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THE INFLUENCE OF B CHROMOSOMES ON THE SUSCEPTIBILITY OF MAIZE TO GAMMA IRRADIATION INDUCED DNA DAMAGE

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THE INFLUENCE OF B CHROMOSOMES ON THE SUSCEPTIBILITY OF MAIZE TO GAMMA IRRADIATION INDUCED DNA DAMAGE

by

Rick Walter Staub

A Dissertation Submitted to the Faculty of the

COMMITTEE ON GENETICS (GRADUATE)

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Rick Walter Staub

entitled The Influence of B Chromosomes on the Susceptibility of Maize to Gamma Irradiation Induced DNA Damage

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Carol Bernstein	March 23, 1984
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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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SIGNED: Rick Walter Staub

This manuscript is dedicated to

Dr. Robert M. Harris

on the occasion of his retirement

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ABSTRACT

Tests were conducted to ascertain whether B chromosomes influence the susceptibility of maize (Zea mays L.) plants to gamma-radiation-induced DNA damage. Isogenic stocks of Black Mexican sweet corn with and without B chromosomes were premeiotically irradiated and DNA damage was assayed by measuring pollen viability.

Higher pollen viabilities relative to non-irradiated control plants were consistently obtained in irradiated plants with B chromosomes when compared to irradiated plants without B's. Furthermore, among plants irradiated with 1250R those with one B chromosome produced the greatest proportion of viable pollen and plants with increasing numbers of B's produced progressively less viable pollen.

An exophenotypic trait elicited by B chromosomes is also reported. Plants with 5 or more B chromosomes often display an aberrant phenotype characterized by longitudinal white leaf stripes and/or narrow leaves. This phenotype intensifies with increasing numbers of B chromosomes and is the first case of a qualitative exophenotypic trait attributable to B chromosomes reported in maize.

INTRODUCTION

The B chromosome of maize has been shown to increase genetic recombination in A chromosomes in a quantitative manner (Ayonoadu and Rees 1968; Rhoades 1968; Hanson 1969; Melnyczenko 1970; Nel 1973; Chang and Kikudome 1974). The presence of B chromosomes also alters the metabolism of the nucleus in a quantitative manner by increasing histone, RNA, and non-histone protein content (Ayonoadu and Rees 1971). In light of these B chromosome influences, it is reasonable to question whether B chromosomes may also increase recombinational repair of DNA damage.

It has been demonstrated in a number of organisms including bacteria, viruses, fungi, Drosophila and Chinese hamster ovary cells, that the ability to undergo homologous pairing and recombination decreases susceptibility to DNA damaging agents (see Bernstein 1983 for review). In fact, it has been suggested that systems of homologous DNA pairing may have evolved primarily as a consequence of the selective advantage of a DNA repair process (Bernstein, Byers, and Michod 1981).

The experiments described herein were designed to determine whether such a recombinational repair mechanism may exist in maize and whether B chromosomes have any effect on this mechanism. Ionizing radiation is utilized as the DNA

damaging agent because it has the potential to produce double-strand lesions. These allegedly could only be repaired by a recombinational repair process which synthesizes a new double-strand segment to replace the damaged segment by utilizing a homologous non-damaged region as a template.

If a recombinational repair process does not exist in maize, then cells with and without B chromosomes should be equally susceptible to gamma rays unless B chromosomes can alter the susceptibility in some other manner. If a recombinational repair process does exist, B chromosomes would be expected to stimulate this process and cells containing B's should be less susceptible to gamma ray damage. Since genetic recombination is increased in a quantitative fashion with increasing numbers of B chromosomes, recombinational repair of DNA damage would be expected to show the same trend.

If there truly is a recombinational repair process in maize and B chromosomes have a positive influence on it, the presence of B chromosomes in an individual would improve reproductive fitness in any environment in which DNA damaging agents are present. This would provide an additional reason for the maintenance of B chromosomes in natural populations.

LITERATURE REVIEW

B Chromosomes

B chromosomes (also called accessory or supernumerary chromosomes) are chromosomes which a) are found in some but not all members of a population and therefore are not necessary for normal development of an organism; and b) are not homologous to the normal members of the chromosome complement and therefore do not pair with them in meiosis (Pearse and Ehrlich 1979). The term B chromosome was first used to describe the common maize supernumerary and distinguish it from the 10 normal A chromosomes (Randolph 1928). A compilation of species in which B chromosomes have been found lists over 1000 plant species and more than 260 animal species (Jones and Rees 1982). B chromosomes are particularly abundant in the flowering plants and insects, but Rees (1974) warns that this is most likely a reflection of the fact that they have been cytologically studied to a larger extent.

In corn, B chromosomes were first described by Kuwada (1915). Their cytological morphology at mitotic metaphase and meiotic mid-prophase has been described in detail (Randolph 1941; McClintock 1933). B chromosomes appear telocentric in root-tip metaphase cells and are

approximately two-thirds the length of the smallest A chromosome (Fig. 1a). They contain 70% more DNA, however, than the average A chromosome (Ayonoadu and Rees 1971). In meiotic pachytene preparations they are also shorter than any A chromosome and are largely heterochromatic. At this stage it is apparent that the B chromosome is not actually telocentric. A very short heterochromatic segment comprises the small arm of the chromosome (Fig. 1 b and c). A larger heterochromatic segment flanks the centromere on the other side, followed by a euchromatic segment containing 6 chromomeres, a large heterochromatic segment, and a distal euchromatic tip.

Accumulation Mechanisms

In organisms in which B chromosomes have been studied, a common motif is accumulation of B's. That is to say, progeny of individuals possessing B's often have a mean B chromosome content which is greater than the parental mean. The actual accumulation mechanisms are understood in over 25 plant species and generally include nondisjunction or preferential segregation or fertilization (Jones 1975).

Nondisjunction

The majority of nondisjunctional accumulation mechanisms involve mitotic divisions of the gametophyte. In rye, for example, nondisjunction of B's frequently occurs at

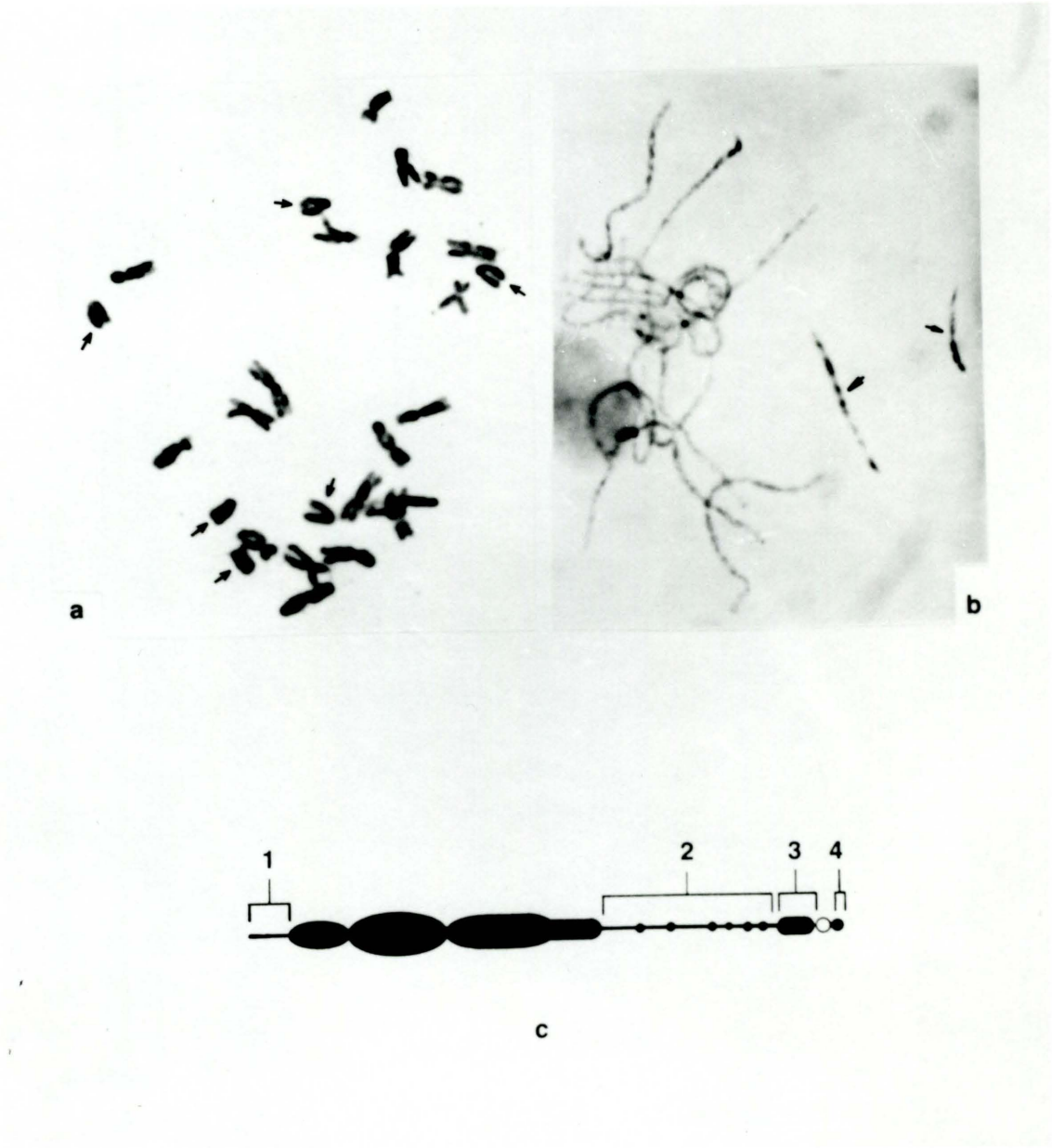


FIGURE 1. a) Mitotic metaphase spread of Black Mexican sweet corn with 6 B chromosomes (arrows).
b) Pachytene spread of Black Mexican sweet corn with 3 B chromosomes. Note that the univalent B chromosome (small arrow) is shorter than the B chromosome bivalent (large arrow).
c) B chromosome pachytene morphology indicating 4 regions influencing nondisjunction.

the first mitotic division of the pollen grain; furthermore, this is a directed nondisjunction in which the nondisjoined B's tend to be included in the generative nucleus (Hasegawa 1934). A similar accumulation mechanism is exhibited in female gametophytes of rye. A directed nondisjunction occurs in the developing embryo sac which accumulates B's in the egg nucleus (Hakansson 1948).

In maize, there is no accumulation mechanism for B chromosomes in female gametogenesis. On the male side however, it has been shown that nondisjunction of B chromosomes occurs at the second pollen mitosis (Roman 1947). In order to demonstrate this, Roman utilized plants homozygous for a reciprocal interchange betweeen the short arm of chromosome 4 and the long arm of the B chromosome as pollen parents. These plants contain the Su (non-sugary endosperm) allele on the segment of chromosome 4 which has been translocated to the B chromosome. These plants were used to pollinate su/su females and approximately half of the resultant kernels had sugary endosperms. Seedlings grown from sugary kernels all contained two B^{4Su} chromosomes as well as a normal 4 and a 4^{B}. Twenty-four non-sugary grains produced seedlings deficient for the B^{4Su} chromosome while three produced seedlings with one B^{4Su}. These data indicate that nondisjunction occurs during pollen development quite frequently (~88%). Furthermore, the non-correspondence of endosperm and embryo

with regard to the B^4 chromosome indicates that nondisjunction must occur during the division of the generative nucleus. It is assumed that normal B chromosomes exhibit identical nondisjunctional patterns as do B-A interchange chromosomes.

This nondisjunctional property of B-A interchange chromosomes has been exploited by maize geneticists as a means for locating recessive genes on a particular chromosome arm. For example, if one testcrosses a series of B-A translocation stocks as males to females possessing the recessive gene to be located, the stock which uncovers the recessive phenotype (due to pseudodominance) must possess, as part of the B^A interchange chromosome, the A chromosome segment on which the locus in question is situated.

There appear to be at least four factors within the maize B chromosome which influence its nondisjunction (Fig. 1c). Factor 1 is located in the distal euchromatic tip while factor 2 is located in the long interstitial euchromatic segment. These factors have a regulatory influence on nondisjunction which operates in a trans manner. Factor 3 is comprised of the heterochromatic segment which is adjacent to the centromere in the long arm and factor 4 consists of the small heterochromatic segment flanking the centromere in the short arm.

In his original study Roman (1947) determined that B^4 interchange chromosomes would disjoin normally at the second pollen mitosis unless the 4^B chromosome was present in the same generative nucleus. Thus he surmised a regulatory element must be located distal to the translocation breakpoint which was within the large heterochromatic block of the B chromosome. Factor 1 was more precisely mapped when Ward (1973a) obtained similar results with TB-8a in which the translocation breakpoint was located at the junction of the large heterochromatic segment and the distal euchromatic tip.

The existence of a second factor influencing nondisjunction in a trans manner was indicated by the isolation of mutant #1866 by treating TB-9b with ethylmethanesulfonate (Carlson, 1978a). In this mutant the proximal euchromatic region of the B^9 chromosome is deleted and the deficient B^9 disjoins normally. When a normal B chromosome is included in the same generative nucleus however, nondisjunctional ability is restored to the deficient B^9. This implies that a trans-acting regulatory element which stimulates B nondisjunction is located in the proximal euchromatic region of the B. Carlson points out that damage to the centromeric region cannot be excluded in mutant #1866. However, further evidence corroborating the existence of a regulatory factor in the proximal euchromatic region has been obtained by

ingenious experiments in which various 10^B translocations were combined with B^{10} interchange chromosomes of different origin (Lin 1978). If the breakpoint in the translocation from which the 10^B interchange chromosome is obtained is proximal to the breakpoint in the translocation from which the B^{10} is obtained, a deficiency for the region between the B chromosome breakpoints is created in the generative nucleus containing these two chromosomes. Lin found that some deletions which included the proximal euchromatic region of the B caused a loss of B nondisjunctional ability.

In rye, B chromosome nondisjunction is due to non-separation of heterochromatic regions flanking the centromere (Jones 1975). B chromosomes in several other plants exhibit similar nondisjunctional mechanisms (Lima de Faria 1976). Nondisjunction in maize at the division of the generative nucleus has not been observed in light microscope preparations due to interference from starch granules. Data indicate, however, that as in B chromosomes of other plants, the heterochromatic regions flanking the centromere serve to facilitate nondisjunction. Carlson and Chou (1981) studied nondisjunction in telocentric B^9 chromosomes which allegedly resulted from misdivision of a B^9 isochromosome. Two different types of telocentric B^9's were obtained and it was discovered that the B^9 isochromosome that produced them was really a

pseudo-isochromosome which lacked the proximal long arm heterochromatin region in one of its arms. Thus, one of the derived 9^B telocentrics contained this heterochromatin and the other lacked it. The one which lacked it was totally incapable of nondisjunction while the telocentric 9^B possessing this heterochromatic region underwent nondisjunction at variable rates. This indicates the necessity of a third nondisjunction factor in the proximal heterochromatin of the long arm of the B. Since the original isochromosome underwent nondisjunction at variable low rates as well as the telocentric 9^B containing factor 3, the existence of a fourth factor influencing nondisjunction is implied. The isolation of TB-10(18) (Lin 1979), in which the B^{10} chromosome is deficient only for the short arm of the B, and in which B^{10} nondisjunction occurs at a low frequency, provides additional evidence for the existence of a fourth factor in the short arm of the B.

Preferential Fertilization

Nondisjunction of B chromosomes at the second pollen mitosis does not alone provide for accumulation of B's in a population. A plant with 1 B chromosome would produce 1/2 zero-B microspores and 1/2 one-B microspores. If nondisjunction occurred at every generative nucleus division, the end result would be 3/4 zero-B sperm nuclei and 1/4 two-B sperm nuclei, resulting in an average of 1/2 B

per gamete. This is the same average that would be obtained if B's disjoined normally at the division of the generative nucleus. If B chromosomes are to accumulate in a population, plants or gametes containing B's must be more successful than those without B's.

Roman (1948) observed that sperm containing two B^A chromosomes fertilized the egg nucleus almost twice as frequently as their zero-B^A compatriots in pollen grains from TB-A plants. He suggested two explanations. First, it is possible that the B^A sister chromatids undergo a directed nondisjunction so that they tend to end up in the sperm which effects fertilization of the egg nucleus. Alternatively, the presence of a B^A (or B) chromosome could provide a sperm with a competitive advantage over those lacking B^A's (or B's).

In an analysis of root-tip chromosome counts from kernels produced on plants lacking B's pollinated by plants with more than one B chromosome, Catcheside (1956) determined that high B chromosome numbers resulted more frequently than would be expected based on random distribution of nondisjoined B's to either sperm nucleus. He therefore proposed that an oriented nondisjunction of B's occurs.

More recent experiments indicate that the alternative explanation is correct (Carlson 1969). Two different B^A interchange chromosomes present in the same

generative nucleus do not show a tendency to nondisjoin to the same pole during mitosis. Moreover, the competitive advantage of a sperm containing B^A chromosomes can be negated by the addition of several intact B chromosomes to the pollen parent.

Competitive Advantage of Pollen Grains Containing B Chromosomes

It seems that nondisjunction at the second pollen mitosis and preferential fertilization of the embryo by the sperm containing B chromosomes are not the only mechanisms utilized to accumulate B's in maize populations. Beckett (1982) has determined that pollen grains containing B^A interchange chromosomes achieve fertilization more frequently than do pollen grains lacking the B^A interchange. In crosses utilizing TB-1 heterozygotes crossed as males to a bz-2 tester stock, it is possible to identify hypoploid (for the B^A interchange) endosperms by their bronze aleurone since the bz-2 gene is distal to the translocation breakpoint in chromosome 1. Hypoploid embryos can be identified in the seedling stage by their distinctive leaf morphology. Beckett found the ratio of total hypoploids in either embryo or endosperm to normal embryos and endosperms to be approximately 54:42. His data indicate that A^BB^A pollen grains either germinate sooner or grow faster than normal pollen.

Whether this is due to factors on the B chromosome or gametophytic factors on the A chromosome segment has not yet been determined. If the competitive advantage is conferred by one or more factors on the B chromosome (which can be determined by utilizing other B-A translocations lacking various regions of the B), then this meiotic drive mechanism would also serve to accumulate B chromosomes in a population.

Effects of B Chromosomes

B chromosomes generally do not qualitatively affect the exophenotype of organisms in which they are found. Only two examples of this are documented. The presence of one B chromosome in Plantago coronopus causes complete male sterility (Paliwal and Hyde 1959) while the presence of a single B in Happlopappus gracilis changes the color of the achene from rust to purple (Jackson and Newmark 1960).

B chromosomes do, however, exhibit a quantitative influence on germination, growth and vigor, flowering time, fertility, and seed weight in various plants, and vigor, hatching, development, larval size, femur length, tibia length, and fertility in various insects (Jones and Rees 1982). The quantitative effects of B's can cause gross exophenotypic abnormalities when B's are present in high numbers.

Even when present in low numbers, however, B's have subtle influences on the metabolism of the host organism. Cell size and mass is increased in rye plants containing B chromosomes (Muntzing and Akdik 1948). Mitotic cycles are lengthened in Lolium, Zea, and Secale (Evans et al. 1972) and in pollen of Sorghum purpureo-sericium (Darlington and Thomas 1941) in the presence of B's. B chromosomes have been shown to influence nuclear metabolism in both rye (Kirk and Jones 1970) and maize (Ayonoadu and Rees 1971). Chiasma frequencies are increased in Festuca mairei, Listera ovata, Pennisetum typhoides, Puschkinia libanotica, Secale cereale, Zea mays, Melanoplus differentialis, Myrmeleotettix maculatus, and decreased in Aegilops speltoides and Lolium perenne (Jones 1975).

Effects of B chromosomes in maize are particularly interesting and pertinent to the research presented here and will be dealt with in greater depth.

Nuclear Metabolism

In microspectrophotometric studies of maize root-tip nuclei with varying numbers of B-chromosomes Ayonoadu and Rees (1971) found that nuclear dry mass, nucleolar dry mass, and histone, RNA, and protein contents were all increased quantitatively with increasing numbers of B's. That this increase was not due simply to structural components of the B chromosomes themselves is illustrated by the odd-even

effect obtained in the measurements. Measurements of all these nuclear parameters except histone content were disproportionately high for nuclei with even numbers of B's. Histone content was disproportionately high in nuclei with odd numbers of B's. This is in agreement with the supposition that histones tend to repress gene function (See Georgiev 1969 for review).

B chromosomes thus significantly alter the metabolism of maize nuclei. Why the effect on RNA and protein levels should be emphasized and the effect on histone levels de-emphasized in the presence of even numbers of B chromosomes remains a mystery.

High Loss Phenomenon

Besides undergoing a high rate of nondisjunction themselves, B chromosomes also cause nondisjunction or chromosome breakage of A chromosomes containing heterochromatic knobs (Rhoades, Dempsey, and Ghidoni 1967; Rhoades and Dempsey 1972). This phenomenon occurs in a particular stock at a very high rate and thus the stock is designated a "high loss" stock.

Rhoades and Dempsey determined that loss of A chromosomes or segments thereof occurs at the second pollen mitosis of spores with two or more B chromosomes. By substituting B chromosomes from another source (BMSC) they found that loss of A segments occurred at a decreased rate.

However by carrying out backcrosses to OB plants of the high loss stock, this rate was increased to that of the high loss stock or beyond. This indicates that B chromosomes in the high loss stock are no different than B chromosomes from other stocks, and that the ability of B chromosomes to cause loss of A chromosome segments is influenced by modifier genes on A chromosomes.

The phenomenon can be explained, according to Rhoades and Dempsey, by assuming that the gene in the distal euchromatic segment of the B chromosomes which is responsible for nondisjunction influences replication of heterochromatic regions. Faulty or late replication in the centromeric region of B chromosomes causes nondisjunction due to inhibition of proper centromere orientation. Faulty or late replication of a distal heterochromatic knob on an A chromosome however, causes sister-chromatid stickiness at this region but centromeres still separate to opposite poles resulting in a dicentric bridge and subsequent chromosome breakage.

Influence on Recombination

As mentioned earlier, B chromosomes influence chiasma frequencies in A chromosome bivalents of many species. Although chiasma frequencies can be decreased in the presence of B's in some species, most species studied show an increase in the presence of B's. Zea mays has been

found to be one of these species in which B chromosomes quantitatively increase A chromosome chiasma frequencies (Ayonoadu and Rees 1968).

Since the genetic map of maize is the most complete of any organism containing B chromosomes, it lends itself well to the study of B chromosome influence on recombination on a genetic as well as a cytological level. Several genetic studies have verified a significant influence of B's on recombination between marker genes on A chromosomes.

Recombination in the c-sh-wx region of chromosome 9 (Fig. 2) and the gl6-lg2-a1-et region of chromosome 3 is increased significantly in the presence of B chromosomes with multiple crossovers being increased particularly and in a quantitative manner according to B chromosome number (Hanson, 1969). The a2-bt-pr region of chromosome 5 also displays increased recombination in a quantitative manner in the presence of 1, 2 or 4 B chromosomes (Nel 1973). Nel also found that recombination was stimulated by B's to a greater extent in male than in female meiocytes. In addition, he found that recombination in the proximal wx-gl15 region of chromosome 9 was increased at the expense of the adjacent sh-wx region in male meiocytes with two B chromosomes.

The most dramatic B-chromosome-stimulated increase in recombination was reported by Rhoades (1968) for a segment of chromosome 3 which had been inserted into

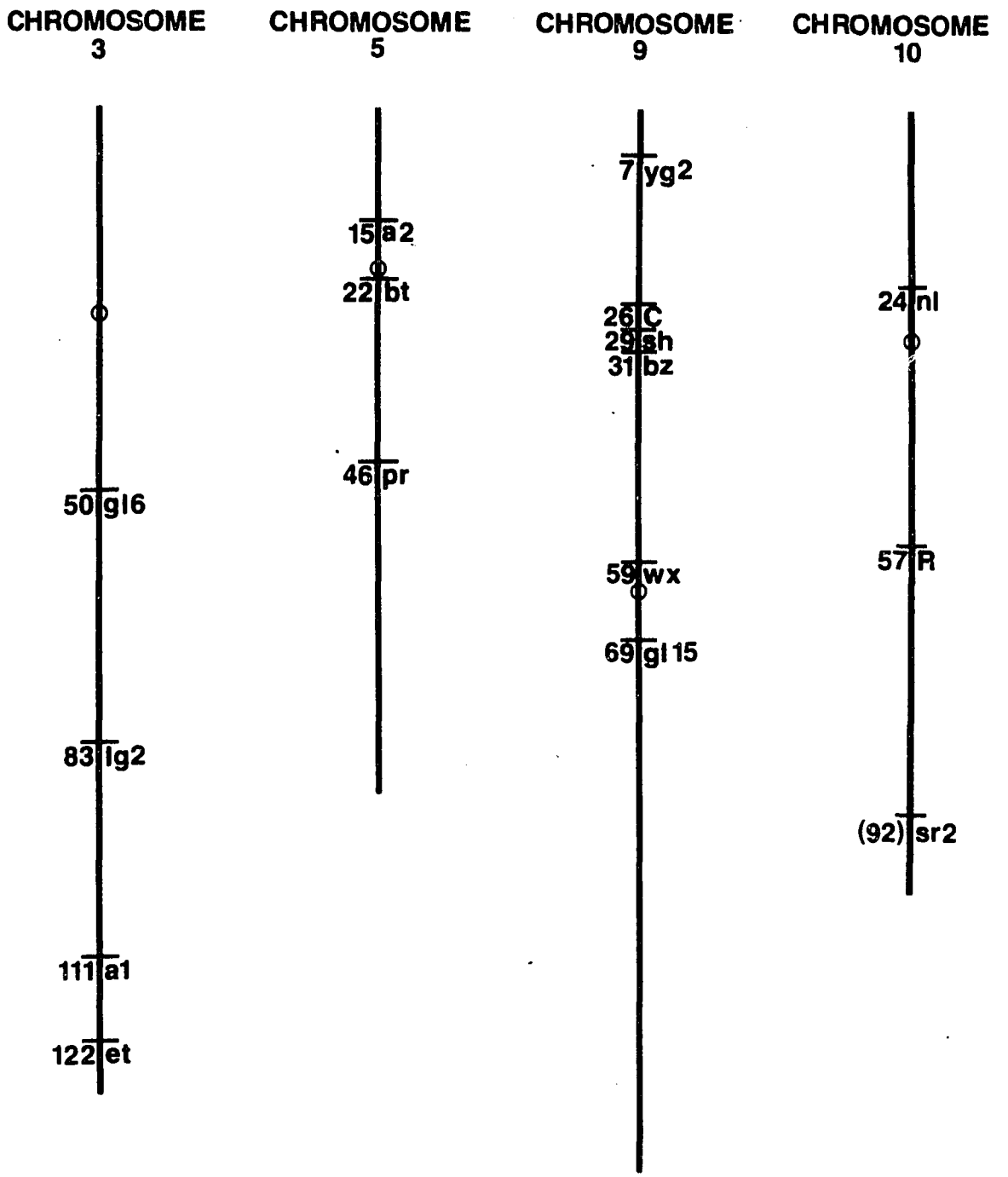


FIGURE 2. Chromosome map locations of loci discussed. () indicates tentative map position; o indicates centromere position.

chromosome 9 between the markers c and wx. In the homozygous condition, this transposition chromosome (Tp9) displayed no more recombination in the c-wx region than did the normal homozygote. In the presence of 1, 2, or 3 B's, however, the recombination rate in this region was more than doubled and a dosage effect was apparent.

These recombination studies by Hanson, Nel, and Rhoades indicate that recombination in regions proximal to the centromere of chromosome 9 may be disproportionately increased in the presence of B chromosomes. This genetic evidence was corroborated by a cytogenetic study by Ward (1976) in which plants with B's showed a greater proportion of proximal to distal chiasmata in A chromosome bivalents than plants without B's.

Chang and Kikudome (1974) subsequently investigated recombination in the yg-sh-bz-wx region of the short arm of chromosome 9 (Fig. 2) in plants which were homomorphic for a small terminal knob on this short arm as well as plants which were heteromorphic for a large and small knob on this short arm. The presence of the large knob significantly influences the manner in which B chromosomes alter recombination. In plants homomorphic for the small knob double recombinants increase at the expense of singles while in heteromorphs, double and single recombinants increase at the expense of non-recombinants. B chromosome influence on recombination was observed to a much greater extent in

megasporocytes than in microsporocytes. Furthermore, an odd-even effect was observed in that plants with odd numbers of B's displayed greater increases in recombination than did plants with even numbers of B's.

This odd-even effect of B chromosomes on recombination has also been observed in rye (Jones and Rees 1967) and Listera (Vosa and Barlow 1970). It has been suggested that activity of B chromosomes may be suppressed when they occur in pairs within a nucleus (Rhoades and Dempsey 1972). Since accumulation mechanisms in many organisms tend to produce progeny with even numbers of B's, Jones and Rees (1969) point out the temptation of suggesting that this means of transmission may be influenced by the differential effect of B's in odd as opposed to even numbers.

In addition to these studies on intergenic recombination, a study on intragenic recombination at the wx locus on chromosome 9 revealed a significant increase in the presence of 1 or 2 B chromosomes (Melnyczenko 1970).

It has been established by interspecies comparisons that the duration of meiosis is directly proportional to DNA content (Bennett 1971, 1977). One component of the B chromosome influence on recombination could, therefore, be increased duration of the meiotic division. B chromosomes do increase the duration of mitotic cycles in Lolium perenne and Secale cereale as well as in maize (Evans et al. 1972).

It can be assumed that meiotic cycles are probably also lengthened. Two aspects of the B chromosome influence on recombination in maize indicate that there is more than a temporal factor involved however. First, the influence on recombination seems to be differential with greater increases in proximal regions. Second, the effect seems to vary with odd and even numbers of B's. It is possible that increased duration of meiosis could produce a greater increase in recombination at regions proximal to centromeres due to initiation of pairing at telomeres via nuclear envelope attachment sites (see Smith 1956; Woollam, Ford and Miller 1966; Wettstein and Sotelo 1967; Moens 1969). If synapsis generally was initiated at the ends of a homologous pair of chromosomes, regions located near the ends would undergo more recombination than those near the middle. Increasing the duration of the meiotic division therefore, would tend to increase recombination in middle regions. The differential effect of odd and even numbers of B's, however, lacks an explanation.

Ward (1973b) attempted unsuccessfully to determine sites on the B chromosome which influenced recombination by utilizing B-A translocations in much the same manner used to detect factors necessary for nondisjunction. He concluded that there are many sites on the B chromosome which influence recombination.

An interesting possibility is that B chromosomes may also influence excision of transposable elements. This excision must involve breakage and rejoining of double helices, but whether the process is similar to that involved in recombination of homologous DNA segments is unknown. Interestingly enough, an experiment investigating this possibility was performed before the mechanisms underlying controlling element activity in maize were understood. Plaut (1953) investigated the effect of B chromosomes on variegation of pericarps produced due to the instability of the $\underline{P}^{vv}$ allele which was subsequently found to be due to the association of the Modulator controlling element Mp with the $\underline{P}^{rr}$ allele (Barclay and Brink 1954). Barclay and Brink also determined that Mp was homologous to the activator (Ac) controlling element which McClintock (1951) had discovered and thus it is an autonomous (or one element) system. Plaut concluded that intensity of variegation produced by the $\underline{P}^{vv}$ allele was not independent of B chromosome number, however no significant correlation could be detected due to great variability allegedly caused by segregation of modifier genes in the highly heterogeneous stocks used. He suggested that a new attack on the same problem seemed warranted when homogeneous stocks were available.

This sort of experiment was indeed reported several years later (Williams 1972). Homogeneous stocks were produced which were heterozygous $\underline{wx}^{m-1}/\underline{wx}$ ($\underline{wx}^{m-1}$ is a Wx allele which has been inactivated by insertion of Mp) and contained from 0 to 5 B chromosomes. These were crossed as males or females with a wx/wx inbred line and the resulting ears were examined for starchy kernels (which would be produced by excision of Mp). Crosses in which $\underline{wx}^{m-1}/\underline{wx}$ plants were used as females were also scored for size of starchy sectors (larger sectors would indicate earlier excision events). B chromosomes were shown to have no influence on either of these parameters thus indicating that excision of Mp in the sporophytic stage is not affected. Concordance of endosperm and embryo for the Wx phenotype was also examined. Non-concordance, which would indicate that the excisional event had occurred during gametophyte development, was significantly greater in plants with B chromosomes when $\underline{wx}^{m-1}/\underline{wx}$ plants were used as females. Thus, although B chromosomes do not seem to affect the excision of controlling elements in the sporophytic stage, they do seem to increase excision during female gametogenesis. Once again, B chromosomes seem to be active only at certain developmental stages.

The influence of B chromosomes on one other type of recombination event has been measured in maize. Chou and Weber (1980) compared the frequency and distribution of

sister-chromatid exchange in root-tips with and without B chromosomes. They found no significant differences and concluded that the mechanism of sister-chromatid exchange appears to be different from that of intergenic recombination. It should be pointed out, however, that the mechanisms could be similar if not identical, but B chromosomes simply have no influence on recombination in these particular cells. The fact that B chromosomes in maize are influential only at certain stages of development is apparent in that they undergo nondisjunction and provoke breakage of A chromosomes at a significant rate only at the division of the generative nucleus, and effect excision of Mp controlling elements at a significant rate in female embryo sac development but not sporophytic development.

Another study that could possibly be viewed as supportive of the idea that B chromosomes are active only at certain stages of development is one by McGirr and Endrizzi (1978) in which the influence of B's on susceptibility to Brome Mosaic Virus (BMV), Maize Dwarf Mosaic Virus (MDMV), Wheat Streak Mosaic Virus (WSMV), and Barley Stripe Mosaic Virus (BSMV) was assayed. If any of these viruses become incorporated into the maize genome in the manner of proviruses, then a B chromosome influence on this effect (either by increasing recombination between the viral DNA and the host genome or by providing additional integration sites) should alter the susceptibility of plants with B

chromosomes. The only influence of B chromosomes in the study was the delay of onset of WSMV-caused necrosis by 15%. If any of these viruses do integrate into the host genome as part of their life-cycle (which is not known) then it is evident that B chromosomes do not seem to influence this integration. This implies that either B chromosomes cannot affect this particular type of recombination event or that they are inactive at this stage of development of the corn plant.

General Effects

The effects of B chromosomes on the phenotype of the host plant have been described in detail by Randolph (1941). He points out that their presence in small numbers can be determined only cytologically. They have no phenotypic effects until there are 10 or more per plant. When they are present in high numbers they cause a reduction in fertility and vigor, production of defective seed, increase in nuclear and cell size, and increased variation in pollen size as well as an increase in aborted pollen percentage.

Randolph points out that these effects become apparent gradually with increasing numbers of B's. Plants with more than 30 B's are virtually sterile. Randolph did obtain one plant with 34 B's and surmised this probably represents the carrying limit of a plant.

Origin

No one has yet traced the origin of a B chromosome in any species. Thus, any theories on their origins remain purely speculative. One point that most researchers would agree upon, however, is that B chromosomes are most likely derived from A chromosomes. Studies of buoyant density and renaturation kinetics of DNA from several species corroborate this conjecture (Rimpau and Flavell 1976; Dover 1976; Chilton and McCarthy 1973; Dover and Henderson 1976; Amos and Dover 1981).

By their very definition, B chromosomes must not be homologous to any A chromosome nor show any appreciable gene activity. If B's are derived from A chromosomes, however, there must exist some mechanism which eliminates homologous pairing of the neophyte B with its A chromosome progenitor as well as a mechanism which genetically inactivates the B. Jones and Rees (1982) point out that heterochromatinization of the B would serve both of these purposes and yet the B chromosomes in many species show no evidence of being either facultatively or constitutively heterochromatic. Other mechanisms must exist which are capable of accomplishing inhibition of pairing and gene activity.

Base composition of DNA preparations from seeds of 0B and 4B plants were shown to differ substantially (van Schaik and Pitout 1966 a and b). Subsequent investigations of buoyant density and base composition of leaf DNA

uncovered no differences between DNA from plants with B's and DNA from OB plants (Rinehart 1966; Pitout and van Shaik 1968). Buoyant density and renaturation kinetics comparisons made with DNA from seedlings with and without B chromosomes (Chilton and McCarthy 1973) also corroborate these subsequent DNA analyses. Buoyant densities of DNA from seedlings with and without B's were identical. Renaturation kinetics indicate that B chromosomes contain mostly repetitive sequence DNA, since no substantial increase in unique sequence DNA was observed in a 5B DNA preparation as compared to a OB DNA preparation. Furthermore, competition studies indicate that these repetitive B-specific sequences cross-react completely with A-specific sequences. Finally, exhaustive attempts to isolate B-specific sequences from libraries created with DNA isolated from B-containing plants have been fruitless (personal communication, Virginia Walbot). Thus it appears that maize B chromosome DNA is very closely related to A chromosome DNA.

From which A chromosome the B chromosome could be derived remains a matter of conjecture. In maize, the most attractive candidate is the abnormal chromosome 10 which is found in a minority of maize varieties. The abnormal chromosome 10 contains a supernumerary segment at the end of the long arm which is about as long as the short arm and consists of a euchromatic region and a larger terminal

heterochromatic region (Longley 1937). The heterochromatic region often folds back on itself at pachytene in heteromorphic bivalents to form what looks like a large knob. Thus the chromosome is called the knobbed chromosome 10 (K10) as opposed to the normal knobless chromosome 10 (k10).

In K10/k10 heterozygotes a preferential segregation occurs in megasporogenesis such that the K10 chromosome appears in the basal megaspore (destined to produce the egg nucleus) more than twice as frequently as the k10 homolog (Rhoades 1942). This preferential segregation does not occur in microsporogenesis. The presence of K10 was also found to induce preferential segregation for other knobbed chromosomes (Longley 1945), the degree of which was correlated with knob size (Kikudome 1959). Preferential segregation occurs only when recombination takes place between the knob and the centromere in megaspore mother cells (Rhoades and Dempsey 1966). In microsporocytes of K10/K10 or K10/k10 plants, secondary centric regions were observed on metaphase I bivalents as well as metaphase II dyads (Rhoades and Vilkomerson 1942). Rhoades (1952) speculates that neocentromere formation could be the underlying cause of preferential segregation in megasporogenesis. Recombination is also increased in K10/k10 and K10/K10 plants with proximal regions being

particularly affected (Rhoades and Dempsey 1957, 1966; Kikudome 1959).

The similarities between the supernumerary knobbed segment of K10 and the B chromosome are striking. They each possess an accumulation mechanism, influence other heterochromatic regions, and increase recombination. It is tempting to suggest that they have a common origin. Ting (1958) observed that the supernumerary chromatin in K10 closely resembles the terminal one-third (containing the centromere) of the B chromosome and suggests that K10 arose via translocation of this B segment to k10. He also reported observing pachytene pairing between K10 and B chromosomes in 2B PMC's heterozygous for K10 (Ting 1959). However, Rhoades and Dempsey (1959) observed no significant association between the K10 and B in 1B PMC's heterozygous for K10. In addition, a meiotic study of haploids containing K10 and one B chromosome indicated no significant bivalent formation between the two chromosomes (Snope 1967).

It has also been suggested that the B chromosome arose as the result of heterochromatinization of the entire K10 chromosome (Ward 1979). He has compared pachytene structures of both chromosomes and found striking similarities in location of heterochromatic blocks and chromomeres (Fig. 10). Ward points out that loss of synaptic homology would have been advantageous if a B

chromosome had arisen in a population segregating the progenitor K10 as a trisomic.

Recent cytological observations of pollen mother cells of maize from Sikkim, a popcorn of the Eastern Himalayas, have revealed a very peculiar phenomenon which may have some bearing on the origin of B chromosomes. A low frequency of abnormal meioses has been observed in which preprophase and early prophase cells lacking cell walls actually fuse resulting in an immediate doubling of chromosome number (Peeters and Wilkes 1983). The complement of the cell that is at the most advanced stage of meiosis remains normal while the invading genome may be eliminated. This elimination does not always occur however, and when it does occur, any number of chromosomes from the invading genome may be eliminated. Stabilized heterochromatic chromosomes identical to B chromosomes have been recovered and these presumably originated in the genome which was partially eliminated. Peeters and Wilkes report obtaining stable diploid lines with 1, 2, and 3 B-like chromosomes.

Ionizing Radiation

Ionizing radiation exerts its effects on DNA in an aqueous environment by formation of reactive species from water molecules which can subsequently react with DNA molecules (Weiss 1944, 1960). Approximately 80% of the radicals produced react with the base moieties of DNA

(Scholes et al. 1960). This portion consists mainly of H atoms and solvated electrons. Studies on model compounds indicate that about 10-20% of the OH radicals produced would be expected to react with the sugar moieties of DNA molecules (Scholes, Ward and Weiss 1960; Jung et al. 1969; Hoard, Hayes and Goad 1974). Chemical scission of the sugar-phosphate backbone as well as base release are results of the interaction of OH radicals with deoxyribose moieties (Von Sonntag and Schulte-Frohlinde 1978).

Backbone scission can occur either by breakage of the phosphate ester bond or cleavage of C-C bonds in the deoxyribose skeleton (5'-4' or 4'-3'). The first of these occurs in the presence or absence of oxygen while the second seems to occur only when oxygen is present (Von Sonntag and Schulte-Frohlinde 1978). Oxygen reacts with H and OH radicals to form highly reactive peroxyl radicals. Strand breakage can be increased by a factor of 2-4 in oxygenated as compared to deoxygenated conditions (Von Sonntag and Schulte-Frohlinde 1978). In a parallel vein, the oxygen content of cells has been shown to be directly proportional to mutational susceptibility to X-rays in Vicia faba (Thoday and Read 1947) as well as Drosophila melanogaster (Hollaender et al. 1951).

The fact that ionizing radiation can affect individual bases in a double helix as well as cause single- and double-strand scissions implies that, besides producing

chromosomal rearrangements, ionizing radiation should also induce intragenic mutations as well as induce reversion of existing mutations. Experiments with Drosophila (Patterson and Muller 1930; Timofeeff-Ressovsky 1932; Muller and Oster 1957; Lefevre and Green 1959; Green 1961) and Neurospora (Giles, de Serres and Partridge 1955; de Serres 1957; Malling and de Serres 1967) indicate that reversions and intragenic mutations can be induced by ionizing radiation.

Curiously, the situation in maize appears different. Extensive irradiation experiments (Stadler 1944; Stadler and Roman 1948; Emmerling 1955; Nuffer 1957; Mottinger 1970) have failed to reveal one case of reversion or intragenic mutation. Mottinger (1970) points out the possibility that the irradiation doses used in these experiments were above the optimal level for inducing point mutations and therefore favored the induction of two-hit mutations such as deletions. He also mentions the possible existence of an efficient repair process for one-hit mutations.

MATERIALS AND METHODS

Seed Stocks

All Black Mexican sweet corn (BMSC) used in experiments discussed herein was derived from seed stocks obtained from Dr. Scott McGirr of the University of Arizona. These stocks in turn were derived by sib-crossing two separate lines of Black Mexican. One line possesses B chromosomes and is referred to as the B line and the other lacks them and is referred to as the A line. Dr. McGirr's original seed stocks were sib 68-213 (BMSC with B chromosomes) and sib 68-209 (BMSC without B chromosomes), which were obtained from the Maize Genetics Cooperative Stock Center in Urbana, Illinois.

Interestingly enough, Black Mexican sweet corn does not hail from Mexico. It has been a commercially available variety in New England for more than a century and was derived from the "black puckers" sweet corn of the Iroquois tribe of native Americans. The misleading nationality was probably added to indicate that it was an exotic variety when it was initially introduced commercially (Sheridan 1982). The kernels are black in color due to homozygosity for the aleurone color genes A, C1, C2, R, and Pr.

The stocks maintained by the Maize Genetics Cooperation Stock Center were originally donated by Dr. Marcus Rhoades who long ago obtained seed stock from Dr. E.W. Lindstrom at Ames, Iowa and continually selfed it before separating it into A and B lines. Thus the BMSC-A and BMSC-B lines are isogenic barring spontaneous mutation (M. Rhoades, personal communication).

All A-line seed used in all experiments was obtained from an initial seed increase performed by intercrossing A-line plants. B-line seed used in experiments 1, 2, 3 and 5 was obtained from an initial seed increase in which B-line plants were selfed and intercrossed in order to increase B chromosome levels in the progeny. B-line seed used in experiment 4 was obtained from a second seed increase in which A-line and B-line plants were intercrossed in order to reduce B chromosome levels in the progeny. This was necessary due to the unexpected numbers of aberrant plants among those containing large numbers of B chromosomes (see Results and Discussion section).

The c1 tester stock used in experiment 6 was a hybrid (Coe K195 - 28 x 219, K55 x W23) obtained from Dr. E. Coe of the University of Missouri. Black Mexican sweet corn used in experiment 6 was the same as used in experiments 1-5.

Planting

All kernels were surface-sterilized in a 10% bleach solution for 10 minutes. They were then soaked in distilled water for 3 hours and planted in Jiffy peat pellets. Pellets containing B-line kernels were placed 3 inches apart in polystyrene flats and surrounded by perlite in order to stimulate luxuriant root growth and facilitate easier separation of plants at the time of root-tip harvesting.

Two to three weeks from the planting date, root-tips were collected from all B-line plants and randomly selected A-line plants and plants were transferred to 6-inch diameter styrofoam pots containing a soil mixture of 3 parts dirt, 2 parts sphagnum peat, and 2 parts perlite.

All plants were grown in one greenhouse and were collectively fertilized with Rapid-Gro fertilizer and ferric oxide when necessary. Malathion and Cygon were used in an attempt to control spider mites.

Cytology

All B-line plants utilized for irradiation experiments were analyzed for B chromosome number by examining root-tip cells. Root-tips were excised at 8-9 A.M. and pretreated for 4 hours in a saturated solution of monobromonapthalene. The tips were then fixed using one of the following methods. Method I:

1. Fix overnight in 3:1 (95% ethanol:glacial acetic acid).

2. Hydrolyze in 1N HCl at 60 degrees C for 1.5 minutes.

3. Squash in propio-carmine with iron alum added.

Method II:

1. Hydrolyze in 1N HCl at 60 degrees C for 1.5 minutes.

2. Store in 0.5% aceto-carmine (0.5% carmine in 45% acetic acid) for at least 4 days.

3. Squash in propio-carmine with iron.

B-line plants were assigned B chromosome numbers after 5-10 spreads without major chromosome overlap were in agreement.

The stability of the B chromosomes in the B-line was verified by examining both root tips and pollen mother cells of 36 plants. Pollen mother cells were scored by fixing immature tassels in 3:1 (95% ethanol:glacial acetic acid) overnight and squashing in propio-carmine with iron.

Irradiation

Irradiations were performed in the cobalt-60 chamber located in the Engineering Building at the University of Arizona. The source strength at the commencement of

irradiation experiments was 406 curies. This had decayed to 343 Curies by the conclusion of all experiments.

All plants were irradiated singly due to the small size of the chamber. Plants in experiments 1, 2, 3, 4, and 6 were irradiated by positioning the plant so that the broad side of the stalk was perpendicular to the direction of gamma rays in order to irradiate all parts of the immature tassel as evenly as possible.

Due to their much larger size, plants in experiment 5 (postmeiotic irradiation) were irradiated by positioning the plants with the stalk horizontal to the ground and thus situating the tassel parallel to the direction of gamma rays. Dose rate was measured by an ion chamber probe attached to a Radocon meter and was adjusted by changing the distance from the source. All plants in experiments 1, 2, 3 and 5 were irradiated at 25 R/minute while those in experiments 4 and 6 were irradiated at 20 R/minute.

Following irradiation, all plants to be scored for pollen viability were coded by letter and returned to the greenhouse. All pollen viability measurements were thus made in a blind manner.

Pollen Collection and Viability Analysis

Pollen viabilities in all experiments were measured by two different staining techniques: fluorescein diacetate (Rotman and Papermaster 1966; Heslop-Harrison and Heslop-Harrison 1970) and I_2-KI. The fluorescein diacetate (FDA) staining solution was prepared daily by adding FDA stock solution dropwise to phosphate buffered saline (PBS) until it became milky. The FDA stock solution (2 mg/ml FDA in acetone) was stored at -20 degrees C. PBS consisted of 5.6g anhydrous Na_2HPO_4, and 4.1g NaCl brought to 1 liter with distilled water and adjusted to pH 7.2. I_2-KI stain was prepared by dissolving 1g potassium iodide in 25ml distilled water and then adding 0.3g iodine flakes. Finally, 25ml glycerine was added.

Pollen samples were taken daily, at 10 A.M.-2 P.M., beginning with the first day of anthesis. The typical anthesis period is 7 days. In the FDA technique, one drop of stain was placed in the middle of a 1 inch by 3 inch microscope slide. Dehisced pollen was then shaken on to the slide and another drop of stain was added to the first. A 22 mm square #1 glass coverslip was then placed over the sample and sealed with paraffin. If no pollen had dehisced but anthers had been extruded, 3 or 4 anthers were removed and the pollen was gently teased into the drop of FDA stain. Samples were then refrigerated and scored later that same day.

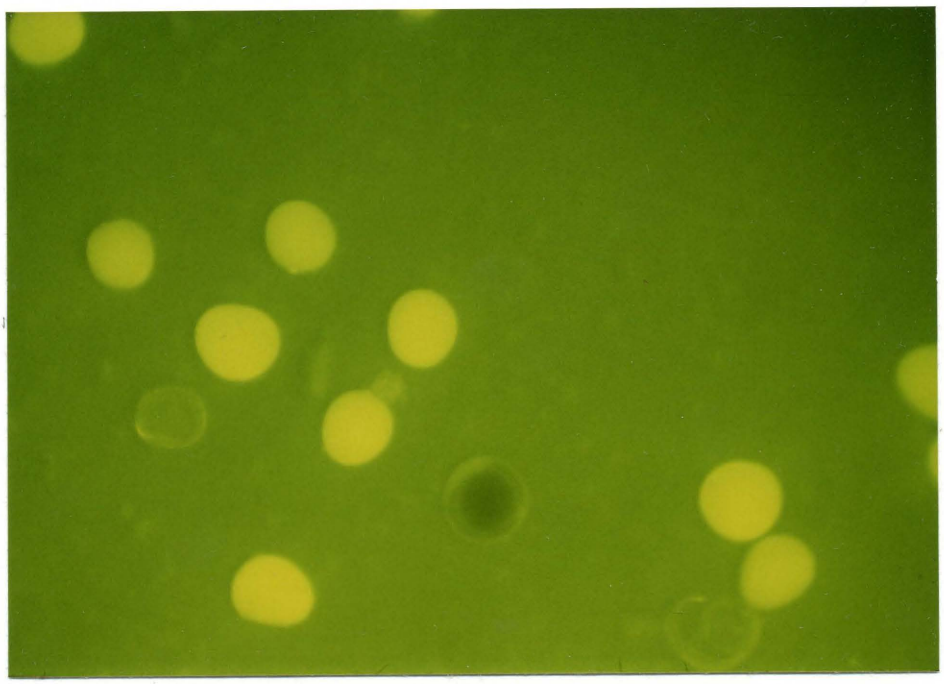
After the FDA pollen sample was taken from each plant, a sample of extruded anthers was gathered and stored in 70% ethanol. These samples were analyzed at a later date for pollen starch content with I_2-KI stain. In the FDA samples, pollen was scored as viable (fluorescent) or non-viable (non-fluorescent) at low power under semi-darkfield conditions on a fluorescence microscope with a Schott BG-12 exciter filter and a Y-8 barrier filter (see Fig. 3a).

The samples stored in 70% ethanol were analyzed by gently teasing a small portion of the pollen from 3 or 4 anthers into a drop of I_2-KI stain and covering with one-half of a 22 mm square No. 1 coverslip. They were observed under brightfield conditions with both a blue and a neutral density filter. Pollen grains containing starch were scored as viable and those devoid of starch were scored as non-viable (Fig. 3b).

Altogether, 576,844 pollen grains were scored in the FDA analysis ($\bar{x}$= 400 grains/sample) for a grand total of 1,032,759 pollen grains scored in all experiments.

Due to high irradiation dose levels in experiment 1, many plants did not extrude anthers. From these plants, 7 anther samples were removed at points evenly distributed on the tassel and subsequently were scored for pollen viability. In experiments 2, 3, 4, and 5 most plants extruded anthers and pollen samples were taken only from

a



b

FIGURE 3. a) Pollen stained with fluorescein diacetate. Fluorescent yellow grains are scored as viable; dull grains are scored as non-viable.
b) Pollen stained with iodine-potassium iodide. Brown or black grains are scored as viable; empty grains are scored as non-viable.

plants with extruding anthers. If a plant extruded no anthers on a given day, no sample was taken.

Analysis of Data

All statistical analyses on experiments 1 through 5 were based on a minimum seven day pollen shedding period. Due to high doses of irradiation in experiment 1, many plants failed to extrude anthers. Anthers were dissected from these plants and the pollen teased from these anthers was analyzed. This pollen showed essentially no viability. Thus in experiments 2 through 5, if a plant failed to extrude any anthers on a given day, pollen viability was scored as zero. Due to lower doses of irradiation in experiments 2, 3 and 4 and the postmeiotic condition of microspores and pollen in experiment 5, this occurrence was much less frequent however.

In all experiments, several non-irradiated control plants with and without B chromosomes were included and average pollen viabilities (V_o) were computed from measurements of daily samples. For each control plant, an average pollen viability percentage was calculated over the seven day dehiscence period. These average pollen viabilities were then averaged for all control plants containing equivalent numbers of B's to yield the V_o value. Pollen viabilities (V) of irradiated plants were compared to the appropriate control to produce V/V_o

values which were used in all statistical analyses. A V/V_o value of 0 may thus represent totally inviable pollen or lack of anther extrusion in experiments 2, 3, 4, and 5. In experiment 1, a V/V_o of 0 may represent these possibilities as well as lack of tassel development or plant death. V/V_o values were grouped in classes only for the presentation of data in tables. All statistical analyses were executed on non-grouped data.

Due to high variability in pollen viability measurements, all measurements were combined within each group to give a large distribution of V/V_o measurements. In experiments 1, 2, 4b, and 5, two sample comparisons were made between plants with and without B chromosomes using the Mann-Whitney two sample rank test (Sokal and Rohlf 1969). In experiments 3 and 4a, samples with varying numbers of B chromosomes were compared using the Kruskal-Wallis one-way analysis of variance (Kruskal and Wallis 1952).

All statistics were computed using the MINITAB statistical program (Copyright - Penn State University 1981).

Determination of Aleurone Mean Cell Number

In order to determine the rate of mutation of C1 to c1 in developing aleurone tissues, the mean number of cells per aleurone was calculated in the following manner:

1. 10 kernels each from bulked c1 x BMSC-A and c1 x BMSC-B were selected and soaked in water for 36 hours.

2. Pericarps were peeled from the area covering the aleurone layer and flattened between two microscope slides.

3. Flattened pericarps were enlarged 5 times on a Bausch and Lomb Transfer scope and traced on paper.

4. Aleurone layers from each of the kernels were scraped into a drop of phosphate buffer, covered with a #1 coverslip, and a group of ten cells was traced using a Zeiss drawing tube. Size was determined using a stage micrometer.

5. Areas of the two groups of 10 pericarps and two groups of 10-cell regions were measured using a Salmoiraghi 236 planimeter.

RESULTS AND DISCUSSION

Experiments 1 through 5 all involve comparison of pollen viability in plants with and without B chromosomes subsequent to gamma irradiation. Radiation treatments in experiments 1 through 4 were administered prior to meiosis in the developing tassel. The radiation treatment in experiment 5 was administered postmeiotically.

It seems obvious that a very wide spectrum of genes is necessary for the development of a viable pollen grain. The utilization of pollen viability stains will thus provide an estimate of radiation-induced DNA damage in these genes.

It must be emphasized that, although the data are described in terms of viability measurement, this in no way implies the measurement given is an indicator of true pollen germinability. Any pollen viability stain can only measure some one or two parameters associated with viability (e.g. nitroblue tetrazolium reduction produces a blue formazan in the presence of succinate, indicating that oxidative respiration is taking place).

The attributes assayed by fluorescein diacetate are the presence of an intact cell membrane and the presence of esterases. According to Heslop-Harrison and Heslop-Harrison (1970), the presumed method of operation of this stain is as follows:

1. Non-polar fluorescein diacetate molecules enter the cell through the plasma membrane.

2. Esterification to fatty acids occurs.

3. Fatty acid esterases break the ester bonds formed, producing polar fluorescein molecules which fluoresce brightly in the yellow-green region of the spectrum upon stimulation by U.V.(~300 nm) and cannot traverse the plasma membrane (Fig. 3a).

Iodine-potassium iodide stain reacts with amylose molecules to produce a blue-black complex or with amylopectin to produce a red-brown complex (Ehrenberg and Eriksson 1966). The presence of amylose is due to the expression of the Wx (starchy) gene in pollen or endosperm. Amylopectin is also present in Wx pollen or endosperm. However, wx (waxy) pollen or endosperm contains only amylopectin in the starch granules (Nelson 1959). 100 kernels each of BMSC-A and BMSC-B were tested and determined to have starchy endosperms. Thus it is assumed that both stocks are fixed with respect to the Wx gene. This suggests that the I_2-KI test could be used to assay mutation rate at a single locus. However, the expression of this gene in pollen grains was found to be variable in BMSC, and in many plants pollen staining gave a continuous spectrum from brown to black which made a Wx to wx mutation rate impossible to

assay. Thus, the I_2-KI test was used as a pollen viability test and pollen grains were scored as either starch-containing or empty. The supposition is that all pollen grains that contain no starch are non-viable (Fig. 3b).

It is important to note that FDA viability measurements and I_2-KI viability measurements are based on totally different parameters and thus would not be expected to give the same viability estimate for a given pollen sample. This was, in fact, demonstrated by Jefferies (1977) by utilizing a technique in which the same pollen grains could be sequentially assayed with the FDA and I_2-KI stains. The two techniques gave essentially independent results. However, both assays should show the same trend in viability measurements of varying samples. This was indeed found to be the case, as illustrated in Fig. 4.

The notable aspect of the data presented in Fig. 4 is the negative correlation of pollen viability with B chromosome number. Randolph (1941) reported an increase in the frequency of aborted pollen grains in plants with large (>10) numbers of B chromosomes. The data reported here, however, show the effect to be a gradual one with increasing number of B's.

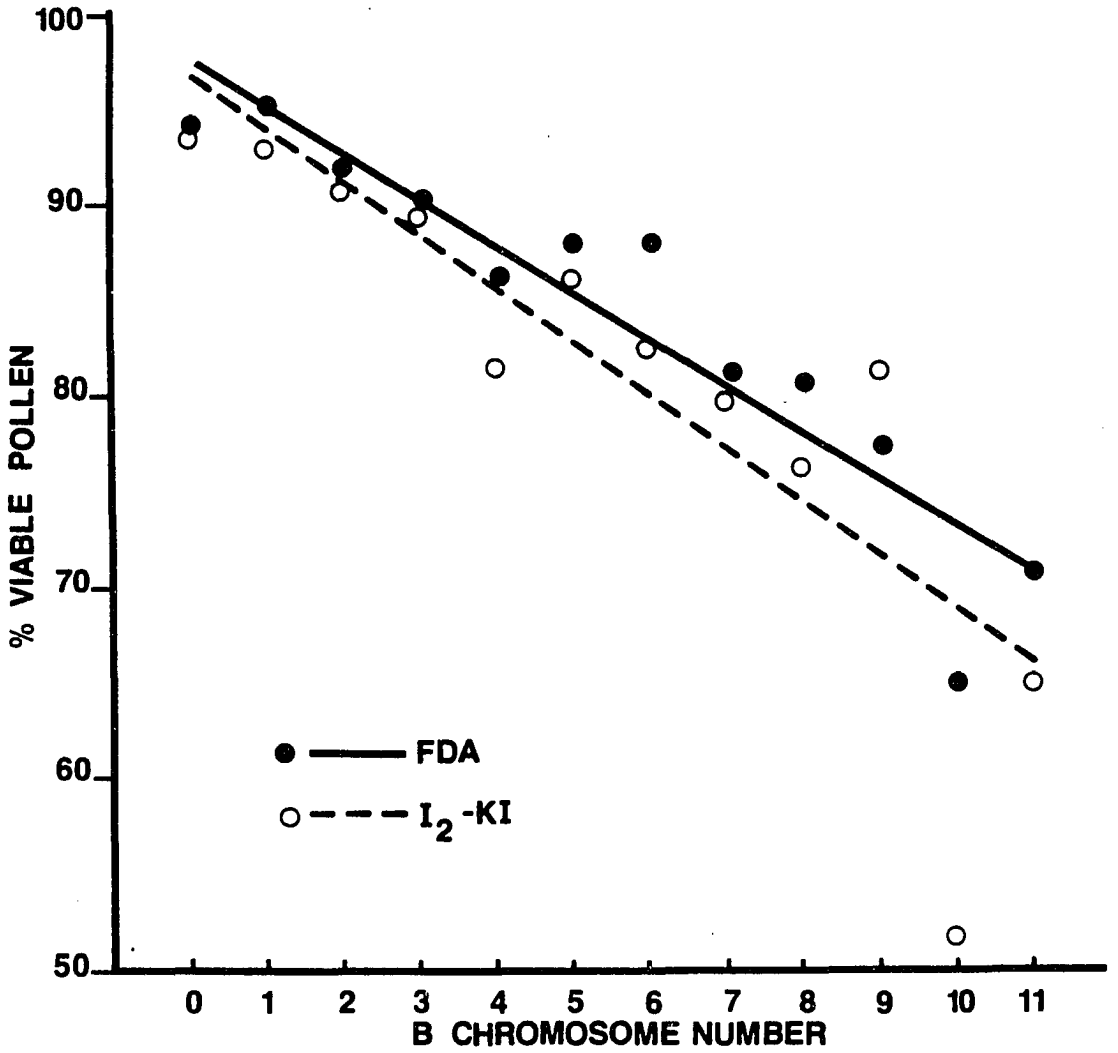


FIGURE 4. Mean pollen viabilities of non-irradiated plants containing 0 to 11 B chromosomes. Viability was assayed using fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) stains. Linear regression lines were calculated using average pollen viabilities for several plants measured over a seven-day pollen shedding period.

How the presence of increasing numbers of B's causes decreases in pollen viability remains a mystery. The B's could exert either a sporophytic or gametophytic effect, or both. For example, a sporophytic effect could result from a B-chromosome-influenced change in the nuclear metabolism (e.g. delayed replication resulting in reduction of endoreduplication of chromosomes) of the tapetal cells within a developing anther locule. This could result in a reduction of pollen viability due to inaccessibility of important metabolites. A gametophytic effect could be produced simply by changes in nuclear metabolism which lead to defective sporogenesis and which are magnified by increasing numbers of B's. A possibility which would result in a gametophytic effect is that B chromosomes induce A chromosomes to undergo nondisjunction or breakage at anaphase I or II of meiosis. This would produce microspores and resultant vegetative nuclei that are genetically deficient and, consequently, non-viable pollen. B chromosomes have, of course, been shown to cause breakage of A chromosomes with heterochromatic knobs at the second pollen mitosis, producing the high-loss phenomenon for marker genes on those chromosomes (Rhoades and Dempsey 1972). However, in the voluminous literature on maize B chromosome cytology (Darlington and Upcott 1941; Blackwood 1956), the occurrence of A chromosome nondisjunction at meiosis was not reported. More recently, however, Kindiger

(1982) has reported that PMC's from plants with B chromosomes display bridge-like structures between homologous A chromosomes at anaphase I of meiosis.

Another interesting aspect of pollen viability measurements on non-irradiated plants is illustrated in Table 1. In this table, average pollen viabilities of 3B plants grown from kernels obtained in the original seed increase (BMSC-B line intercrossed) are compared to those of plants grown from kernels obtained from the seed increase performed to decrease B chromosome numbers (BMSC-B x BMSC-A and BMSC-A x BMSC-B). There is a significant difference in average pollen viabilities of 3B plants obtained from the two seed increases. In this particular comparison, all plants exhibiting decreased pollen viability were from the same cross (7B x OB).

There are two possible explanations for this observation. First, it may be that modifier genes differ in the two BMSC stocks. This would imply that the two stocks are, in reality, not isogenic as has been assumed. The second possibility is that there may exist an imprinting phenomenon such that when B chromosomes are transmitted through the female reproductive system their detrimental effects are more pronounced than when transmitted through the male reproductive system. Unfortunately, no data is available on pollen viability from reciprocal crosses and this possibility could not be tested. Crosses are now being

Table 1. Average pollen viabilities of plants with 3 B chromosomes obtained from BMSC-B x BMSC-B and BMSC-B x BMSC-A seed increases. Each value represents mean % viable pollen grains over seven-day dehiscence period as measured with fluorescein diacetate.

BMSC-B x BMSC-B	BMSC-B x BMSC-A
95.22	87.62
92.12	87.96
95.21	84.19
94.82	86.47
93.64	78.87
94.98	
94.64	
$\bar{x}$=94.83	$\bar{x}$=85.02

t=5.42

P<0.01

made to test this hypothesis. The fact that the accumulation and high loss mechanisms operate only in male gametogenesis indicates that there are differences in nuclear metabolism between male and female gamete-producing cells which can strikingly influence B chromosome behavior.

If some imprinting mechanism existed on the female side which increased deleterious effects of B's, or alternatively, on the male side which decreased them, it would be logical to assume that some type of molecular change is instigated in the B chromosome itself. A change of this type is a common occurrence in mammalian cells in which one X chromosome in each cell is inactivated to form a heterochromatic Barr body (Lyon 1962). This change takes place in embryonic female cells only and recent evidence (Mohandas et al. 1981) suggests that DNA methylation is responsible for inactivation of the X chromosome.

It is generally true, at least in vertebrates, that DNA methylation and gene activity are inversely related (Razin and Riggs 1980). Most 5-methylcytosine, which is the only methylated base commonly found in eukaryotic DNA, is located in satellite DNA (Salomon et al. 1969; Miller et al. 1974; Harbers et al. 1975). Satellite DNA in turn is found predominantly in highly condensed heterochromatin (Pardue and Gall 1970). B chromosomes in maize, like inactivated mammalian X chromosomes, are highly heterochromatic and thus quite possibly highly methylated.

More interestingly, DNA methylation in prokaryotic systems influences replication, recombination and mutagenesis. In the bacteriophage ØX174, blocking methylation at one specific site inhibits DNA synthesis (Razin 1978). According to Razin and Riggs (1980), the sequence GATC is always methylated under normal growth conditions in E. coli and occurs ten times more frequently than expected at the origin of replication. Also, replication of the E. coli genophore is halted after one round of synthesis if methylation is inhibited (Sugimoto et al. 1979; Meyer et al. 1979). In E. coli, methylase-deficient mutants, which exhibit reduced GATC sequence methylation, also are more sensitive to mutagens, have a higher spontaneous mutation rate, and display a hyperrecombination phenotype (Marinus and Morris 1975). In bacteriophage lambda, deficient methylation of cytosine at CC$\frac{A}{T}$GG sites also stimulates recombination (Korba and Hays 1982). It should be noted here that B-chromosomes are known to induce hyperrecombination in selected regions of the maize genome (Hanson 1969; Nel 1973; Rhoades 1968a and b; Chang and Kikudome 1974) and it has been hypothesized that both nondisjunction of B's and the high loss phenomenon at the second pollen mitosis are due to faulty DNA replication in heterochromatic regions (Rhoades and Dempsey 1972). The relationship between B chromosomes and induction of mutation remains to be investigated in this work. Any

relationship between maize B chromosomes and DNA methylation remains purely speculatory at this point, however.

Experiment 1

In experiment 1a, plants with and without B's were irradiated premeiotically with 2500 rads. A five plant sample containing no B chromosomes was compared to an eleven plant sample containing varying numbers of B chromosomes ($\bar{x}$=5.5 B's/plant). The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 2 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values indicates a decrease in susceptibility to gamma radiation damage. Although a significant difference between the distributions of the two samples is detected in the I_2-KI analysis, a 2500R dose is too high to collect meaningful pollen viability data which can be used to compare BMSC-A and BMSC-B plants. The reason for this is that many of the plants shriveled and died within 2 weeks of the irradiation. It was observed, however, that all plants without B chromosomes died (five plants lacking B's died resulting in 35 V/V_o measurements of 0). In the sample with B chromosomes, however, some plants survived the irradiation and produced some viable pollen.

Table 2. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with ($\bar{x}$=5.5) and without B chromosomes irradiated premeiotically with 2500R in experiment 1a. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS	FDA		I_2-KI	
CENTER	WITHOUT B's	WITH B's	WITHOUT B's	WITH B's
0.0	35	65	30	51
0.1	0	1	0	1
0.2	0	3	0	1
0.3	0	0	2	1
0.4	0	3	2	4
0.5	0	0	1	3
0.6	0	1	0	7
0.7	0	1	0	4
0.8	0	1	0	0
0.9	0	2	0	3
1.0	0	0	0	2
N	35	77	35	77
MEDIAN	0.0	0.0	0.0	0.0
MANN-WHITNEY P VALUE	0.1		0.04	

In experiment 1b, plants with and without B's were irradiated premeiotically with 1500R. A five plant sample containing no B chromosomes was compared to a six plant sample containing varying numbers of B chromosomes ($\bar{x}$=6.33 B's/plant). The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 3 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values indicates a decrease in susceptibility to gamma radiation damage. The distributions of samples with and without B chromosomes show a highly significant difference ($P<.0001$) in either assay system. The distribution of the V/V_o values for B chromosome plants is shifted toward higher viabilities.

It is observed also in experiment 1b that a 1500R dose is too high to give meaningful pollen viability data because many of the plants did not extrude any anthers, resulting in a high proportion of V/V_o values in the 0.0 class. Pollen that was analyzed required teasing from dissected anthers.

The results of experiments 1a and 1b indicate that plants containing B chromosomes may be less susceptible to gamma-ray-induced DNA damage than plants lacking B's. Since the doses used produced such devastating effects in irradiated plants, lower doses were used in subsequent

Table 3. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with ($\bar{x}$=6.3) and without B chromosomes irradiated premeiotically with 1500R in experiment 1b. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS CENTER	FDA		I_2-KI	
	WITHOUT B's	WITH B's	WITHOUT B's	WITH B's
0.0	28	21	27	12
0.1	1	0	1	0
0.2	0	3	1	1
0.3	1	0	0	1
0.4	0	2	1	1
0.5	3	1	1	3
0.6	0	2	3	4
0.7	1	5	1	6
0.8	1	3	0	6
0.9	0	3	0	7
1.0	0	4	0	1
1.1	0	0	0	3
N	35	44	35	45
MEDIAN	0.0	0.18	0.0	0.66
MANN-WHITNEY P VALUE	.0001		<.0001	

experiments so that most plants would shed pollen and more accurate comparisons of pollen viability could be made.

Experiment 2

In experiment 2a, plants with and without B chromosomes were irradiated premeiotically with 500R. A five plant sample containing no B chromosomes was compared to a five plant sample containing varying numbers of B chromosomes ($\bar{x}$=6 B's/plant). The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 4 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values indicates a decrease in susceptibility to gamma radiation damage. A highly significant difference is detected between the samples as measured by FDA ($P<.001$) or I_2-KI ($P<.0001$) with the distribution of V/V_o values shifted to a higher level in the sample of plants containing B chromosomes.

In experiment 2b, plants with and without B chromosomes were irradiated premeiotically with 1000R. A five plant sample containing no B chromosomes was compared to a five plant sample containing varying numbers of B chromosomes ($\bar{x}$=6 B's/plant). The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 5 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values

Table 4. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with ($\bar{x}$=6) and without B chromosomes irradiated premeiotically with 500R in experiment 2a. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS CENTER	FDA		I_2-KI	
	WITHOUT B's	WITH B's	WITHOUT B's	WITH B's
0.0	1	1	1	1
0.1	0	0	0	0
0.2	0	0	0	0
0.3	0	0	0	0
0.4	0	0	0	0
0.5	0	0	0	0
0.6	0	0	0	0
0.7	0	0	0	1
0.8	2	0	5	1
0.9	8	6	21	10
1.0	27	20	11	15
1.1		11		13
1.2		3		1
N	38	41	38	42
MEDIAN	0.98	1.02	0.92	1.02
MANN-WHITNEY P VALUE	.0008		<.0001	

Table 5. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with ($\bar{x}$=6) and without B chromosomes irradiated premeiotically with 1000R in experiment 2b. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS CENTER	FDA		I_2-KI	
	WITHOUT B's	WITH B's	WITHOUT B's	WITH B's
0.0	1	1	1	0
0.1	0	0	0	0
0.2	0	1	0	0
0.3	0	1	0	0
0.4	0	2	0	0
0.5	0	6	0	0
0.6	0	3	1	2
0.7	0	10	3	0
0.8	3	3	8	4
0.9	12	5	18	8
1.0	21	4	6	17
1.1		2		10
1.2		1		2
N	37	39	37	43
MEDIAN	0.96	0.96	0.88	0.98
MANN-WHITNEY P VALUE	0.48		<.0001	

indicates a decrease in susceptibility to gamma radiation damage. The distributions of V/V_o values as analyzed by FDA showed no significant difference. In the I_2-KI analysis, however, the two distributions were significantly different, with the V/V_o values of the B chromosome sample shifted to a higher level.

Experiment 3

In experiment 3, plants with and without B chromosomes were irradiated premeiotically with 1250R. Five plant samples containing 0, 1, and 3 B chromosomes were compared. The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 6 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values indicates a decrease in susceptibility to gamma radiation damage. Highly significant ($P_{FDA}<.01$, $P_{I_2\text{-KI}}<.01$) Kruskal-Wallis H values were obtained in both FDA and I_2-KI analyses indicating that the distributions of the three groups differ. It is obvious from the frequency distributions of V/V_o values that the OB sample was significantly shifted toward lower V/V_o values. Thus, this experiment indicates a dramatic effect of B chromosomes on susceptibility to gamma radiation damage.

Table 6. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with 0, 1, or 3 B chromosomes irradiated premeiotically with 1250R in experiment 3. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS CENTER	FDA			I_2-KI		
	0B	1B	3B	0B	1B	3B
0.0	26	1	8	26	2	8
0.1	1	4	1	0	0	0
0.2	1	3	1	0	0	0
0.3	1	1	1	0	0	0
0.4	2	3	4	0	0	0
0.5	2	1	0	0	0	0
0.6	1	1	3	1	3	2
0.7	1	2	2	2	5	8
0.8	0	2	8	4	14	11
0.9	0	15	7	2	12	6
1.0	0	3	0	0	0	0
N	35	36	35	35	36	35
MEDIAN	0.0	0.83	0.63	0.0	0.78	0.74
KRUSKAL-WALLIS H VALUE		41.21 P<.01			33.14 P<.01	

Experiment 4

Experiment 4a consists essentially of a repeat of experiment 3 in which plants with and without B chromosomes are irradiated with 1250R. Six plant samples containing 0, 2, 4, and 6 B chromosomes were compared. The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 7 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values indicates a decrease in susceptibility to gamma radiation damage. Kruskal-Wallis H values indicate no significant difference in location of these V/V_o distributions ($.1<P_{I_2\text{-KI}}<P_{FDA}<.5$). The incongruence of the results in experiments 3 and 4a will be investigated subsequently in a combined analysis.

Experiment 4b consists of a repeat of experiment 2b in which plants with and without B chromosomes are irradiated premeiotically with 1000R. Six plant samples containing 0 or 3 B chromosomes were compared. The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 8 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values indicates a decrease in susceptibility to gamma radiation damage. No statistically significant difference is detected between location of the distributions of V/V_o values for the two samples.

Table 7. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with 0, 2, 4, or 6 B chromosomes irradiated premeiotically with 1250R in experiment 4a. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS CENTER	FDA				I_2-KI			
	0B	2B	4B	6B	0B	2B	4B	6B
0.0	14	2	11	8	12	2	11	8
0.1	1	2	1	4	2	0	0	1
0.2	1	4	0	2	0	1	0	1
0.3	0	4	3	3	3	0	0	2
0.4	1	5	2	6	0	1	0	6
0.5	3	4	3	5	0	8	4	4
0.6	7	7	2	5	2	4	4	3
0.7	8	4	9	3	6	8	3	9
0.8	5	5	6	10	11	10	7	4
0.9	3	5	7	0	4	5	5	4
1.0	2	3	0	0	5	6	6	3
1.1							3	1
1.2							1	
N	45	45	44	46	45	45	44	46
MEDIAN	0.57	0.56	0.63	0.44	0.73	0.72	0.72	0.60
KRUSKAL-WALLIS H VALUE		3.24 P=.3-.5				5.51 P=.1-.2		

Table 8. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with 0 or 3 B chromosomes irradiated premeiotically with 1000R in experiment 4b. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS CENTER	FDA		I_2-KI	
	0B	3B	0B	3B
0.0	1	0	1	0
0.1	0	1	0	0
0.2	0	1	0	0
0.3	0	0	0	0
0.4	0	2	0	0
0.5	2	2	0	2
0.6	3	4	1	7
0.7	8	4	2	6
0.8	12	8	14	7
0.9	10	13	11	12
1.0	3	12	10	8
1.1		4		8
N	39	51	39	50
MEDIAN	0.82	0.86	0.88	0.87
MANN-WHITNEY P VALUE	0.047		0.779	

Experiment 5

In experiment 5, plants with and without B chromosomes were irradiated postmeiotically with 1500R. A five plant sample containing no B chromosomes was compared to a five plant sample containing varying numbers of B's ($\bar{x}$=8.4 B's/plant). The pollen viability data as analyzed by FDA and I_2-KI stains is presented in Table 9 in the form of frequency distributions. A shift in the frequency distribution toward higher V/V_o values indicates a decrease in susceptibility to gamma radiation damage. No statistically significant difference is detected in the location of the distributions of V/V_o values of the two samples.

Analysis of the Combined Data

Inspection of the data in experiment 3 alone, with a dose of 1250R, leaves little doubt that plants with one or three B chromosomes are significantly less susceptible to gamma ray induced DNA damage than plants without B chromosomes. However, experiments 4a and 4b, with doses of 1250R and 1000R respectively, indicate that there is no significant influence of B chromosomes on susceptibility to the deleterious effects of gamma rays. It might be argued that the archesporial and/or pollen mother cells in the developing tassels of the plants lacking B chromosomes in experiment 3 were by chance at a highly radiosensitive stage

Table 9. Frequency distributions of pollen viability (V/V_o) measurements taken daily over minimum seven-day period of anther extrusion from plants with ($\bar{x}$=8.4) and without B chromosomes irradiated postmeiotically with 1500R in experiment 5. Table indicates number of measurements of pollen viability that occurred in each class with fluorescein diacetate (FDA) and iodine-potassium iodide (I_2-KI) staining methods.

V/V_o CLASS CENTER	FDA		I_2-KI	
	WITHOUT B's	WITH B's	WITHOUT B's	WITH B's
0.0	14	15	7	4
0.1	23	10	6	9
0.2	4	5	13	7
0.3	3	6	9	6
0.4	0	0	7	7
0.5	2	0	2	4
0.6	1	3	3	1
0.7	0	1	0	2
0.8	0	0	0	0
0.9	0	0	0	1
1.0	0	1	0	0
N	47	41	47	41
MEDIAN	0.10	0.09	0.24	0.27
MANN-WHITNEY P VALUE	0.38		0.38	

of development, thus yielding a high amount of pollen inviability subsequent to irradiation. After all, V/V_o values in the OB sample in experiment 4a are distributed much more closely to the samples containing B chromosomes.

If it is assumed that tassels of OB plants in experiment 3 were by chance at a highly radiosensitive developmental stage, the OB data from experiment 3, which is assumed to be giving artificial significance, can be excluded from the statistical analysis and the remaining data from 1B and 3B plants can be combined with the data from OB, 2B, 4B, and 6B plants in experiment 4a. A Kruskal-Wallis one-way analysis of variance on these combined data (Table 10) indicates ($P_{FDA}<.025$, $P_{I_2\text{-}KI}<.05$) that B chromosome number does affect V/V_o in plants irradiated with 1250R. If the data is reorganized so that 1B plants are compared to all other plants in an a posteriori Kruskal-Wallis analysis, a highly significant difference is detected ($P_{FDA}<.005$, $P_{I_2\text{-}KI}<.01$) between the two samples. The data thus imply that 1B plants are significantly less susceptible to the deleterious effects of gamma rays.

Due to the non-normal distribution of V/V_o values in these samples the mean V/V_o value cannot be used as a predictor for individual pollen samples. However, it can be used as an estimator of radiation-induced damage to the entire population of pollen grains representing each

Table 10. Frequency distributions of pollen viability (V/V_o) measurements taken daily over a minimum seven-day period of anther extrusion from plants with 0, 1, 2, 3, 4, or 6 B chromosomes irradiated premeiotically with 1250R in experiments 3 and 4a. 1B and 3B samples are from experiment 3 while 0B, 2B, 4B, and 6B samples are from experiment 4a.

V/V_o	FDA						I_2-KI					
CLASS CENTER	0B	1B	2B	3B	4B	6B	0B	1B	2B	3B	4B	6B
0.0	14	1	2	8	11	8	12	2	2	8	11	8
0.1	1	4	2	1	1	4	2	0	0	0	0	1
0.2	1	3	4	1	0	2	0	0	1	0	0	1
0.3	0	1	4	1	3	3	3	0	0	0	0	2
0.4	1	3	5	4	2	6	0	0	1	0	0	6
0.5	3	1	4	0	3	5	0	0	8	0	4	4
0.6	7	2	4	2	9	3	6	5	8	8	3	9
0.7	8	2	4	2	9	3	6	5	8	8	3	9
0.8	5	2	5	8	6	10	11	14	10	11	7	4
0.9	3	15	5	7	7	0	4	12	5	6	5	4
1.0	2	3	3	0	0	0	5	0	6	0	6	3
1.1											3	1
1.2											1	
N	45	36	45	35	44	46	45	36	45	35	44	46
MEDIAN	.57	.83	.56	.63	.63	.44	.73	.78	.72	.74	.72	.60
KRUSKAL-WALLIS H VALUE			13.41 P<0.05						12.75 P<0.05			

B chromosome level. If mean V/V_o values are plotted against B chromosome number, an interesting correlation is observed (Fig. 5). The 1B sample displays a significantly greater mean V/V_o value than the 0B sample and mean V/V_o values for subsequent B chromosome levels decrease linearly (r_{FDA}=.963, $r_{I_2\text{-}KI}$=.951) from that point.

It is interesting to note that, according to the regression line, mean V/V_o values drop below the level for 0B plants at approximately the 5B level. Thus the picture that emerges is that low numbers of B's in some way decrease the susceptibility of a plant to the type of damage caused by gamma rays.

Experiments 1 and 2, although not designed to properly test this idea, do provide support for it. In all experiments the B chromosome samples showed a tendency toward higher V/V_o values than did the 0B samples and often they differed significantly from the 0B samples.

Experiment 6

In experiment 6 the effect of B chromosomes on mutation rate at one locus (the C1 locus on chromosome 9) was investigated by crossing BMSC-A and BMSC-B plants (premeiotically irradiated with 1000R as well as non-irradiated) as pollen plants to a c1 tester stock. No whole kernel mutations were detected in the C1 gene in any

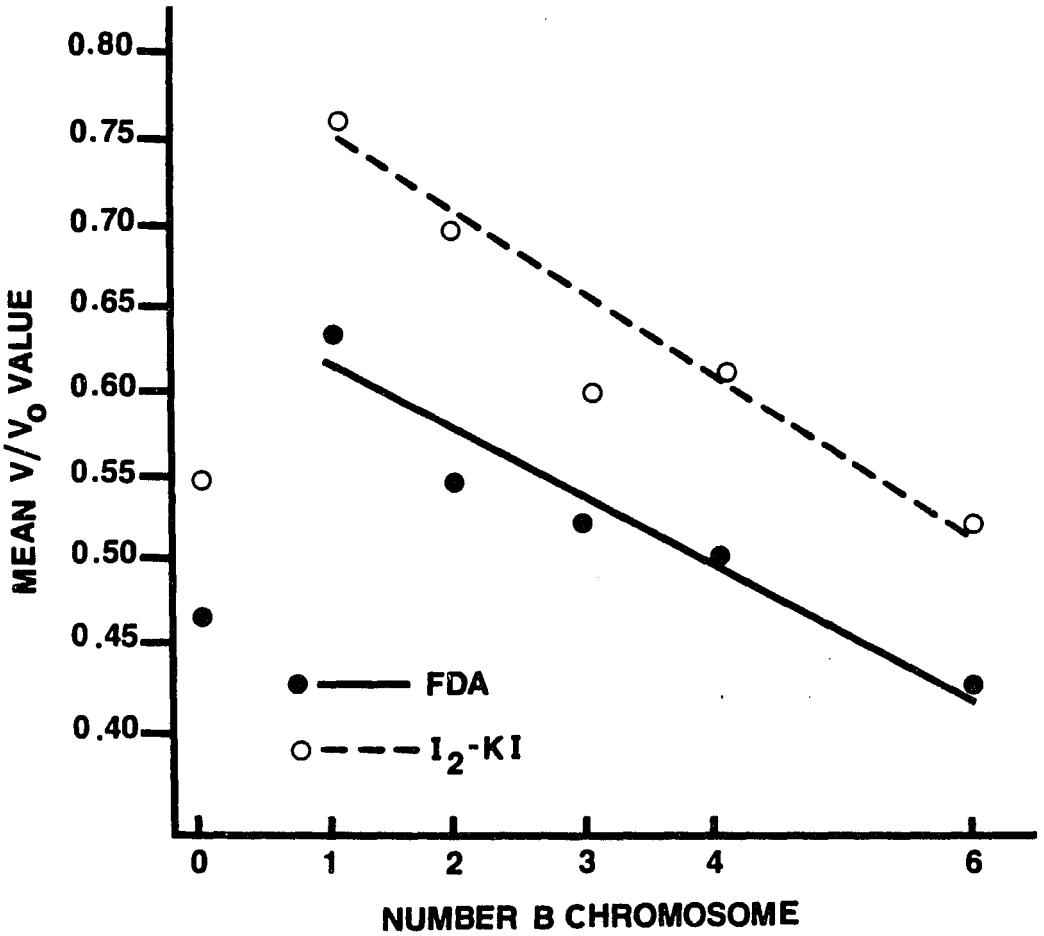


FIGURE 5. Mean V/V_o values for pollen from plants irradiated premeiotically with 1250R in experiments 3 and 4a (0B mean V/V_o from experiment 4a data only). Regression lines fitted using data from plants with B chromosomes only.

cross. The sample sizes for kernels from crosses involving irradiated plants were much too small (evidently due to low pollen viability) to draw any conclusions about the differential effect of gamma rays in plants with and without B's on the C1 to c1 mutation rate. Sectored kernels did, however, appear in all crosses (see Fig. 6a). It is assumed that sectored kernels result from the spontaneous mutation of the C1 gene (transmitted by the BMSC) to c1 in the aleurone cells. The amount of sectoring would depend upon the time the mutation occurred in the development of the aleurone layer. All data are summarized in Table 11.

The data from crosses of non-irradiated BMSC onto the c1 tester reveal a significant ($P<.01$) difference in spontaneous mutation rate at the C1 locus between plants with and without B chromosomes. Plants with B chromosomes ($\bar{x}$(weighted for kernel production) = 2.63 B's/plant) show a reduced spontaneous C1 to c1 mutation rate.

When the BMSC plants are irradiated premeiotically with 1000R, however, the populations with ($\bar{x}$= 2 B's/plant) and without B's show no significant difference in spontaneous mutation rate. In fact, neither irradiated population differs significantly from the non-irradiated BMSC-A population. However, kernels produced by crossing premeiotically irradiated BMSC-B onto the c1 tester display a significantly higher spontaneous mutation rate than those produced using non-irradiated BMSC-B plants as pollen

a

b

FIGURE 6. a) Sectored kernel observed on ear from c1 x C1 cross. The non-pigmented sector is putatively due to inactivation of the C1 allele.
b) Black, yellow mottled, yellow, white mottled, and white kernels from ear on plant RS1252 after pollination by RS1251.

Table 11. Aleurone C1 to c1 mutation rates in kernels obtained by pollinating c1 tester stocks with pollen from non-irradiated as well as premeiotically irradiated Black Mexican sweet corn.

POLLEN PARENT	# B's/ POLLEN PARENT	GAMMA RAY DOSE	SECTORED KERNELS	SOLID COLORED KERNELS	MUTATIONS /CELL($\times 10^{-8}$)
BMSC-A		0R	15	3861	1.465 .025
BMSC-B	2.63	0R	4	4310	0.351 .007
BMSC-A		1000R	1	289	1.305 .025
BMSC-B	2.0	1000R	3	626	1.805 .035

CONTINGENCY X^2

BMSC-A^{0R} vs.	BMSC-B^{0R}	$X^2=7.64$	$P<0.01$
BMSC-B^{0R} vs.	BMSC-B^{1000R}	$X^2=5.73$	$P<0.025$

parents. The implication is that B chromosomes confer upon a plant a reduced susceptibility to spontaneous mutation (at least at the C1 locus). That this reduced susceptibility is eradicated by premeiotic irradiation of the B-containing plants is puzzling. Perhaps whatever sequences in the DNA of the B chromosome that are required to produce this reduced susceptibility to spontaneous mutation at the C1 locus are themselves particularly susceptible to gamma ray damage. Due to the fact that B chromosomes possess no genes necessary for pollen germination, the gametophytic seive that screens out most gross chromosomal mutations produced by premeiotic DNA lesions would have no effect on the B chromosomes. It is therefore possible that although B chromosomes have been passed on through the pollen, they have essentially lost their function with respect to decreasing mutation susceptibility at the C1 locus.

Aberrant Plants

This study was initially designed to compare plants with high numbers of B chromosomes to plants lacking them with regard to gamma ray susceptibility. During the course of these experiments, numerous plants in the B-line turned out to be grossly aberrant and useless for irradiation experiments. The general phenotype of these plants was one of white longitudinal striping (Fig. 7). Many of these plants also displayed perverse developmental patterns. They

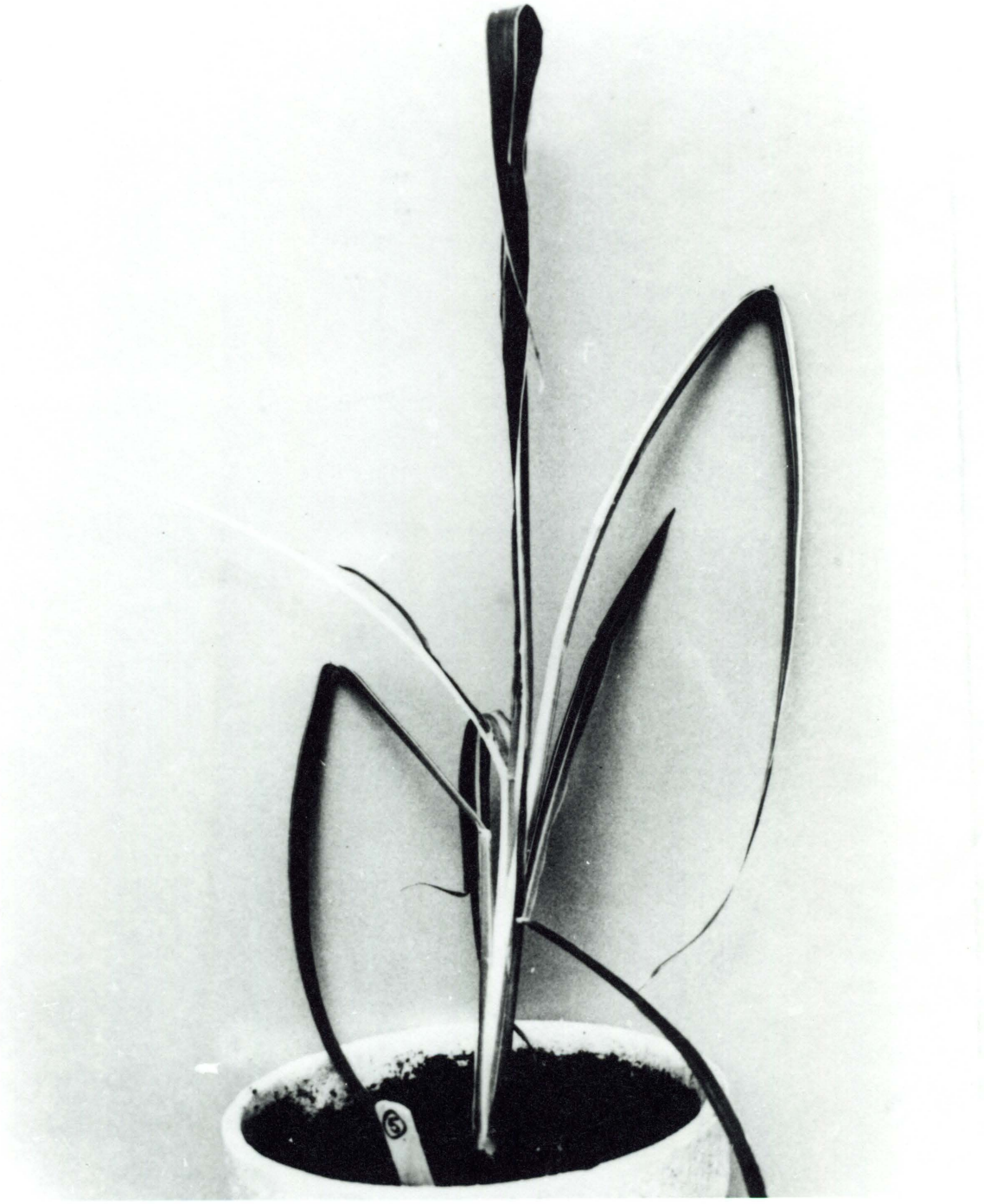


FIGURE 7. Aberrant striped-leaf plant with a high number of B chromosomes.

were often very stunted; had extremely narrow leaves; had extremely gnarled leaves which would not unfold properly; possessed male inflorescences with a reduced number of florets; displayed reduced seed set; or would grow in a prostrate manner. Individual plants exhibited any number of these traits.

The striping generally would not manifest itself until at least the 3-leaf stage and sometimes not until the 7-leaf stage. Quite commonly the striping would disappear in later-developed leaves. Many striped plants would also develop normal tillers.

Root tip cytology was performed on many striped and nonstriped plants from the BMSC-B line and the results are presented in Fig. 8. From the data presented, it is obvious that striping never occurs in plants with less than five B chromosomes and always occurs in plants with more than 8 B chromosomes.

The correlation of striping with high B chromosome number is impressive. Furthermore, striped plants were divided into three groups: mildly striped, striped, and heavily striped. Assignment to groups was made before the plants were analyzed for B chromosome number. The results of this grouping correlated with B chromosome number are illustrated in Table 12. It is evident that striping is influenced by B chromosomes in a quantitative fashion. This is true for most effects of B chromosomes studied in all

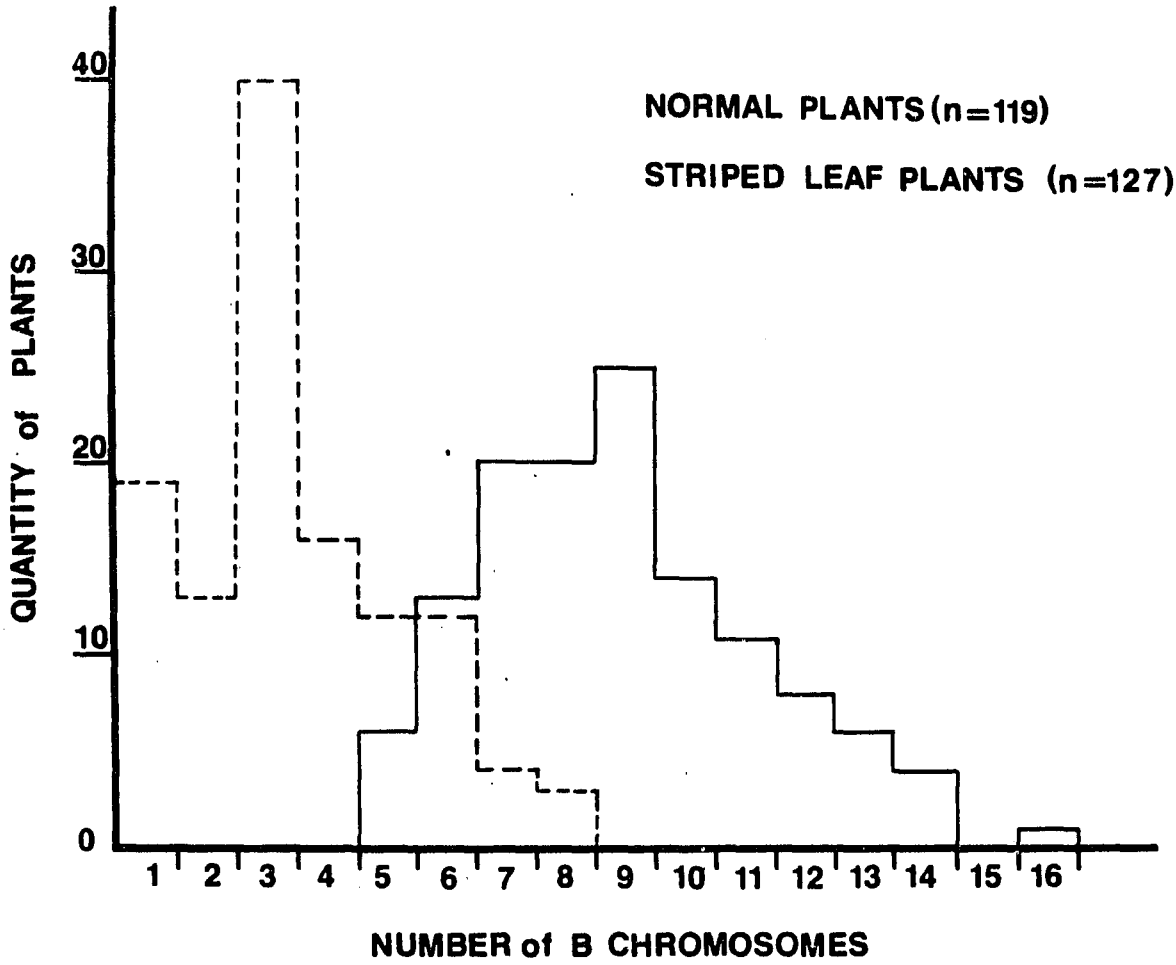


FIGURE 8. Number of B chromosomes in striped-leaf and normal (non-striped leaf) plants.

Table 12. Correlation of B chromosome number with intensity of leaf striping in aberrant plants.

MILDLY STRIPED	MEDIUM STRIPED	HEAVILY STRIPED
N=35	N=74	N=21
$\bar{x}$=6.91	$\bar{x}$=9.03	$\bar{x}$=11.19
SD=1.22	SD=2.09	SD= 1.66

Assigning MILDLY STRIPED = 1
MEDIUM STRIPED = 2
HEAVILY STRIPED = 3 as abscissa values,

REGRESSION EQUATION

$Y = 2.13X + 4.77$

$r^2 = 0.363$

ANALYSIS OF VARIANCE

DUE TO	DF	SS	MS=SS/DF
REGRESSION	1	248.25	248.25
RESIDUAL	128	425.95	3.33
TOTAL	129	674.20	

organisms (Jones and Rees 1982). Exceptions to the rule occur in Plantago coronopus, in which one B chromosome provokes complete male sterility (Paliwal and Hyde 1959), and in Happlopappus gracilis, in which the normal reddish-brown color of the achene is altered to a deep purple color by the presence of one B chromosome (Jackson and Newmark 1960).

Indeed, most exophenotypic effects of B chromosomes are subtle as well as quantitative. Characters such as seed germination rate, growth and vigor, flowering time, fertility, and seed weight are influenced in plants, and vigor, hatching time, development, larval size, femur length, tibia length, and fertility are influenced in various insects (Jones and Rees 1982).

The striping phenotype described here is quantitative as shown but is also qualitative in that plants with less than five B chromosomes never exhibit the phenotype. This is the first striking exophenotypic characteristic attributed to B chromosomes in maize.

An explanation for this phenomenon is not easily produced. One plausible explanation is that the presence of 5 or more B chromosomes in a leaf cell nucleus can cause the segregational loss of A chromosomes or segments thereof. Such a loss would be analogous to the high loss phenomenon described by Rhoades and Dempsey (1972) for segments of A chromosomes containing heterochromatic knobs which occurs at

the second pollen mitosis. They attribute this loss to late replication of heterochromatic regions induced by the distal portion of the B chromosome which Roman (1947) had shown was essential for nondisjunction of B chromosomes themselves. Subsequently, Ward (1973a) located the responsible region more precisely to the distal euchromatic tip of the B chromosome.

Rhoades and Dempsey determined that loss of A chromosome segments occurred only in the presence of two or more B chromosomes in the generative nucleus. Similarly, the striping phenotype occurs only in the presence of 5 or more B chromosomes in a diploid cell. In their study of the high loss phenomenon they found no evidence of increased rate of loss in spores with more than two B's. The striping phenomenon seen here, however, differs from the high loss phenomenon in that it is quantitative.

If B chromosomes are inducing loss of A chromosomes or A chromosome segments, one might expect this loss to be manifested in pollen mother cells of aberrant plants. However, in the course of this study, 36 striped-leaf and non-striped-leaf plants were analyzed mitotically and meiotically for B chromosome number. Of these, only one showed instability and that plant possessed 2 B's in root tip cells and only one B in pollen mother cells. Seven striped-leaf plants were analyzed and all seven showed stability for numbers of A and B chromosomes present. If

loss of A chromosomes is causing striping in the leaves the loss may be limited to cells in those leaves. Chromosome counts in PMC's were made on diakinesis and metaphase I cells. Pachytene chromosomes were not observed, so the loss of A chromosome segments cannot be ruled out. If a phenomenon of the high loss sort is responsible for striping it must be consistently occurring for each chromosome of a homologous pair in order to produce the complete loss of a particular segment and result in striping. This seems a bit unlikely.

A possible explanation for the striping phenomenon invokes the presence of controlling elements in the BMSC-B stock. One of the most well-understood controlling element systems is a nonautonomous one described by McClintock (1951) which consists of a regulator element (Ac=activator) and an operator element (Ds=dissociation). The first attribute assigned to Ds was its ability, in the presence of Ac, to induce chromosome breakage at the site where Ds itself is located. Subsequently, McClintock found that Ds could transpose to other sites (generally on the same chromosome). This transposition would also only occur in the presence of Ac. If Ds transposed to a site within a structural gene or at an adjacent regulatory site for that gene, a recessive mutant allele would quite frequently result. This allele would be stable in the absence of Ac but quite unstable in its presence due to the putative

excision of Ds resulting in the reformation of the original ancestral allele. Such alleles created by transposition of Ds are designated mutable alleles due to their high reversion rate in the presence of Ac. McClintock (1951) has designated chromosome breakage caused by Ds a Type I event and excision of Ds a Type II event.

If controlling elements are involved in the etiology of striping, a regulator element would be located on the B chromosome and the operator (renamed "receptor" by Fincham and Sastry (1974) to avoid confusion with the operators of bacterial systems) would be located on an A chromosome. A Type I event would be initiated at the receptor in the presence of the regulator elements, and this would only result in observable leaf striping when five or more regulator elements are present. The Type I event might be fairly common but would only result in lack of chlorophyll production when it occurred on both receptor-containing homologs in one cell.

If indeed this system did exist, it would be a developmentally-triggered one in that striping never occurs until at least the third leaf and can develop any time up to about the nine-leaf stage. McClintock's studies with the Ac-Ds system indicated a developmentally-triggered pattern of both Type I and Type II events that was dependent in an inverse fashion on the dose of Ac in endosperm tissue. This

system would require mutational events to be directly dependent on the dose of the regulator element.

Rhoades (1966) described a regulator element which he named Dt and which conditions mutability at the a1 locus in a dose dependent fashion. Increasing the dose of Dt in the endosperm from one to three copies progressively increases the a1 to A1 mutation rate (presumably by excision of a responding element; a Type II event). Interestingly enough, Rhoades and Dempsey (1982, 1983) have recently reported the discovery of three new controlling elements--Mrh, Mut, and Ac2--in stocks being used to study the high loss phenomenon. The unstable allele bz2-m, which is responsive to McClintock's Ac, is also responsive to Ac2, but in a dose dependent fashion. Aleurones homozygous for bz2-m and containing one dose of McClintock's Ac display a coarse pattern of Bz sectors on a bz background indicative of early mutational events in the development of the aleurone. When two doses of Ac are present the mutational events occur later and several small Bz dots appear on a bz background. Three doses of Ac cause mutational events to occur very late and few if any Bz dots result. Rhoades' and Dempsey's Ac2, however, induces mutational events (i.e. excision of Ds) rather late in the development of the aleurone no matter what the dose. The frequency of Type II events is highly correlated with the dose of Ac2 however. Zero or one dose of Ac2 produces no Bz dots. Two doses of

Ac2 produces an average of a dozen Bz dots per kernel, while three doses produces an average of 300 Bz dots per kernel. Similarly, Mrh and Mut display increasing mutation rates at higher dose levels.

It is important to note that these new mutable systems had their origins in plants that contained a great deal of heterochromatin. Both the Ac2 and Mrh controlling elements were derived from a plant containing 4 B chromosomes as well as numerous knobs (including K10). Plants that produced the new controlling elements were being subjected to chromosome breakage via the high loss mechanism. Very similarly McClintock's (1951) discovery of controlling elements occurred in plants whose chromosomes were being subjected to the breakage-fusion-bridge cycle which also produced numerous cytological alterations to heterochromatic areas in other chromosomes besides chromosome 9 (which was involved in the B-F-B cycle). Rhoades' Dt element was also located in the heterochromatic knob (Rhoades 1945) on the short arm of chromosome 9. It is also interesting to note that Rhoades (1941) originally isolated Dt and the a1 allele which responds to it, when it turned up spontaneously in Black Mexican sweet corn.

If indeed there is a correlation between heterochromatin breakage and release of transposable elements, then stocks containing B chromosomes may have great potential for producing mutable systems. Randolph

(1941) described a considerable number of B chromosome derivatives which appeared in his B chromosome stocks over the years presumably as a result of B chromosome breakage. He named these derivatives C, D, E, and F chromosomes in order of decreasing size. Longley (1956) has shown that B chromosomes display dyscentric pairing in which the distal and proximal sections of the long arm are paired by a fold-back. This type of pairing has been observed in B chromosome trivalents in the BMSC-B stock used in these experiments (see Fig. 9). This pairing could be of a nonhomologous nature of the sort reported by McClintock (1933), or it could involve homologous pairing of repeated segments of the B chromosome. If recombination occurs between sister chromatids in an inverse repeat region, a dicentric chromosome would be formed which could initiate a B-F-B cycle and create a variety of B chromosome derivatives. This cycle may also induce activity of mutable systems.

If the Black Mexican sweet corn stock with B chromosomes used in these experiments does indeed contain any mutable systems, a search for recessive mutants by crossing to appropriate tester stocks should be fruitful. In fact, of the approximately 10,000 seed produced in seed increases, two different mutant alleles influencing aleurone pigmentation have already been uncovered capriciously.

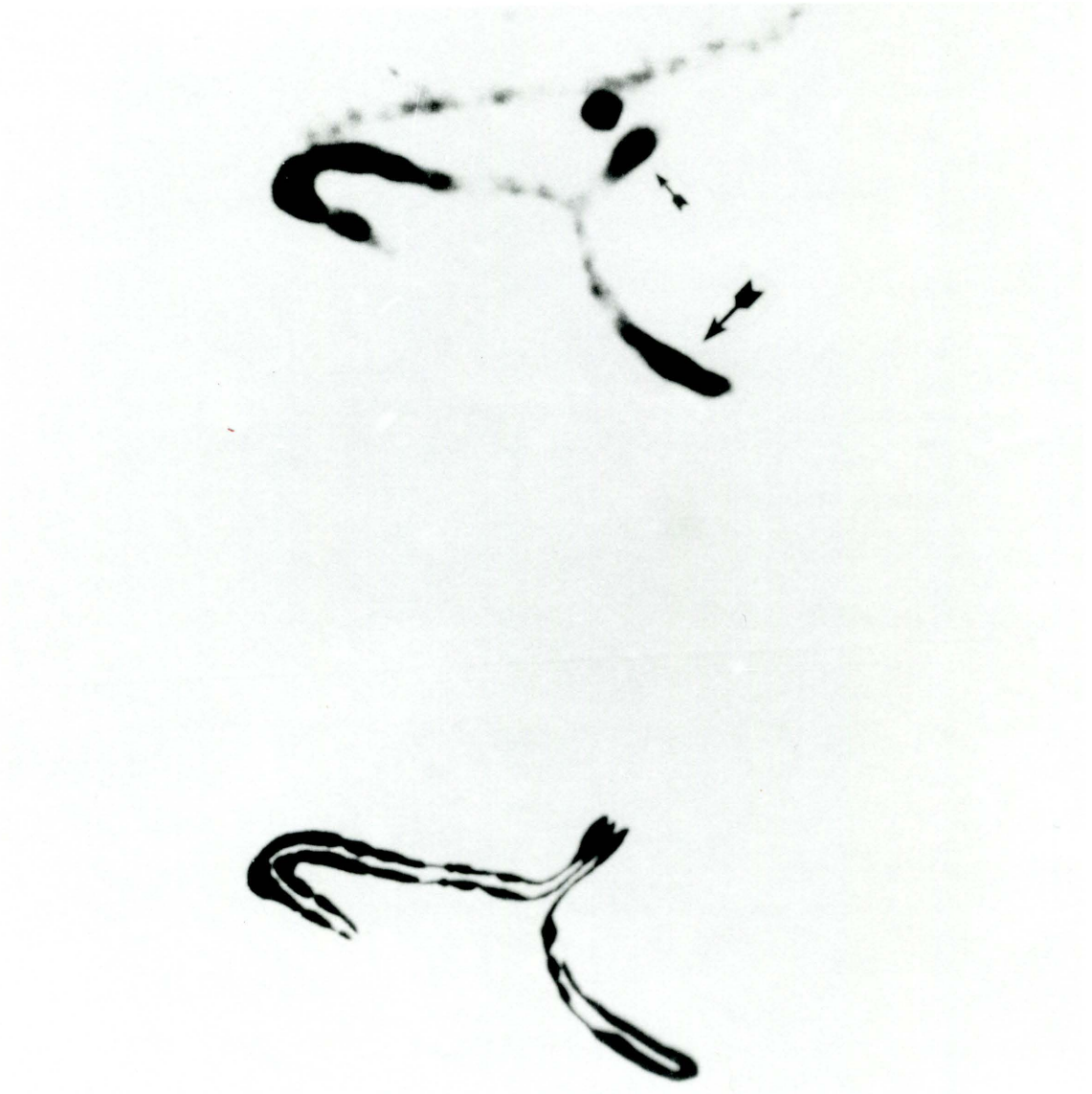


FIGURE 9. B chromosome trivalent at pachytene in PMC from a plant with 3 B chromosomes. Large arrow indicates region of assumed fold-back pairing in the large heterochromatic block of the long arm in one B. Centromeric regions (small arrows) appear to be associated in triplicate.

The first mutant was isolated when plant 1006-(601,928), which contained 10 B chromosomes and had striped leaves, produced on selfing, an ear with 37 black and 11 colorless kernels. None of 1006-(601,928)'s sibs produced any colorless kernels which indicates that the mutation occurred in 1006-(601,928). Crosses to marker stocks indicate that an A1 to a1 mutation has occurred.

The second mutant was isolated when plant No. 214 (containing 2 B chromosomes) was pollinated with pollen from plant No. 913 (possessing 9B chromosomes), producing an ear with 46 normal black kernels, 12 yellow kernels with black spotting and 17 white kernels with black spotting in the aleurone layer.

Two of the yellow mottled kernels produced plants RS1251 and RS1252. Plant RS1251 had 6B chromosomes and mildly striped leaves while RS1252 had 10B chromosomes and striped leaves. Pollen from RS1251 was applied to silks of RS1252. The resulting ear produced 24 black, 12 yellow, 1 white, 10 mottled yellow, and 5 mottled white kernels (see Fig. 6b for kernel phenotypes). Note that this highly inbred Black Mexican strain is segregating for yellow vs. white endosperm which can only be discovered when formation of aleurone pigment is inhibited.

Due to the decreased fertility of plants with high B chromosome number there exists a relative paucity of data. However, analysis of these data leads to some rather obvious conclusions. The gene R at position 57 on the long arm of 10 is necessary for the production of aleurone pigment (Coe and Neuffer 1977). Two or three doses of R produces a colored aleurone while one dose produces a mottled aleurone. Kermicle (1970) has shown that expression of the R allele is altered upon transmission through the male resulting in the mottled phenotype. If a mutation occurred in the R gene (or a dominant gene with a similar effect) causing 214 to be Rr, and either 913 or 214 is Yy while the other is yy, then 1/2 black:1/4 yellow mottled:1/4 white mottled kernels would be expected on the ear produced on 214 if R and Y are not linked. The data fit this hypothesis (X^2=4.52, P_{2df}=.1-.2).

Plants RS1251 and RS1252, being derived from yellow mottled kernels, must be genotypically RrYy. Thus, crossing RS1251 with RS1252 should produce 1/2 black:3/16 yellow:3/16 yellow mottled:1/16 white:1/16 white mottled kernels. Again, the data obtained satisfy this hypothesis (X^2=3.18, P_{4df}=.5-.7).

Until further allele tests are performed the identity of this mutant aleurone color allele cannot be conclusively identified as r, but it certainly behaves in an identical manner.

The a1 mutant does not demonstrate mutable behavior. The putative r mutant tentatively seems to be behaving in a non-mutable fashion. Obviously, more tests need to be performed on these isolated mutants before any statements can be made about the existence of a mutator system in this stock. The occurrence of two spontaneous mutations among less than 10,000 kernels and the existence of alternative endosperm color alleles (putatively Y and y) in this highly inbred stock seem, however, to at least indicate the possibility that the BMSC-B stock is exhibiting a high mutation rate.

Another possible explanation for the striping phenotype is that B chromosomes physically interfere with interactions of A chromosomes. The possibility that physical proximity of chromosomal regions is involved in gene regulatory processes has been suggested (see Lewin 1981); much evidence supports the supposition that chromosomes occupy specific locations within the nuclear architecture (Avivi and Feldman 1980; Horn and Walden 1978; Maguire 1983). Along similar lines, Rhoades and Dempsey (1972) also suggested that B chromosomes may physically interfere with movements of A chromosomes. If indeed B chromosomes do interfere with regulatory communication in A chromosomes, they should be nonrandomly distributed in the nucleus, since they are able to cause this consistent striping phenotype from plant to plant. It is possible that

there are only a limited number of slots within the nuclear matrix which are available to B chromosomes and additional B's tend to interfere with normal nuclear processes. Clearly, more data are needed on distribution of B's within the nucleus before any statements can be made regarding their physical interactions with A chromosomes.

If it is assumed that the B chromosome of maize was derived from an A chromosome, it is possible that B's contain DNA sequences which are quasi-homologous to sequences in the ancestral A chromosome. These could interfere with normal regulatory processes of certain genes on the A chromosome. Ward (1979) has proposed that the B chromosome of maize was derived from the K10 chromosome (an unusual version of chromosome 10 with a large heterochromatic knob terminally located on the long arm). He has compared pachytene structures of both chromosomes and found striking similarities in location of heterochromatic blocks and chromosomes (Fig. 10).

It may be only a coincidence, but chromosome 10 contains two loci at which recessive mutant alleles condition narrow leaves and leaf striping, the two predominant phenotypic traits of aberrant plants in the B chromosome line. These are the nl (narrow leaf) locus which maps to position 24 in the short arm of 10 and sr2 (striate-2) which maps close to the end of long arm of 10 (Neuffer, Jones, and Zuber, 1968). It could be reasonably

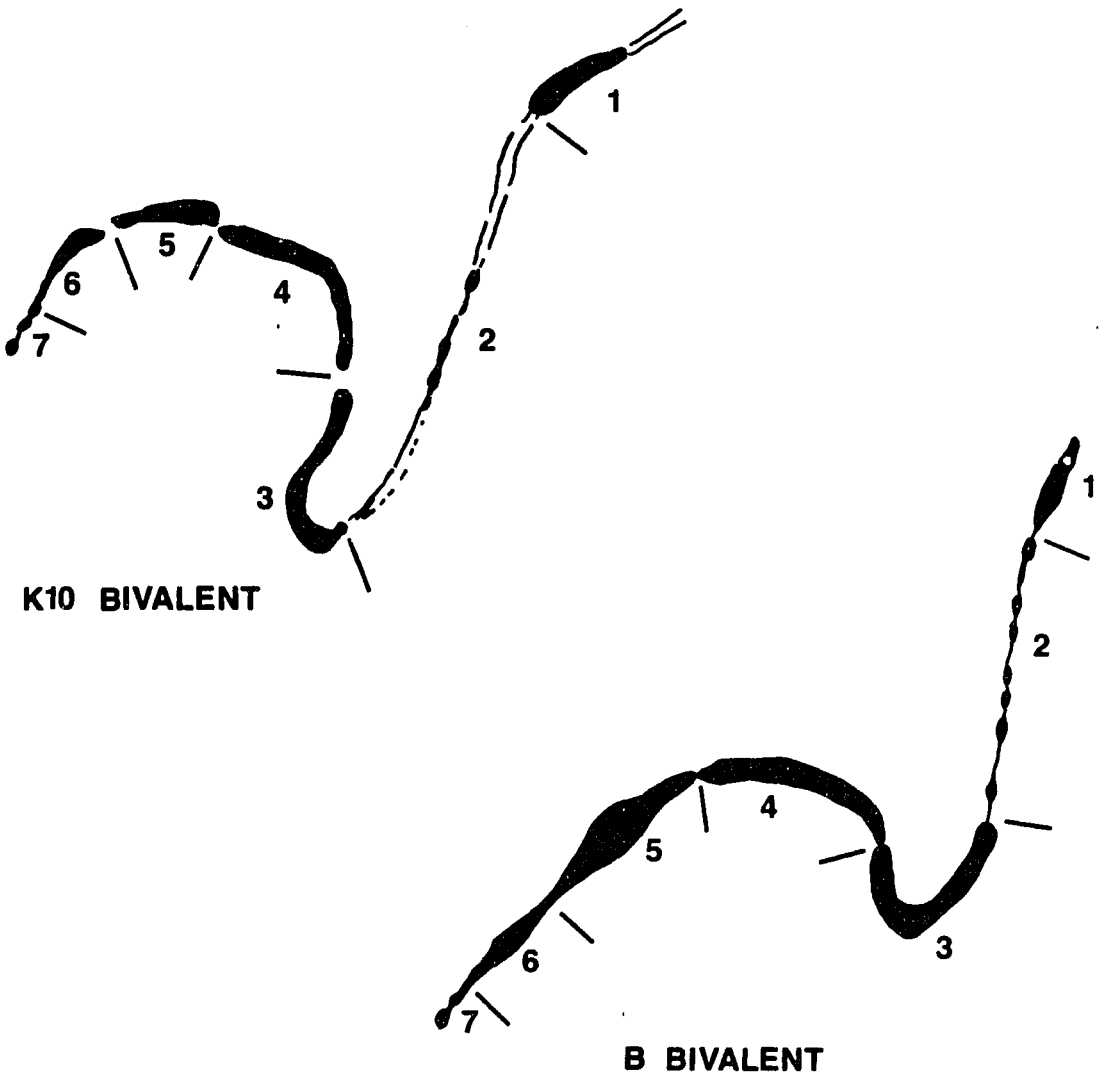


FIGURE 10. Comparison of B and K10 pachytene bivalents (after Ward 1979).

argued that there is a large number of mutant genes which produce leaf striping (e.g. sr1, sr3, f1, ij, j1, j2) that have been mapped to several different chromosomes, and to single out sr2 as the culprit smacks of lynch-gang tactics. The narrow leaf (n1) gene, however, is the only one of its kind which has been mapped in maize, and it is on the putative ancestral chromosome 10.

It is plausible, for example, that the B chromosome contains sequences which are quite similar to the $n1^+$ and $sr2^+$ sequences present on each chromosome 10. Let us assume that the sequences are transcribed into pre-mRNA molecules that cannot be processed into functional mRNA's. They still, however, tie up the processing machinery in the nucleus, competing with pre-mRNA transcripts of the $n1^+$ and $sr2^+$ genes. If the B chromosome transcripts are present in large enough quantities, normal expression of $n1^+$ and $sr2^+$ genes will be inhibited. Saturation of the processing machinery to the point where a noticeable exophenotypic effect is produced would not occur until at least five B chromosomes were residing in a nucleus.

It is interesting to note that Ayonoadu and Rees (1971) also found a cutoff level of five B chromosomes when they investigated RNA/DNA ratios in maize root meristem nuclei containing varying numbers of B's. The ratio in plants with one to four B's was greater than that in plants lacking B's, but once five B chromosomes were present in the

nucleus the ratio was back at the zero level and decreased with increasing numbers of B's. Thus, the presence of one to four B chromosomes seems to be stimulating transcription. It is unknown whether this increased transcription is occurring from B chromosome or A chromosome DNA, although it appears that rRNA synthesis may be responsible for a good proportion of the increase. Once five B chromosomes are present in the nucleus, it appears that RNA production decreases. It is also true that the striping phenomenon never occurs in a plant with less than five B chromosomes.

Irrespective of the etiology of the aberrant striping phenotype, an explanation for its sudden appearance in this highly inbred Black Mexican sweet corn stock is sorely lacking. This same stock has been utilized by countless numbers of researchers and yet this striking exophenotypic trait, so highly correlated with B chromosome number, has never been reported.

It is interesting to note that 14 of 29 plants used in the initial BMSC-B seed increase contained more than 5 B chromosomes, yet no aberrant plants were observed. Of the 29 plants, three contained 9 B chromosomes yet displayed no striping.

Although the aberrant striped plants are hard to explain, the correlation between B chromosome number and striping should prove useful in separating populations of plants into high and low B subpopulations in experiments

designed to determine effects of B chromosomes. This will eliminate much time-consuming cytology.

Relationship of B Chromosome Number to Gamma Ray Susceptibility

Great care was taken in experiments 1-4 to assure that plants were irradiated at a stage in which germ cells were not yet at the pachytene stage of meiosis. Before any irradiations were performed, several populations of plants were grown and individual plants were sequentially dissected to determine the best technique for assaying proper date of irradiation.

The developing tassel displays a logarithmic growth rate just prior to initiation of meiosis in the oldest PMC's. This growth rate continues until the youngest PMC's have almost completed meiotic divisions. There is approximately a seven-day difference in the initiation of meiosis in the youngest and oldest PMC's. This is in agreement with the observation that individual Black Mexican sweet corn plants in these experiments shed pollen for approximately seven days and with the observation that typical maize plants shed pollen for a week or more (Kiesselbach 1949). When this logarithmic growth is initiated, a 35 mm elongation of a tassel in 24 hours is not uncommon. By cytological analysis, it was found that

tassels 90 mm or shorter generally contained all prepachytene germ cells.

Since growth of tassels was so rapid at this stage, however, tassels of meiotic estimator plants which were between 25 and 90 mm were considered indicative of readiness for irradiation. Interestingly, this stage showed a weak correlation with plant size within populations. It showed a much better correlation with plant development as determined by leaf emergence. However, leaf emergence could not be used as an indicator across populations which were planted at different times of the year. For example, if a population was planted at a time of year in which days were long the plants might reach the 11- or possibly even 12-leaf stage by the time the tassel was of irradiation age. If growing conditions were not so good, plants would only reach the 10-leaf stage by the time of irradiation.

The ideal irradiation protocol would be one which irradiates all PMC's at the leptotene stage of prophase. Since the germ cells in the tassel display such a developmental gradient, this is not possible. The technique used assures that all germ cells are pre-pachytene at the time of irradiation but due to this developmental gradient as well as environmental variability, some germ cells may be at the PMC stage while others may still be at the archesporial stage. Thus, some induced DNA damage may actually be of a clonal type.

The data presented in this section have strong implications. In all experiments, plants with B chromosomes consistently displayed higher mean V/V_o values than plants lacking B chromosomes. In experiments 3 and 4, in which plants were divided into groups by B chromosome number, the data were striking (Fig. 5 and Table 10). Plants with one B chromosome were significantly resistant to the deleterious effects of gamma radiation. Plants with more than one B chromosome were not significantly more resistant than plants lacking B chromosomes. However, the trend is that increasing numbers of B chromosomes gradually reduce the resistance to gamma radiation induced DNA damage. When a plant contains 5 or 6 B chromosomes per cell, the susceptibility appears to be the same as that of plants without B chromosomes.

This presents a very important question. How is it that the presence of a B chromosome provides a reduction in susceptibility to gamma radiation induced DNA damage that is gradually negated by increasing the number of B chromosomes?

The idea that constitutive heterochromatin may serve to protect the rest of the chromatin in the nucleus from mutagens, clastogens, and viruses has been proposed (Hsu 1975). Hsu points out that studies in Peromyscus eremicus indicate a layer of condensed chromatin lining the inner nuclear membrane in interphase cells. This layer of condensed chromatin appears to contain constitutive

heterochromatin, although it may also contain some facultative heterochromatin; and, according to Hsu, it could form an excellent barrier to mutagens, clastogens and viruses. Thus the heterochromatin would act as a "bodyguard" to the euchromatin of the nucleus. Hsu also points out that the availability of maize stocks with and without B chromosomes provides an ideal experimental situation in which to test the bodyguard hypothesis. Autoradiographs of interphase nuclei containing B chromosomes which have incorporated ^{3}H-thymidine suggest that the B's also are located at the periphery of the nucleus (Himes 1967).

If B chromosomes are indeed located at the periphery of the interphase nucleus, then one would expect them to have no effect on susceptibility to penetrating ionizing radiation. If, however, the chromatin of B chromosomes were interspersed throughout the nucleus, it is possible that B's would confer a certain amount of protection from gamma rays to the euchromatin. For example, the B chromosome DNA could scavenge free radicals within the nuclear milieu, thereby reducing DNA damage to A chromosomes.

If B chromosomes serve a bodyguard function against gamma ray damage, V/V_o values obtained in experiments 3 and 4 would be expected to show a positive correlation with B chromosome number. On the contrary, V/V_o values tend to be negatively correlated with B chromosome number. The

data imply that B chromosomes do not serve as bodyguards to A chromosomes.

It has been pointed out previously that B chromosomes alter the metabolism of nuclei in which they reside (Ayonoadu and Rees 1971). This suggests the possibility that, by altering the chemical composition of the nucleus, B chromosomes could reduce the amount of free radicals and thus decrease radiation-induced DNA lesions.

Finally, it is possible that B chromosomes have no influence on induction of DNA lesions by gamma radiation, but instead increase a plant's ability to repair those lesions. The correlation of radiation resistance with DNA repair capacity has been demonstrated in a number of organisms including Drosophila (Tikhomirova 1980), silkworms (Tazima 1968), yeast (Resnick 1976; Saeki et al. 1980), and Micrococcus radiodurans (Kitayama and Matsuyama 1971).

Repair of DNA lesions has been described in numerous organisms and appears to be a universal phenomenon. (For good reviews see Hanawalt et al. 1978, Hanawalt et al. 1979, Lehmann and Karran 1981.) Ionizing radiation can cause single-strand lesions or double-strand scissions. Repair mechanisms for single-strand lesions are well-known and include photoreactivation, excision repair, post-replication recombinational repair, and SOS or error-prone repair. On the other hand, very little is known about the repair of double-strand scissions.

Essential to the repair of any double-strand scission is the existence of some means for maintaining the broken ends of the double helix in close proximity. Chromosomal proteins in both prokaryotes and eukaryotes could provide this stabilization.

It is feasible that a double-strand break could simply be repaired by a ligase which rejoins the broken ends. However, this requires the proper substrates (e.g. a 3' hydroxyl and 5' phosphate). While approximately 10-20% of ionizing-radiation-induced breaks produce 3' phosphate and 5' hydroxyl ends, it can also be assumed that some breaks produce phosphate groups at both the 5' and 3' ends of the break, since phosphate free sugars as well as damaged nucleosides are liberated during irradiation of DNA solutions (Von Sonntag and Schulte-Frohlinde 1978). The latter type of break produces a one-nucleotide gap.

Larger gaps could result from the exonuclease degradation of the free ends. DNA degradation following irradiation has been observed in both prokaryotes and eukaryotes (Mattern et al. 1973). If gaps exist in the same region on both strands of a double helix, accurate repair of that region requires a homologous double helix to be used as a template.

Repair of double-strand breaks in DNA would thus be facilitated by a recombinational mechanism which would enable gaps to be filled by replication of strands from a homologous double helix. Resnick (1976) has proposed a model for repair of double-strand breaks involving heteroduplex formation and resultant reciprocal or non-reciprocal recombination.

It should be pointed out here that the interrelationship between DNA repair and genetic recombination has been demonstrated in a number of organisms including many bacteriophages and viruses, E. coli, Saccharomyces cerevisiae, Ustilago maydis, Drosophila melanogaster and cultured Chinese hamster ovary cells (for an excellent review see Bernstein 1983).

The strong correlation between DNA repair and recombination has in fact evoked the suggestion that the repair of DNA damage may have been a selective factor equal in importance to the generation of genotypic diversity in the evolution of homologous pairing and recombination of DNA molecules (Bernstein 1977; Bernstein, Byers and Michod 1981). The idea that DNA repair may have been a factor in the evolution of recombination has also been expressed by Felsenstein (1974) and Maynard Smith (1978) on the basis of more theoretical evolutionary considerations.

Of particular interest is the fact that double-strand breaks in E. coli as well as yeast can be repaired and that this repair is dependent on recombination. In E. coli K12, double-strand breaks produced by gamma irradiation were repaired when four to five genomes per cell were present. Repair did not occur with only 1.3 genomes per cell. Furthermore, recA$^-$ cells (which are unable to undergo genetic recombination) did not repair double-strand scissions (Krasin and Hutchinson 1977). In yeast, rad 52 mutants cannot repair double-strand scissions induced by ionizing radiation and are unable to undergo recombination or mating-type switching (Resnick and Martin 1976; Prakash et al. 1980; Game et al. 1980; Malone and Esposito 1980). Furthermore, during meiotic DNA synthesis they accumulate breaks in parental DNA which are single-stranded and may represent unresolved recombination intermediates (Resnick et al. 1981).

Information on the relationship of DNA repair and recombination in plants is scarce. The only relevant data have been obtained from barley, in which a desynaptic mutant displayed significantly more chromosome reunions in root-tip cells of X-irradiated seed than did its normal counterpart (Riley and Miller 1966). Eriksson and Tavrin (1965) summarize data on radiosensitivity in several organisms during meiosis and point out that the most sensitive stages are most frequently post-zygotene. This summary includes

data from Tradescantia, Vicia, Trillium, and Lilium and they report experiments which indicate the most radiosensitive stages in maize (as measured by Wx to wx mutation in pollen grains) to be pachytene and diplotene. Although chromosome condensation may have a significant influence on irradiation susceptibility, homologous pairing may also and these data are in agreement with the idea that repair of DNA lesions can occur when chromosomes are homologously paired.

In light of the fact that B chromosomes in maize alter metabolism of the nucleus (Ayonoadu and Rees 1971) and are capable of stimulating recombination (Ayonoadu and Rees 1968; Rhoades 1968a and b; Hanson 1969; Nel 1973; Ward 1973b; Chang and Kikudome 1974), it is reasonable to expect that they may have a stimulatory effect on recombinational repair of DNA damage. The data obtained in the experiments presented here are in agreement with this expectation to an extent. However, chiasma frequency and map distances between selected loci respond to B chromosomes in a quantitative fashion while radioresistance does not. This discrepancy is in want of an explanation.

An explanation becomes apparent when the effect of B chromosomes on pollen viability in control plants is considered (Fig. 4). Increasing numbers of B chromosomes progressively reduce pollen viability. Since pollen viability is also used to measure susceptibility to irradiation, a positive interaction between the effects of B

chromosomes and radiation on pollen viability is quite possible.

For example, if B chromosomes actually reduce pollen viability by inducing meiotic breakage of A chromosomes, as postulated earlier in this section, then premeiotic irradiation might well intensify this effect by creating more potential breakage points. If such a positive interaction exists, then the data indicate that the presence of one or more B chromosomes increases the recombinational repair capacity of a plant.

It is also important to note that postmeiotic irradiation in experiment 5 produces no significant difference in the distribution of V/V_o values of plants with and without B chromosomes (Table 9). These data must be interpreted with caution, however, since the mean number of B's per plant was 8.4. This group of plants might not be expected to differ from a OB sample even when irradiated premeiotically.

Once again, in the postmeiotic irradiation, B chromosome plants showed a tendency toward higher V/V_o values. Together with the results of experiment 6 (Table 11), in which spontaneous C1 to c1 mutation in aleurone cells was shown to decrease in the presence of B chromosomes, these results imply that B chromosomes may provide protection against DNA damage in non-meiotic as well as meiotic cells. It is possible that recombinational

repair of DNA lesions also occurs in somatic cells if sister chromatids could be used as templates.

Possible Selective Advantage of Low Numbers of B's

It has been mentioned previously, and is illustrated in Figure 5, that V/V_o values gradually decrease with increasing numbers of B's when plants are irradiated premeiotically with 1250R. At the 5 or 6 B chromosome level, the mean V/V_o value is reduced to the level of 0B plants.

It is also true that striping never occurs in plants with less than 5 B chromosomes. Plants with increasing numbers of B's show an increased intensity of striping as well as other developmental abnormalities.

The presence of B chromosomes also has a stimulatory effect on RNA and protein synthesis (Ayonoadu and Rees 1971). The RNA/DNA ratio is higher in nuclei containing 1-4 B chromosomes (with an odd-even effect) than in 0B controls; however, when 5 or more B's are present, this ratio is reduced to a level at or below that of 0B nuclei.

Darlington and Upcott (1941) reported the tendency of B chromosomes to be lost in meiotic divisions and postulated some selective force acting to increase the number of B's in a population in order that loss may be compensated and an equilibrium reached. The data presented here are in agreement with the idea that a low number of B

chromosomes per plant may confer a selective advantage on a plant or a population of plants whereas higher numbers of B's seem to be detrimental. Furthermore, there is a strong implication that negative influences on fitness become apparent at the 5 or 6 B chromosome level.

It has been suggested (Ostergren 1945; Rhoades and Dempsey 1972) that the populational maintenance of B chromosomes can be explained by their parasitic nature. Thus, the deleterious effects of high numbers of B's are countered by accumulation mechanisms. There is little doubt that this situation exists in maize. However, it is suggested that increased population variability due to increased genetic recombination as well as decreased mutational susceptibility of plants with B chromosomes may have had important roles in the maintenance of B chromosomes in natural populations of maize or teosinte.

SUMMARY

Experiments were performed in order to test the effect of B chromosomes on the susceptibility of maize to gamma-radiation-induced DNA damage. Isogenic stocks of Black Mexican sweet corn were premeiotically irradiated and DNA damage was assayed by measuring pollen viability.

Samples of plants with B chromosomes consistently displayed higher post-irradiation pollen viabilities when compared to non-irradiated controls than did plants lacking B's. When samples with varying numbers of B chromosomes were irradiated with 1250R, 1B plants displayed the highest pollen viabilities and those with 2, 3, 4 or 6 B's showed progressively less viability. The pollen viability of 1B plants was significantly different than that of 0B plants.

Possible mechanisms for the differential susceptibility to gamma radiation of plants with and without B chromosomes are discussed. A bodyguard effect of the heterochromatic B chromosomes is not supported by the data. The possibility that B chromosomes may increase the ability to repair gamma-radiation-induced DNA damage is suggested. Whether this repair is due to a recombinational process is not ascertainable with certainty from results presented here, although it remains an attractive possibility.

The appearance of aberrant plants in the Black Mexican sweet corn stock with B chromosomes is also reported. The most striking aspect of the aberrant plants is the appearance of white longitudinal leaf stripes. Aberrant plants often have narrow leaves in addition to leaf stripes. Cytological studies of root tips from plants in this stock reveal that aberrant plants always have 5 or more B chromosomes. Furthermore, striping varies quantitatively with B chromosome number.

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