and Barton and David used a derived "L" statistic. Both of these statistics made use of a variance ratio and thus required that the underlying distribution of distances between chromosomes be normal. Neither group of investigators determined the shape of the underlying distance distribution.

Feldman (1966) showed that the underlying distribution of distances between 2 chromosomes that are randomly associated was not normally distributed. The results of Barton and David (1962) and Schneiderman and Smith (1962) should be reexamined using appropriate non-parametric statistics for the analysis before their conclusions and arguments can be considered convincing.

Merrington and Penrose (1964) used the same L-statistic developed by Barton and David (1963) and could
not show any differences of association between homologous and non-homologues. The analysis and interpretation are subject to the same criticisms as that of Barton and Davids.

Irwin (1965) proposed that any chromosome could associate with another chromosome with a probability \( p \). The probability of any additional chromosomes also associating with the previously associated chromosomes was also considered likely with the same probability \( p \). Irwin found no differences in the association of either homologues or non-homologues at metaphase. Additionally he found no association among the members of the D and G groups of chromosomes.

Irwin's statistics require that all chromosomes on the metaphase plate 'behave' similarly; that is, one chromosome does not preferentially associate with another chromosome. Previous investigators had demonstrated that chromosomes of the D and G groups in human were preferentially associated. As this is contrary to Irwin's initial assumption that all chromosomes associate with an equal probability, Irwin's approach need not be considered further.

Galparin (1968) examined somatic association in cultured human cells in metaphase using a circular distance transformation. A computer program generated histograms from the frequency classes of the standardized dis-
tances between homologous and non-homologous chromosomes. The histograms were compared by means of Chi-squared analysis.

Galparin found that the D and G group chromosomes were significantly more associated when compared to the frequency of associations of all 46 chromosomes taken together. It may be a source of considerable error to compare a distance distribution based on 10 observations (within the D and G groups) to one derived from 46 observations (among all chromosomes) unless appropriate notice is taken of the weights of the individual points which Galparin did not.

Prior to the publication of Feldman et al. (1966), the underlying distribution of distances between 2 randomly associating metaphase chromosomes had been considered normally distributed. Feldman suggested that the theoretical distribution for the distances between 2 points distributed at random throughout a sphere of a radius "r", as developed by Hammersley (1950) and Lord (1954), might more accurately estimate the distribution of distances between 2 chromosomes at metaphase. Feldman used a corollary of this basic theorem which involved 2 points from a random distribution in a 3-dimensional sphere projected to a 2-dimensional circle. This corollary is analogous to the situation where 2 chromosomes are distributed at random in the nucleus and the nucleus is squashed flat.
Employing this approach Feldman (1966) found that the observed frequency distribution of distances between non-homologous telocentric chromosomes (with non-homologous centromeres) was the same as predicted by the random distribution of 2 points in a circle. The observed frequency distribution of distances between the same or opposite arm telocentrics was found to be significantly less than that predicted by the random expectation.

The 'non-normality' of the underlying distribution of distances between 2 points distributed at random in a sphere projected into 2 dimensions was noted by Feldman. The necessity of using non-parametric statistics to compare the observed and expected data was pointed out.

Since the exposé by Feldman et al (1966) the same type of statistical approach has been used by several investigators. Noteworthy contributions include: Avivi et al (1968, 1969, 1970); Miles (1968); Sadasavish et al (1969); Horn (1970); Dubec and McGinnis (1970); Fedak and Helgason (1970); and Darvey and Driscoll (1972a).

The major objection to the use of this type of statistic is that the investigator is obliged to use circular metaphase spreads to satisfy the underlying statistical assumption of measuring the distribution of 'n' points distributed at random in a sphere and projected vertically to a plane surface.
2.16 Somatic Association and the Use of Cold Treatment and Colchicine as Antimitotic Agents:

With the exception of this study and Miles' (1968) the only methods of accumulating metaphases for a study of somatic association have been cold treatment (0° to 5° C for 12 to 24 hrs.) as used by: Feldman et al (1966); Avivi et al (1968, 1969, 1970); Sadasiviah et al (1969); Dubec and McGinnes (1970); Fedak and Helgason (1970); and Darvey and Driscoll (1972a), or the use of colchicine or colcemide (all studies of mammalian chromosomes, e.g. Ferguson-Smith and Handmaker (1961), Cohen and Shaw (1967), Cohen (1972)). Investigators who used colchicine to arrest metaphase found no somatic association of homologous chromosomes outside of the D and G group associations in the human (Merrington and Penrose (1964), Cohen and Shaw (1967), Cohen (1972)). When cold treatment was used in plant material to block mitosis at metaphase, homologous chromosomes were significantly more associated than were non-homologous chromosomes.

Avivi et al (1969) utilized the telocentric marker chromosome system in wheat. When colchicine was used to disrupt the spindle to accumulate metaphase cells, the homologous and non-homologous chromosomes were randomly associated. But, when Avivi et al (1969) used a cold
treatment (0°C for 24 hrs.) to block the cells at metaphase, homologous telocentrics were associated and non-homologous telocentrics were not.

Avivi et al (1969) suggested that the loss of observed somatic association of homologous metaphase chromosomes after treatment of the cells with colchicine implied that a spindle-fiber like material was responsible for attaching the interphase chromosomes to the nuclear membrane. Observations of DuPraw (1966) and Coming and Okada (1969, 1970) have shown that the chromosomes are attached to the nuclear membrane by fibrils which are dissolved with DNAase and are not spindle fiber proteins.

As will be shown later in this study, cold treatment and chemical antimitotic agents similar in action to colchicine affect somatic association in different fashions.

Cohen et al (1972) examined muntjac (2N=6) metaphase preparations for the somatic association of homologous chromosomes. These mammalian cells had been blocked at metaphase with colchicine. Cohen found that the homologous chromosomes were no more associated with each other than with non-homologous chromosomes. However, Heneen and Nichols (1972) also examined somatic association in the muntjac. They used neither antimitotic agent nor cold to arrest the cells at metaphase. In these untreated cells, the homologous chromosomes were
significantly more associated at metaphase than were non-homologues. Also in the same study, the chromosomes were observed arranged by pairs of homologues around the equator of the nuclear membrane in polar prophase preparations.

In the experiments reported in this thesis, a cold treatment was employed for the majority of the somatic-association arrest data. The effects of other chemical antimitotic agents on the association of homologous chromosomes is also examined.

In summary, the use of colchicine to block spindle formation at metaphase consistently destroys any somatic association of homologous chromosomes that may have been present. The use of a cold treatment to accumulate the cells at metaphase allowed at least some association of the homologues to be retained.

2.17 Somatic Association and An Organized Interphase Nucleus:

It has been suggested that the observed somatic association of homologous metaphase chromosomes is a reflection of a pair by pair association during interphase (Feldman, 1966; Sved, 1966; Coming, 1968; Avivi et al, 1969; Brown and Stack, 1968; Stack and Brown, 1969). It was also proposed that, in order to maintain this association, the chromosomes were attached to the inner
lamella of the nuclear membrane during interphase.

Early light microscope studies by Flemming (1880) showed that the chromatin of *Allium odorum* was closely associated with the boundaries of the interphase nucleus. Further observations by Flemming, in higher organisms showed that the chromosomes appeared at the edges of the nucleus at prophase.

White (1937) irradiated meiocyte cells of *Orthoptera* and found that the chromosome did not appear broken until late prophase or after the breakdown of nuclear membrane. Sax (1942) proposed that the chromosomes of *Tradescantia* were also attached to the nuclear membrane by their centromeres. He found that radiation induced breaks were not randomly distributed throughout the chromosome arms. The breaks were more numerous in the proximal region of each chromosome arm. Sax suggested that there was an increase in uncoiling tension in the proximal regions of the chromosome arm as a consequence of the attachment of the centromere to the nuclear membrane. He hypothesized that the increased tension was responsible for more breaks being recovered in the proximal region of the chromosome.

Brenner (1953) centrifuged rat liver cells and sectioned the pellet parallel to the centrifugal force axis. He stained sections with hematoxylin and verified the presence of DNA by staining alternate sections with
Feulgen. Brenner found the bulk of DNA in a mass at the centrifugal pole. Fine Feulgen positive strands, ran from all over the nuclear membrane to this chromatin mass. Brenner concluded that chromosomes were attached to the nuclear membrane during interphase.

Person (1953) in a light microscope examination of the chromosomes in one cell cycle of Allium cepa somatic cell nuclei suggested that the chromosomes of Allium were organized at anaphase into a polarized arrangement which persisted until prophase. Person noted that the centromeres of the Allium chromosomes were closely grouped together at one pole of the nucleus, while the arms of the chromosomes were extended toward the opposite pole of the nucleus. Person described an apparent movement of the centromeres along the nuclear membrane to an equatorial position in the nucleus at late prophase. As the reader shall see later, this phenomenon is evident in corn somatic cells and assumes prominence in a rationalization of the results observed in this study.

Carlson (1956) performed a careful micromanipulation of the mitotic prophase of grasshopper neuroblasts. His observations showed the attachment by middle prophase to the nuclear membrane of the chromosomes by their centromeres but not by their telomeres.

Thus, it was well established from light microscope
studies before 1960 that the chromosomes were ordered in the nucleus to the extent that they were attached to the nuclear membrane, apparently in some spacial orientation to one another. This attachment occurred at anaphase and persisted through interphase to prophase. The attachment was by chromosome fibers.

DuPraw (1965), Fawcett (1966), Comings and Okada (1970) have demonstrated a layer of 250 Å fibrils attached around the annuli or nuclear pores of the inner lamella of the nuclear membrane. DuPraw (1965) showed that these fibers were nucleoprotein fibers attaching the chromosomes to the annuli of the nuclear membrane in the Honey bee. The attachment to the membrane was sufficiently strong to break the 250 Å fiber before the fiber would disengage from the nuclear membrane. Treatment with DNAase destroyed the attachment to the nuclear membrane; treatment with trypsin did not.

Sparvoli et al (1965) examined serial sections through prophase nuclei of Tradescantia paludosa. The chromosomes were found attached to the nuclear membrane by both ends during prophase. Observations of telophase nuclei again showed the attachment of the ends of the chromosomes to the nuclear membrane. The attachment of chromosome telomeres to the nuclear membrane has also been observed by Woolan et al (1967) in mouse spermato-
cytes, by Moses (1968) in *Locusta migratoria* and by Gillies (1972) in *Neurospora crassa*.

Roth (1967) noted the association of metaphase chromosomes and nuclear membrane fragments in the giant amoeba. Large fragments of the nuclear membrane were observed migrating to the poles, ahead of the chromosomes. By early telophase the chromosomes were completely surrounded by a new nuclear membrane which incorporated some of the fragments of the old membrane during its formations.

Comings and Okada (1970a, b) noted that isolated metaphase and anaphase mammalian chromosomes frequently had fragments of nuclear membrane attached at various and apparently random places along the arms of the chromosomes. Considerable tension was exhibited in some of the fibers indicating that the attachment of the chromosome fiber to the annuli of the nuclear membrane was not entirely fortuitous.

Avivi et al (1970) proposed that the chromosomes were attached to the nuclear membrane by a spindle-like material which was depolymerized with colchicine. The preceding observations of DuPraw (1965) and Comings and Okada (1970a, b) have shown that a 250 Å DNA containing chromosome fibril is responsible for the attachment to nuclear membrane.

Comings (1968) suggested a rational for an organ-
ized interphase nucleus in which chromosomes would be attached to the nuclear membrane at multiple points along the chromosomes. The ends of homologous chromosomes would be attached in proximity on the nuclear membrane. The attachment sites of the chromosome would be specific for each chromosome pair. Comings (1968) proposed that sites of initiation of chromosomal DNA synthesis were the attachment points of the chromosomes to the nuclear membrane. The somatic association of a pair of homologues would be a reflection of the side by side attachment of the homologous telomeres of chromosomes found during the preceding interphase.

Wagenaar (1968) showed that during early prophase in *Allium cepa* and *Crepis capillaris* the centromeres of all the chromosomes were attached to one side of the nucleus and all the chromosome ends project to the other side of the nucleus. Similar observation had been made fifteen years earlier by Person (1953) in *Allium*. Wagenaar's observations of a satellited pair of chromosomes of *Crepis capillaris* (2N=6) indicated that the homologous telomeres of homologous chromosomes were attached to the nuclear membrane in proximity to each other. He also proposed that all the chromosomes were attached end to end to form a circle. Similar results were reported in the 1920's with *Crepis* but were dismissed by Darlington (1965) who showed that poor fixation techniques caused a collapse of
the telomeres which gave rise to end to end chromosome fusions.

Heneen and Nichols (1972) demonstrated the pair by pair attachment of homologous pairs of chromosomes to the nuclear membrane in muntjac (2N=6). Comings and Okada (1970b) showed that the prophase chromosomes of the muntjac were attached at multiple sites along the nuclear membrane.

In summary, the interphase nucleus is apparently highly organized. The chromosomes may be arranged with the centromeres at one pole of the nucleus and the ends or telomeres extending toward the opposite pole. Person (1953) has observed that the chromosome orientation or direction of polarization also corresponds to the polarization of the next somatic cell division.

In addition, the chromosomes are attached to the annuli of the inner lamella of the nuclear membrane by their centromeres, telomeres and at a multiplicity of other points along each chromosome.

The chromosomes may also be attached in a specific arrangement relative to each other e.g. pair by pair arrangements of homologues in the muntjac. This implies some mechanism for ordering the nucleus which is either mechanically (chromosome size, arm ratio etc.) or genetically determined, or both.

As will be shown later a pair by pair arrangement of homologues on the nuclear membrane may be replaced
by a symmetrical arrangement of the chromosomes when
the number of chromosomes (2N) is higher than 6. This
study proposes that the chromosomes of maize are opposite
their homologues in a symmetrically ordered nucleus but
not necessarily paired with their homologue along their
entire lengths.

2.18 Somatic Association and Meiotic Chromosome Pairing:

There are a number of mutant stocks in a variety
of organisms such as wheat, corn and tomato which result
in abnormal chromosome pairing behavior at meiosis.
While these reports are too numerous to catalogue here,
it is sufficient to note that a variety of meiotic mutants
have been studied and are available in corn.

The degree of synapsis in corn can be reduced with
a deletion or mutation at the asynaptic (as) locus in
chromosome 1. (Beadle, 1933; Barker and Morgan, 1969 ).
Meiosis can be completely eliminated by the homozygous
recessive allele of the gene ameiotic (am) of chromosome
5 (Singh 1965).

Riley and Chapman (1958) and Feldman (1966) showed
that in wheat homologue recognition prior to pairing was
regulated by a gene located in the long arm of chromosome
5B. Feldman (1966 ) showed that the somatic association
of homologous telocentric chromosomes was also affected
by varying doses of 5B L.
Stack and Brown (1968) and Brown and Stack (1969) proposed that premeiotic chromosome recognition and pairing occurred prior to meiosis. They suggested that the pairing was a result of random contacts between homologues prior to meiosis. Stack and Brown proposed that once the chromosomes paired at any time prior to meiosis, the chromosomes remained paired through pachynema.

Kasha and Burnham (1965), Burnham (1972) and Maguire (1972) have shown with corn that chromosome pairing at meiosis must be initiated at the ends of the chromosomes and followed soon after by pairing and synapsis at other points along the chromosome.

Sved (1966) theorized that the attachment of homologous telomeres of homologous arms of chromosomes was a necessary feature of the interphase nucleus to prevent chromosome tangling, a high frequency of non-homologous chromosome pairing and to minimize the number of interlocking bivalents found during meiosis.

Moens (1969) observed the pairing of and initiation of a synaptonemal complex between 2 previously closely co-aligned (0.3μ) telomeres of homologous chromosomes in the last premeiotic interphase of Locusta migratoria. This co-alignment would limit chromosome pairing to the last premeiotic interphase confirming the predictions of Sved (1966) but in contradiction to the results of Brown and Stack.
Palmer (1972) found no evidence for chromosome pairing prior to the last premeiotic interphase in normal and ameiotic stocks of maize.

Buss and Henderson (1971) found a high frequency of interlocking bivalents involving the longest 2 autosomal pairs of Locusta migratoria following a brief heat shock during the last premeiotic interphase. They postulated that the time of chromosome co-alignment or pairing prior to synapsis had been affected by the heat shock. Buss and Henderson (1971) and Feldman (1972) have suggested that chromosome pairing during meiosis is a 2 step process. The first step involves the recognition of homologous chromosomes and a close co-alignment with one another. The second step would involve intimate pairing brought about by a synaptonemal complex.

Most of the evidence from the literature would support the arguments for a 2 step event. Homologues may be attached in proximity on the nuclear membrane prior to the last premeiotic interphase. The close co-alignment and pairing of homologues seems reserved for the last premeiotic interphase. These co-alignment and synaptic events are most likely under genetic control at least in corn (Beadle, 1933; Singh, 1965; Feldman, 1966). The mitotic somatic association of homologous chromosomes probably reflects the minimum possible separation of chromosomes short of co-alignment prior to meiosis.
The evidence presented suggests that the long-term random contact and pairing mechanism proposed by Brown and Stack (1969) and Stack and Brown (1968) is not a general one and that it is operative only in *Plantago ovato* and *Haplopappus gracilis*.

2.2 **Mechanisms of Spindle Disruption:**

The spindle apparatus is a protein polymer of 6 S subunits that are similar to the muscle protein actin (Stephen 1968). There are a large number of ribosome-like particles containing RNA assembled in bead-like chains between the spindle fibers and in close association with them (Goldman and Rebuhn 1969). These particles may function in the assembly of spindle proteins or alternatively they may be a remnant of polyribosomes from the cytoplasm trapped during spindle formation. The high energy nucleotide phosphates, ATP (Mazia et al 1961) and GTP (Stevens et al 1967) are associated with the mitotic spindle. The spindle proteins have ATPase activity associated with them (Weisenberg and Taylor 1968, Mazia et al 1972).

Without a functional spindle apparatus the chromosomes do not move to opposite poles in a regular mitotic division (Molé-Bajer 1969). Based on the site of action there are two main categories of agents that arrest cells at metaphase by creating a non-functional spindle. As
ATP or GTP is required for a stable and functional spindle apparatus, any agent which prevents ATP formation by poisoning the cell's respiratory chain (e.g. CN⁻) will prevent spindle activity and stop mitosis (Takatori, 1966). A surfactant agent such as Digitonin which uncouples surface bound energy systems also inhibits mitosis (Underbrink and Olah 1968).

A second class of antimitotic agents includes those chelating agents which sequester the spindle subunits, preventing their polymerization. Colcemide, an alkaloid, and its relative synthetic compound colchicine, bind to the 6S subunits of the spindle elements (Borisy and Taylor 1967, 1967a; Brinkley et al 1967). 8-hydroxyquinoline (cited from Sharma et al 1972) and mono-bromo-napthalene (cited from Sharma et al 1972) are also types of allosteric modifiers (Sharma and Sharma 1972). Sufficient but sub-lethal concentrations of these chelating agents will depolymerize an existing spindle apparatus.

A third class of agent is temperature shock. Inoue (1952) noted that application of a cold treatment (0° C) to a dividing cell resulted in a rapid loss of spindle activity and birefringence in polarized light. Cold treatment (2° C for 5 minutes) will disrupt an already formed spindle apparatus by depolymerizing the microtubules and microtubule cross-linking elements (Roth 1967). Cold treatment (0° C to 5° C) could also arrest
ATP and ATPase activity in the spindle apparatus. If ATP is necessary to stabilize the spindle apparatus, the inhibition of ATP production and ATPase activity would result in the breakdown of spindle-fiber polymers. The prevention of ATP binding to the spindle protein, or of ATPase activity associated with the spindle protein by a surfactant uncoupling agent, by cold or by a metabolic poison, could result in a depolymerized spindle apparatus by inhibiting ATP stabilization of the spindle polymers.

2.3 Corn as an Ideal Organism for the Study of Somatic Association:

At first glance, a plant such as Haplopappus gracilis (2N=4) or Crepis capillaris (2N=6) or an animal (mammal) such as Muntjac (2N=6♀) with a low chromosome number would appear to be more suited than corn (2N=20) for a study of somatic association and nuclear order. Brown and Stack (1968) in Haplopappus, Waganaar (1968) in Crepis and Heneen and Nichols (1972) in Muntjac have looked for and found the somatic association of homologous chromosomes in these organisms. Further, Heneen and Nichols (1972) have shown that chromosomes of the muntjac at prophase are arranged in pairs of homologues about the edge of the nucleus.

In an organism where the nucleus is more complex in organization because more chromosomes have to be accom-
modated, such a simple pairwise association of homologous chromosomes at metaphase and during interphase might not be possible. Corn has a more complex nucleus than muntjac or *Haplopappus* by virtue of the greater number of chromosomes in the nucleus. This may require the nucleus to order the chromosomes in a more complex fashion and allow additional insights into the mechanisms by which the nucleus is ordered.

The traditional approach to somatic association has been to study the spacial relationship of chromosomes relative to each other at metaphase. This approach has necessitated the assignment of an identity to homologous pairs of chromosomes in some clear and repeatable fashion to allow one to measure the same spacial relationship from one cell to the next. Few organisms with as many chromosomes as corn have chromosomes sufficiently distinct from one another to allow unequivocable assignment of an identi-fication to all chromosome pairs. In good preparations of somatic metaphase, all the chromosomes of corn can be identified. This has been shown biometrically by Chen, 1969. This makes corn an ideal organism with a complex interphase nucleus in which somatic association can be studied.

Additional advantages of corn for a study of somatic association are that there is a large catalogue of aberrant stocks including over 1000 mapped reciprocal
translocations which are readily available. Finally, there are the mutants of maize which affect the gene regulation of chromosome behavior during mitosis and meiosis. These may allow one to more closely examine the importance of the ordered somatic interphase in relationship to the process of chromosome pairing.
CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Protocol:

In order to examine the question of somatic association in maize we approached the problem in the following ways: We surveyed three antimitotic agents: cold arrest (50°C for 24 hrs.), 2% monobromonapthalene (aqueous) for 3 hours or 8-hydroxyquinoline (0.5%) for 3 hours in order to determine which method of accumulating metaphase figures would allow us to best amine the problem of somatic association. We selected cold arrest as the most suitable for arresting at metaphase as no somatic association was found after pretreatment with 8-hydroxyquinoline or monobromonaphthalene. A sample of 200 metaphase cells from 4 'normal' stocks of maize with different genetic backgrounds were accumulated using cold shock to arrest the spindle-fiber apparatus. From this sample, a basis was derived for comparing the effects of cytogenetic aberrations on the somatic association of homologous chromosomes at metaphase.

The 14 stocks used in this study, a stock description and the type of antimitotic agent used on each stock
are listed in Table 3.1.

Among the aberrant stocks available from the maize genetics arsenal is one in which a large heterochromatic knob addition to the distal portion of chromosome 10L is included. This knobbed chromosome is called Kl0 and the normal chromosome 10 is referred to as kl0 in the heterozygous knob stock (Kl0/kl0). An additional source of heterochromatin available is the B chromosome. The effects of the addition of heterochromatin on the pattern of somatic association of chromosomes at metaphase with the addition of zero (kl0/kl0); 1 Kl0 (Kl0/kl0); 2 Kl0's (Kl0/Kl0) and 2B chromosomes to the normal maize chromosome complement were examined.

The effect of the accommodation of an extra chromosome on the ordering of the chromosomes in the interphase nucleus was examined by the use of a primary trisomic stock, trisomic 2. The disomic 2 segregants of the same cross were also examined. The changes in the ordering of the nucleus induced by the presence of a homozygous reciprocal translocation (T/T) between 9S and 10S (9-10b) and a translocation heterozygote (T/N) between 8L and 2L (2-8) were examined. These stocks are diagrammed in Figure 3.1.

Hamerton (1970) suggested that polar metaphases, observed with a low frequency in somatic mitosis, are a result of the failure of the centromeres of the chromo-
<table>
<thead>
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<th>Stock Description</th>
<th>Antimitotic Agent</th>
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<tbody>
<tr>
<td>A</td>
<td>Asynaptic (As/As)</td>
<td>Cold shock (5°C for 24 hr.)</td>
</tr>
<tr>
<td>B</td>
<td>4752-2 Disomic 2</td>
<td>&quot;</td>
</tr>
<tr>
<td>C</td>
<td>Seneca-60</td>
<td>&quot;</td>
</tr>
<tr>
<td>D</td>
<td>Seneca-60 (prometaphase)</td>
<td>&quot;</td>
</tr>
<tr>
<td>E</td>
<td>k10/k10 (OK10)</td>
<td>&quot;</td>
</tr>
<tr>
<td>F</td>
<td>K10/k10 (1K10)</td>
<td>&quot;</td>
</tr>
<tr>
<td>G</td>
<td>K10/K10 (2K10)</td>
<td>&quot;</td>
</tr>
<tr>
<td>H</td>
<td>2 B chromosomes</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>4752-2 Trisomic 2</td>
<td>&quot;</td>
</tr>
<tr>
<td>J</td>
<td>Translocation heterozygote 2-8</td>
<td>&quot;</td>
</tr>
<tr>
<td>K</td>
<td>Translocation homozygote 9-10</td>
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<td>Seneca-60</td>
<td>8-hydroxyquinoline</td>
</tr>
<tr>
<td>M</td>
<td>Seneca-60</td>
<td>monobromonaphthalene</td>
</tr>
<tr>
<td>N</td>
<td>K10/k10</td>
<td>8-hydroxyquinoline</td>
</tr>
</tbody>
</table>
Fig. 3.1

A. Stock J.

A diagramatic representation of the translocation chromosomes of translocation stock T_{2-8}.

B. Stock K.

A diagramatic representation of the translocation chromosomes of translocation stock T_{9-10}. 

somes to detach from the nuclear membrane at prometa-
phase. The author felt that an increase in the number of
polar metaphases observed could lead to a direct means of
examining the relative spacial relationships as represent-
ative of interphase.

An attempt to induce a higher frequency of polar
metaphases was made using cycloheximide (0.02%) at the
suggestion of Van't Hoff (1972 personal communication).

3.2.1 Preparation of Slides:

Two samples, each of 25 of each stock seeds, were
plated out in 15 cm. petrie plates on moistened filter
paper. The seeds were germinated in the dark at 27°C
until the primary roots were 5 to 8 cm. long (72 to 96
hrs.). All root-tips selected for use in the study were
treated as listed in Table 3.1. The root-tips were ex-
cised and fixed immediately after treatment in 3:1
ethanol-acetic acid for 24 hrs at room temperature (22°C
to 25°C).

Immediately following fixation, the root-tips
were hydrolized and stained with Feulgen following the
protocol of Chen (1969). Root-tips which could not be
stained and squashed within 24 hrs. of fixation were dis-
carded and the preparation of a replacement sample was
begun. One root-tip was squashed per slide and an aver-
age of 3 cells satisfactory for analysis were obtained
from each slide. As not all preparative attempts were successful, an average of one cell per seed germinated was obtained.

3.22 Photography:
Slides were scanned in contiguous, adjacent, but non-overlapping fields beginning at the lower left hand corner of the coverslip and continuing backward and forward across the slide until the scan was complete. All metaphase figures in which the chromosomes were well spread in a circular distribution were photographed. Additional criteria which had to be satisfied included the chromosomes had to be non-overlapping in the same focal plane at 1200X magnification and the satellites on both chromosomes 6 had to be clearly visible.

Photographs were taken with a Zeiss photomicroscope II equipped with a planapo 100/1.3 oil immersion lens. Kodak High Contrast Copy Film and a green filter (530 nm.) were used. Film was developed with Kodak D-19 developer and prints were made with an Agfa Varioscope (35/44) enlarger. A Kodak Ekatmatic Processor was used to make working, 8 x 10 N-surface prints.

3.23 Chromosome Measurement
The first 50 prints of metaphase spreads which satisfied the following criteria were used for any one
stock:

1. All the chromosomes fell within the limits of an 8 x 10 photographic sheet at 5220X magnification;

2. All the chromosome limits, telomeres, centromeres and satellites were clearly defined; and

3. None of the chromosomes were missing, distorted or folded and all the chromosomes were in the same focal plane.

The chromosomes were assigned a numerical identity from 1 to N in a random sequence, where N was the number of chromosomes present in the somatic metaphase figure.

The centromere and long and short telomere positions of each chromosome were marked by superimposing a sheet of Keuffel and Esser millimeter graph paper (12 5285) over the photograph (Plate 3.1). Arbitrary cartesian coordinates were established and the (x,y) coordinates of each of the 3 points for each of the N chromosomes in each of the 50 cells of each of the 14 stocks [36,000 (x,y) coordinates] were recorded and punched on computer cards.

The metaphase photographs were projected onto a sheet of Bristol Board (30,000X total magnification) using a Delinoscope Opaque Projector. The central axis of each chromosome arm was traced from each telomere to the limits of the centromere. These lines were measured
PLATE 3.1

An example of the method by which the \((X,Y)\) coordinates of the centromere and the telomeres were recorded by the overlay of cartesian coordinates over the photograph of metaphase.
by a millimeter rule or a map wheel device. A total of 28,000 chromosome arms were measured.

The measurement data was punched on computer cards and processed using computer program SA-1 (Table 3.2). Program SA-1 assigned an identity to each chromosome based on the relative arm lengths, the arm ratios and the presence of any other distinguishing morphological features such as the presence of a satellite. An independent, visual identification of the chromosomes was also made. The resultant assignment of chromosome identities was compared to those assignments made by the computer. Whenever a difference in identity assignment occurred, the original photograph was referred to and the difference was rationalized.

3.24 Biometrical Analysis:

Standardized distance functions between each of the centromeres, the long telomeres and the short telomeres of all possible combinations of chromosomes were accumulated over the 50 metaphase cells for each experiment using programs SA-2. The standardized function used was:

\[ \frac{d(x_a - x_b)}{d(BIG)} \] .................................(1)

Where:

\( d(x_a - x_b) \) was the distance between any 2 chromosomes 'a' and 'b' and \( d(BIG) \) was the greatest distance found between any two chromosomes in the cell. The dis-
<table>
<thead>
<tr>
<th>Program Designation</th>
<th>Program Description</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA.1</td>
<td>Assigns an identity to chromosomes from their arm lengths and arm ratios.</td>
<td>J.D. Horn</td>
</tr>
<tr>
<td>SA.2</td>
<td>Calculates the standardized distances between all possible chromosome combinations and accumulates them over &quot;n&quot; cells. Each possible distance distribution is made cumulative and tested against the expected random distribution generated by SA-3. The Kolmogorov-Smirnov non-parametric best fit test is used.</td>
<td>J.D. Horn</td>
</tr>
<tr>
<td>SA.3</td>
<td>Generates an expected frequency distribution of the distances between any two random points in a sphere reduced to a circle in two dimensions.</td>
<td>J.D. Horn</td>
</tr>
<tr>
<td>SA.4</td>
<td>Ranks the distances between all homologous chromosomes and the center of the cell for &quot;n&quot; cells.</td>
<td>J.D. Horn</td>
</tr>
</tbody>
</table>

* All programs were written in Fortran IV.
tance functions was set equal to 1.0 when the distance between chromosomes a and b was the greatest distance between any two chromosomes in the cell. It approached zero when the chromosomes a and b lay side by side so that the points of measurement were very close together.

There are $N^2$ possible distance distributions between all the chromosomes taken pairwise. In corn ($N=10$) for a normal stock, of the 100 possible distance distributions which can be measured between all chromosomes, 90 were measured between non-homologous chromosomes. These measurements were repeated 3 times for each chromosome; once for the centromeres and once for the distance between the telomeres of homologous long and short arms. Thus, a total of 180,000 distance measurements were considered in this study.

Computer program SA-2 (Table 3.2) used:

$$d(X_a-X_b) = \sqrt{(X_a-X_b)^2 + (Y_a-Y_b)^2} \quad \text{(2)}$$

yielding the distance between any 2 points a and b in two dimensions. The distance between any 2 points $(X_a, Y_a)$ and $(X_b, Y_b)$ is described by the X and Y coordinates of the two points.

The standardized distances between all 100 possible chromosome combinations for each of the centromeres and the two telomeres were accumulated independently over a sample of 50 cells for each treatment. The standardized distances derived between any 2 chromosomes for any one point of measurement (e.g. between 2 cento-
meres or between 2 telomeres of long arms) were classified into 20 frequency classes between 0 and 1.0 with a frequency interval of 0.05. The frequency of distances between any 2 specific chromosomes is often referred to as the distance distribution between those chromosomes in this study.

This distance distribution was compared to the theoretical distribution of 2 randomly located points in a sphere that is projected into 2 dimensions. The formula describing the expected random distribution was derived by Hammersly (1950) and Lord (1954) and used by Feldman et al (1966) can be represented by:

\[ f(x) = \frac{16 \cos^{-1} x - x(1-x^2)^{-\frac{1}{2}}}{2^{\frac{3}{2}}} \]  

The resultant distance distribution expected between 2 chromosomes which were randomly associated in the nucleus and then squashed into a plane surface are given in Figure 3.2a, b.

The distance distribution observed between any 2 chromosomes was compared to the expected (random) by the Kolmogorov-Smirnov non-parametric statistic (Sokal and Rolf 1969).

The data from the analysis of the distance distributions between all pairwise combinations of the 10 chromosomes in normal maize as measured at metaphase were collected and recorded in a manner which would allow Analysis of Variance techniques to be applied. A Randomized Complete Block Design (10 x 14) was chosen. No stock had
Fig. 3.2

A. The expected distribution of distances between two points distributed at random in a sphere, projected vertically into two dimensions.

B. The cumulative frequency plot of the frequency distribution of Figure 3.2A.
B. DISTANCE BETWEEN HOMOLOGUES
less than 10 pairs of chromosomes and each cell in the
Block recorded the contributions of 50 individual
measurements.

Calculations by Galperin (1968) had indicated
that 50 cells would provide a sufficiently accurate
sample to estimate the actual distribution of the chromo-
somes at somatic metaphase. The fact that all distance
distributions between all chromosome pairs was being made
and analyzed increased our confidence that a sample of 50
cells was sufficient to pick up any major differences
that may exist between the spacial relationships of
chromosomes at somatic metaphase.

3.25 An Ordered Metaphase Plate:

If certain pairs of homologous chromosomes were
found toward the center of the metaphase plate, they
would appear associated because they are always distribu-
ted within a smaller area than the rest of the chromo-
somes. Any pairs of homologous chromosomes which occu-
pied the more central regions of every metaphase plate
would also appear associated with the non-homologous
chromosomes of this area as well as with their own homolo-
gue. Non-homologous associations were computed in pro-
gram SA-2 (Table 3.2).

An attempt was made to rank each pair of homolo-
gous chromosomes based on the mean decimal fraction of
the radius of each circular metaphase that each pair of chromosomes (homologues) lay from the center of the metaphase spread of chromosomes.

3.26 A Direct Method of Determining the Relative Interphase Arrangement of Chromosomes by Direct Cytological Observation

Seeds of Seneca-60 (a single cross hybrid commercial sweet corn (su₁/su₁) were germinated for 4 days in the dark at 27°C. Half the sample of root-tips were excised and fixed in 3:1 ethanol-acetic acid. The remaining intact primary roots were immersed up to the seed in 0.02% cycloheximide for 15 minutes at 23°C. The cycloheximide treatment was followed by a 5 minute wash in running distilled water. The intact treated seedlings were returned to the 27°C incubator for 40 minutes to allow those cells in late G₂ and prophase at the time of treatment to proceed to metaphase.

The root-tips of one half of the cycloheximide treated sample were fixed in 3:1 ethanol-acetic acid. The remaining half of the treated sample were fixed in glutaraldehyde and osmium-tetroxide for 3 hours and embedded in Epon-812-araldite plastic mixture for sectioning for the electron microscope following the procedure of Luft (1961). Pictures were taken on a Phillips EM-75.
CHAPTER 4

EXPERIMENTAL RESULTS

4.1 **Karyotypes:**

A composite picture of several examples of the types of cells used in this study is presented in Plate 4.1. Karyotypes of the normal stock C (Seneca-60) and a prometaphase cell of Seneca-60 (Stock D) are shown in Plate 4.2. In addition, plate 4.2 also presents karyotypes from cells of the aberrant stocks: J(T2-8), F(K10/k10), G(K10/k10), H(2B's), I(Trisomic 2) and K(T9-10b). The reader should note that each type of aberrant is readily identified at somatic metaphase, offering a control on each study. Unfortunately the ease with which each aberration can be identified made blind coded studies impractical in most cases.

4.2 **Biometrical results:**

Fourteen experiments, stock codes A to N (Table 3.1), were performed.

Each of the 3,600 distance relationships accumu-
PLATE 4.1

A composite picture of 4 cells used in this study.

<table>
<thead>
<tr>
<th>Plate identification</th>
<th>Stock code</th>
<th>Stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>F</td>
<td>k10/K10</td>
</tr>
<tr>
<td>b</td>
<td>C</td>
<td>Seneca-60</td>
</tr>
<tr>
<td>c</td>
<td>H</td>
<td>2 B chromosomes</td>
</tr>
<tr>
<td>d</td>
<td>D</td>
<td>prometaphase</td>
</tr>
</tbody>
</table>
PLATE 4.2

The following eight pages are sample karyotypes of the normal and aberrant stocks used in this study. The stock identification on each plate is that of the particular stock used.

<table>
<thead>
<tr>
<th>Plate Identification</th>
<th>Stock Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seneca-60</td>
<td>C</td>
</tr>
<tr>
<td>Seneca-60 prometaphase</td>
<td>D</td>
</tr>
<tr>
<td>$T_{2-8}$ (T/N)</td>
<td>J</td>
</tr>
<tr>
<td>K10/k10</td>
<td>F</td>
</tr>
<tr>
<td>K10/K10</td>
<td>G</td>
</tr>
<tr>
<td>2B chromosomes</td>
<td>H</td>
</tr>
<tr>
<td>4752-2 Trisomic 2</td>
<td>I</td>
</tr>
<tr>
<td>$T_{9-10b}$ (T/T)</td>
<td>K</td>
</tr>
</tbody>
</table>
MAIZE CYTOGENETICS LABORATORY
UNIVERSITY OF WESTERN ONTARIO

NAME: JOHN HORN
STOCK: 72-8
FILM NO.: 79-16

IDENTIFICATION: Translocation Heterozygote

KARYOTYPE

1 2 3
4 5 6
7 8 9
10

B
NAME: JOHN HORN
STOCK: 2 B Chromosomes
FILM NO.: 73-29

IDENTIFICATION

KARYOTYPE

1  2  3
4  5  6
7  8  9
10  B
NAME: JOHN HORN

STOCK: T/T T9-10b

FILM NO.: 94-7

IDENTIFICATION: Translocation Homozygote

KARYOTYPE:

1  2  3
4  5  6
7  8  9
10 B
lated between 0 and 1.0 as a consequence of the manner in which the measurements between any chromosome pair were standardized (see section 3.24). Each distance relationship between any 2 chromosomes accumulated over 50 cells was also recorded as a cumulative frequency plot. The 3600 distance plots are on permanent file in the maize cytogenetics data bank at the University of Western Ontario.

The means of the distance distributions are presented as standardized values between 0 and 1.0 in Table 4.1, 4.2 and 4.3 for the mean distance found between homologous centromeres, telomeres of homologous long arms and telomeres of homologous short arms respectively.

The graph tests for normality of the distributions of means in each of Table 4.1, 4.2 and 4.3 are presented in Figure 4.1. A linear plot indicates a normal distribution of means. The underlying distribution of these means of the frequency plots between homologous pairs of chromosomes was found to be normal for each of Table 4.1, 4.2 and 4.3 by the graph test results (Sokal and Rohlf 1969). Having demonstrated normalcy of the distribution of the means of the distances between homologous centromeres and telomeres of homologous long and
<table>
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<td>.419</td>
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1 standardized units as a decimal fraction of a nucleus of a radius 1.0
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<td>.458</td>
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<td>.477</td>
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<td>.453</td>
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<td>.426</td>
<td>.433</td>
<td>.405</td>
<td>.448</td>
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</tbody>
</table>

1 standardized units as a decimal fraction of a nucleus of a radius 1.0
TABLE 4.3

MEAN DISTANCE BETWEEN TELOMERS OF HOMOLOGOUS SHORT ARMS

<table>
<thead>
<tr>
<th>Stock</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.424</td>
<td>0.311</td>
<td>0.370</td>
<td>0.394</td>
<td>0.405</td>
<td>0.410</td>
<td>0.357</td>
<td>0.387</td>
<td>0.420</td>
<td>0.288</td>
</tr>
<tr>
<td>C</td>
<td>0.498</td>
<td>0.400</td>
<td>0.375</td>
<td>0.381</td>
<td>0.449</td>
<td>0.425</td>
<td>0.350</td>
<td>0.417</td>
<td>0.407</td>
<td>0.402</td>
</tr>
<tr>
<td>D</td>
<td>0.443</td>
<td>0.459</td>
<td>0.471</td>
<td>0.358</td>
<td>0.475</td>
<td>0.380</td>
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<td>0.459</td>
</tr>
<tr>
<td>E</td>
<td>0.421</td>
<td>0.381</td>
<td>0.355</td>
<td>0.402</td>
<td>0.375</td>
<td>0.398</td>
<td>0.393</td>
<td>0.421</td>
<td>0.388</td>
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</tr>
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<td>F</td>
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<td>0.407</td>
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<tr>
<td>G</td>
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<tr>
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<td>0.376</td>
<td>0.433</td>
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<td>0.354</td>
<td>0.426</td>
<td>0.402</td>
<td>0.426</td>
</tr>
<tr>
<td>I</td>
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<td>0.353</td>
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<td>0.391</td>
<td>0.449</td>
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<td>0.324</td>
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<tr>
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<td>0.450</td>
<td>0.372</td>
<td>0.439</td>
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<tr>
<td>K</td>
<td>0.458</td>
<td>0.396</td>
<td>0.388</td>
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<td>0.464</td>
<td>0.417</td>
<td>0.347</td>
<td>0.412</td>
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<tr>
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<td>0.381</td>
<td>0.442</td>
<td>0.498</td>
<td>0.410</td>
<td>0.414</td>
<td>0.428</td>
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</tbody>
</table>

1 standardized units as a decimal fraction of a nucleus of a radius 1.0.
Fig. 4.1

A. Graph test of means of distance distributions between homologous centromeres.
B. Graph test of means of distance distributions between telomeres of homologous long arms.
C. Graph test of means of distance distributions between telomeres of homologous short arms.
short arms, the use of parametric statistics for analysis of the data was justified.

A 2-way Analysis of Variance (ANOVA) was performed on the means presented in each of Table 4.1, 4.2 and 4.3. The block (chromosome) and treatment (stock) means for Tables 4.1, 4.2 and 4.3 are presented in Tables 4.4, 4.5 and 4.6 for centromere mean data, long telomere mean data and short telomere mean data respectively.

2-way ANOVA tables for the centromere mean data, the long telomere mean data and the short telomere mean data are presented in Tables 4.7, 4.8 and 4.9 respectively.

The reader should note that the F-values derived from the ANOVA of the mean distances between homologues in Table 4.7 are significant for both blocks and treatments. This result indicates that the type of treatment used (aberrant stock or antimitotic agent) is an important variable in the experimental protocol. The results also indicate that the degree of association observed between homologous chromosomes (blocks) is also variable. That is, homologous chromosomes respond differentially to the treatments used as measured by the degree of somatic association measured between homologues.

F-tests (treatment) based on the ANOVA results for the means of distances between the telomeres of homologous long arms (Table 4.8) and between the telo-
### TABLE 4.4

<table>
<thead>
<tr>
<th>Block Means</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>38.8</td>
<td>41.0</td>
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<td>39.9</td>
<td>39.9</td>
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<table>
<thead>
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<th>Treatment Means</th>
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<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>38.1</td>
<td>38.9</td>
<td>45.6</td>
<td>39.9</td>
<td>38.8</td>
<td>39.9</td>
<td>40.4</td>
<td>37.3</td>
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</table>
### Table 4.5

**Block and Treatment Means for Long Telomere Mean Data**

#### Block Means

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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
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<td>39.3</td>
<td>39.1</td>
<td>40.4</td>
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<td>39.2</td>
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<td>39.9</td>
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</tbody>
</table>

#### Treatment Means

<table>
<thead>
<tr>
<th>A</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
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<td>40.1</td>
<td>38.6</td>
<td>40.0</td>
<td>42.1</td>
<td>37.4</td>
<td>41.7</td>
<td>38.8</td>
<td>43.2</td>
</tr>
</tbody>
</table>
### BLOCK AND TREATMENT MEANS FOR SHORT TELOMERE MEAN DATA

#### Block Means

<table>
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<tr>
<th>Chromosome</th>
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<th>4</th>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
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<td>38.1</td>
<td>40.5</td>
<td>42.4</td>
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<td>39.0</td>
<td>41.6</td>
<td>39.0</td>
<td>38.3</td>
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</table>

#### Treatment Means

<table>
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<tr>
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<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
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<td>41.0</td>
<td>43.9</td>
<td>39.1</td>
<td>38.7</td>
<td>39.5</td>
<td>40.9</td>
<td>37.4</td>
<td>40.5</td>
<td>38.6</td>
<td>42.5</td>
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</tbody>
</table>
### TABLE 4.7

2-WAY ANALYSIS OF VARIANCE TABLE FOR CENTROMERE MEAN DATA

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>9</td>
<td>479.1</td>
<td>53.2</td>
<td>3.88**</td>
</tr>
<tr>
<td>Treatments</td>
<td>13</td>
<td>872.8</td>
<td>67.1</td>
<td>4.89**</td>
</tr>
<tr>
<td>Error</td>
<td>117</td>
<td>1,613.6</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>2,975.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**significant F-value at p<0.01**
### TABLE 4.3

2-WAY ANALYSIS OF VARIANCE TABLE FOR LONG TELOMERE MEAN DATA

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>9</td>
<td>208.9</td>
<td>23.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Treatments</td>
<td>10</td>
<td>548.3</td>
<td>54.8</td>
<td>4.3**</td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>1,129.4</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>1,886.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**significant F-value at p<0.01
<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>9</td>
<td>510.7</td>
<td>56.7</td>
<td>2.5*</td>
</tr>
<tr>
<td>Treatments</td>
<td>10</td>
<td>408.0</td>
<td>40.8</td>
<td>2.9*</td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>1,236.3</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>1,955.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant F-value at p < 0.05
meres of homologous short telomeres (Table 4.9) indicated that the telomeres of homologous chromosome arms showed different degrees of association within different stocks or treatments. The telomeres of all chromosome arms (blocks) were associated to the same extent across all stocks.

A Duncan's Multiple Range Test (DMRT) was performed on the pooled mean distances among homologous centromeres (BLOCK) and between the pooled treatment means listed in Table 4.4 to determine which differences among chromosomes and between treatments were significant. The results of the DMRT are scored in Table 4.10 (block means) and Table 4.11 (treatment means).

There is a tendency for the chromosomes (Table 4.11) of stocks L, M, N and D to be less associated with their homologues than in the other 10 stocks. The stocks L, M and N were treated with a chelating type of anti-mitotic agent prior to fixation (see Table 3.1) and stock D with cold treatment prior to fixation. Stock D cells were in prometaphase at the time of fixation and were collected from the same slides as those metaphase cells of stock C. The chromosomes of stock C (metaphase) are clearly more associated with their homologues than are the chromosomes of Stock D (prometaphase). The chromosomes must associate somehow with their homologues at metaphase and are not associated with their homologues
TABLE 4.10

DUNCAN'S MULTIPLE RANGE TEST* ON BLOCK MEANS FROM CENTROMERERE DATA

\[
\begin{align*}
&\text{Ranked Block (chromosome) Means} \\
&\begin{array}{cccccccccc}
2 & 10 & 3 & 9 & 7 & 6 & 4 & 8 & 1 & 5 \\
37.9 & 38.6 & 38.8 & 39.3 & 39.9 & 39.9 & 41.0 & 42.7 & 43.0 & 43.1
\end{array}
\end{align*}
\]

\[S \sigma = 1.52 \]
\[p = 0.05\]

* Means underscored by a line are not significantly different
TABLE 4.11

DUNCAN'S MULTIPLE RANGE TEST* ON TREATMENT MEANS FROM CENTROMERE DATA

\[ S_x = 1.03 \]
\[ p \leq 0.05 \]

<table>
<thead>
<tr>
<th>Ranked Treatment Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>I  A  B  F  C  K  G  E  H  J  N  L  M  D</td>
</tr>
<tr>
<td>37.3 37.4 38.1 38.8 38.9 39.1 39.8 39.9 40.4 41.0 42.2 43.0 44.8 45.6</td>
</tr>
</tbody>
</table>

* Means underscored by a common line are not significantly different.
immediately prior (about 4 minutes at 27°C) at prometaphase. The author will return to this important point in the discussion as any model of an organized nucleus must account for this observation.

The use of a cold treatment prior to fixation permits a much greater frequency of homologue associations to be detected at metaphase. As some of the stocks contain aberrations which also may affect the observed association of chromosomes, a separate ANOVA was carried out with 4 normal cold arrested stocks A, B, C and E, 8-hydroxyquinoline arrested stocks, L and N and monobromonaphthalene arrested stock M. The results of the analysis of the normal cold and chemical arrested stocks follow (Table 4.18) but are anticipated in Table 4.11. The chromosomes of a cold treated stock (A, B, C and E) are more associated at metaphase than are the homologues of a stock (L, M, N) treated with a chemical chelating agent. A similar result was reported for colchicine and cold treatment by Avivi et al (1969).

All the chromosomes of maize are not associated with their homologues to the same extent at metaphase (Table 4.10).

Duncan's Multiple Range Test were also performed on the block and treatment means of the pooled mean distances among the telomeres of homologous long arms (Table 4.5) and among the telomeres of homologous short arms (Table 4.6). The results of the DMRTs of the block means
TABLE 4.12

DUNCAN'S MULTIPLE RANGE TEST* FOR BLOCK MEANS AMONG LONG TELOMERES

\[ S_x = 1.18 \]
\[ p \leq 0.05 \]

<table>
<thead>
<tr>
<th>Ranked Block (chromosome) Means</th>
<th>9</th>
<th>3</th>
<th>7</th>
<th>2</th>
<th>10</th>
<th>6</th>
<th>4</th>
<th>5</th>
<th>8</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38.7</td>
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<td>41.7</td>
<td>41.9</td>
<td>43.2</td>
</tr>
</tbody>
</table>

* Means underscored by a common line are not significantly different.
**TABLE 4.13**

DUNCAN’S MULTIPLE RANGE TEST* FOR TREATMENT MEANS AMONG LONG TELOMERES

\[ S_x = 1.12 \]
\[ p = 0.05 \]

<table>
<thead>
<tr>
<th>Ranked Treatment Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>37.4</td>
</tr>
</tbody>
</table>

*Means underscored by a common line are not significantly different.*
### TABLE 4.14

**DUNCAN'S MULTIPLE RANGE TEST** FOR BLOCK MEANS AMONG SHORT TELOMERES

<table>
<thead>
<tr>
<th>Block (chromosome)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.9</td>
</tr>
<tr>
<td>2</td>
<td>38.1</td>
</tr>
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<tr>
<td>9</td>
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<td>10</td>
<td>42.9</td>
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</tbody>
</table>

*Means underscored by a common line are not significantly different.*
**TABLE 4.15**

**DUNCAN'S MULTIPLE RANGE TEST FOR TREATMENT MEANS AMONG SHORT TELOMERES**

- $S_x = 1.17$
- $p = 0.05$

<table>
<thead>
<tr>
<th>Ranked Treatment Means</th>
</tr>
</thead>
<tbody>
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<tr>
<td>37.4</td>
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</tbody>
</table>

* Means underscored by a common line are not significantly different.
and treatment means of the distances between telomeres of homologous long arms are presented in Tables 4.12 and 4.13 respectively. The results of the DMRTs of the block means and treatment means of the distances between of homologous short arms are presented in Tables 4.14 and Tables 4.15 respectively.

The reader will note that analysis of the block and treatment means derived from the means of measurements among the telomeres of homologous long arms and among the telomeres of homologous short arms did not contribute any information in addition to that derived from the Duncan's Multiple Range analysis of the block and treatment means derived from the means of measurements between the centromeres of homologous chromosomes. In view of the role that Sved (1966) and Comings (1968) proposed for the telomeres as the agents of association and pairing for the homologues of chromosomes during somatic interphase and in meiosis, the fact that the homologous telomeres are no more associated than are the homologous centromeres is somewhat unexpected. This result is similar to the observation reported by Sadasiviah et al (1969). It may reflect the attachment of the centromeres but not the telomeres of prophase chromosomes observed by Carlson (1956). As the chromosomes coil most from prophase to metaphase, if the telomeres are not attached to the nuclear membrane but the centromeres are,
the observed position of the telomeres are probably a reflection of the centromere's relative positions rather than the relative position of the telomeres during the preceding interphase.

As the analysis of the distances between telomeres of homologous arms did not contribute any additional information, only the centromere distance distribution means and subsequently the centromere distance distribution cumulative frequency plots were reported in this study. The frequency class information for the analysis performed on the distance found between telomeres of homologous long arms and telomeres of homologous short arms are on permanent file in the Maize Cytogenetics Laboratory Data Bank in the Department of Plant Sciences at the University of Western Ontario. The results of the Duncan's Multiple Range test performed on the means of distances among homologous centromeres of chromosomes across all 14 stocks had indicated that cold treatment to arrest mitosis at metaphase produced more chromosome associations than did the use of 8-hydroxyquinoline or monobromonaphthalene (Table 4.11). Thus further extensive analyses of cold treatment stocks were conducted.

In order to evaluate the response of individual chromosome associations (among homologues) to a cold shock or to the use of a chelating antimitotic agent such as 8-hydroxyquinoline and monobromonaphthalene we selected
the 4 'normal' stocks A, B, C and E all blocked at metaphase with a cold treatment to form one group for the analysis. Two other normal stocks L and M and one aberrant stock N (K10/k10) blocked at metaphase with 8-hydroxyquinoline or monobromonaphthalene formed the second group in the analysis.

The results of a 2 way Analysis of Variance on each of the two groups of stocks are presented in Tables 4.16 (cold treatment) and Table 4.17 (chemical antimitotic agent). A 2 way Analysis of Variance was also performed on the 2 groups pooled and the results are presented in Table 4.18.

The results of the F-test for blocks from the results of the ANOVA for cold arrested metaphase (Table 4.16) indicated that homologous chromosomes of maize were not all associated to the same extent at metaphase. We interpret this result to indicate that some chromosomes lay significantly closer to or farther apart from their homologous partners at metaphase than did other chromosomes to their homologues. However it should be noted from the F-test results of the treatment means that degree of association between chromosomes was the same from treatment to treatment.

Table 4.19 presents the results of Duncan's Multiple Range Test on the block (chromosome) means of the 4 normal cold shock arrested stocks A, B, C and E
**TABLE 4.16**

2-WAY ANOVA TABLE FOR CENTROMERE MEAN DATA OF 4 COLD ARRESTED* NORMAL STOCKS.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>9</td>
<td>536.3</td>
<td>134.0</td>
<td>32.6***</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>35.5</td>
<td>3.9</td>
<td>0.9 NS</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>112.2</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>39</td>
<td>.684.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** significant F-value at p=0.001.

NS - not significant.

* antimitotic agent used to block mitosis at metaphase.
TABLE 4.17

2-WAY ANOVA TABLE FOR CENTROMERE MEAN DATA OF 3 CHEMICAL ARRESTED* STOCKS.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>9</td>
<td>174.9</td>
<td>.19.4</td>
<td>1.6 NS</td>
</tr>
<tr>
<td>Treatments</td>
<td>2</td>
<td>37.0</td>
<td>18.5</td>
<td>1.5 NS</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>211.9</td>
<td>11.7</td>
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<tr>
<td>Total</td>
<td>29</td>
<td>435.0</td>
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<td></td>
</tr>
</tbody>
</table>

NS - not significant

* antimitotic agent used to block mitosis at metaphase
### TABLE 4.18

1-WAY ANALYSIS OF VARIANCE TABLE FOR CENTROMERE MEAN DATA OF 4 COLD ARRESTED AND 3 CHEMICAL ARRESTED NORMAL STOCKS.

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<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>458.8</td>
<td>76.4</td>
<td>4.6***</td>
</tr>
<tr>
<td>Error</td>
<td>63</td>
<td>1,046.8</td>
<td>16.6</td>
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</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>1,505.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*antimitotic agent used to block mitosis at metaphase.

*** significant F-value at p<0.005
as tabulated in Table 4.16. It is apparent that half the chromosomes of maize, chromosomes 10, 2, 7, 3 and 4 are significantly more associated with their homologous partners than are chromosomes 9, 6, 5, 8 and 1. It should be noted that this pattern of association was consistent, not only from cell to cell within a stock but also between stocks as reflected in the significant F-test score for blocks (chromosomes) and non-significant F-test score for treatments (Table 4.16).

Table 4.17 presents a summary of the Analysis of Variance which was performed on the chromosome distance means of those 3 stocks L, M and N which were treated with 8-hydroxyquinoline (L and N) or monobromonapthalene (Stock M) to accumulate metaphase figures. The results of the F-tests in Table 4.17 indicated that all of the chromosomes of maize were associated to the same extent when either 8-hydroxyquinoline or monobromonapthalene were used to block cells at metaphase.

When an Analysis of Variance was performed on 7 stocks of the cold arrested and chemical arrested metaphase cells, Stocks A, B, C and E and Stocks L, M and N respectively, the F-test values based on the results of the ANOVA (Table 4.18) showed significant variation in the degree to which chromosomes were associated with their homologues at metaphase. A Duncan's Multiple Range test was performed on the treatment means from the ANOVA
<table>
<thead>
<tr>
<th>Ranked Block (Chromosome)</th>
<th>Means</th>
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<td>9</td>
<td>39.7</td>
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<tr>
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<td>41.2</td>
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<td>8</td>
<td>41.7</td>
</tr>
<tr>
<td>1</td>
<td>43.5</td>
</tr>
</tbody>
</table>

*Sx=0.65  
P<0.05

* Means underscored by a common line are not significantly different.
TABLE 4.20

DUNCAN'S MULTIPLE RANGE TEST* ON THE TREATMENT MEANS OF 4 COLD AND 3 CHEMICAL ARRESTED NORMAL STOCKS

\[ S_X = 1.53 \]
\[ p = 0.05 \]

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>E</th>
<th>N</th>
<th>L</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.4</td>
<td>38.1</td>
<td>38.9</td>
<td>39.8</td>
<td>42.2</td>
<td>43.0</td>
<td>44.0</td>
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</tbody>
</table>

* means underscored by a common line are not significantly different.
shown in Table 4.19 and the results are tabulated in Table 4.20. The chromosomes of stocks A, B, C and E are seen to be more associated with their homologues than are the chromosomes of stocks L, M or N.

From an examination of the means of the distances between 2 homologous chromosomes at metaphase two significant features become apparent. Firstly, that the method of accumulating metaphase figures (cold treatment v.s. chemical agent) will affect the observed association of homologous chromosomes at metaphase. Secondly, when the amount of somatic association of chromosomes (homologues) detected is increased by the use of an agent such as cold shock, there are 2 distinct groups of chromosomes as defined on the basis of their association with their homologous chromosome. One group, composed of chromosomes 10, 2, 7, 3 and 4 are significantly more associated with their homologous partner chromosomes than are the chromosomes 9, 6, 5, 8 or 1. Henceforth, this grouping is referred to as the 'normal' pattern of association in this study.

The shape of the frequency plot of the distances (distance distribution curve between homologous chromosomes) can also be informative and provide additional insights that may not be apparent from an examination of the mean distance between homologous chromosomes. Figure 3.2b presents a cumulative frequency plot of the expected
distribution of distances found between 2 points distributed at random in a sphere of a diameter 1.0 if that sphere was projected onto a plane surface. We used this as a standard expected curve and compared each of the cumulative frequency plots to this curve. Each of the 20 frequency classes for the observed distance distribution plots between the centromeres and between telomeres of homologous long and short arms were tested for deviations from the expected distribution of Figure 3.2. A deviation greater than could be expected by chance alone at the 95% confidence limit (p = 0.05) was accepted as a significant deviation. The total number of frequency classes which showed a significant deviation from the expected within any one frequency plot were scored. The results are summarized in Table 4.21, 4.22 and 4.23 for the distributions of distances between homologous centromeres, telomeres of homologous long arms and telomeres of homologous short arms respectively.

The reader will note that those chromosomes which showed a significantly smaller mean value for the distances between homologous chromosomes, that is; chromosomes 10, 2, 7, 3 and 4 (Table 4.19) also have the bulk of the significant deviations from the random expectation. From an examination of the means of the distances between homologous chromosomes, this is a significant result as it was not evident previously whether homologous chromo-
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</tr>
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</tr>
</tbody>
</table>
somes were randomly or non-randomly associated at metaphase. An example of the correlation between the 2 types of analysis (shapes of the distributions and means of the distributions) follows.

The reader will recall that the homologues of chromosome 2 and chromosome 10 were significantly more associated than were the homologues of Chromosome 1 as measured by a Duncan's Multiple Range Test (Table 4.19). Examination of Table 4.21 will show the number of significant deviations from random was higher for the distances found between the homologous of chromosome 10 (35 deviations) and chromosome 2 (55 deviations) than those distances found between the homologous centromeres of chromosome 1 (12 deviations). The reader may further recall that the type of antimitotic agent used; cold treatment (Stocks A, B, C and E) or chemical agent (Stocks L, M and N) affected the degree of association exhibited between homologues within a normal stock (Table 4.20).

From the analysis performed thus far it is apparent that some of the chromosomes of maize (10, 2, 7, 3 and 4) are significantly more associated with their homologues at metaphase than are other chromosomes (9, 6, 5, 8 or 1). The next question which was addressed by the author was; are either of these two groups of chromosomes, as defined on the basis of more closely or less closely associated, also non-randomly distributed at metaphase? If so, is
the reduction in the number of significant homologue associations observed (Table 4.21, stocks L, M, N) after the use of a chemical antimitotic agent as compared to cold shock (Table 4.21, stocks A to K with the exception of stock D (prometaphase)), also a change from a non-random spatial orientation of homologues to a random one?

To examine this question, the author has plotted the cumulative frequency curves of the distance distributions between homologous centromeres of chromosomes 2, 10 and 1 after cold arrest and after 8-hydroxyquinoline arrest of the spindle function. These 2 cumulative frequency curves for each homologue pair are plotted against the random distribution as presented in Figure 3.2. The new plots are shown in Figures 4.2, 4.3 and 4.4.

From the data given above I conclude that the use of a cold shock to collect cells at metaphase permits the non-random association of homologues to be detected. Similarly the use of antimitotic agents such as 8-hydroxyquinoline or monobromonaphthalene randomizes the associations observed between homologous chromosomes.

Even if a cold shock is used to block mitosis, not all the chromosomes of maize are non-randomly associated with their homologues at metaphase. This is an observation of some significance when applied to the concept of an ordered somatic nucleus. I will later argue that this non-random association of only some of the homologues...
Fig. 4.2

A comparison of the distances found between the homologous centromeres of chromosome 2 after cold arrest and 8-hydroxyquinoline were used as antimitotic agents.

Legend

------------- theoretical random distribution
- - - - - - - cold arrest at metaphase
- - - - - - - - 8-hydroxyquinoline arrest at metaphase
Fig. 4.3

A comparison of the distances found between the homologous centromeres of chromosome 10 after cold arrest and 8-hydroxyquinoline were used as antimitotic agents.

Legend

------------- theoretical random distribution

---- cold arrest at metaphase

----- 8-hydroxyquinoline arrest at metaphase
Fig. 4.4

A comparison of the distances found between the homologous centromeres of chromosome 1 after cold arrest and 8-hydroxyquinoline were used as antimitotic agents.

Legend

--------- theoretical random distribution

--- cold arrest at metaphase

--- 8-hydroxyquinoline arrest at metaphase
is not the case during interphase.

Cells at prometaphase (identified as Stock D Table 3.1) and metaphase (identified as Stock C Table 3.1), but in fact collected from the same slides, were analyzed for the association of homologues. A cold shock was used as the antimitotic agent. The cumulative frequency curves based on distances observed between the homologous centromeres of chromosome 2, in a representative example in each of stocks C and D is shown, in Figure 4.5. The chromosomes of Stock C are associated in the typical, normal pattern derived from Table 4.19. The prophase chromosomes of maize are not associated with their homologues. A rationalization of this result is given in the discussion section, as this is a key result for the establishment of a model for the interphase nucleus.

Reference to figure 4.3 will remind the reader that the homologues of chromosome 10 are normally associated at metaphase if cold treatment (5°C for 24 hrs.) is used to block mitosis. The addition of a large piece of heterochromatin (K10) to the distal region of 10L ((Stock F(K10/k10) and Stock G(K10/K10); see Plate 4.2)) significantly altered the association recorded from chromosomes K10/K10 at metaphase. Figure 4.6 diagrams the dose dependent change in association observed with the addition of 1 and 2 heterochromatic knobs to the distal region of 10L. It is clear that the chromosomes K10/K10 are not associated at metaphase in distinction to the
Fig. 4.5

A comparison of the observed distance distribution between the centromeres of homologous chromosomes 2 in stocks C and D. The cells from which these data were derived were taken from the same slides.

Legend

--------------- Theoretical expected random distribution of the 2 homologues.

Cumulative frequency plot of distances between centromeres of chromosome 2 in stock C,

Cumulative frequency plots of distances found between centromeres of chromosome 2 in stock D.
previously recorded association of k10/k10.

The K10 knobs are largely heterochromatic and stain densely with Feulgen during interphase. This property permits an unusual opportunity to observe the interphase position of the distal region of the chromosome K10. It allows a direct means of evaluating the significance of somatic association of homologues at metaphase as it relates to the interphase position of the homologues. Plate 4.3 presents 4 interphase nuclei showing the relative positions of the K10 knobs during interphase. A visual comparison of the densely staining K10 knobs and the diffusely staining B chromosomes is also provided in Plate 4.3. The 2K10 knobs are closely associated during interphase whenever both K10's can be observed clearly. Yet the 2K10 chromosomes are not associated at metaphase (Figure 4.6). Some mechanism must exist which will dissociate the 2K10 chromosomes by metaphase and re-associate them at anaphase. This point will be further amplified later.

The presence of one K10 (K10/k10) or 2 K10 (K10/K10) chromosomes affects the distribution of chromosome 6 as observed at metaphase. In the absence of K10, chromosome 6 is randomly associated at metaphase. In the presence of K10, a dose dependent increase in the association of the satellited chromosomes 6 can be recorded. This dosage effect is shown in Figure 4.7. This result is similar to that observed by Miles (1968) for her
Fig. 4.6. A comparison of the distances found between 0K10\textsuperscript{(k10/k10)}, 1K10\textsuperscript{(K10/k10)}, 2K10\textsuperscript{(K10/K10)} in cold arrested cells.

Legend

\begin{itemize}
  \item [\textbullet\textbullet\textbullet\textbullet ] Distance between homologous centromeres of normal chromosome 10\textsuperscript{(k10/k10)}
  \item [\textbullet\textbullet\textbullet\textbullet ] Distance between homologous centromeres of 1 abnormal 10 and 1 normal 10.
  \item [\textbullet\textbullet\textbullet\textbullet\textbullet ] Distance between homologous centromeres of 2 abnormal chromosomes 10\textsuperscript{(K10/K10)}
\end{itemize}
PLATE 4.3

Plate 4.3 a, b, c, and d show the interphase position of the knobed chromosome K10. (K10/K10; stock G.)
The two knobed chromosomes 10 are obviously associated during interphase. Other knob associations are also visible.
Plate 4.3e and f are interphase nuclei of the 2B chromosome stock, stock H. The 2B chromosomes are not clearly defined during interphase.
Fig. 4.7

A comparison of the distance distributions found between homologous chromosomes 6 in the presence of 0K10 (stock E), 1K10 (stock F) and 2K10 (stock G).

Legend

............... Theoretical random distribution

--- Distance between homologous chromosomes 6 with 0 K10.

----- Distance between homologous chromosomes 6 with 1K10.

---- Distance between homologous chromosomes 6 with 2K10.
observations of the effects of K10 on chromosome 6 associations. K10 can also affect other chromosome associations, as reported in section 4.24.

The addition of an extra chromosome 2 as a primary trisomic 2 (Stock I; Table 3.1) did not change the mean association of chromosome 2 from that recorded from the control Stock B (Table 3.1). Figure 4.8 shows the cumulative frequency plots derived from the distances observed between the 2 homologues of chromosome 2 of Stock B and the 3 homologues of chromosome 2 of Stock I. Seeds for Stock B and Stock I were siblings from the same cob, segregating 2N and 2N+1 respectively.

The association of other homologous chromosomes in the Stock I (Table 4.21) trisomic 2 (2N+1) complement, were also notably affected, particularly chromosomes 1, 5, 6, 7 and 9. Marked increases were observed in the association among homologues. Examples of these changes from the control stock B are presented in Figure 4.9 for chromosomes 1 and Figure 4.10 for chromosomes 6.

4.23 An Ordered Metaphase Plate

An attempt was made to rank the 10 pairs of homologous chromosomes of maize relative to one another based on the mean distances that each homologous pair lay from the center of the metaphase spread. The mean distances from the center of the metaphase spread for any pair of chromosomes (homologues) was scored as a decimal
Fig. 4.8

Cumulative frequency plots of the distances found between the homologues of chromosome 2 in stock B (4752-2 disomic 2) and stock I (4752-2 trisomic 2).

Legend

.............. Theoretical random distribution

Stock B (disomic 2)

Stock I (trisomic 2)
Fig. 4.9

Cumulative frequency plots of the distances found between the centromeres of homologous chromosomes 1 of stock B (4752-2 disomic 2) and stock I (4752-2 trisomic 2). An increase in homologue association was observed in the presence of the trisomic 2.

**Legend**

```
.............  Theoretical random distribution
---          Stock B (disomic 2)
----          Stock I (trisomic 2)
```
Cumulative frequency plots of the distances found between the centromeres of homologous chromosomes 6 of stock B (4752-2 disomic 2) and stock I (4752-2 trisomic 2). An increase in homologue association was observed in the presence of the trisomic 2.

**Legend**

---

Theoretical random distribution

---

Stock B (disomic 2)

---

Stock I (trisomic 2)
fraction of the radius (1.0) of the circular metaphase spread.

The mean decimal scores of the 50 distances of each homologous pair of chromosomes from the centers of the metaphase plates of each stock are presented in Table 4.24. No definite pattern was observed. No one or several pairs of homologous chromosomes were scored consistently closer to the center of the metaphase spread. In particular, those chromosomes 10, 2, 7, 3 and 4 which showed a significant association with their homologous partner in the studies reported in section 4.2 were not grouped near the center of the metaphase plate. This result ruled out the possibility that somatic association of these chromosomes was the sole result of the tendency of these chromosomes to lie centrally grouped in the metaphase plate. Therefore, the observed somatic association of chromosomes must have some other mechanistic basis.

4.24 Random Somatic Associations of Non-Homologous Chromosomes

Our present study involved an analysis of the spatial relationships of each somatic metaphase chromosome to every other chromosome in a pair by pair fashion. This type of analysis permitted an additional method of evaluating the phenomenon of somatic association of metaphase chromosomes. The analysis could provide a comparison
<table>
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<tr>
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<td>.72</td>
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<td>.49</td>
<td></td>
</tr>
</tbody>
</table>

* where the radius of each metaphase was standardized to 1.0
between the frequency of non-random associations observed among homologous chromosomes compared to the frequency of non-random associations observed between any other specific non-homologous chromosomes.

If chromosomes are randomly associated at metaphase, then each chromosome should associate with every other chromosome with the same frequency. Or, for every significant association observed among 2 homologous chromosomes there should be 9 other significant associations observed between those 2 homologues and all other non-homologous chromosomes.

By exploiting our computer-based analysis programs, cumulative frequency plots of the distribution of distances between the centromeres of the 10 pairs of homologous chromosomes and each of the 9 possible combinations of non-homologues taken as homologous pairs were prepared from each of the 14 stocks A to N. Similar cumulative frequency plots were prepared between homologous and non-homologous telomeres of the long arms. Also, similar plots were prepared for the short telomere associations in eleven of the 14 stocks. It was considered inappropriate to present each of the 3240 frequency plots considered in this study. These plots remain on permanent file in the data bank of the Maize Cytogenetics Laboratory, in the Department of Plant Sciences at the University of Western Ontario.
The large number of combinations of comparisons of all possible frequency plots of the distribution of distances between homologous against non-homologous chromosomes over all stocks ruled out a meaningful analysis by direct comparison. As an alternative, each distance distribution between non-homologous was compared to an expected distribution of distances. The expected distribution was derived from the consideration of the non-homologous chromosomes as randomly associated points in a circle.

The number of frequency classes for any chromosome that was significantly different from the expected \( p=0.05 \) are recorded in Table 4.26. The results presented in Table 4.26 may be compared directly to those tabulated in Table 4.21. It should be noted that the number of frequency classes which were significantly different from the random expectation was greater for the distances derived among homologues as compared to between non-homologous chromosomes by 238 to 2 in the normal cold arrested stocks A, B, C and E. The majority of non-homologous chromosome associations occurred in Stock \( F(K10/k10) \) and Stock \( K \) (T9-10b) (48 of 54 across all 14 stocks).

Table 4.27 presents a breakdown of the significant non-homologous chromosome associations by participant chromosomes. In Stock \( F \), the bulk of the non-homologous associations are observed between the chromosomes \( k10/K10 \)
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<tr>
<th>Stock</th>
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<th>Homologues</th>
<th>Non-homologues</th>
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<tr>
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<td></td>
<td>S</td>
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<tr>
<td></td>
<td>S</td>
<td>24</td>
<td>9</td>
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</tbody>
</table>
TABLE 4.26 (continued)

NUMBER OF FREQUENCY CLASSES THAT WERE SIGNIFICANTLY DIFFERENT FROM THE EXPECTED RANDOM DISTRIBUTION FOR ALL CHROMOSOMES TAKEN PAIRWISE IN EACH STOCK. (p≤0.05)

<table>
<thead>
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<th>Stock</th>
<th>Point of measurement*</th>
<th>Homologues</th>
<th>Non-homologues</th>
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<td>L</td>
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</tr>
<tr>
<td></td>
<td>S</td>
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<td>0</td>
</tr>
<tr>
<td>M</td>
<td>C</td>
<td>1</td>
<td>0</td>
</tr>
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<td></td>
<td>L</td>
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<td></td>
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<tr>
<td></td>
<td>S</td>
<td>6</td>
<td>0</td>
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</tbody>
</table>

* C - centromere
  L - long telomere
  S - short telomere
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<td>8-10</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>F_S</td>
<td>10-6</td>
</tr>
<tr>
<td>G_C</td>
<td>6-4</td>
</tr>
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<td>I_C, I_L, I_S</td>
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<td>K_L</td>
<td>10-7, 10-4, 9-4, 3-6</td>
</tr>
</tbody>
</table>

* L - long arm telomeres  
C - centromeres  
S - short telomeres
and the chromosomes 5, 6 and 3.

It is interesting to note that Douglas (1972) has shown that the replication patterns of chromosomes 3 and 5 were altered in the presence of k10/K10. Chromosomes 3 and 5 also demonstrate preferential segregation during meiosis in the presence of K10 if one of the chromosomes 3 or 5 is knobbed (Rhoades (1959), p. 57; (1965), p. 89).

One of the effects of K10 (Stock F) on chromosome 6 includes the demonstration that the 6's are more associated in presence of K10 than they are in the absence of K10 (Figure 4.6). The analysis of non-homologous chromosome associations (Stock F, Table 4.27) showed that the 2 chromosomes 6 and the 2 chromosomes 10 (K10 and k10) were spatially associated. Stock K, the translocation homozygote (Table 4.27) also contributed to the observed associations of non-homologous chromosomes. The only non-homologous associations that were noted between the interchange chromosomes 9 and 10 were observed between the short arms of each of the interchange chromosomes; precisely and unequivocably the rearranged arms. This observed non-homologous association of the interchange arms may reflect a mechanism for homologue recognition carried over from the translocation heterozygote state.

If the chromosomes are fixed in arrangement on the nuclear membrane then the presence of a translocation heterozygote should alter the relative spatial inter-
relationships of the non-homologous chromosomes involved in the interchange. In order to test this hypothesis, we selected the translocation heterozygote T2-8. (The interchange Stock J is diagramed, Figure 3.1). It should be emphasized that all 4 chromosomes of the interchange are readily indentified at metaphase. Reference to Table 4.21, Stock J shows a changed association pattern among homologues, especially the loss of association noted between chromosome 2 and T2. No non-homologous associations were found.

The choice of the T2-8 translocation may have been fortuitous one because of the length of the interchange chromosome 2. The additional length of chromosome 2L might allow 2L to associate with the interchange chromosome 8 from almost anywhere in the nucleus. The hypothesis should be tested further, perhaps with T9-10b (T/N)translocation heterozygote.

In summary, the association of homologous chromosomes is more frequent than that observed between non-homologous chromosomes. However, not all the chromosomes of maize are associated with their homologues at metaphase. Those that do associate, do so in a predictable manner, herein called a 'normal association pattern'. Chromosomes which are not associated at metaphase nevertheless may be associated during interphase as was shown for K10.
4.3 Cytological Observations of Mitosis in Maize:

Observations of non-arrested, fixed and squashed root tips lead to the conclusion that the metaphase plate (Plate 4.4c) was the same length as the diameter of the prophase and interphase nuclei. Measurements were taken of cells at a standardized magnification and the results are presented in Table 4.28. The mensuration data confirmed the cytological observation that the interphase and prophase nuclei and the metaphase plate are the same size. This has important implications for the movements of the chromosomes from prophase to metaphase as will be developed in Chapter 5.

Additional information of fixed and squashed material indicated that the chromosomes of maize apparently moved during prophase from the pole to pole orientation seen in Plate 4.5;a, through the 'middle' prophase orientation seen in Plate 4.5;b, to an equatorial position seen in Plate 4.6;a. The polarized structuring of the maize nucleus could also be seen at early prophase (Plate 4.6;b). A close association of the terminal knob regions (arrows) of some chromosomes with the nuclear membrane can also be seen in Plate 4.6;b.

Roots were fixed in glutaraldehyde-osmium for 3 hrs. and embedded in araldite-epon 812 mixture following the procedure of section 3.28 with the exception that no cycloheximide pretreatment was used.

Observations of sectioned material on a Phillips
PLATE 4.4

The cell cycle in root-tip cells of maize.

a. Interphase nucleus
b. Prophase (middle)
c. Metaphase
d. Anaphase
e. Telophase (early)
f. Telophase (late)
<table>
<thead>
<tr>
<th>Stage</th>
<th>Mean (μm)</th>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td>15.4</td>
<td>±2.1</td>
<td>200</td>
</tr>
<tr>
<td>Prophase</td>
<td>15.8</td>
<td>±1.8</td>
<td>200</td>
</tr>
<tr>
<td>Metaphase</td>
<td>15.2</td>
<td>±1.5</td>
<td>200</td>
</tr>
<tr>
<td>Early Telophase</td>
<td>14.7</td>
<td>±2.9</td>
<td>50</td>
</tr>
<tr>
<td>Telophase</td>
<td>10.1</td>
<td>±1.1</td>
<td>50</td>
</tr>
</tbody>
</table>
PLATE 4.5

Early prophase of maize somatic cell.
a. and b.; prophase showing the polar orientation of the chromosomes.
c. and d.; prophase showing the association of the chromosomes with the nuclear boundaries.
PLATE 4.6

a. the equatorial arrangement of somatic prophase chromosomes in maize. The association of the centromereic regions of some chromosomes with the nuclear boundary is shown.

b. the polarized arrangement of an early prophase nucleus is shown. The arrows indicate areas of close association of the terminal knobs of four chromosomes with the nuclear membrane.
EM-75 electron microscope showed that the chromatin material was attached to the nuclear membrane during early prophase (Plate 4.7a, b). An apparent telomere attachment of a chromosome to the nuclear membrane (arrow) can also be seen in Plate 4.7b.

Observations of 'normal' metaphase figures such as seen in Plate 4.8a, made it apparent that chromosomes of maize were arranged in a linear fashion at metaphase.

Approximately 5% (Table 4.29) of the observed metaphase figures (control) were polar metaphases similar to that shown in Plate 4.8c.

Root-tips of Seneca-60 which had been treated with cycloheximide (0.02%) for 15 minutes, grown at 27° C for 40 minutes and fixed and embedded according to the protocol of section 3.28. The protocol ensured that only late G2 and early prophase cells were being treated.

Observations of sectioned material with the electron microscope indicated that the nuclear membrane had not broken down by the time the metaphase plate had formed. Normally the chromosomes detach from the nuclear membrane coincident with the fragmentation of the nuclear membrane during prometaphase (Bajer and Malé-Bajer 1969). The presence of the nuclear membrane at metaphase in the cycloheximide treated cells was coincident with an increase (9X) in the frequency of polar metaphases scored (Table 4.29). Examples of the polar metaphases observed are
PLATE 4.7

Electron micrographs of maize mitotic nuclei.

a. Early prophase showing chromosome material associated with the nuclear membrane.
b. Middle prophase with the chromosomes still attached to the nuclear membrane. An apparent telomere attachment is shown by the arrow.
c. and d. Sections through a mitotic plate after treatment with 0.02% cycloheximide for 15 minutes. The nuclear membrane is still intact as a nuclear-cytoplasmic surface but is not clearly defined in these preparations.
PLATE 4.8

Metaphase plate configurations observed in normal and cycloheximide (0.02%) treated root-tips of Seneca-60.

a. normal metaphase from an untreated root.
b. anaphase in an untreated root-tip of corn. The squashing force was oriented so that the two groups of daughter chromosomes have been flattened from one end.
c.,d., and e.; Examples of the polar metaphase plates from cycloheximide treated roots.
f. A normal linear and a polar metaphase from Seneca-60 roots treated with cycloheximide (0.02%) for 15 minutes.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cycloheximide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Frequency</td>
<td>513</td>
<td>318</td>
</tr>
<tr>
<td>Uncertain</td>
<td>78</td>
<td>42</td>
</tr>
<tr>
<td>Polar Frequency</td>
<td>21</td>
<td>252</td>
</tr>
<tr>
<td>Percent Normal</td>
<td>83.6</td>
<td>51.9</td>
</tr>
<tr>
<td>Percent Uncertain</td>
<td>12.0</td>
<td>6.8</td>
</tr>
<tr>
<td>Percent Polar</td>
<td>4.4</td>
<td>41.3</td>
</tr>
</tbody>
</table>
given in Plate 4.8; c, d and e.

Infrequently, our observations of Stock C showed an unusual anaphase configuration. This is shown in Plate 4.8; b. A tangential squashing force applied to the pole of an anaphase cell has permitted the 2 sets of anaphase chromosomes to be viewed from one pole. The similarity of configuration of the normal untreated anaphase (Plate 4.8; b) to the induced (0.02% cycloheximide) polar metaphases of the same Plate 4.8; c, d and e should be noted.

The radiating-arm configuration of anaphase (Plate 4.8; b) with the close coalignment of some of the chromosome arms is similar to the anaphase configuration noted by Boss (1954) in the anaphase of the newt.

Plate 4.9; a to d presents 4 cycloheximide treated mitotic cells demonstrating a polar metaphase configuration. The overall chromosome configuration of each polar figure is strikingly similar. Where individual chromosomes could be identified they were. It is apparent that some somatic association of the chromosomes is present, e.g. the association of the satellited chromosomes 6 in Plate 4.9; b and c. This homologue association of the somatic chromosomes most likely occurred at anaphase when the radial arm configuration was established.
PLATE 4.9
Apparent homologue pairing in somatic cells of maize following treatment with cycloheximide (0.02%) for 15 minutes.
CHAPTER 5

DISCUSSION

5.1 Somatic Association:

The majority of the studies of the non-random distribution of chromosomes at metaphase have been done with cultured mammalian cell preparations. These studies all used colchicine as the antimitotic agent. Yet, Avivi et al (1969) in wheat, Cohen et al (1972) in muntjac and Heddle (personal communication) in humans found no somatic association of the chromosomes after the use of colchicine as a spindle disrupting agent.

In this study, we have shown that the use of the chelating type of spindle disrupting agents such as monobromonapthalene and 8-hydroxyquinoline resulted in a random association of the chromosomes at metaphase. This observation is similar to the report by Avivi et al (1969) in which colchicine was used as the antimitotic agent.

Studies on the somatic association of chromosomes which employed colchicine as the antimitotic agent reported the random association of all metaphase chromosomes

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other than those within the D and G groups (Merrington and Penrose (1966), Cohen and Shaw (1967), Galparin (1968) and Heddle (personal communication)). The lack of observed associations may really be an experimental artifact induced by the use of colchicine.

The somatic association of the satellited nucleolus organizer (NO) chromosomes, the D and G group chromosomes in the human karyotype is well documented even after the use of colchicine (Ferguson-Smith and Handmaker (1961, 1963), Shaw (1961), Cohen and Shaw (1967) and Galperin (1968)). Nankin (1970) showed that the frequency of association of these NO chromosomes in cultured human cell lines was correlated with the frequency of fusion of the nucleoli observed at interphase. Nankin found that up to 89% of the interphase nuclei showed a nucleolar fusion within 24 hrs. of culturing the cells.

Morgan (1971) reported only 0.02% nucleolar fusions at interphase in maize and Darvey and Driscoll (1972) reported 57% nucleolar fusions in wheat. Miles (1968) and Palmer (1971) in maize and Darvey and Driscoll (1972a) in wheat showed that the satellited NO chromosomes of these organisms were not associated at metaphase in somatic cells. Our study clearly confirms and places on a much more rigorous basis the claim that a random association of the satellited chromosomes 6 exists even in the presence of cold arrest.
The most probable cause of the observed somatic associations of the human satellited chromosomes is their participation in a NO function followed by a high frequency of nucleolar fusions in culture. When the frequency of observed nucleolar fusions is lower as it is in non-cultured cell systems such as a maize or wheat root-tip, the satellited chromosomes are randomly associated. It is logical, then, to discriminate between association caused by NO fusion, and association apparently representative of programmed pattern in the interphase nucleus.

Studies which used cold treatment to arrest at metaphase such as Feldman et al (1966a), Avivi et al (1969), Horn (1969) and Sadasiviah et al (1969) found that chromosomes were associated with their homologous partners at metaphase. Heneen and Nichols (1972) found the pair by pair somatic association of the 3 homologous pairs of chromosomes in the muntjac in polar metaphase preparations. No antimitotic agent was used.

The study presented here examined all the possible associations of all homologous and non-homologous chromosome combinations. Analyses of this magnitude have not previously been performed in another experimental system. Chromosomes were found to be non-randomly associated with their homologues and randomly associated with non-homologues. Less than 5% of the associations between non-homologues were statistically significant deviations (p<0.05). The
non-homologous associations most frequently involved aberrant or interchange chromosomes inserted into the normal complement.

Feldman et al (1966), and Avivi et al (1969, 1970) extrapolated the results of their studies, based on a series of observations of the distance distributions found between several abnormal (X-ray synthesized telocentrics) chromosomes, to suggest that all the chromosomes of wheat were more closely associated with their homologues than with non-homologous chromosomes.

Our study has shown that not all the metaphase chromosomes of maize are associated with their homologous chromosomes to the same extent (Table 4.19). In particular, chromosomes 10, 2, 7, 3 and 4 are significantly more associated with their homologues than are chromosomes 9, 6, 5, 8 and 1. The same chromosomes were associated not only within a stock but between the 4 different normal stocks (Table 4.19).

We have presented evidence for the existence of a mechanism which will dissociate 2 closely associated interphase chromosomes (K10) during prophase. This mechanism must separate the homologues of a chromosomes pair sufficiently so that they may appear non-randomly distributed at metaphase (Figure 4.4). The same mechanism must have the capacity to reassociate the 2 homologous chromosomes by the next anaphase prior to attachment to
the nuclear membrane.

A mechanism similar to that operating with K10/K10 could also function in the case of other chromosomes which do not show somatic association at metaphase. Maguire (1967), based on observations of the heteropy-knotic knob regions of chromosomes in maize (KYS) stock, suggested that all homologous chromosomes were associated pairwise during interphase. The association of the knobs at interphase, as confirmed by the evidence presented in Plate 4.3, indicated that the knobs and thus the chromosomes, were not fused but coaligned. These observations support the suggestion of the more universal action of the association dissociation phenomenon at metaphase as demonstrated in this study with stock G.

Our cytological observations of the chromosomes at anaphase and in cycloheximide induced polar metaphases indicated that the chromosomes were oriented in a manner such that all the centromeres were attached to one pole of the nuclear membrane. When the centromeres were prevented from moving from this polar configuration as they normally do during early to middle prophase (Persson 1953), a high frequency of apparent homologue pairing was observed. We conclude that this is additional evidence that all of the chromosomes are coaligned with their homologue during somatic interphase.

It has been proposed by Sved (1966) and Comings
that the chromosomes are ordered in the nucleus in such a manner that the homologous chromosomes are attached to the nuclear membrane at specific, genetically determined sites of attachment. These sites are presumably limited in number and specific for each chromosome. In maize, there would be a presumed requirement for a minimum of 10 such unique sites. These sites would permit homologues to coalign as they must attach to these sites in one area of the nuclear membrane during telophase or early interphase.

The release of the chromosomes from the nuclear membrane during prophase could allow the chromosomes to become randomized with respect to their homologues at prophase. While our results are compatible with this postulate in as much as no somatic association of chromosomes with their homologues was observed at prometaphase, Stock D, Table 4.11, it is difficult to rationalize the specific site model of chromosome attachment with all of the experimental observation of this study.

5.2 A Limited Number of Non-specific Sites for Chromosome Attachment to the Nuclear Membrane

If the chromosomes of maize are spacially fixed in homologous pairs to the inner lamella of nuclear membrane during interphase, the problem of how the homologues are paired up prior to attachment of the chromosomes to the nuclear membrane must be approached.

Sved (1966) and Comings (1968) have proposed that
the inner lamella of the nuclear membrane has a number of sites of chromosome attachment. Some fraction of these sites on a particular area of the NM would be specific for the centromeres and telomeres of each unique chromosome in the complement. Recognition of these unique sites by a specific chromosome prior to the chromosome's attachment to the nuclear membrane would allow the association of homologous chromosomes. Specific membrane sites unique for each chromosome, and a means of chromosome recognition of its own particular sites are necessary for this scheme of chromosome ordering in the nucleus. Chromosome recognition of its own particular sites of membrane attachment, resulting in the association of homologous chromosomes, must obviously occur after the synthesis of the daughter cell nuclear membrane is complete.

Sved (1966) and Comings (1968) also proposed that the coalignment of homologous chromosomes in the somatic interphase nucleus is a necessary prerequisite for the meiotic pairing of homologous chromosomes without a high frequency of interlocking bivalents and tangled chromosomes.

If the somatic association of homologous chromosomes at metaphase is a reflection of the homologues association during interphase, and if the somatic interphase association of homologous chromosomes is important
for the meiotic pairing of chromosomes, then the addition of a third homologue of a chromosome as a primary trisomic stock, will have a predictable result. That is the third homologue of a trisomic will also show somatic association with its other two homologous chromosomes at metaphase as a reflection of the attachment of all three homologous chromosomes (to a common area of the nuclear membrane) during interphase.

If all the chromosomes of the maize complement have specific attachment sites to the nuclear membrane, then these specific sites probably exist in excess for each unique chromosome in order to accommodate the third homologue of a trisomic stock. These additional sites should be in proximity to the two homologous chromosomes of the disomic state. As these 'extra' sites presumably exist for all ten chromosomes of the maize complement if they exist for one, these 'extra' specific sites must be spread over some additional area of membrane above that required in the disomic state, in order to accommodate a trisomic chromosome on the membrane. The trisomic state of a complement should be accommodated in a nucleus without affecting the spacial interrelationships of the other chromosomes on the nuclear membrane particularly if positions of the attachment sites of the chromosomes were already determined on the membrane prior to chromosome attachment to the nuclear membrane.

The results of the addition of an extra chromo-
some 2 in Stock I, showed that the three homologues were closely associated (disomic $\bar{x} = .334$, trisomic $\bar{x} = .327$, Table 4.3).

This discrete, critical cytogenetic test suggests strongly that the chromosomes 2 were attached in the same region of the nuclear membrane and presumably, as argued earlier, were associated during interphase. This result is in accordance with a 'specific site' model in which a surplus of sites exists in each area reserved for a unique chromosome in this case, chromosome 2.

The addition of the extra chromosome should have in no way affected the spacial relationships observed between other non-homologous chromosomes attached to their specific areas of the membrane 'buffered' by an excess number of attachment sites, if the 'specific site' model is valid. From the results tabulated in Table 4.3 and Table 4.7 for Stock I (compare to control stock B) it may be seen the addition of the extra chromosome has resulted in the crowding of the rest of the chromosomes into a more confined area. This crowding was quantified in the newly observed somatic association of chromosomes as 1, 5, 6 and 9 (Table 4.21 stock I; Figures 4.8, 4.9) which were not associated in the disomic 2 state (Table 4.19). No increase in non-homologous associations were recorded (Table 4.26) for the trisomic stock I.

This crowding or compensation effect will be at
a maximum if the chromosomes reassociate with their homologues at anaphase prior to the attachment of the chromosomes to the membrane at telophase as the nuclear envelope is at a minimum in size and just encloses the chromosomes (Roth 1967, Whaley et al. 1960). The 'specific site' model necessitates that the ordering of the nucleus occurs after the nuclear membrane (NM) is formed at telophase. Electronmicrographs of the telophase nuclei of maize (Whaley et al. 1960) clearly shows chromosome association with the NM at or before the time of the synthesis of the new nuclear membrane. Therefore, chromosome organization has to take place during anaphase before the nuclear membrane is reformed at telophase.

The observation that this crowding effect of the addition of an additional chromosome in stock I increases the association of other chromosomes only with their homologues is strong evidence that the chromosomes of maize are already ordered into homologous pairs at anaphase prior to attachment to the nuclear membrane. A similar cytological observation was made by Boss (1954) at anaphase in the newt.

These results have also indicated that the sites of chromosome attachment to the nuclear membrane are non-specific. If the chromosomes were sited or attached to specific areas only, then the addition of an extra chromosome should not have affected the association of many of the other non-homologous chromosomes. We would propose
that the recorded association (Table 4.21) between homologues of chromosomes other than chromosome 2 in a trisomic 2 stock, indicated that the ordering of the nucleus took place at anaphase when the chromosomes were crowded together and before the chromosomes were attached to the nuclear membrane. We consider this evidence critical for the projection of a concept of an ordered nucleus, in as much as it renders the specific site concept invalid. Thus the concept of specific sites of chromosome attachment is not relevant to the development of a model of the ordered nucleus in maize.

5.3 A Model for an Organized Nucleus:

Observations of anaphase nuclei showed that the chromosomes are orientated in a polar fashion. In Plate 4.5 it can be seen that the centromeres of the chromosomes are orientated towards the equator of the dividing cell and the centromeres of each group of daughter chromosomes are orientated toward one spindle pole. As the nucleus progresses into late anaphase and early telophase the centromeres are pulled into close proximity by the spindle. The chromosomes attach to the nuclear membrane at this time (telophase) (Roth 1967, Whaley et al 1960).

Vanderlyn (1948) and Person (1953) in Allium cepa observed that subsequent cell divisions were orientated along the axis of polarization of the nucleus. In our
material, the chromosomes are polarized in the nucleus at early prophase (Plate 4.5, Figure 5.2A). As the nuclear cycle proceeds from prophase to prometaphase, the centromeres of the chromosomes separate from the centromere 'knot' and move to an equatorial position (plate 4.5a and b, Figure 5.1). This phenomenon of centromere movement during prophase was also proposed by Person (1953) in Allium cepa root tips and by Moens (1969) in Locusta migratoria spermatocytes.

Treatment of late G₂ and early prophase cells of maize with 0.02% cycloheximide blocked the movement of the chromosomes from the polar centromere knot to an equatorial position (Table 4.29, Plate 4.8). As the manufacture of new membrane to move the centromeres may involve protein synthesis, which cycloheximide blocks, I would propose that the centromere movement from the polar region to the equatorial position is being blocked by the addition of cycloheximide.

If the chromosomes retain the centromere knot configuration through to metaphase, a polar metaphase results. In this latter configuration, chromosomes which did not show somatic association at normal metaphase such as the satellited chromosomes 6, are associated (Plate 4.9). Clearly, this association must have been accomplished before the chromosomes were attached to the nuclear membrane during the previous telophase.
Fig. 5.1

A drawing to show the movement of the centromeres along the nuclear membrane in somatic cells of maize.

A. Early prophase.
B. Middle prophase.
C. Prometaphase.
Fig. 5.2

An hypothetical cell cycle in a 3 chromosome organism showing how an organized nucleus similar to that of maize can be divided and still remain ordered.

A. The polarized nucleus of interphase and early prophase. Note that homologous chromosomes are paired at least at the centromere regions.

B. Middle prophase. The homologous pairs of chromosomes have moved apart somewhat as the centromeres migrate along the nuclear membrane.

C. Prometaphase. The chromosomes have moved to an equatorial position. Homologous chromosomes are sufficiently separated as to appear randomly associated.

D. Metaphase. Somatic association of one pair of chromosomes (solid) can be seen.

E. Anaphase. Reassociation of homologues is occurring as the centromeres compact together to fit the smaller area of the anaphase pole.

F. The two resultant daughter nuclei. Both show identical organization. This organization is also identical to that of Fig. 5.2A. Note that homologous chromosomes are paired again.
Normally, the chromosomes move apart from their homologues as the chromosomes move from the polar centromere knot configuration of early prophase, to an equatorial position at prometaphase. This movement is recorded as the loss of somatic association observed in Stock D at prometaphase (Table 4.11, Figure 4.5). This loss of association of homologues at prometaphase presents an enigma. If one postulates that all the chromosomes are associated with their homologues during interphase, and by observation at least some of the chromosomes show homologue associations at metaphase, then why is there no association observed between homologues at prometaphase?

The enigma is resolved by considering the restrictions on the movements of the chromosomes from interphase to metaphase as diagramed in figure 5.2. The movement of the centromeres from the polar knot configuration during early prophase to the equatorial position during prometaphase will result in an apparent loss of association of homologues. This due to the 'spreading out' of the distribution of centromeres as the greater area of the equatorial configuration permits (Plate 4.6a). Thus the apparent enigma can be resolved and becomes a predicted event by the model presented in Figure 5.2.

The somatic association of certain chromosomes with their homologues is a reflection of a fixed axis of orientation of the metaphase plate formed in 1 dimension (linear) from a previously 2 dimensional (circular)
structuring of the nucleus at prometaphase. The association of homologues at somatic metaphase may be coincident with a pair by pair association of chromosomes with their homologues during interphase but is not a direct reflection of this co-alignment. The observed somatic association of homologues reflects rather the events of division of an ordered nucleus.

Because of the equatorial alignment of the centromeres of the chromosomes around the edge of the nucleus and midway between the 2 spindle poles as proposed diagrammatically in Figure 5.1c (see also Plate 4.4b), the chromosomes can only move laterally to form the metaphase plate. Cytologically this movement appears as a migration of the chromosomes to the center of the nucleus. As the length of the metaphase plate is virtually identical (Table 4.28), the diameter of the prophase nucleus, the paths of centromere movement to form the plate must be parallel to one another and perpendicular to the long axis of the metaphase plate.

The movement of the chromosomes to the anaphase pole will result in the chromosomes being telescoped back along their axis of movement to the metaphase plate. The diameter of the anaphase group of chromosomes is "less" than the length of the metaphase plate (Table 4.28) and therefore the chromosomes must be telescoped "sideways" to allow the reduction in length of the chromatin mass to
come about. This "squeezing" will restore the prometa-
phase chromosome ordering of the centromeres of the
chromosomes relative to one another if the chromosomes
are displaced back along the lines along which they came
to form the metaphase plate.

The drawing together of the centromeres of the
chromosomes at late anaphase is mechanistically dif-
ferent and functionally opposite to the separation of the
centromeres observed through prophase. Clearly, anaphase
movement must be (to fit our model) an ordered, programed
movement to yield a pattern in which the chromosomes
would be paired up again by their homologous centromeres.
Homologous telomeres would be in proximity as well but
at the opposite nuclear pole to the centromeres.

The movements described above are diagramed in
Figure 5.2 in a hypothetical 3 chromosome organism.

When the movements of the chromosomes are dia-
gramed through one cell division as in Figure 5.2 it is
apparent that certain chromosomes such as chromosome 3,
are associated at metaphase with their homologous chromo-
some. The other chromosomes 1 and 2 are not. This type
of mechanism can account for the fact that not all chromo-
somes are associated with their homologues at metaphase.
In other terms, this is a mechanism for the dissociation
and reassociation of chromosomes observed in this study.
The reassociation of the chromosomes at anaphase prior to
chromosome attachment to the nuclear membrane may occur
simply as a consequence of the "squeezing down" of the
length of the metaphase plate to fit the anaphase config-
uration as the centromeres aggregate at the spindle pole.

A consequence of the type of organization described
above is that no specific sites or recognition factors
are required to coalign the chromosomes during interphase.
The chromosomes would remain in the polarized and paired
anaphase configuration throughout interphase to the next
prophase when the chromosomes are again separated prior
to metaphase, by the centromere migration previously
outlined.

In conclusion, then, we would propose that the
chromosomes of maize are spatially organized throughout
the nuclear cycle, with each classical cytogenetic 'stage'
of interphase, prophase, metaphase, anaphase and telophase
having its own discrete but interrelated chromosome spat-
ial organization.
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