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CYTOGENETICS OF GAMETOGENESIS IN HAPLO 9 OF GOSSYPIUM HIRSUTUM L.

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CYTOGENETICS OF GAMETOGENESIS IN HAPLO 9 OF *GOSSYPIUM HIRSUTUM* L.

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Elbert Lewis Myles, Jr.

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

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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.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

J E Endrizzi	10/22/84
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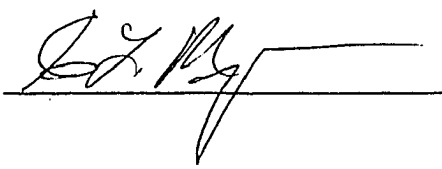
SIGNED: 

Dedicated in Remembrance

of

Everett, Janice, Enoch,

Janica and Everett II

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ABSTRACT

Plants monosomic for chromosome 9 of Gossypium hirsutum, in addition to Haplo 9 plants, produce many kinds of aneuploid progenies consisting of monosomics, trisomics, multiple monosomics, and different monosomic-trisomic combinations. Cytological analysis of megasporagenesis was conducted to determine the stage and the mechanism that would account for the production of the different kinds of aneuploid progeny.

Two new cytotypes involving chromosome 9 were isolated and identified as monotelodisome 9S (Telo 9S) and monoisodisome 9S (Iso 9S), both of which produce different kinds of aneuploids very similar to Haplo 9. Monotelodisomic 9L plants do not produce various kinds of aneuploid progeny like Haplo 9, Telo 9S and Iso 9S. These observations indicate that control of chromosome segregation is on the long arm of chromosome 9. There was no significant difference between Telo 9S and Iso 9S in the kinds and frequencies of aneuploids, but there was a significant difference between these two and Haplo 9. Haplo 9, Telo 9S and Iso 9S showed no significant difference in the chromosomes subtracted or added to the chromosome complement of their respective aneuploid progenies.

Analysis of female gametogenesis in TM1 or control plants showed that premeiotic division occurred when bud size is 6.0 - 6.6mm; meiosis occurred when bud size is 6.3 - 7.2mm; the first mitotic division of the megaspore occurred when bud size is 6.9 - 7.8mm, and the second and third mitotic divisions of the megaspore occurred when bud size is 7.8 - 8.5mm. The premeiotic, meiotic, and post meiotic divisions were normal.

Female gametogenesis was cytologically analyzed in Haplo 9 plants and it was observed that there was a higher frequency of gametophytes with fewer post meiotic divisions and that there was nonsynchrony of the post-meiotic divisions in a number of the developing female gametophytes. It is assumed that these irregularities are due to the absence of chromosome 9.

It is concluded that the long arm of chromosome 9 has control over chromosome segregation, and that most likely nondisjunction occurs throughout female gametogenesis, but confined primarily to the three mitotic divisions following meiosis.

Genetic tests showed that eight mutant genes were not located on chromosome 9. The aneuploids had lower seeds per boll than normal disomic plants.

CHAPTER 1

INTRODUCTION

The genus Gossypium is divided into two major groups of species: Old World and New World diploids (2n=2x=26) and allotetraploids (2n=4x=52). The genomes of the diploids are categorized as A through G (Beasley, 1942; Phillips and Strickland, 1966; Edwards and Mirza, 1979). The allotetraploid species are composed of the A and D subgenome chromosomes which form 26 bivalents during meiosis. Chromosomes of the A subgenome are about twice the size of the chromosomes of the D subgenome (Davie, 1933). Two of the allotetraploids are cultivated for their seed and fiber and one of these, G. hirsutum L. [$2(AD)_1$ genome], is grown worldwide because of its high yielding capacity.

Over the years, a considerable body of data has been developed on the cytogenetic relationships of the species in the genus Gossypium. In recent years, cytogenetic research has been concentrated in the cultivated allotetraploid species G. hirsutum for the development of chromosome stocks consisting of aneuploids and reciprocal translocations. These stocks have been used to identify and number the A and D subgenome chromosomes of the allotetraploid G. hirsutum. The A subgenome chromosomes are numbered 1 through 13 and the D subgenome chromosomes are numbered 14 through 26.

In species with the diploid number of chromosomes, the loss of a chromosome is more deleterious than the addition of a chromosome. Individuals with a 2n-1 chromosome complement among diploids are relatively rare unless the chromosome involved is very small, such as chromosome IV in Drosophila, or largely heterochromatic (Bridges, 1916, 1928; Hamklao, 1973). Polyploid species, however, can tolerate the loss of one or more chromosomes because they consist of duplications for two or more genomes. The allotetraploid G. hirsutum can withstand various chromosome deficiencies simply because of genomic duplications. Currently, 19 of the 26 chromosomes of G. hirsutum have been identified as either monosomes or telosomes. These include chromosomes 1 through 10 and 12 of the A subgenome chromosomes 14 through 18, 20, 22, 25 and 26 of the D subgenome (Endrizzi and Ramsay, 1979).

In G. hirsutum, a monosomic plant (2n-1) when crossed with a normal disome (2n) or self-pollinated produces progeny consisting almost always of disomic plants and monosomic plants for the chromosome involved. On rare occasions plants appear among the progeny which are monosomic for a different chromosome. When this occurs it is commonly referred to as univalent shift (Person, 1956). The monosomics of wheat also show the phenomenon of univalent shift which can occur in anywhere from zero to two percent of the progeny and in almost all cases involves the shift of a single chromosome (Burnham, 1954; Persons, 1956).

A plant that was monosomic for a large A subgenome chromosome of G. hirsutum later designated as Mo24 was produced by pollen irradiation of a multiple marker line T586 and pollinating a multiple recessive line T582 with the irradiated pollen (Endrizzi and Ramsay, 1980). The monosome was later identified as chromosome 9 in a cross with a reciprocal translocation (T_{9-17}) involving chromosome 9 and 17 of the A and D subgenomes respectively. Plants haploid for chromosome 9 (Haplo 9 or Mono 9) produce progeny which have the lowest recovery rate of the monosome as compared to all other monosomes of cotton (Malek-Hedayt, 1981). Furthermore, the progeny resulting from either self-pollination or outcrossing Haplo 9 plants show in addition to Haplo 9 plants a high incidence of other aneuploid types of plants. The aneuploid cytotypes range from 2n-1 to 2n-1-1-1-1-1-1-1-1-1 for example.

In the cytological analysis of pollen mother cells of Haplo 9 plants, meiosis is essentially normal in that chromosome pairing at metaphase I is almost always 25 bivalents and a univalent; also divisions I and II appear normal. At the quartet stage, four normal size microspores are observed with an occasional micronucleus due to exclusion of the univalent chromosome. Any aneuploid gametes in addition to the ones which are deficient for the chromosome 9 that might form due to the rare occurrence of abnormal pairing or chromosome nondisjunction in microsporogenesis would not likely be functional in self-pollination because of certation. Furthermore, the different kinds of aneuploids always occur in crosses of Haplo 9 as the female parent with a male

parent possessing the standard or normal chromosome complement. Thus it is believed that the aneuploid gametes are the results of irregular chromosomal segregation in either meiosis or mitosis during megasporogenesis.

It is assumed that the gametes involved in the production of the aneuploid types are those that are deficient for chromosome 9 which were produced following division I of meiosis. If that is the case, it suggests that chromosome 9 carries a gene(s) controlling normal chromosome segregation and/or cellular division, whose absence causes irregular chromosome segregation, resulting eventually in the formation of aneuploid zygotes. The present study was conducted to determine the stage and the mechanism for the abnormal cytological effects that occur in Haplo 9 resulting in the various kinds of aneuploid gametes. In addition, average seed-set per boll was determined in Haplo 9 and telosomes and an isosome of chromosome 9. Also, several marker genes were tested with Haplo 9 to determine whether one or more were associated with chromosome 9. The objective of the latter study was to identify a marker gene on chromosome 9 so that the marker gene could be used to obtain information on the segregation of chromosome 9 in Haplo 9 plants in gamete formation and its occurrence among the progeny.

CHAPTER 2

LITERATURE REVIEW

Genetic Control of Cell Division

Dividing and non-dividing cells pass through a regular sequence of events commonly referred to as the cell cycle. Completion of the cycle requires varying periods of time depending on the type of cell and external factors such as temperature and available nutrients. It is well understood that the cycling process is under genetic control. (Hartwell, Cullotti and Reid, 1970; Esposito and Esposito, 1974; Evgen'ev and Sidorova, 1976). The genetic control of the structural and physiological features of nuclear and cell division is exemplified in a number of organisms.

Meiotic Mutants

The systematic isolation of mutants has been initiated in a wide range of organisms, including both plants and animals. The analysis of cell cycle mutants in the yeast Saccharomyces cerevisiae has reached a more advanced stage of sophistication than in other eucaryotic systems (Simchen, 1978). The complex cellular interactions that regulate meiosis in multicellular organisms and the encumbrances of gamete development are lacking in this single celled organism. Yet the basic processes of meiotic DNA replication, chromosome pairing, genetic recombination, interference, polarity and chromosome segregation are all present and similar to those of higher organisms.

The mechanism by which many mutations affect the meiotic process in yeast has been studied extensively. Most of the mutants show a marked reduction in viability upon incubation at restrictive temperatures, after which the mutation is defined by specific procedures that employ genetic and physiological tests. Such tests were performed by Esposito and Moens (1974) in their identification of two recessive meiotic mutants, spo2 and spo3, in that there was no ascus formed on sporulation medium at restrictive temperature. Yeast mutants that are homozygous for spo2 fail to sporulate at the restrictive temperature and also have unduplicated spindle pole bodies. In these diploids, meiosis I is defective in that nuclear division is accomplished but migration of nuclear material does not occur. The result is one ascospore with 4 nuclei and 3 ascospores without nuclei. In spo3 diploids, sporulation is morphologically normal until the second meiotic division. At this time nuclear budding lags relative to closure of the prospore walls which leads to anucleate immature ascospores; also ascospores are formed containing bits of nucleoplasm. In addition to spo2 and spo3 there are 12 other loci that effect the segregation pattern of nuclear materials during meiosis (Johnson and Game,1978; Culotti and Hartwell, 1971). These mutants are similar in that the kinetic functions are modified or impaired. Spindles may be deformed or absent, nuclear segregation may be deviant or unorderly, and spore wall formation may be limited or erratic. The mutants, mei1, mei2, mei3 (Roth,1973), spo7, spo8, and

spo9 (Esposito and Esposito,1969) have normal growth but at restrictive temperatures fail to exhibit any of the meiotic parameters such as DNA synthesis, recombination, or spore formation. The exact nature of the lesions is unknown for these mutants.

The recessive mutant spoll was originally identified as a temperature-sensitive sporulation-deficient mutant and inhibition of sporulation was assumed to be caused by the lack of meiotic DNA synthesis at 34°C. Simchen and Hirschberg's (1977) observations have shown that meiotic development was relatively normal but division of the unreplicated chromosomes lead to aneuploid spores which degenerate rapidly producing the apparent sporulation deficient phenotype after 36 hours on sporulation medium. Moens and Kundu (1982) in their investigation with spoll found that the axial cores of the synaptonemal complex (SCs) do not synapse even though the presence of polysynaptonemal complex suggests a normal synthesis of the synaptonemal did occur. Spoll thus appears to possess the basic element for SC formation, however, the unduplicated chromosomes fail to synapse. Moens and Kundu surmized that the development of the meiotic kinetic apparatus, the axial cores and poly SCs,is not dependent on DNA synthesis.

DeLange and Griffiths (1980a) conducted a cytological study of three recessive meiotic mutants in Neurospora crassa (2n=14): asc(DL95), asc(DL243) and asc(DL879). Individuals homozygous for asc(DL95) had 40% to 70% abortion of post-meiotic products. There was reduced pairing

during the zygotene/pachytene stage and at metaphase I as many as 14 univalents were seen. Normal segregation of chromosomes during meiosis I was usually followed by an irregular second meiotic division such as spindle overlap, lagging of chromosomes, apparent slow separation of dividing nuclei,and movement of spindle-pole bodies to the same poles. These irregularities apparently lead to the distribution of unequal amounts of chromatin to the ascospores. In stocks that are homozygous for asc(DL243), meiosis I was normal with seven bivalents during diplotene, which divided normally. However, second division figures show one spindle-pole body with a large amount of chromatin attached and the other with little or no chromatin. Spore abortion here was 90 to 98%. Stocks that are homozygous for the mutant asc(DL879) had a drastic decrease in pairing at pachytene and spore abortion of usually 70%. Even though equal amounts of chromatin often segregated at anaphase I, the second division and the post-meiotic divisions had an unequal amount of chromatin.

A number of mutant genes have been identified that affect more complex multicellular eucaryotes. Of all the mutants that affect the cycle, those that have an influence on chromosome pairing seem to be the most prevalent. The failure of chromosome pairing in first meiotic prophase has been termed asynapsis or desynapsis. Asynapsis is described as complete absence of pairing whereas desynapsis implies brief or partial pairing between homologues.

Gottschalk and Klein (1976) analyzed 57 recessive mutant genes showing genetically conditioned meiotic abnormalities in Pisum sativum (n=7). Forty-one mutant genes are characterized as asynaptic or desynaptic in that the frequency of bivalents observed during metaphase I ranges from two to seven. These synaptic mutants affect both microsporogenesis and megasporogenesis. Thirteen mutants were characterized as male steriles in that the recessive genes caused degeneration of chromosomes during microsporogenesis. The remaining three mutant genes showed less specific meiotic disturbances and seemed to be environmentally influenced.

Beasely and Brown (1942) identified a two-factor inherited synaptic mutant in an interspecific Gossypium hybrid. The F_2 progeny of G. hirsutum x G. barbadense gave a ratio of 15 fertile to 1 sterile and the sterile plants were shown to be asynaptic (really desynaptic). The F_1 progeny showed normal pairing, therefore, since there are no apparent pairing irregularities, the absence of structural abnormalities is implied. Further proof of genic action was given upon doubling the genomes of the asynaptic mutant, which failed to restore normal pairing or fertility.

Two single genes as^1 as^2, that cause desynapsis in sorghum (Sorghum bicolor, 2n=20) were investigated by Krishnaswamy and Meenakshi (1957). Plants homozygous for as^1 averaged 6.5 bivalents per cell and plants homozygous for as^2 averaged 0.6 bivalent per cell. Ross,

Sanders and Franzke (1960), identified a third desynaptic allele ($\underline{as}^3$) in sorghum which showed 0 to 12 univalents at anaphase I. They also observed the occurrences of unpaired strands at zygotene and pachytene, which provided evidence showing that abnormalities at the time of synapsis were responsible for univalents seen at first metaphase.

Kitada and Omura (1984) identified three recessive genes which exhibit genetic control of meiosis in rice (2n=20). The mutant genes MM-19, MM-4 and MM-16 were produced by treatment with N-methyl-N-nitrosourea after which three sterile plants were selected for analysis and evaluation. Each of the three plants, homozygous for any one of the mutant genes appeared normal; however, the mean seed fertility was 41.66% in MM-4 plants, 0.3% in MM-16 plants, and 0% in MM-19 plants. Cytological analysis of PMCs included observations of the amount of pairing, number of bivalents present per cell and chiasma frequencies. The amount of pairing in plants homozygous for genes MM-4 or MM-16 show a range of pairing from cell to cell in that some cells had variable pairing and others had no pairing. In plants homozygous for the mutant gene MM-19, there was no pairing, which is indicative of an asynaptic mutant, whereas MM-4 and MM-16 are desynaptic mutants. The mean chiasma frequency per cell for MM-4, MM-16 and MM-19 were 13.38, 7.15 and 0 respectively. There were no bivalents in MM-19 plants; as for MM-4 and MM-16, the mean bivalents formed were 1.18 and 1.11 respectively. Each plant heterozygous for an individual recessive gene when selfed gave a 3:1 ratio, thus indicating that each phenotype is controlled by one gene. When homozygotes of MM-4 and MM-16 were crossed,

the F_1 progeny was normal and the F_2 progeny was 9 normal to 7 synaptic mutants, therefore, since 9:7 is an independently assorting ratio for two loci, MM-4 and MM-16 are not allelic.

Swaminathan and Murty (1959) found that there was no pairing at pachytene in two plants of Nicotiana in which there was failure of bivalent formation at diakinesis. This failure of pairing was attributed to the occurrence of a recessive spontaneous mutation.

In Lycopersicon esculentum, Soost (1950) performed genetic studies with five asynaptic mutants showing that they were inherited as non-allelic recessives. Cytological investigation of PMCs with two of the five genes (as_1 and as_4) revealed that temperature plays an important role in the degree of pairing in that an increase in temperature lead to an increase in asynapsis.

Stringham (1970) presented genetic data on three synaptic mutants of Brassica campestris that showed they were controlled by different recessive genes. The degree of asynapsis in PMCs varied from complete failure of pairing in two of the mutants (as_1 and as_2) to partial asynapsis in the third (as_3). Also, plants homozygous for as_3 seem to show little or no embryo sac abortion as observed in the amount of seed set in open-pollinated fields. Similar data were not available for the other two mutant genes.

Beadle (1930 and 1933) gives a detailed description of a recessive asynaptic gene in maize. There was little pairing throughout all the prophase I substages which conforms to the conditions of asynapsis.

In later studies, a more variable behavior was observed to result from the homozygosity of the recessive allele. Beadle described a partial but not complete failure of first metaphase association as not due to a failure of initial synapsis but to an abnormal early separation of synapsed homologues. Miller (1963) confirmed the variable response in the asynaptic homozygotes which showed complete failure of pachytene pairing in some plants and partial pairing in others.

Variable pachytene pairing owing to a recessive gene has been described in a number of other organisms including Hordeum vulgare (Enns and Larter, 1960); Oryza sativa (Chao and Hu, 1960); Secale cereale (Prakken, 1943); Colovasia antiguorum (Krishnan Magoon and Bai, 1970), Locusta migratoria (Diez and Puertas, 1984); Rumex acetosa (Love, 1943); Crepis capillaris (Richardson, 1936), and Oenothera (Catcheside, 1939).

Similar to asynaptic chromosomal behavior, complications also arise when the possible causes of desynapsis are considered. For asynapsis the decision is unequivocal in that there is a direct failure of chromosome pairing. Desynapsis may arise for a number of alternative causes, one of which is the failure of chiasma formation; and where this effect is genetic, the occurrence of univalents at first metaphase could result from the activities of genes that directly control the frequencies of positions of chiasmata. Another cause could be the relaxation of pairing between normally synapsed chromosomes before crossing over takes place.

Sears and Okamoto (1958) demonstrated that homologous pairing in Triticum aestivum is genetically controlled. They showed that when chromosome 5B is absent in hybrids of Triticum aestivum and Triticum monoccum, homoeologs paired in a rather high frequency. They concluded that this chromosome carried genes that suppressed homoeologus pairing. Riley, Chapman and Kimber (1960), also experimenting with T. aestivum and T. monoccum, found that when the 5B chromosome was absent, a higher frequency of quadrivalents and trivalents were observed. They also concluded that homoeologus chromosome pairing was suppressed when chromosome 5B was present. Further investigation with the full complement and complements deficient either for the complete chromosome 5B or the long arm (5BL) or short arm (5BS) showed that the gene controlling pairing was confined to the long arm. Continuing the investigations with chromosome 5B demonstrated that the long arm of this chromosome suppressed pairing in all chromosomes, homologous as well as homoeologous (Feldman, 1966 and Feldman and Mello-Sampayo, 1967). In di-isosomic 5BL plants, the amount of univalents observed was 1.14 per cell, however, in tri-isosomic 5BL plants the number of univalents observed was 14.20 to 16.74 per cell. Riley and Chapman (1958) previously had shown that chromosomes 5A and 5D have loci which are homoeologous to those located on chromosome 5B. Feldman's (1966) experiments revealed that chromosomes 5A and 5D have genes that promote homologous pairing whereas the 5B locus inhibits homoeologous pairing. Feldman suggests that the 5B gene

was derived as an antimorphic mutation from an association-promoting gene like those carried by its homoeologues.

Genetic pairing control of recombination has been identified in Culex tritaeniorhynchus in that there is no genetic recombination in females when the mutant gene golden, located on the X chromosomes, is present (Baker and Rabbani, 1970).

Another abnormality that appears to have genic control is chromosome stickiness which has been reported in maize (Beadle, 1932) and in Capsicum (Shopova, 1966). Stickiness has been observed to be associated with asynapsis in Capsicum (Morgan, 1963) and in Avena hybrids (Holden and Mota, 1956). Perhaps the best explanation of the possible molecular events that could cause chromosomal stickiness and other associated abnormalities is provided by the work of Hotta, Ito and Stern (1966). They have demonstrated that replication of a small proportion (0.3%) of DNA is delayed from the S period of the premeiotic interphase to early prophase I (zygotene - pachytene). They also found that interruption of the synthesis of this small amount of DNA through the use of deoxyadenosine at different times from early zygotene to pachytene caused chromosome breakage at prophase I and degeneration of the cells prior to metaphase I and anaphase II. In addition, such inhibition caused breakage at metaphase I and anaphase II as well as chromosome stickiness. Stern and Hotta (1973) concluded that the continuity of the DNA of the chromosome is interrupted by gaps which they called axial

elements. According to them, the DNA synthesized during zygotene is located in the axial element of the chromosome and when replicated results in the formation of two functional chromatids. Prophase fragments are attributed to the incomplete replications of the axial elements. They stated that, "If the gaps in the DNA filament resulting from an interrupted synthesis are not repaired, the structure of the chromosome would be abnormal in the region of the gaps, and if repair occurs by random linking of free ends, sticky chromosomes would result." The phenomenon of chromosome stickiness may be due to inhibition of DNA synthesis during prophase I. Unfortunately, failure of DNA synthesis cannot account for all cases involving chromosome stickiness because not all cases are accompanied with fragementation as demonstrated in Collinsia tinctoria (Mehra and Rai, 1970). Beadle (1932) reported stickiness in both meiotic and mitotic cells in maize.

Carapetian and Rupert (1977) conducted cytological investigations of male-female sterility in Carthamus tinctorius. They found in the study of microsporogenesis in 64 plants of an F_2 generation, that seven plants were sterile because of abnormal chromosome behavior, while the remaining 57 plants were normal in every respect. The abnormalities found in the seven plants were chromosome stickiness leading to anaphase bridges, multipolar divisions, and the formation of three to nine cell quartets. Carapetian and Rupert concluded that sterility was conditioned by three genes which was based on the ratio 57:7.

Sterility in Cucurbita maxima was observed by Hutchins (1944), and based on the 3:1 ratio observed in the F_2 generation, concluded that sterility was controlled by one recessive gene. Singh and Rhodes (1961) conducted a cytological study of this mutant and found that the effect of this gene was the inhibition of meiotic division of PMCs.

Irregularities during the formation of spindle fibers have also been reported as a cause of sterility. Clark (1940) observed divergence of meiotic spindle formation during microsporogeneis in Zea maize. Instead of the spindle converging at the two poles, they were parallel or divergent. Although disjunction of bivalents is normal, the chromosomes failed to converge at the poles. The result is that each chromosome or group of chromosomes forms a nucleus. Consequently, there may be more than four spores per tetrad and most (42 - 95%) microspores are multinucleate. The cause of such spindle abnormalities is attributed to the recessive gene dv (divergent spindle). Pollen is semi-sterile which is attributed to aneuploidy resulting from completion of meiosis II. Spindle abnormalities exhibiting similar behavior has been described in Agropyron (Tai, 1970) and Clarkia (Vasek, 1962).

Blakeslee (1935) reported on sterility in Datura stramonium that was due to a single mutant gene dy (dyad) which manifests itself in megasporogenesis and microsporogenesis. The first meiotic division of PMCs is normal, but the second division does not occur which means the chromosome complement of each cell is doubled. The cells enter into a prolonged period of interkinesis, after which chromosome replication occurs and the cell divides mitotically, resulting in the formation

of 2n gametes. Other stage-specific abnormalities which are controlled by a recessive gene have been described in tomato (Clayberg, 1959) where there is a precocious centromere division during meiosis I, and in Agropyron (Tai, 1970) which results in multipolar nuclei.

Vosa and Bingham (1979) have demonstrated in crosses of 4n x 2n alfalfa plants that the resulting diploid progeny can produce 2n pollen grains. The cytological mechanism of 2n pollen formation was found to be disorientation of spindles at metaphase II, of which 38% of PMCs showed the spindle disorder. Both n and 2n pollen were produced by all diploids examined. Normal spindles at metaphase II were oriented such that they defined the poles of a tetrahedron and resulted in normal tetrahydral arrangement. Disoriented spindles were basically parallel to each other and resulted in formation of dyads and occasionally triads. Dyads developed into two 2n pollen grains; triads developed into one 2n and two 1n pollen grains.

First and second division restitution was reported in male and/or female gametophytes of potatoes (Wang, Peloquin and Meniburu, 1971), wheat (Wagenaar, 1968), and corn (Rhoades and Dempsey, 1966; Nelson and Clary, 1952). The corn investigation differs from the others in that the genic control of first division restitution was functional only in the female gametophyte and described as semi-steriles. Another instance where a meiotic mutant is only exhibited in females was reported by Iwanaga and Peloquin (1979) in Solanum tuberosum. They reported that

when the chromosome synaptic mutant (sy) gene is homozygous, all megaspores are desynaptic to varying degrees which results in chromosome loss. Ultimately, the result is total gametic abortion. When two heterozygotes are crossed, megaspore mother cells exhibit a ratio that is Mendelian. This was the first investigation that demonstrated cytologically the occurrence of desynapsis in the female gametogenesis. The mutant gene manifests itself as 100% penetrance in all ovules that were assumed to be homozygous recessive.

In Drosphila there is a recessive gene located on chromosome 3 which causes a high frequency of chromosome nondisjunction (Gowen and Gowen, 1928, 1933). Homozygous c(3)G females produce virtually no meiotic recombinants but produce a very high frequency of nondisjunction at the first meiotic division. Homologues do not segregate at random and the different chromosome pairs of the complement do not disjoin independently. Homozygous males show no meiotic irregularities.

Smith and King (1968) found that synaptonemal complexes, normally present in early oocytes are absent in c(3)G homozygotes. Hinton (1966) observed that in Drosophila which are heterozygous at this locus exhibit somewhat higher than normal recombination, whereas the deficiency mutant Df(3R)88(F) which is deficient for the $c(3)G^{+}$ locus exhibit lower than normal recombination. In experiments conducted by Hall (1972), two different mutant alleles of c(3G) were used, $c(3)G^{17}$ and $c(3)G^{68}$. Homozygous $c(3)G^{17}$ individuals had a gametic frequency of X, second or third chromosome nondisjunction of approximately 30% and

nondisjunction of the fourth chromosome of 20%. The frequency of nondisjunction in $\underline{c}(3)G^{18}$ homozygotes was 0.4 for the X chromosome and 0.3 for chromosome 4. Hall reports that chromosome loss induced by both mutants are similar and at least 200 times above that expected from spontaneous nondisjunction.

In Drosphila the nondisjunction recessive mutant $\underline{ca}^{nd}$ is an allele of claret eye (ca). This mutation was first discovered in D. simulans by Sturtevant (1929) and later in D. melanogaster by Lewis and Gencarella (1952). The gene is located on chromosome 3 and has no effect on meiosis in males nor heterozygous females. In Drosphila simulans, Wald (1936) cytologically examined a meiotic mutant designated $\underline{ca}^{nd}$ and found that spindles of the first meiotic division were distorted and that meiosis resulted in the production of supernumerary nuclei. Lewis and Gencarella (1952) observed in D. melanogaster that when the gene was homozygous in females 42% of the progeny are normal. The remaining 58% consisted of exceptional males and females that were Haplo 4, Haplo 4 mosaics, other types of mosaics, gymandromorphs, triploid females, and triploid intersexes. Crossing over is approximately normal in $\underline{ca}^{nd}$ females but there is an exceedingly high incidence of nondisjunction of all pairs of homologs in the first meiotic division; however, homologs separate more often than 50%. Chromosome loss is also frequent during mitotic divisions. The recessive paternal loss gene (pal) is very similar to $\underline{ca}^{nd}$. This gene, when homozygous in male Drosphila allows normal formation of gametes as well as fertilization; however,

during cleavage, there is selective elimination of paternal chromosomes leading to the formation of chimeras and exceptional progenies (Baker, 1975).

Sandler et al. (1968) identified two females and two male specific meiotic mutants in D. melanogaster. The female-specific recessive mutant mei-S282 was found to be on chromosome 3 and causes failure of pairing and a polarized reduction in crossing over (most pronounced distally) among regularly disjoining chromosomes. The partially dominant female-specific mutation mei-S332 is located on chromosome 2 and increases disjunction of the sex chromosome and chromosome 4. The two male-specific mutants are mei-58 and mei-081, both of which are recessive. The mei-58 gene increased nondisjunction in chromosome 4 which occurs during meiosis I as revealed by cytological analysis. The mutant gene mei-081 is found on chromosome 4 and causes nondisjunction of all chromosomes.

Sandler's (1972) investigation of three recessive sex linked mutants, mei-9, mei-9^b, and mei-218 in D. melanogaster revealed that all three decreased recombination and increased nondisjunction for all chromosome pairs. Also mei-9 and mei-9^b were found to be allelic in that heterozygotes for these two genes exhibit high frequencies of nondisjunction in chromosomes 1 and 4 (24% and 11% respectively). The mutant mei-28 suppressed recombination in all chromosomes of both males and females in a progeny of 61 files. Carpenter and Sandler (1974) experimented with meiotic mutants in Drosphila and concluded that of

the 14 known recombination-defective loci, all reduce crossing over and increase nondisjunction of all four chromosome pairs. Their observations suggest that the segregational abnormalities resulting from recombination mutants may be a secondary consequence of reduced recombination as observed in desynaptic mutants. However, one must realize that chromosomes carry genes that govern their segregation patterns as well as their physiological environment; thus, irregularities of one (gene or physical environment) can alter the other.

Diez and Puertas (1984) identified three desynaptic mutants in Locusta migratoria. Cytology of the spermatocytes in the mutant males showed a significant decrease in chiasma frequency from diplotene to metaphase I. The inheritance pattern of the mutations was not established because the males died before sexual maturity or only females appeared in the progeny.

A very large breeding experiment with canaries was conducted by Durham (1926) involving 1300 birds which carried the z-linked traits pink (bb) and black eyes (B-), however, no genetic analysis was made. Sittman (1983) reinvestigated Durham's work and found that crosses between pink-eyed males and black-eyed females produced unexpected pink-eyed sons and daughters. These results were attributed to primary nondisjunction of the sex chromosome in meiosis II of spermatogenesis. Genic control is indicated because of the abnormally high incidence of nondisjunction progeny which ranged from 13 to 19 percent.

Hulten Solari and Skakkebaek (1974) conducted cytological investigations of testicular material of a sterile human male that was the offspring of a marriage between first cousins. This was the first meiotic mutant described in man. The sterile male had a normal mitotic karyotype including a normal banding pattern. However, meiosis was completely arrested at the primary spermatocyte level with the most advanced stage being meiotis I. Leptotene seemed normal, however, zygotene and pachytene displayed a number of unpaired chromosomes. Of the 100 pachytene cells observed, no XY bivalent was seen. Chromosome counts at diplotene ranged from 37 to 46. Electron microscopy of the synaptonemal complex revealed a central element but lateral fibrils were absent. Chromosome counts of 16 spermatogonial cells showed 12 with 46 chromosomes and the remaining 4 cells with 96, 44, 43, and 41 chromosomes respectively. Hulten et al. (1974) concluded that sterility was caused by a low chiasma frequency induced by a recessive gene.

Mitotic Mutants

Mitosis, like meiosis, is under genetic control by specific gene or gene groups. Meiosis is a much more complicated and delicate process than mitosis. Consequently, genetically controlled variations in cell division are much less frequent for mitosis than for meiosis. As in meiosis, S. cerevisiae is an appropriate organism for the genetic study of the mitotic cell cycle. The organism undergoes the same sequence of events during cell division in both the haploid and diploid states.

Therefore, recessive mutations that impair the ability of the cell to complete cell division can be isolated in the haploid stage and the ability of two mutations exhibiting complementation can be studied in the diploid stage. Also the genetic system of S. cerevisiae has been extensively studied and mutational analyses are easily made.

Hartwell (1971) identified two recessive temperature sensitive mutants in yeast, both of which function during DNA synthesis. The two mutants cdc4 and cdc8 occur in two unlinked complementation groups. The mutant cdc4 functions in the initiation of DNA replication and cdc8 is necessary for the continuation of DNA synthesis. Neither mutant is capable of cell division at the restrictive temperature because of the inhibition in DNA replication, but can undergo cell division at the restrictive temperature following the completion of DNA replication at the permissive temperature. It was concluded that cell division appears to be dependent either upon DNA replication or the successful completion of nuclear division. Hartwell and Leland (1973) examined 148 temperature sensitive mutants of yeast. Complementation studies ordered these recessive mutations into 32 groups and tetrad analysis revealed that each of these groups defined a single nuclear gene. Cell cycle mutants 22, 28 and 32 had one to four nuclei per cell at termination of growth at the restrictive temperature. In cdc mutants 3, 10, 11 and 12, the number of nuclei per cell ranged from 2 to 16. Most of the mutants caused cells to lyse after extended periods of incubation at the restrictive temperature. This lysis was assumed to be the result of

continued nuclear division without cell division as most mutants continue to enlarge at the restrictive temperature. Other mutants showed no growth at the restrictive temperature and have been identified as defective in DNA synthesis. The three recessive mutants cdc28, 21 and 7 are defective in the initiation of DNA synthesis in that replication does not begin at the restrictive temperature; however, if any one of these mutants is allowed to replicate at the permissive temperature, then shifted to the restrictive temperature, cell division is accomplished without difficulty. This response to temperature is similar to cdc4 and 8 described above. The cdc mutant genes 4, 7, 8 and 28, function both in mitosis as well as in meiosis which is logical in that DNA synthesis occurs in both processes. However, all genes that function during mitosis do not necessarily function during meiosis.

Maine, Sinha and Tye (1984) isolated a recessive mutant in yeast which is responsible for the maintenance of normally mitotically stable minichromosomes. The minichromosomes contain the gene ARS (autonomously replicating sequence) and a specific centromeric sequence (CEN 5). Maine et al. characterized 40 minichromosome maintenance defective mutants (Mcm$^-$) which constitute 16 complementation groups. There were two classes of Mcm$^-$ mutants which were identified as specific and nonspecific. The specific class of mutants is defective only in the maintenance of the minichromosomes that carry the ARS irrespective of the centromeric sequence present. The response of this class of mutants to three restrictive temperatures, 23^{o}, 30^{o} and 35^{o}

temperatures, was chromosome loss after the first mitotic division which was caused by a failure of DNA replication. The nonspecific class of mutants is defective in the maintenance of all minichromosomes irrespective of ARS and centromeric sequences. The mutants were tested at the three temperatures 23^{o}, 30^{o} and 35^{o}. The results were 88% loss after the first mitotic division at all temperatures. The nonspecific class is thought to include mutants that are affected in the segregation and replication of the minichromosomes.

The investigation of Resnick et al. (1981) revealed that the yeast mutant rad 52 gene plays a central role in recombination. It seems that this locus mediates ultraviolet and ionizing radiation-induced mitotic recombination, spontaneous mitotic and meiotic recombination and possibly recombination involved in mating switching.

Another example of a gene that affects both meiosis and mitosis is the asc (DL 243) gene in Neurospora discussed under the section on meiotic mutants. This gene's mode of action was unequal distribution of chromatin materials which was observed in meiosis II and in the subsequent mitotic divisions. Delange and Griffiths (1980) compared this mutant with the two Drosphila sex specific mutant genes pal and ca^{nd} which causes nondisjunction. As described earlier, the pal gene causes nondisjunction in the zygote and it does so only if the male parent was homozygous. The ca^{nd} gene causes nondisjunction during meiosis in homozygous females. Griffith thought that a component that interacts with the centromeric region of the chromosomes is absent in these mutants.

However, one cannot exclude the possibility that chromosome elimination in these mutants may be caused by defective spindle pole bodies or spindle plaque formation.

Finch and Bennett (1982) recorded chromosome elimination in the development of the female gametophyte and the embryos of interspecific hybrids involving Hordeum vulgare with H. marinium and H. bulbosum. In the cross of H. vulgare with H. marinium there was elimination of H. marinium chromosomes from the embryo and of H. vulgare chromosomes from the endosperm. In crosses of H. vulgare with H. bulbosum, it was H. bulbosum chromosomes that were eliminated, which occurred in the embryo. The authors observed that the eliminated chromosomes had smaller centromeres which they attributed to centromere inactivation. They proposed that the suppression of centromere activity was due to methylation of cytosine bases which prevented the attachment of the centromeres to spindle fibers. This would cause the chromosomes to be lost during cell division.

Chromosome elimination studies of the interspecific hybrid Hordeum vulgare x Hordeum bulbosum have been conducted by a number of investigators (Lang, 1971; Subrahmanyam and Kasha, 1973; Bennett, Finch and Beardsly, 1976; Thomas and Pickering, 1983a, b). The amphidiploid (2n=28) hybrids exhibit chromosome instability in that any hybrid plant with a chromosome complement of both bulbosum and vulgare chromosomes will selectively eliminate the bulbosum genome during gametogenesis and early embryogenesis. Thomas and Pickering (1983a) found that when

the amphidiploid hybrid (2n=28) consisted of chromosomes from the Vada cultivar of H. vulgare, elimination of the H. bulbosum chromosomes is greatly diminished in that as many as 86% of the progeny retained the chromosomes of H. bulbosum. Cytology of the PMCs revealed that the chromosome number of the progeny ranged from 24 to 30 chromosomes with a mean of 27.25. Cytology of root-tips was also performed on the amphidiploid containing the Vada cultivar and it was found that the somatic cells had a consistent chromosome number of 28. When the amphidiploid contained the chromosomes of H. vulgare originating from the Emir cultivar, elimination of the bulbosum chromosomes was greatly increased in that less than 1% of the progeny retained the chromosomes of H. bulbosum. The range of chromosome numbers of the progeny was 14 to 30; and 57% of the PMCs contained chromosome fragments of degraded chromosomes. Cytology of root-tips was performed and the chromosome number was consistently 14 per cell, of which all were H. vulgare. Reciprocal crosses gave similar results. Thomas and Pickering concluded that the Vada cultivar contained gene(s) that suppress elimination of the H. bulbosum chromosomes.

Evgen'ev and Sidorova (1976) performed experiments in Drosphila virilis and Drosphila litoralis (2n=12), similar to those done by Thomas and Pickering in Hordeum. When D. virilis females are crossed to D. litoralis males, chromosome 6 of D. litoralis was eliminated in 42% of the progeny. However, in the reciprocal crosses, elimination did

not occur. The elimination of the chromosome occurred mitotically during the first cleavage stage.

Localization of the gene that controls the elimination of chromosome 6 was determined through the use of marker genes by establishing different strains of D. virilis which were heterozygous for each chromosome of D. litoralis. When D. virilis was heterozygous for chromosomes 2 or 4, there was a decrease in elimination frequency to 5.5 and 17.2% respectively. Genetic analysis revealed that the observed maternal effect on mitosis is controlled by recessive genes located on the 2nd and 4th chromosomes of D. virilis.

Liras et al. (1978) isolated and identified in yeast the recessive chromosome loss (chl) gene. This gene, when homozygous in diploid yeast will induce nonrandom chromosome loss of chromosomes 1, 3, 8, and 16 during mitotic division. Chromosomes 1 and 3 were lost in 1% of all cells and chromosomes 8 and 16 were lost in 0.3% of cells.

Beadle (1931, 1933) identified the polymitotic gene of maize. This recessive gene (po) has its effect during postmeiotic cell divisions. During the first microspore division the chromosomes do not replicate and split, but cytokinesis occurs in rapid succession separating the chromosomes into small cells. The results of these polymitotic divisions are many small cells with 0 to 10 chromosomes. As many as five cell divisions have been observed. The consequence of such polymitosis is complete male sterility. On the female side, gametophytes are viable, containing the proper chromosome complement. Similar

polymitotic behavior has been reported in Rhumohra aristata (Bhavamandan, 1971), Alopecurus myosuveides (Johnsson, 1944), and Chlorophytum elatum (Koul, 1970).

Chromosome instability was observed in a strain of Lathyrus sativus (2n=14) (Lavania, 1982). This particular strain shows intra- and inter-plant chromosome variations. Cytological examinations revealed approximately 60% of root-tip cells with different chromosome numbers, ranging from 2n=14 to 2n=11. Although cytology was performed on somatic tissue, the resulting progeny were occasionally triploid. This may indicate that gene(s) may function in both mitotic and meiotic cells.

Chromosome instability has also been observed by Nirmala and Rao (1984) in their studies of Coix gigantea (2n=20). Upon observation of meiosis in pollen mother cells (PMCs) they found an intraplant chromosome variation from 2n=7 to 2n=29 with varying numbers of fragments, univalents and bivalents. Inter- and intra-plant variation was established. The intra-plant variations affected the fitness of the plant. The average chromosome number per cell was 17.25 of which 28% of these cells contained chromosome fragments. The abnormalities probably originated during the mitotic divisions in somatic cells. Nirmala and Rao surmised that the instability was a demonstration of genomic plasticity which would ultimately increase the diversity of the species.

Bloom's syndrome is a genetic condition in man that exhibits chromosome instability during mitosis (German, 1964; German, Archibald

and Bloom, 1965). Analysis of fibroblast and blood lymphocyte cultures revealed recombination between homologous and nonhomologous chromosomes. Also, there were high frequencies of sister strand exchange, centric and acentric chromosome fragments, anaphase bridges, and abnormal DNA synthesis that were observed.

As discussed above, there are a number of genes that function in the cell cycle but the order of these processing events cannot be determined very easily in most species, because there are too few suitable mutations available or because there are technical problems in analyzing very complex systems.

In Zea maize, a mutant designated r-X1 is a small deficiency involving the R locus on chromosome 10. This mutant is of particular interest because it elicits a behavior similar to that of Haplo 9 plants. The r-X1 deficiency was first induced by Stadler (1933) via pollen irradiation. Although used experimentally by other investigators, more extensive investigations were performed by Weber (1970, 1974) and Plewa and Weber (1973, 1975). The r-X1 deficiency is lethal in the homozygous state and can only be transmitted through the female. When females, heterozygous for this deficiency, are pollinated, the resulting progeny are 11% trisomic, 11% monosomic and 78% disomic. Zero to 1% of the progeny are doubly or triply monosomic (Weber, 1970). These results show that the chromosome with the deficiency induces a high rate of irregular disjunction, indicating that the normal gene is necessary for normal chromosome disjunction. All ten chromosomes of maize have been

isolated as monosomics which indicates that the r-X1 deficiency lacks chromosome specificity. Weber has stated that the mode of action for this deficiency is post-meiotic in which the nondisjunction occurs during mitotic divisions in the development of the female gametophyte. Females heterozygous for this deficiency (+/r-X1) produce two kinds of gametes, one containing the chrommsome with the deficiency and the other containing the normal chromosome. If the r-X1 deficiency occurs in the functional megaspore, the following mitotic divisions will eventually lead to aneuploidy. This series of events probably occurs in this fashion for all monosomics recovered from the heterozygous parent in that all monosomic progeny contain the r-X1 deficiency (Plewa and Weber, 1975). Cytological investigations of megasporogenesis were not conducted because of difficulty in identification of the abnormal segregation patterns (Weber, personal communication). However, in experiments conducted by Weber using endosperm markers, there was no homology between the endosperm and the embryo in absence of a specific chromosome(s). This occurs because loss of a chromosome in the endosperm is not accompanied by the loss of the same chromosome in the embryo (Plewa and Weber, 1973). The Haplo 9 plants of cotton in the present study, which are deficient for the entire chromosome, exhibit a cytological behavior very similar to that of the r-X1 mutant of maize. In both cases, a rather high frequency of aneuploid gametes involving a wide range of chromosomes are produced.

CHAPTER 3

MATERIALS AND METHODS

Plants monosomic, telosomic and isosomic for chromosome 9 were cytologically investigated to determine their effect on cell divisions leading to gamete formation. It was pointed out earlier that haplo 9 plants produce various kinds of aneuploid progeny. Upon performing cytological investigations of haplo 9 plants, it was noted that the meiotic divisions of pollen mother cells were normal. Therefore, it was assumed that the abnormal divisions in haplo 9 plants producing the aneuploid progeny occurred during megasporogenesis. This assumption was reinforced by the effects of r-X1 deficiency in corn which causes the formation of aneuploid progeny because of abnormal nuclear divisions during the development of the female gametophyte.

The initial seed stocks included monosome 9, monotelodisome 9L, and two homozygous translocations that were obtained from J. E. Endrizzi, Plant Sciences Department, University of Arizona Agricultural Experiment Station, Tucson, Arizona. All cotton monosomes, telosomes and isochromosomes that are maintained in the cotton cytogenetic collection have been or are in the process of being transferred to the genetic background of the highly inbred standard line designated as TMI. The line

that carries the teleocentric for the long arm of chromosome 9 is designated Telo 9L.

In G. hirsutum, misdivision of monosomes occurs either during the first or second meiotic division (Brown, 1958; Endrizzi, 1983). The results are generally the production of an isochromosome or a telocentric chromosome, both of which were obtained in earlier studies and in the present study of the progeny of haplo 9 plants. A telosome and an isochromosome for the short arm of an A subgenome chromosome that arose from haplo 9 during the present study were isolated and identified in tests with translocations involving chromosome 9. The identification number of the plant which had the initial telosome was C1-4-1982. Subsequent progeny generations derived from crossing the telosome to the standard TMI are designated as the Telo 9S line. The identification number of the plant with the initial isochromosome was B25-9-1981. Later progeny generations derived from crossing the isochromosome to the standard are designated Iso 9S line.

The two homozygous translocation stocks that were used to identify the telosome and the isochromosome were, T_{3-9}, 8-30-5 and T_{9-25}, 2870. The translocation stock, T_{3-9}, consisted of an interchange between the A subgenome chromosomes 3 and 9. The other translocation, T_{9-25}, involved both an A and D subgenome chromosome 9 and 25 respectively. The newly isolated isocentric and telocentric chromosomes for the short arm of an A subgenome chromosome were associated with the two

translocations in tests that will be described in Results and Discussions. Since these two cytotypes are associated with two translocations and the two translocations had chromosome 9 in common, this alone established that the two new cytotypes involve the short arm of chromosome 9.

In cross pollination, the flowers of the female parent were emasculated one day before the flowers open or bloom. Immediately following emasculation, a short section of a soda straw was placed over the stigma to protect it from foreign pollen. On the following day, at which time the stigma is receptive, the emasculated flowers were pollinated. The soda straw was replaced over the stigma and the flower was tagged. Self pollinations were made by sealing the tip of flowers one day prior to blooming with cellulose acetate. They were then tagged.

Seed Selection

Figure 1 shows that in crossing a monosomic plant as the female parent with a normal male plant in cotton, two types of progeny are recovered, 2n-1 and 2n. As seen in Figure 1, it is expected that monosomic plants should be recovered in the same frequency as the disomic plants when pollinated with a normal disome. However, seeds which are monosomic for chromosome 9 are recovered at a very low frequency. This is also the case in self-pollination of the monosomic plants. Here again, only the two cytotypes are recovered, 2n-1 and 2n. Nullisomes are not recovered in self-pollination of monosomic plants in cotton,

Female Monosomic Plant		Male Disomic Plant	
2n-1 Gametes	x	2n Gametes	Progeny
n-1		n	2n-1
n		n	2n

Figure 1. Illustration of Monosomic Inheritance When Crossed as a Female With a Normal Disomic Male.

There are two different types of gametes formed in the female, one missing a chromosome (n-1) and the other with a full complement of chromosomes (n). The resulting progeny are of two types: the monosomic plants which result from the union of normal gametes from the male and abnormal gametes (missing one chromosome) from the female, and the normal disomic plants resulting from the union of normal gametes.

indicating that only "n" pollen is functional. Since the frequency of recovered mono 9 plants is very low, there is an obvious need for a system that would increase the frequency of aneuploids. It has been shown that lower seed weights are indicative of trisomic conditions in barley (Ramage and Day, 1960) and of six monosomics in cotton (Douglas, 1972). Malek-Hedayat (1981) performed studies of this nature on larger samples of monosomic cotton and reported that by selecting the lower weight seed the frequency of monosomic plants were increased. For haplo 9, the frequency was increased to 18% from a low of 4.38%. This increase was accomplished by weighing a sample of seeds from haplo 9 plants, after which the means and the standard deviation were calculated. To obtain the maximum amount of aneuploids, seeds with weights ranging from the minimum weight to minus one standard deviation of the mean weight of total seeds were selected for planting. I used this method in an attempt to increase the frequency of haplo 9 plants. Figure 2 contrasts the recovery percentage of aneuploids among weighed and unweighed seeds over a five-year period in the present study. Although the recovery rate is erratic, aneuploids retrieved are consistently higher in weighed than unweighed seed.

Seed Production

The four cytotypes, monosome 9, monoisodisome 9 short, and monotelodisome 9 long and short, were crossed as female parents with TMI for analysis of seed production. Fifty to 76 bolls were taken from

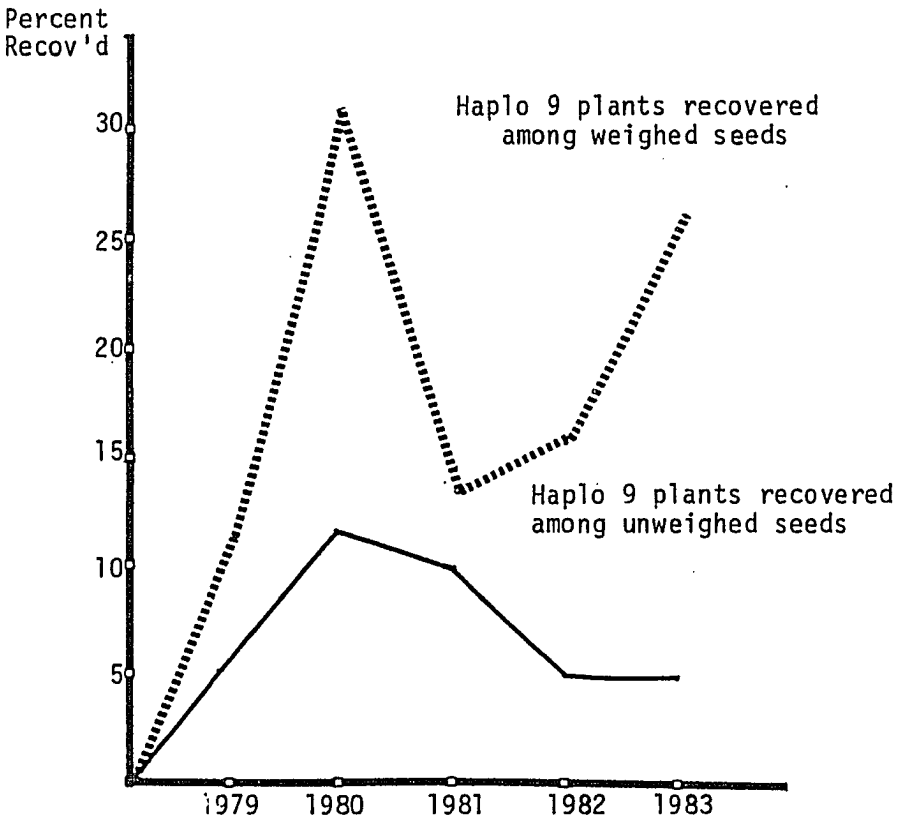

Figure 2. Contrast of Recovery Rates of Haplo 9 Plants Among Weighed and Unweighed Seeds.

All seeds from a boll are weighed and the standard deviation is determined, after which seeds that are 1 standard deviation below the means are selected. Unweighed seeds are randomly selected. Comparison of each year for recovering Haplo 9 plants is consistently higher when seeds are selected for lower weights.

several plants of each cytotype and several normal plants in the progeny rows. Seeds were separated from the fiber and a count was taken for each boll. Bolls were also taken from the standard TM1 to use as an additional control.

Marker Genes Used In Test For Association With Haplo 9

Selected marker genes were crossed into Haplo 9 plants to determine whether one or more of the marker genes were located on chromosome 9.

It was assumed that if one of the marker genes resided on chromosome 9, the marker gene would provide a method of tagging chromosome 9 to determine its behavior in gamete formation in Haplo 9 plants. The genetic stocks and the mutant genes each carry are as follows: (1) AG170, $\underline{gl_1}\underline{gl_1}$, $\underline{v_1}\underline{v_1}$, cucu, fgfg; (2) G257, CrpCrp; (3) AG144, $\underline{P_1}\underline{P_1}\underline{p_2}\underline{p_2}$, $\underline{Y_1}\underline{Y_1}$. The latter stock is G. barbadense, whereas the former two are G. hirsutum. The mode of inheritance, the phenotype of the homozygote, the species origin and the reference which initially described the mutant, are given in Table 1.

In the isogenic multiple marker stock AG170, there were four mutant genes: cup leaf, frego bract, glandless stems and bolls, and virescent leaf. The recessive gene for cup leaf (cu) manifests itself as a slightly smaller leaf with shallow base, deep lobing and leaf margins curved upward and toward the center of the leaf. The gene for frego bract (fg) is inherited as a recessive and expressed in the bracteoles surrounding buds. The bracteoles are convoluted and coiled around the bud, which becomes more twisted as the bud matures. The gene for frego bract is located on chromosome 3. Nevertheless, its segregation behavior was scored in the test with Haplo 9 and will be discussed briefly.

Table 1. Marker Genes Used In Tests For Their Possible Association With Chromosome 9.

Gene Symbol	Inheritance	Phenotype	Origin	Reference
cu	recessive	cup leaf	hirsutum	Lewis, 1954
gl_1	recessive	glandless stem and boll	hirsutum	McMichael 1954
fg	recessive	frego bract	hirsutum	Green, 1955
v_1	recessive	virescent plant	hirsutum	Kohel, 1972
Crp	dominant	crumpled leaf	hirsutum	Kohel, 1973
Lc_yLc_z	dominant two factor	brown lint	hirsutum	Kohel, 1978
P_1P_2	dominant two factor	orange pollen	hirsutum & barbadense	Harland, 1929
Y_1	dominant	yellow petal	hirsutum & barbadense	Turcotte and Feaster 1963

The gene for glandless (gl_1) is a recessive trait that specifically refers to the absence of gossypol (a dark chemical compound found within glands on the plant) in stems, petioles and bolls thereby making these plant structures appear as glandless. The recessive gene virescent (v_1) is expressed in the leaves and stems which appear virescent in color.

The isogenic stock G257 is homozygous for the dominant gene Crumpled leaf (Crp). Unlike most dominant alleles in cotton, the appearance of heterozygous crumpled plants is only slightly different from the homozygous dominant condition. In both instances, the leaf phenotype appears extremely corrugated and wrinkled with little or no lobing. Also, plants are very short and slow growing.

The isogenic stock AG201 has the two-factor inherited trait for brown lint, Lc_yLc_z. The genes are expressed in the fiber or lint giving it a brown color. The brown color is dependent upon the number of dominant genes present in that a plant with four dominant genes has lint color that is much darker phenotypically than a plant having fewer dominant genes.

In the isogenic stock AG144 are the two gene factors for pollen color, P_1 and P_2. The color of the pollen grain is determined by the number of dominant genes present; also gene interaction is clearly observed. The homozygous dominant for both gene pairs, $P_1P_1P_2P_2$, produces yellow pollen; $p_1p_1P_2P_2$ and $P_1P_1p_2p_2$ procuces cream and neocream pollen respectively, and $p_1p_1p_2p_2$ produces orange pollen. Also in this

stock is the dominant gene for petal color, $\underline{Y_1 Y_1}$. This gene produces yellow flower petals: however, when heterozygous, the flower petals are slightly less yellow than that of the homozygous dominant.

For the above mutant genes, the contrasting or normal genotypes which are common to haplo 9 plants and to Gossypium varieties and the standard TMI are as follows: CuCu (normal leaf shape), FgFg (normal bracts), $\underline{Gl_1 Gl_1}$ (glanded plants), $\underline{V_1 V_1}$ (normal green leaves and stems), crp crp (normal leaf), $\underline{lc_y\ lc_z}$ (white lint), $\underline{p_1 p_1 P_2 P_2}$ (cream pollen), and $\underline{y_1 y_1}$ (cream petal).

For many recessive genes in cotton, dominance of the normal allele is incomplete. In a few cases, it has been observed that when the recessive allele is hemizygous, the phenotype of the plant is intermediate like that of the heterozygous F_1, or the phenotype is almost normal in appearance, in which case the recessive allele is hemizygous ineffective in its expression. Therefore, the usual procedure is to analyze testcross or F_2 populations for the final determination for the possible association of the recessive mutant with the monosome.

With a few exceptions, seeds were planted in peat pellets and grown under greenhouse conditions. After a period of approximately three weeks the seedlings were transferred to the field. Pollinations were made onto monosome plants using the standard TMI or the marker lines as the pollen source. The F_1 seeds were weighed and selected for planting as described above and all F_1 plants were scored for the phenotypes of mono 9 and the disome and for the phenotypes of the marker genes in the

crosses with the genetic stocks. In the cross with genetic stocks, both mono 9 and disomic F_1's were test-crossed and/or self-pollinated to produce seed which were planted in the greenhouse or the field for scoring for association of the markers with chromosome 9. Segregating population for the dominant marker brown lint were scored twice, once in the greenhouse and again in the field.

Cytology of Pollen Mother Cells

The chromosome complement of plants was determined by analyzing metaphase I of pollen mother cells (PMC). The four different cytotypes of chromosome 9 were pollinated with the standard line TMI for cytological analysis of the resulting progeny. Haplo 9 plants that were from crosses to genetic marker lines were also analyzed to verify that they were monosomic. Also telocentric and isochromosome for the short arm of chromosome 9, which were recovered in the progeny of Haplo 9 plants in this study, were crossed to two translocation stocks and analyzed cytologically for positive identification of the aberrant chromosome. Figures 3a and c represent a diagrammatic illustration of cytological observations which demonstrated the normal pairing behavior of the telocentric and isocentric chromosomes when crossed with a standard complement. In these cases, pairing consists of 25 bivalents and a monotelodisome or monoisodisome bivalent. Figures 3b and d are the expected pairing behavior of the isocentric and telocentric chromosomes when crossed with a translocation. If the telo or isocentric chromosome

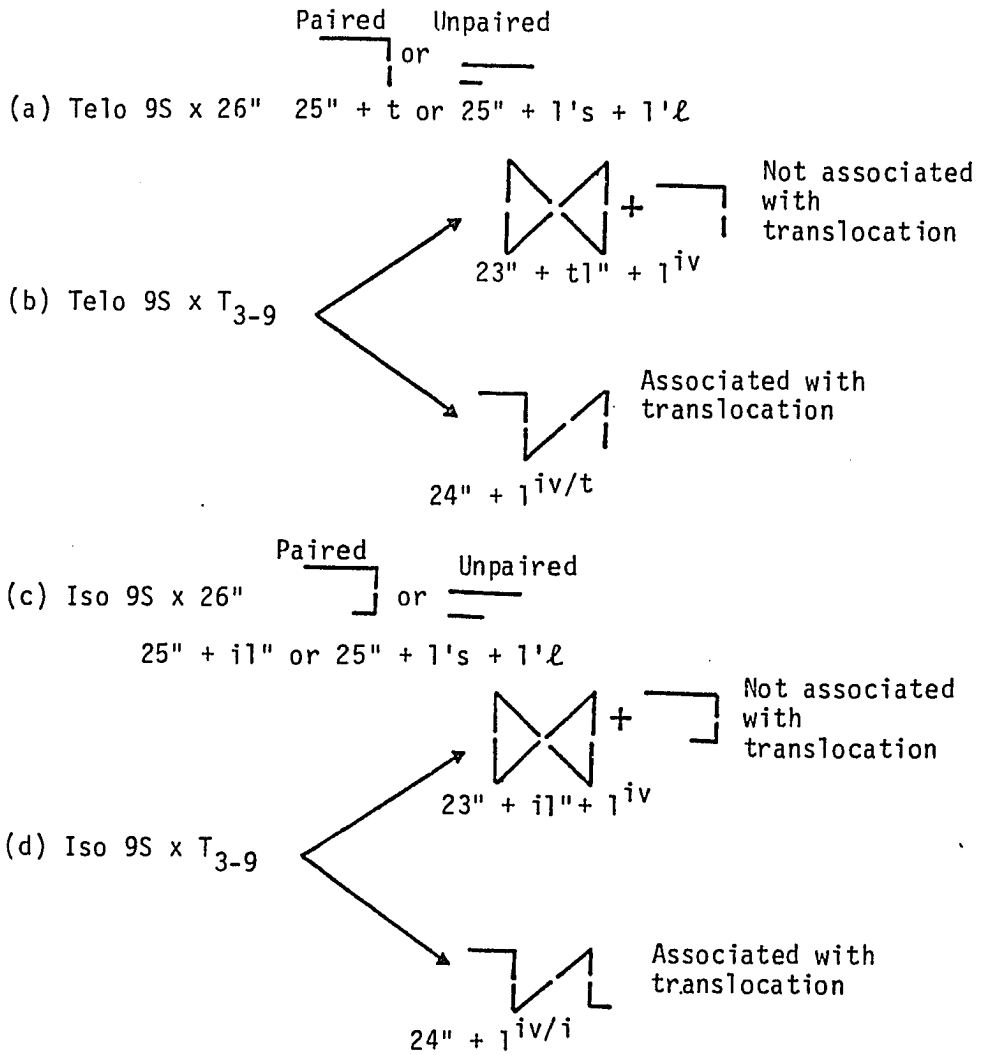

Figure 3. Protocol Used to Identify Telo 9 Short and Iso 9 Short, (a) and (c).

When the Telosome or Isosome are viewed cytologically, they may be paired and seen as unequal bivalents, or unpaired, and seen as 2 univalents, 1 large and 1 small, (b) and (d). When a known translocation is crossed to an unknown Telocentric or Isosomic chromosome, there is only one of two possible results seen cytologically among the resulting progeny. If there is no association when a ring of four and an unequal bivalent is seen; however, if there is homology between the Telo or Iso with any chromosome of the translocation, a chain of four chromosomes, one of which is either the telocentric or the isocentric chromosome, is observed.

is not one of the chromosomes in the translocation, then 23 bivalents, a quadrivalent and a monotelodisome or monoisodisome bivalent will be observed. If the telo or isosomic chromosome is homologous to a chromosome in the translocation, 24 bivalents and a chain of four chromosomes will be observed, in which one of the chromosomes in the chain will be either the telocentric or the isosomic chromosome.

Experiments conducted in the cotton cytogenetics laboratory at the University of Arizona had revealed that meiosis occurs in the pollen mother cells when the size of the flower bud is between 3.8 and 4.5 mm in width. Flower buds of this range size were collected from selected plants for cytological analysis. The fixative used was a modified version of Farmer's solution consisting of seven parts 95% ethanol to three parts of glacial acetic acid. Following the collection of the flower buds, the calyx and corolla were removed to maximize the exposure of the anthers to the killing and fixing agent. The buds were placed in plastic embedding capsules and labeled as to plant number. The specimens were refrigerated for 18 to 24 hours prior to cytological analysis.

PCMs were analyzed using the squash technique. Iron propiocarmine was used to stain the chromosomes of metaphase I configurations to determine the chromosome complement and pairing behavior.

Megasporogenesis and Early Embryogenesis

Analysis of the development of female gametogenesis was performed by cytological observation in paraffin sections. These analyses were

conducted to determine whether abnormal nuclear divisions were occurring during female gametogenesis. In addition, data were taken to determine the relationship of bud size with meiotic and mitotic stages of female gametogenesis. Flower buds were collected from each cytotype and the breadth of each measured with Vernier calipers. The calyx, corolla and anthers were removed to expose the ovary to the killing and fixing agent. The ovaries were placed in plastic embedding capsules and labeled with respect to bud size and cytotype. Ovaries were fixed for 24 to 48 hours in Bouin's fixative, after which they underwent a 24-hour rinse before dehydration and infiltration with paraplast. They were then refrigerated until they could be embedded. After embedding in paraplast, serial section ranging from 10 - 20 microns were made. Because of the perpendicular arrangement of the ovules within the buds, cross sections were made through the ovaries. All slides were individually cleaned with 95% ethanol and coated with a 2% solution of gelatin. Paraffin sections were affixed to slides and allowed to dry for two days. The paraffin was removed with xylol and hydrated in decreasing concentrations of ethanol. Sections were stained according to Johansen (1940) with safranin and crystal violet.

Scoring of the female developing gametophyte was performed according to the stage of cell or nuclear division, whether the division was normal or not and the position of each cell or nucleus in the gametophyte. In Haplo 9 plant, ovules were also scored with respect to the presence of an embryo and dividing endosperm cells.

CHAPTER 4

RESULTS AND DISCUSSION

Seed Production

The amount of seed set per boll was determined for Haplo 9, Telo 9L, Telo 9S and Iso 9S plants with their respective 2n progeny as well as TM1, the normal standard for G. hirsutum. Average seed set was determined in samples ranging from 50 to 76 bolls collected from several plants of each cytotype and the controls. These results are given in Table 2. The pooled t-test was used to determine significant differences among the cytotypes. The mean seed set for TM1 plants was approximately 30 seeds per boll which did not show any significant difference with any of the 2n progeny. Haplo 9 plants only had a seed set of 15.5 per boll, which is approximately half of the expected. Telo 9L, Telo 9S and Iso 9S had a mean seed set of 25.6, 22.8 and 24.2 seeds per boll respectively. All four cytotypes involving chromosome 9 showed a significant difference from plants with a normal chromosome complement. This indicates that complete absence of chromosome 9 as well as a deficiency for either arm, long or short, reduces viability of the embryo, as evidenced by the amount of seed set. Also, the relationship between seed set and amount of the deficiency was inversely proportionate. To explain further, Table 2 shows no significant difference between the

Table 2. Results of Seed Set Investigations of TM1 and Haplo 9, Telo 9L, Telo 9S, Iso 9S and Their Respective 2n Progeny.

Cytotype	Number of Plants	Number of Bolls	Mean Seed Set Per Boll	Standard Deviation	Standard Error	Pooled t test
TM1	10	75	30.030	3.342	0.503	a*
H9	5	60	15.500	3.694	0.477	b
2n	5	58	29.845	2.980	0.391	a
Telo 9L	8	76	25.632	2.998	0.344	c
2n	8	53	29.950	3.011	0.440	a
Telo 9S	2	50	22.846	3.800	0.431	d
2n	5	50	29.541	3.741	0.401	a
Iso 9S	6	75	24.187	3.451	0.399	d
2n	7	50	30.180	3.273	0.493	a

* lines that differ significantly from each other in seed set are indicated by different letters.

telosome and the isosome for the short arm of chromosome 9 whereas Telo 9L and Haplo 9 differ significantly with all other cytotypes in the mean number of seed per boll. Haplo 9 showed lower seed number per boll than the Telo 9S and Iso 9S. The latter two showed lower seeds per boll than Telo 9L plants which had fewer seeds per boll than the 2n and TM1 plants. All four cytotypes involving chromosome 9 differed significantly from TM1 and their 2n sibs in amount of seed set per boll. These results on seed set per boll indicate when an n gamete became the functional megaspore, nuclear division and gamete formation were normal and that when a deficient gamete became the functional megaspore, nuclear division and gamete formation were likely to be abnormal which would affect ovule viability. These data show that chromosome 9 plays an important role during gametogenesis as well as embryogenesis.

Analysis For The Association Marker Genes on Chromosome 9

The results of the tests for association of recessive and dominant genes with chromosome 9 are given in Table 3. In the cross of Haplo 9 plants with marker genes, at least one Haplo 9 plant must be recovered among the F_1 progeny in order to make a correct assessment of a marker gene's presence on chromosome 9. As outlined in Figures 4 and 5, the progeny resulting from a controlled cross involving a monosome is predictable.

As shown in Figure 4, if a recessive marker gene is located on chromosome 9, then all F_1 Haplo 9 progeny should express the recessive

Table 3. Results of Test For Association of Marker Genes With Haplo 9 in Crosses of Haplo 9 Plants With Genetic Marker Stocks

Marker Genes	Phenotype	Number of F_1 plants recovered of each cytotype and its phenotype		No. of testcross plants of each F_1 cytotype in each phenotypic class		No. of F_2 plants of each F_1 cytotype in each phenotypic class	
		2n-1	2n	$2n\text{-}1F_1$	$2nF_1$	$2n\text{-}1F_1$	$2nF_1$
Cu	Normal	2	43			18	22
cu	cupleaf	0	0			6	5
Gl_1	Glanded	2	43			14	18
gl_1	glandless	0	0			10	9
Fg	Normal	2	43			18	22
fg	frego bract	0	0			6	5
V_1	Normal	2	43			15	19
v_1	virescent	0	0			9	6
Crp	Crumpled	4	15	33	22	93	180
crp	normal	0	0	40	28	83	93
Lc_yLc_z	Brown lint	1	29	31	35	40	24
lc_ylc_z	white lint	0	0	46	28	59	14
P_1P_2	Yellow pollen	1	29			19	35
P_1p_2	Orange pollen	0	0			3	5
p_1p_2 *	cream pollen	0	0			9	12
Y_1	Yellow petal	1	29			24	37
y_1	cream petal	0	0			8	15

*or p_1p_1

phenotype and all F_1 disomic progeny should express the normal or dominant phenotype. However, a recessive gene may be on the monosome and not express the typical mutant phenotype because of the hemizygous condition. In this case, the phenotype of the hemizygous F_1 will appear very similar to heterozygous plants or very similar to homozygous normal plants in which case the recessive allele is hemizygous ineffective. Because of the possibility that a recessive gene may not express its phenotype unless homozygous, it was necessary to analyze the testcross or the F_2 progeny of the monosomic line to determine whether the gene was associated with Haplo 9.

All testcross progeny in the study were derived from crossing the F_1s to normal disomic lines. If a 1:1 ratio is obtained in the testcross or a 3:1 ratio is obtained in the F_2 of Haplo 9 F_1's, then it can be concluded that the gene in question is not on the monosome. But if in the testcross, all progeny have the recessive phenotype when the Haplo 9 F_1 is used as the male, or in the F_2, all 2n plants have the recessive phenotype and all 2n-1 have a normal phenotype, then it can be concluded that the gene is on the monosome and that the gene is hemizygous ineffective.

In the case of dominant marker genes which were used in crosses with Haplo 9, all monosomic and disomic F_1 progeny will express the dominant phenotypes (Figure 5). If the gene is on the monosome, all of the F_2 progeny of the monosomic F_1 would express the dominant phenotype,

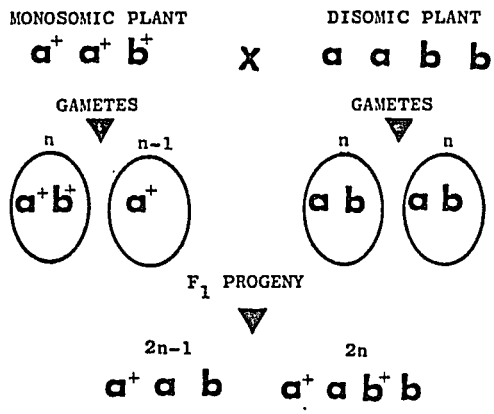

Figure 4. Crossing Procedure For Locating Recessive Mutant Genes on Monosomic Chromosomes.

The genes a and b are located on different chromosomes. The $a^+a^+b^+$ genotypic designation of the monosomic plant indicates that the a^+ allele is located on a disomic pair of chromosomes and the b^+ allele is located on the monosomic chromosome. The monosomic line carries the normal or the wild type allele and the disomic tester line carries the recessive alleles. Two kinds of F_1's will consist of the 2n F_1 plants which have the phenotype of both dominant alleles as expected, and the 2n-1 F_1 plants which have the phenotype of the normal a^+ alleles and recessive (hemizygous) phenotype of the b allele. This shows that the b^+ locus, but not the a^+ locus, is located in the monosomic chromosome.

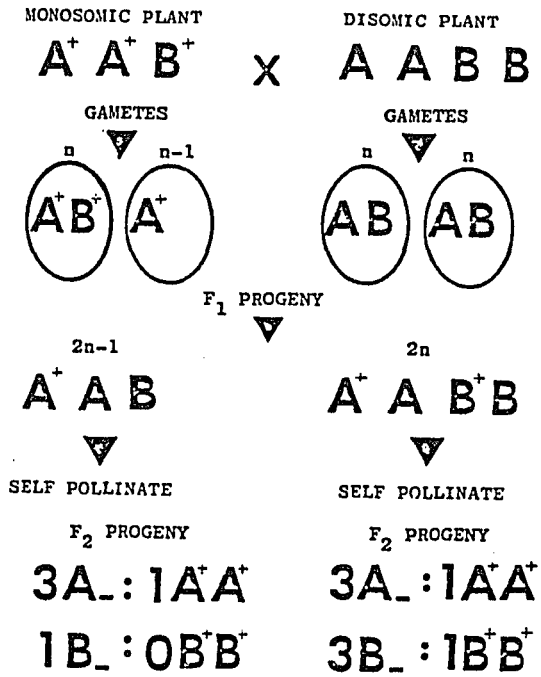

Figure 5. Crossing Procedure For Locating Dominant Mutant Genes On Monosomic Chromosomes.

The genes A and B are located on different chromosomes. The $A^+A^+B^+$ genotypic designation of the monosomic plant indicates that the A^+ allele is located on a disomic pair of chromosomes and the B^+ allele is located on the monosomic chromosome. The monosomic line carries the normal or wild type allele and disomic tester line carries the dominant alleles. Two kinds of F_1's will be produced, one is 2n-1 and the other is 2n. Both types express the dominant marker genes as expected. The monosomic and disomic are self-pollinated, after which the segregation behavior will be different among the F_2 progeny. For the A locus, a ratio of 3A-: $1A^+A^+$ is expected in the F_2 of both cytotypes. For the B locus, however, a ratio of 3B-: $1B^+B^+$ is expected in the F_2 of the disomes, but plants with only the B phenotype are expected in the F_2 of the monosomics, i.e., plants with the B^+ or normal phenotype will not be observed. This shows that the B^+ locus but not the A^+ locus is located on the monosomic chromosome.

whereas the F_2 progeny of the disomic F_1 would segregate in a 3:1 ratio of dominant to recessive phenotypes. If the gene is not on the monosome, all monosomic and disomic F_1 progeny will express the dominant phenotypes (Figure 5). However, the F_2 progeny of the monosomic F_1 and the F_2 progeny of the disomic F_1 would segregate in a 3:1 ratio of dominant to recessive phenotypes.

The recessive gene cup leaf (cu) was used as the pollen parent in crosses with Haplo 9 plants. The results of this test are given in Table 3. Two of the 45 plants were monosomic for chromosome 9 and all plants expressed the heterozygous leaf phenotype. Furthermore, the F_2 progeny of the 2n F_1, segregated into 22 Cu- plants and 5 cucu plants which is essentially a 3:1 ratio (x^2=.318; 1Df P = .50 - .70). The F_2 of the 2n-1 F_1 consisted of 18 dominant to 6 recessive plants (x^2=0; 1Df, P >.99) which is very similar to the F_2 data of the 2n F_1 plants. These data indicate that the gene for cup-leaf is not on chromosome 9.

Forty-five plants were scored in the F_1 progeny resulting from crosses of Haplo 9 plants with the marker gene glandless (gl_1) (Table 3). As shown in Table 3, two plants were monosomic for chromosome 9, none of which expressed the glandless phenotype, which establishes that the gene for glandless is not on chromosome 9. In addition, F_2 progeny of the 2n-1 F_1 segregated into 14 Gl_1- and 10 gl_1gl_1 which does not fit a 3:1 ratio (x^2= 4.06; 1 Df P = .01 - .05); however, since both phenotypes are present in which the dominant phenotype predominates, this establishes

that $\underline{gl_1}\underline{gl_1}$ is not on chromosome 9. These data are very similar to the F_2 segregation data of the 2n F_1, where the ratio of dominant to recessive phenotypes is 18:9 respectively (Table 3). These results fit a 3:1 ratio (x^2 = 1.24; 1 Df, P = .20 - .30).

Forty-five plants were scored in the F_1 progeny resulting from the cross of Haplo 9 plants with the marker gene frego bract (fg) (Table 3). The two monosomic and 43 disomic plants expressed the normal phenotype for bracteoles which signifies that the gene for frego bracteoles was not on chromosome 9. Additional proof was observed in the F_2 progeny of 2n-1 F_1 which segregated into 18:6 of normal to frego bract respectively. These results fit a 3:1 ratio (x^2 = 0; 1 Df, P >.99). The data obtained in the F_2 of the 2n-1 F_1 cross are very similar to those obtained in the F_2 of the 2n F_1 cross in that the F_2 ratio was 22 normal to 5 frego plants. These results also fit a 3:1 ratio (x^2 = .61; 1 Df, P = .30 - .50).

Forty-five plants were scored in the F_1 progeny resulting from the cross of Haplo 9 plants with the marker gene virescent leaf (v_1) (Table 3). The two monosomics as well as the 43 disomic F_1 plants expressed the normal green-leaf phenotype, which indicates that the gene for virescent is not on chromosome 9. This was shown to be the case in the test with the F_2 populations of both the monosomic F_1 and the disomic F_1. The former segregated into 15 normal green: 9 virescent plants [x^2(3:1) = 1.5; 1 Df, P = .20 - .30] whereas the latter segregated into 19 normal green: 6 virescent plants [x^2(3:1)=0.13; 1 Df, P=.90 - .95].

The Crumpled leaf mutant gene (Crp) is dominant to its allele for normal leaf (crp), therefore, both the F_1 monosomic and disomic progenies in the cross with Crumple-leaf expressed the crumpled leaf phenotype (Table 3). When dominant genes are used, the analysis for the association of the dominant character requires the scoring of the test-cross and/or the F_2 population of both the monosomic and the disomic F_1's. The testcross and F_2 results are given in Table 3 where it can be seen that the 2n-1 F_1 plant produced testcross progeny segregating 33 Crumpled 40 to noncrumpled or normal phenotypes, which is essentially a 1:1 ratio (x^2 = 0.671; 1 Df, P = .30 - .50). This establishes that the gene for Crumpled leaf is not on chromosome 9. If it were, all testcross progeny would have been Crumpled. The 2n F_1's as expected also segregated in the testcross into a 1:1 ratio (22 Crumpled: 28 normal; x^2 = .72; 1 Df, P = .30 - .50). The F_2 population of the 2n-1 F_1 Crumpled plants segregated like the control 2n F_1 plants, again establishing that the Crumpled allele is not associated with chromosome 9 (Table 3). The F_2 monosomic progeny and disomic progeny segregation ratios deviate significantly from the expected 3:1 ratios; however, this pattern of segregation is similar to that found by Kohel, (1973 and 1978). He found that in six F_2 populations using the dominant gene Crumpled leaf, only two had an acceptable segregation ratio of 3:1, one had a probability greater than 0.99, and the other three had a low probability of 0.1 - 0.2. In the present study, the results established that the gene for Crumpled leaf is not present on chromosome 9.

The two genes for brown lint, $\underline{Lc_y}\underline{Lc_z}$, are dominant which requires analysis of testcross and/or F_2 populations for possible association with chromosome 9. Kohel (1978) has reported that $\underline{Lc_y}$ and $\underline{Lc_z}$ genes are closely linked with a map distance of approximately 29.5 between the two. Therefore, in the monosomic test the two alleles are treated as a segregating unit even though there will be some recombination between the two when in the disomic condition. However, since my primary concern was whether the two genes are located on chromosome 9, the segregating testcross and F_2 populations were scored as to whether the individual plants had brown or white lint. In the cross of brown lint with Haplo 9, 30 F_1 plants were recovered, one of which were monosomic for chromosome 9 (Table 3). All F_1 progeny expressed the brown lint phenotype. The 2n-1 F_1 testcross progeny segregated 31 brown lint to 46 white lint, which fits a 1:1 ratio (x^2 = 2.99; 1 Df, P = .05 - .1). The 2n F_1 testcross progeny also segregated into a 1:1 ratio with 35 brown lint and 28 white lint. (x^2 = .77; 1 Df P = .30 - .50). The F_2 results of selfing the 2n-1 F_1 were 40 plants with brown lint and 59 plants with white lint. As described earlier, these two genes are linked and a 3:1 ratio was not expected; however, if the genes for lint color were on chromosome 9, then all the F_2 progeny would have expressed the brown lint phenotype. The F_2 results for the 2n F_1 were 24 plants with brown lint to 14 plants with white lint. These data establish that the $\underline{Lc_y}\underline{Lc_z}$ genes for brown lint are not on chromosome 9.

The inheritance of pollen color shows an epistatic relationship as described earlier. In the present study cream pollen ($\underline{p}_1\underline{p}_1\underline{P}_2\underline{P}_2$) was used as the monosomic female parent and orange pollen ($\underline{P}_1\underline{P}_1\underline{p}_2\underline{p}_2$, Ag114) was used as the pollen source. Thirty F_1 plants were obtained, of which one was monosomic for chromosome 9. The 30 F_1 plants had yellow pollen which is determined by the dominants $\underline{P}_1$ and $\underline{P}_2$. The gene, $\underline{P}_1$ has already been determined to be on chromosome 5; therefore, this leaves only $\underline{P}_2$ as possibly being on chromosome 9. If $\underline{P}_2$ is on chromosome 9, then the genotype of Haplo 9 plants will be $\underline{P}_1\underline{p}_1\underline{P}_2$ - and the 2n F_1 plants will be $\underline{P}_1\underline{p}_1\underline{P}_2\underline{p}_2$, both of which express the phenotype for yellow pollen. Thus, if P_2 is on chromosome 9, then in the F_2 of Haplo 9 F_1's, only two phenotypic classes are expected, yellow pollen (P_1-p_2-) and orange pollen ($p_1p_1P_2$-), rather than the three phenotypic classes, yellow, orange, and cream which occurs in the F_2 segregation of the normal disomic F_1. Therefore, the occurrence of plants with cream pollen in the F_2 population of 2n-1 F_2's is indicative that P_2 is not on chromosome 9. The F_2 results of self-pollinating the 2n-1 F_1 plant were 19 yellow pollen, 3 orange pollen and 9 cream pollen which fits the expected 9:3:4 ratio (x^2 = 1.703, 2 Df, P = .50 - .70). The F_2 results for the 2n F_1 were 35 yellow pollen, 5 orange pollen and 12 cream pollen which also fits the 9:3:4 ratio (x^2 = 3.52; 2 Df, P = .20 - .10). Therefore it can be concluded that the gene P_2 is not on chromosome 9.

The same 30 plants that were analyzed for pollen color were also analyzed for the association of the dominant gene yellow pollen (Y_1) with Haplo 9. As pointed out above in the cross of Haplo 9 x AG114, there was only one F_1 plant that was monosomic for chromosome 9. All F_1 plants expressed the yellow petal phenotype. The 2n-1 F_1 plants segregated in the F_2 generation in a 3:1 ratio with 24 Yellow petal plants to 8 cream petal plants (x^2 = 0, 1 Df, P > .99). The 2n F_1 when selfed also produced a segregation ratio of 3:1 with 37 yellow petal plants and 15 cream petal plants (x^2 = .410, 1 Df, P = .50 - .30). Therefore it is concluded that the gene for Yellow petal is not located on chromosome 9.

Evidence presented here establishes that the recessive genes for cup leaf (cu), glandless (gl_1), frego bract (fg) and virescent (v_1) are not on chromosome 9. The dominant genes for Crumpled leaf (Crp), brown lint (Lc_yLc_z), Pollen color (P_2) and Yellow petal (Y_1) are also not on chromosome 9.

Telesome and Isosome for the Short Arm of Chromosome 9

Monosomes occasionally misdivide and form an isochromosome or a telocentric chromosome. In the analysis of progeny from Haplo 9 plants crossed with the standard TM1, two new cytotypes were identified, a monotelodisome and a monoisodisome. Visual cytological analysis of the metaphase I chromosomes showed that the telo and isosome were the short arm of a large A subgenome chromosome. Normally chromosome 9

can be seen cytologically in Haplo 9 plants as consisting of a short arm and a long arm. Since the telosome and isosome came from a Haplo 9 plant, it was assumed that they involved chromosome 9. Further proof that they indeed are arm deficiencies of chromosome 9 will be presented later.

One of the plants in the progeny of Haplo 9 that contained one of the above cytotypes was a monotelomonoisodisome for two large A subgenome chromosomes. It was suspected that one of these aberrant chromosomes was chromosome 9. By crossing this plant to TM1, the Iso and Telo chromosomes were separated. The telosome was the short arm of a large A subgenome chromosome and the phenotype of the plant was very similar to Haplo 9, indicating that the telosome was Telo 9S. Tests with translocation proved that the telosome was Telo 9S, and will be discussed below.

Cytological analysis of PMCs from Telo 9S plants has revealed that Telo 9S and the standard chromosome do not consistently form a monotelodisomic bivalent in pollen mother cells. Frequently the two chromosomes occur as two univalents. The same is true for Iso 9S plants. Table 4 gives the frequency of pairing as seen cytologically for both cytotypes. In Telo 9S plants, a total of 152 cells were scored of which 83 showed bivalent pairing and 69 cells had two univalents consisting of the standard chromosome and the isochromosome. These data show that in 55 percent of the metaphase I cells, Telo 9S is paired with its homologue. In Iso 9S plants, a total of 514 cells

were scored and bivalent pairing was observed in 235 cells and the remaining 279 cells showed two univalents consisting of the standard chromosome and the isochromosome. These data show that only 46 percent of cells had bivalent pairing of the two homeologs. It is understandable that the isosome would show less pairing than the telosome because both arms of the isosome are identical and the two will frequently pair with each other rather than with the normal homolog. Both cytotypes show instability in pairing, therefore, there are different types of gametes that can be formed: (1) n, (2) n + Iso 9S or n + Telo 9S, (3) n-1, i.e., the gamete is monosomic for chromosome 9 and (4) n gametes which include the telosome and isosome. This instability of pairing was observed in the crosses of these two cytotypes with TM1 and the translocations.

Identification of Telo 9S and Iso 9S

In the crosses of Telo 9S and Iso 9S with homozygous T_{9-25}, which consists of the large A subgenome chromosome 9 and the small D subgenome chromosome 25, the telosome and isosome were associated with the large chromosome in the translocation heterozygote.

Figure 6 shows the results of crossing the homozygous reciprocal translocation involving chromosome 9 and 25 with the monotelodisome 9S. The results of cytological analysis of 85 PMCs showed that 24 or 28% of cells had a chain-of-four chromosomes in which one of the chromosomes was the telosome (Figure 6b). In 54 or 64% of the cells the translocation complex occurred as a trivalent and a univalent in which the

Table 4. Frequency of Pairing Of The Standard Chromosome With The Telocentric Chromosome 9S and The Isochrome 9S in Monotelodisomic 9S and monoisodisomic 9S Plants.

Cytotype	Number of Cells Observed	Number and Percent of Cells With Paired Chromosomes	Number and Percent of Cells With Unpaired Chromosomes
Monotelo-disome 9S	152	83 54.605	69 45.395
Monoiso-disome 9S	514	235 45.720	279 54.280

telosome was a member of the trivalent (Figure 6c). In 7 or 8% of the cells, pairing of the chromosomes in the translocation complex consisted of two types in which the telesome in these two types of pairing was present either as a univalent (Figure 6e) or as a heteromorphic bivalent (Figure 6f). In all instances where pairing of the telosome was observed, it was noted that the telosome paired with the larger chromosomes of the translocation complex. Because chromosome 9 is much larger than chromosome 25, it establishes that the telocentric chromosome is of chromosome 9.

Figure 7 shows the results of crossing the homozygous reciprocal translocation involving chromosomes 3 and 9 with the monotelodisome 9S. The results of cytological analysis of 126 PMCs showed that 61 or 48% of the cells had a chain-of-four chromosomes in which one of the chromosomes was the telosome (Figures 7a, b). In 61 or 48% of the cells, the translocation complex occurred as a trivalent and a univalent in which the telosome was either a member of the trivalent (Figure 7c) or it was the univalent chromosome (Figure 7d). In 6 or 7% of the cells, pairing of the chromosomes of the translocation complex was a bivalent and two univalents consisting of two types; in one type the telosome was present as a univalent (Figure 7e) and in the other type it was present as a heteromorphic bivalent (Figure 7f).

It was demonstrated in the analysis of the cross of the telocentric with the T_{9-25} translocation that the telosome paired with the large A subgenome chromosome 9 rather than the small D subgenome chromosome 25, establishing that the telocentric is chromosome 9. In the

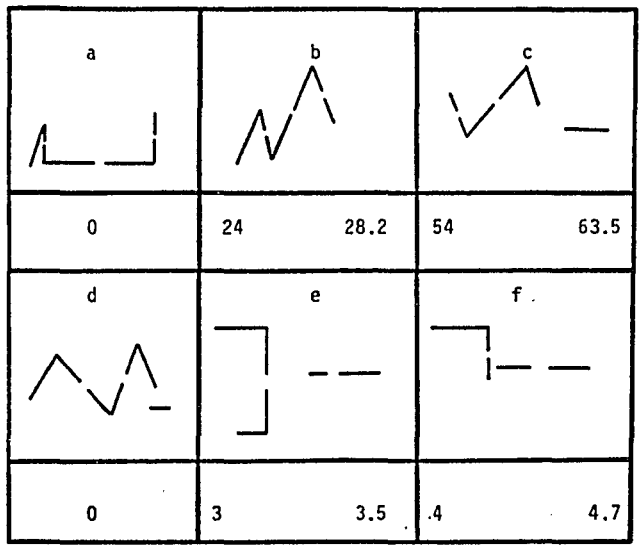

Figure 6. Monotelodisomic Plants Were Crossed With Plants Homozygous For a Reciprocal Translocation Involving Chromosomes 9 (subgenome A) and 25 (subgenome D).

Analysis of chromosome pairing revealed that the telocentric chromosome was one of the four chromosomes of the translocation complex in which it was observed to pair with the large translocated A subgenome chromosome 9. (a-f) are diagrammatic illustrations of metaphase I pairing configurations which assisted in the identification of the telocentric chromosome as involving chromosome 9. The numbers in the left and right sides of the squares are respectively the number and percent of cells observed of each pairing configuration. All cells had 24 bivalents in addition to the pairing types illustrated above. (a) No cells were observed in which the chain of four chromosomes occurred as an adjacent configuration. (b) 24 or 28% of the cells had an alternate chain of four chromosome. (c) 54 or 64% of the cells had a univalent translocated chromosome plus a trivalent with the Telo 9S. (d) No cells were observed in which Telo 9S occurred as a univalent plus a trivalent. (e) 3 or 4% of the cells had two univalents and a heteromorphic bivalent consisting of the telocentric chromosome and a translocated chromosome.

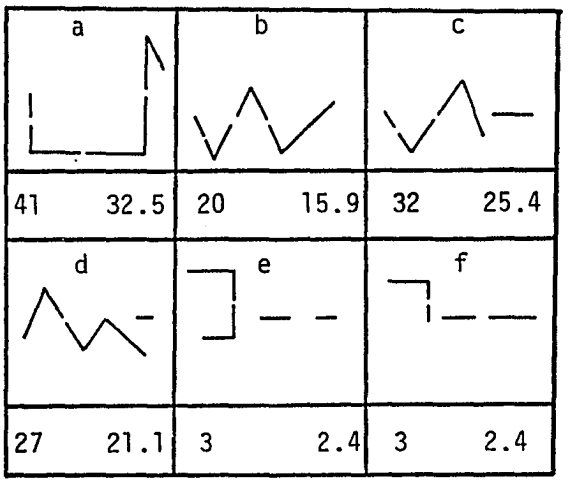

Figure 7. Monotelodisomic Plants Were Crossed With Plants Homozygous For a Reciprocal Translocation Involving Chromosomes 3 and 9 (both are of subgenome A).

The numbers in the left and right sides of the squares are respectively the number and percent of cells observed in each pairing configuration. Analysis of chromosome pairing revealed that the telocentric chromosome was one of the four chromosomes of the translocation complex and by deduction as described in the text, it was concluded that the telocentric chromosome paired with the translocation chromosome 9. (a-f) are diagrammatic illustrations of metaphase I pairing configurations which assisted in the identification of the telocentric chromosomes recovered from H9 plants. All cells had 24 bivalents in addition to the pairing types illustrated above. (a and b) 61 or 47% of the cells had a chain of four chromosomes in which one of the chromosomes was the telosome: (c and d) 59 or 25% of the cells in the translocation complex occurred as a trivalent and a univalent in which telosome was either a member of the trivalent (c) or it was the univalent (d). (e and f) 6 or 7% of the cells, pairing of the translocation complex was a bivalent and two univalents; in these two cases, the telosome was present as a univalent (e) or as a heteromorphic bivalent (f).

analysis of the crosses of the telosome with the two translocations T_{3-9} and T_{9-25}, the telosome was observed paired with both translocations; therefore, since the two translocations have only chromosome 9 in common and the telosome pairs with two, then the telosome must be Telo 9S.

Figure 8 shows the results of crossing the homozygous reciprocal translocation involving chromosomes 9 and 25 with the monoisodisome 9S. The results of cytological analysis of 35 PMCs showed that 12 or 34% of the cells had a chain-of-four chromosomes in which one of the chromosomes was the isosome (Figures 8a, b). In 18 or 51% of the cells the translocation complex occurred as a trivalent and a univalent in which the isosome was either a member of the trivalent (Figure 8c) or it was the univalent chromosome (Figure 8d). In 5 or 14% of the cells, pairing of the translocation complex involving the isosome was a bivalent and two univalents in which the isosome was one of the univalents (Figure 8e). When the isosome paired with another chromosome, its associated homolog was always a large chromosome, therefore, since the large chromosome is 9, the isosome must be chromosome 9.

Figure 9 shows the result of crossing the homozygous reciprocal translocation involving chromosomes 3 and 9 with the monoisidisome 9S. The results of cytological analysis of 112 PMCs showed that 58 or 52% of cells had a chain-of-four chromosomes, one of which was the isochromosome (Figures 9a, b). In 43 or 38% of the cells, pairing of the translocation complex occurred as a trivalent and a univalent in which

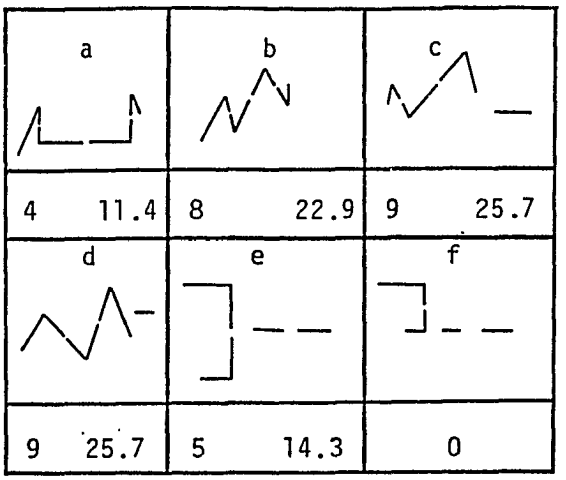

Figure 8. Monoisodisomic Plants Were Crossed With Plants Homozygous For a Reciprocal Translocation Involving Chromosomes 9 (subgenome A) and 25 (subgenome D).

Analysis of chromosome pairing revealed that the isochromosome was one of the four chromosomes of the translocation complex in which it was observed to pair with the large translocated A subgenome chromosome 9. The numbers in the left and right sides of the squares are respectively the number and percent of cells observed in each pairing configuration. (a-f) are diagrammatic illustrations of metaphase I pairing configurations which assisted in the identification of the isochromosome recovered from H9 plants. All cells had 24 bivalents in addition to the pairing types illustrated above. (a) 4 or 11.4% of the cells had an adjacent chain of four chromosomes, (b) 8 or 22.9% of the cells had an alternate chain of four, (c) 9 or 25.7% of cells had a univalent and a trivalent with the telocentric chromosome, (d) 9 or 25.7% of cells had a telocentric univalent plus a trivalent, (e) 5 or 14.3% of the cells had a telocentric chromosome as a univalent plus a bivalent, (f) no cells were observed with that pairing combination.

the isosome occurred either as one member of the trivalent or as a univalent (Figures 9c, d). In the remaining 4 or 10% of the cells, the translocation complex occurred as a bivalent and two univalents, one of which was the isosome. The isochromosome was observed paired with the two translocations, T_{9-25} and T_{3-9}, both of which have chromosome 9, thus, the isochromosome must be Iso 9S.

Cytotypes Recovered in Progeny of Aneuploids of Chromosome 9

In a population of 101 plants from crossing monotelodisome 9L with TM1, 68 plants were disomes and 33 plants were monotelodisome 9L. Piror to this study, progeny from outcrossing monotelodisomic 9L with TM1 were grown over a period of several years and only disomes and monotelodisomes 9L were recovered (Endrizzi, personal communication). It is apparent from these results that the progeny of monotelodisome 9L plants did not contain any cytologically aberrant plants other than the parental cytotype, monotelodisome 9L. Only disome and monotelodisome 9L progeny are recovered.

The progeny from outcrossing and self-pollination of Haplo 9, monotelodisome 9S and monoisodisome 9S plants include, in addition to the parental aneuploid type, many other aneuploid or cytotypes. The aneuploid types and their frequency, which are discussed below, are given in Tables 5, 6 and 7.

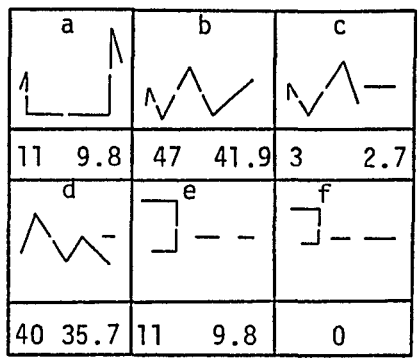

Figure 9. Monoisodisomic Plants Were Crossed With Plants Homozygous For a Reciprocal Translocation Involving Chromosomes 3 and 9 (both chromosomes are of subgenome A).

The numbers in the left and right sides of the square are respectively the number and percent of cells observed in each pairing configuration. Analysis of chromosome pairing revealed that the isochromosome was one of the four chromosomes of the translocation complex, and by deduction as described in the text, it was concluded that the isochromosome paired with the translocated chromosome 9. (a-f) Diagrammatic illustrations of metaphase I pairing configurations which assisted in the identification of the isochromosome recovered from H9 plants. All cells had 24 bivalents in addition to the pairing types illustrated above. (a) 11 or 10% of the cells had an adjacent chain of four chromosomes, (b) 47 or 42% of the cells had an alternate chain of four chromosomes, (c) 3 or 3% of the cells had a univalent and a trivalent with the isochromosome, (d) 40 or 36% of the cells had an iso univalent plus a trivalent, (e) 11 or 10% of the cells the translocation complex occurred as a bivalent and two univalents, one of which was the isochromosome, (f) No cells were observed with that pairing combination.

Table 5. Number and Percentage of Different Cytotypes Recovered from 1967 to 1983 in Outcrossing and Self-pollination of Haplo 9 (25" + 1'ℓ) Plants.

Cytotypes	No. of Plants	Percentage	Cytotypes	No. of Plants	Percentage
26"	1143	93.230	23" + 1ℓ + 1ℓ + 1ℓ	2	0.163
[a]25" + 1'ℓ	34	2.773	23" + 1's + 1'ℓ + 1ℓ	2	0.163
[b]25" + 1'ℓ	1	0.082	23" + 1's + 1ℓ + 1'ℓ + f	1	0.082
25" + 1's	2	0.163	23" + 1"ℓ + 1"ℓ + 1"ℓ	1	0.082
[c]25" + t1"	3	0.245	22" + 1'm + 1'ℓ + 1'ℓ + 1'ℓ	1	0.082
[d]25" + i1"	2	0.163	22" + 1's + 1'ℓ + 1'ℓ + 1'ℓ	1	0.082
[e]25" + i1"	1	0.082	22" + 1's + 1's + 1'm + 1'ℓ	1	0.082
25" + 1"'ℓ	2	0.163	21" + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ	1	0.082
25" + 1"'m	1	0.082	21" + 1"'s + 1'm + 1'ℓ + 1'ℓ + 1'ℓ	1	0.082
25" + 1"'s	2	0.163	20" + 1'm + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ	1	0.082
24" + 1'ℓ + 1'ℓ	8	0.711	20" + 1's + 1's + 1's + 1'ℓ + 1'ℓ + 1'ℓ	1	0.082
24" + 1's + 1'ℓ	2	0.163	17" + 1's + 1's + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ	1	0.082
[f]24" + t1" + i1"	1	0.082	[g] Unanalyzed	10	0.881
			Total	1226	

a = Haplo 9.
b = ℓ, m and s refer to chromosome size: large, medium and small, respectively.
c = monotelodisome 9L.
d = monoisodisome 9L.
e = monoisodisome 9S.
f = monotelomonoisodisome; it was this plant in which Telo 9S was later isolated.
g = chromosome complement could not be determined.

Table 6. Number and Percent of Different Cytotypes Recovered From 1982 to 1983 in Outcrossing and Self-pollination of Monotelodisome 9S (25" + t1") Plants.

Cytotypes	Number of Plants	Per-centage
26"	117	78.146
26" + f	1	0.660
[a]25" + t2"'	5	3.311
[b]25" + 1"'s	4	2.649
[c]25" + t1"	8	5.298
25" + 1's	2	1.325
25" + 1'ℓ	1	0.660
24" + t1"+ 1's	1	0.660
24" + t1"+ 1'ℓ	4	2.649
24" + 1's + 1'ℓ	1	0.660
24" + 1'ℓ + 1'ℓ	2	1.325
23" + 1's + 1'ℓ +1'ℓ	1	0.660
23" + 1'ℓ + 1'ℓ + 1'ℓ	1	0.660
22" + 1's + 1'ℓ +1'ℓ+ 1'ℓ	1	0.660
[d]Unanalyzed	2	1.325
Total	151	

a = monotelotrisome, 9S

b = ℓ, m and s refers to chromosome size, large, medium and small respectively.

c = monotelodisome 9S.

d = chromosome complement could not be determined.

Table 7. Number and Percent of Different Cytotypes Recovered From 1981 to 1983 in Outcrossing and Self-pollination of Monoisodisome 9S (25"+i1") Plants.

Cytotype	No. of Plants	Percentage
26"	206	73.10
[a]26" + i1"	14	4.98
[b]25" + i1"	22	7.829
[c]25" + 1'ℓ	3	1.068
25" + 1's	4	1.424
25" + 1'" ℓ	2	0.712
24" + i1"+ 1'ℓ	7	2.491
24" + i1"+ 1'"m	1	0.356
24" + i1"+ 1'" ℓ	1	0.356
24" + i1"+ 1's	4	1.424
24" + 1'ℓ + 1'ℓ	2	0.712
23" + i1"+ 1'ℓ + 1'ℓ	4	0.1424
23" + 1's + 1'ℓ + 1'ℓ	3	1.068
23" + 1's + 1'ℓ + 1'ℓ + f	1	0.356
22" + i1" + 1'ℓ + 1'ℓ + 1'	1	0.356
22" + 1's + 1'ℓ + 1'ℓ + 1'ℓ	1	0.356
22" + 1'"s + 1'ℓ + 1'ℓ + 1'ℓ	1	0.356
21" + i1" + 1'ℓ + 1'ℓ + 1'ℓ + 1'ℓ	1	0.356
[d]Unanalyzed	2	0.712
Desynaptic	1	0.356
Total	281	

a = monoisotrisome 9

b = monoisodisome 9

c = ℓ, m and s refer to chromosome size, large, medium and small, respectively.

d = chromosome complement could not be determined.

In the above, it is apparent that the different types of aneuploids appear only in the progeny of Haplo 9, monotelodisome 9S and monoisodisome 9S, and not in the progeny of monotelodisome 9L. It can be concluded from these results that in the absence of the long arm of chromosome 9, different cytotypes are recovered. This suggests that the long arm of chromosome 9 carries gene(s) which control normal chromosome segregation in some stage prior to gamete formation in Haplo 9, monotelodisome 9S and monoisodisome 9S plants.

Table 5 lists all of the cytotypes recovered in crossing and self-pollination of Haplo 9 plants from 1967 to 1983. It can be seen in the table that in a total plant population of 1226 plants, 1143 or 93.2% are normal or disomic plants, and 34 or 2.77% are Haplo 9 plants.

As mentioned earlier, monosomes will occasionally misdivide, thus monotelodisome 9L, monotelodisome 9S and a fragment, later identified as Iso 9S were all initially found among Haplo 9's progeny. Table 5 shows that there are 40 plants that are either Haplo 9, monotelodisome 9L or 9S and monoisodisome 9S which consisted of 3.2% of the total population. One other plant had 24" + t1" + i1". The t1" in this plant was isolated and identified as monotelodisome 9S. Three plants were recovered that were isosomic for the long arm of a chromosome which would indicate that it was of the A subgenome which could have included isosomes of the long arm of chromosome 9. When the frequency of disomes, Haplo 9, monotelodisome 9S and 9L, and monoisodisome 9S and unanalyzed

plants are subtracted from the total population, this gives a frequency of 2.7% of other cytotypes that were recovered in the progeny of Haplo 9. The aneuploid cytotypes included simple monosomes, multiple monosomes, trisomes, and monosome-trisome combinations (Table 5).

The unanalyzed plants were morphologically abnormal and could not be analyzed cytologically. These plants ranged in size from very small to those that were near normal in size but consisted of grossly aberrant morphological plant characteristics; many were completely male and female sterile. It was assumed that all of these had an abnormal chromosome complement which would account for their abnormalities. If the unanalyzed plants are included with the "aneuploids" not involving chromosome 9, the frequency of other aneuploid cytotypes is 3.5%.

Monotelodisome 9S was isolated in 1981, therefore, its progeny were available for study in 1982 and 1983. It can be seen in the analysis of 151 plants in Table 6 that monotelodisome 9S behaves cytologically very similar to that of Haplo 9 plants in that a number of different aneuploid types were recovered. Had a larger population been studied, it is likely that the more extreme types of aneuploids as observed in the progeny of Haplo 9 would have been recovered. The disomic or normal plants constitute the majority (117 or 78%) of the cytotypes. There were 13 or 9% of the plants that were either monotelotrisomic or monotelodisomic 9S. The remaining 21 or 13% of the plants include the unanalyzed plants and the various aneuploid types, consisting of simple monosomics, double, triple, and quadruple

monosomics as well as trisomics. In five of the aneuploid plants, Telo 9S was observed as part of the chromosome complement. The two unanalyzed plants were very small, morphologically abnormal, and male and female sterile. It was assumed that the plant abnormalities were due to anomalous chromosome numbers.

Monoisodisome 9S was isolated in 1980 and from 1981 to 1983, 281 plants were observed (Table 7). Here again, it can be seen that monoisodisome 9S produces a progeny very similar to that of Haplo 9 and Telo 9S in that a high frequency of plants are recovered which are aneuploids.

Two hundred and six, or 73% of the plants were disomic and 36, or 13% of the plants were either monoisotrisomic or monoisodisomic 9S. The remaining 39 or 14% consisted of various kinds of aneuploid, unanalyzed and desynaptic plants. The aneuploids were similar to the aneuploids produced in the progeny of both Haplo 9 and Telo 9S in that a wide range of different cytotypes were found. In 19 of the 41 plants, Iso 9S was part of the aneuploid chromosome complement. The two unanalyzed plants were morphologically abnormal as well as male and female sterile. It was assumed that these plants had an anomalous chromosome complement which contributed to the abnormalities. The desynaptic plants showed a variable amount of pairing which interfered in determining the exact chromosome number. It was assumed that the lack of pairing was due to an abnormal chromosome complement.

It is apparent from the data in Table 5, 6 and 7 that the progeny of Haplo 9, monotelodisome 9S and monoisodisome 9S plants included a high frequency of different aneuploid types. A chi-square contingency test performed with the three cytotypes gave a significant chi-square value (x^2 = 51; 2 Df, P >.01). A pairwise chi-square contingency test was also performed and the results showed a significant difference between the progenies of Haplo 9 and Telo 9S (x^2 = 23; 1 Df, P >.01). Also a significant difference was found between Haplo 9 and Iso 9S (x^2 = 142; 1 Df, P >.01). However, there was no significant difference between Telo 9S and Iso 9S progenies (x^2 = 0.105, 1 Df, P = .70 - .90). Since the kinds and frequencies of aneuploid progenies of Telo 9S and Iso 9S plants were similar but differed significantly from the kinds and frequencies of aneuploid progenies of Haplo 9, this indicates that possibly the short arm of chromosome 9 has an effect on chromosome disjunction, or it could be due to sample size. The population of Telo 9S and Iso 9S consisted of 151 and 281 plants, whereas the population of Haplo 9 consisted of 1226 plants. It is possible that larger populations of Telo 9S and Iso 9S comparable to that of Haplo 9 would include a wider variety of aneuploids similar to those in the progeny of Haplo 9.

It is apparent from the data given in Tables 5, 6 and 7 that many of the progenies of Haplo 9, monotelodisome 9S, and monoisodisome 9S plants are cytologically aberrant.

The size of the chromosomes of the 13A subgenome is considered to be large, while those of the 13D subgenome are considered to be small

by cotton cytologists. The chromosomes of the A subgenome are approximately two times the size of the D subgenome chromosomes; however, there is a graduation in chromosome size in both genomes. Because of this graduation in size, the smallest A subgenome chromosome is not distinctly larger than the largest D subgenome chromosome of G. hirsutum. In this case, the size is neither distinctly large nor distinctly small, but intermediate or median in size, in which case such chromosomes are categorized as median with respect to overall chromosome size in the total complement. According to Endrizzi (personal communication), most simple aneuploids which were initially classified as median in size were shown in later tests to be A subgenome chromosomes.

Table 8 gives the number and percentage of the total chromosomes that were found extra (trisomic) or deleted (monosomic) with reference to size in the progenies of Haplo 9, monotelodisome 9S and monoisodisome 9S plants. As described earlier, the large chromosomes are of the A subgenome and the small chromosomes are of the D subgenome; however, the medium size chromosomes may be of the A or D subgenome. In the progeny of Haplo 9, there was a total of 89 chromosomes that were observed as univalents and trivalents. Univalents occurred far more frequently (91%) than trivalents (9%). The data also show that deficiencies for the large A subgenome chromosomes occurs far more frequently (76.54%) than deficiencies for the small D subgenome chromosomes.

Among the progeny from Telo 9S plants, 22 chromosomes were observed as univalents and four chromosomes were trivalents which constitutes 85% and 15% respectively (Table 8). It is also apparent that

deficiencies for the large A subgenome chromosomes occur more frequently (82%) than deficiencies for the small D subgenome chromosomes.

Among the progeny of Iso 9S plants there were 53 chromosomes observed as univalents and nine chromosomes observed as trivalents which constitute 85% and 15% respectively. A high frequency (81%) of deficiencies for the large A subgenome chromosomes also occurred in the progeny of monoisodisome 9S (Table 8).

The results given in Table 8 are very similar in that all three cytotypes show the elimination of chromosomes more than the addition of chromosomes in their progeny and that the elimination involved primarily A subgenome chromosomes. A chi-square contingency test shows that the frequencies of univalents and trivalents that occurred among the progenies of the three cytotypes are not significantly different ($x^2 = 1.96$; 2 Df P = .50 - .70).

It is apparent from the discussion of the data in Tables 5 to 8 that the progenies of Haplo 9, monotelodisome 9S and monoisodisome 9S contain a rather high frequency of aneuploid plants consisting primarily of deleted chromosomes and that the deleted chromosomes are primarily of the A subgenome.

These types do not occur in the progeny of the monotelodisome for the long arm of chromosome 9. Thus it can be concluded that the absence of the long arm of chromosome 9 is responsible for the occurrence of the numerous kinds of aneuploid types. Furthermore, it can also be concluded that the long arm of chromosome 9 carries genetic

Table 8. Number and Percentage of Chromosomes With Respect to Size, Not Involving the Parental Cytotype, That Were Added and Subtracted From the Chromosome Complement in Progenies of Haplo 9, Monotelodisome 9S and Monoisodisome 9S. + = Addition, - = Deficient.

Cytotype	Large		Medium		Small		Total
	+	-	+	-	+	-	
Haplo 9	3	62	1	4	4	15	89
Telo 9S	0	18	0	0	4	4	26
Iso 9S	3	43	1	0	5	10	62
							177

factor(s) which play a significant role in regulating normal chromosome segregation, and in its absence, normal chromosome segregation is disrupted, resulting in many gametes with deficiencies and/or duplication of chromosomes and parts of chromosomes.

Megasporagenesis in TM1 and Haplo 9 Plants

The central purpose of the gametophytic study was to identify the time of aberrant divisions that give rise to aneuploid progeny of Haplo 9, monotelodisome 9S and monoisodisome 9S plants. If the abnormal division is post-meiotic in these three cytotypes and the control of chromosome segregation patterns lie in the long arm of chromosome 9, then there is theoretically a 50% probability that a functional megaspore will or will not have a normal chromosome 9.

If the megaspore receives a normal chromosome, then it will give rise to normal chromosome segregation and normal gametogenesis. If the megaspore does not have a normal chromosome 9, or it has a Telo 9S or an Iso 9S chromosome, then the megaspore may undergo abnormal nuclear divisions. All stages of megasporogenesis were examined in Haplo 9 and normal (TM1) plants to determine any inconsistencies that may occur during development of the female gametophyte in the former.

The size of the flower bud was recorded in relation to the stages of cellular divisions in the development of the female gametophyte. The size of the bud refers to its diameter or broadest girth.

Table 9 gives the relationship of bud size with the number and position of premeiotic and postmeiotic cells leading to gamete formation within ovules of TM1 plants.

In our observations it was found that when the bud size was approximately 3.5 - 4.0 mm the archesporial cells was first observed at the chalazal end of the ovule (Table 9). The archesporial cell continues to enlarge until the bud reaches 6.0 - 6.3mm at which time there is a mitotic division. One of these cells (the parietal cell) divides several times and the remaining cell becomes the functional megaspore mother cell.

The meiotic divisions occur when the bud size is 6.3 - 6.6mm (Table 9). Soon afterward, the three megaspores degenerate and the remaining megaspore undergoes three mitotic divisions. The first mitotic division of the megaspore occurs when the bud size is 7.2 - 7.5mm. One of the nuclei moves to the micropylar end of the gametophyte after which the second and third mitotic division occurs.

When the bud size is 8.3 - 8.5mm, four nuclei can be observed at the chalazal end and four nuclei at the micropylar end. One nucleus from each end of the gametophyte migrates toward a region just below the middle of the female gametophyte to form the polar nuclei. The antipodals at the chalazal end degenerate shortly afterward, leaving a five celled gametophyte, which occurs primarily at bud size of 8.7mm and larger (Table 9).

Table 9. Relationship of Bud Size With Number and Position of Nuclei Within Ovules of TM1 Plants.

Bud Size (mm)	Number of Ovules	Number of Nuclei per Putative Gametophyte	Position of Nuclei in Gametophyte		
			Chalazal	Middle	Micropyle
3.5 - 4.0	22	1	X		
4.0 - 4.5	18	1	X		
4.5 - 5.0	11	1	X		
5.0 - 5.5	15	1	X		
5.5 - 6.0	32	1	X		
6.0 - 6.3	62	1 - 2	X		
6.3 - 6.6	58	1 - 4	X		
6.6 - 6.9	180	1 - 4	X		
6.9 - 7.2	201	1 - 4	X		
7.2 - 7.5	122	1 - 2	X		
7.5 - 7.8	53	1 - 2	X		
7.8 - 8.1	102	1 - 4	X		X
8.1 - 8.3	105	2 - 8	X	X	X
8.3 - 8.5	56	4 - 8	X	X	X
8.5 - 8.7	25	5 - 8	X	X	X
8.7 - 9.0	42	5		X	X
9.0 - Flower	66	5		X	X

Table 10 shows the relationship of bud size in Haplo 9 plants with the development of the female gametophyte. In comparing the data in Tables 9 and 10, it can be seen that gametogenesis in TM1 and Haplo 9 plants follow the same sequence of cellular developmental events up until about 8.1mm bud-size stage. From this bud size and on, differences between TM1 and Haplo 9 in cellular development become evident. From the 8.1mm bud size to the flower stage, there appears to be two major differences. One is that cellular division in Haplo 9 appears to be less frequent than in TM1 plants. The second difference, which is a major one, occurs when the bud size is greater than 8.7mm. Here it can be seen that TM1 plants do not have ovules with cells at the chalazal end, whereas the ovules in Haplo 9 plants still have cells present at the chalazal end.

Table 11 shows the results of the premeiotic, meiotic and post metaphase I observations of TM1 and Haplo 9 plants. A total of 599 and 1047 ovules in TM1 and Haplo 9 respectively were analyzed.

Cells in the premeiotic stage of 241 and 383 ovules were analyzed in TM1 and Haplo 9 plants respectively (Table 11). The premeiotic cells in Table 11 refer to all cells observed from the first appearance of the archesporial cell through the formation of the megaspore mother cell. No observable abnormalities occurred in either TM1 or Haplo 9 cells in early and late prophase of the premeiotic division. Furthermore, no observable differences were seen between the two in the arrangement or position of nuclear materials. During telophase, cell divisions

Table 10. Relationship of Bud Size With Number and Position of Nuclei Within Ovules of Haplo 9 Plants.

Bud Size (mm)	Number of Ovules	Number of Nuclei per putative gametophyte	Position of Nuclei in Gametophyte		
			Chalazal	Middle	Micropyle
3.5 - 4.0	28	1	X		
4.0 - 4.5	25	1	X		
4.5 - 5.0	25	1	X		
5.0 - 5.5	30	1	X		
5.5 - 6.0	48	1	X		
6.0 - 6.3	96	1 - 2	X		
6.3 - 6.6	304	1 - 4	X		
6.6 - 6.9	386	1 - 4	X		
6.9 - 7.2	105	1 - 4	X		
7.2 - 7.5	188	1 - 2	X		
7.5 - 7.8	85	1 - 2	X		
7.8 - 8.1	104	1 - 4	X		X
8.1 - 8.3	203	1 - 8	X	X	X
8.3 - 8.5	163	1 - 8	X	X	X
8.5 - 8.7	51	1 - 8	X	X	X
8.7 - 9.0	40	1 - 5	X	X	X
9.0 - Flower	180	1 - 5	X	X	X

were always transverse to the plane of sectioning in both cytotypes. One of the cells continues to divide several more times and the other cell became the functional megaspore mother cell. Cytokinesis was normal in that two equal sized cells were formed. Again, no observable differences were seen in either TM1 or Haplo 9 plants.

A total of 196 and 237 prophase I cells were analyzed in TM1 and Haplo 9 plants respectively (Table 11). In both cytotypes, this stage was found to be highly synchronous among ovules and chromosomes were easily observed. Generally, most if not all the nuclei in ovules of an ovary were found to be at that same prophase I stage. No observable differences were seen in either TM1 or Haplo 9 plants.

At the metaphase I stage, 19 cells were analyzed in TM1 and 17 cells were analyzed in Haplo 9 plants. All were normal in that the chromosomes were aligned in the center of the spindle apparatus. However, in monotelodisome 9S, five ovules at metaphase I were examined and one had a bivalent that was aligned off the metaphase plate. All other ovules of this ovary had completed meiosis in that the meiotic products were observed in linear tetrads. A chromosome count of the abnormal cell in Iso 9 and of all other cells at metaphase I in TM1 and Haplo 9 could not be made with any degree of accuracy because these were serial sections that were perpendicular to the spindle axis and the chromosomes were closely associated with each other on the metaphase plate.

A total of 143 and 410 ovules that were in post-metaphase I stage of development were observed in TM1 and Haplo 9 respectively.

Table 11. Observations of Premeiotic and Meiotic Divisions Within Ovules of TM1 and Haplo 9 Plants

Number of Ovules	Stage of Development	Number of Abnormal Ovules
TM1		
241	Premeiotic	0
196	Prophase I	0
19	Metaphase I	0
143	Post Metaphase I	0
599		
Haplo 9		
383	Premeiotic	0
237	Prophase I	0
17	Metaphase I	0
410	Post Metaphase I	0
1047		

Two of 410 ovules observed in Haplo 9 plants were in metaphase II, of which no abnormal chromosome segregation was found. There were no ovules of TM1 that had nuclei at metaphase II. All tetrads formed were linear and no micronuclei or multipolar nuclei were observed.

Since 50% of the telophase I nuclei of Haplo 9 are expected to be n-1, we had assumed that the second meiotic divisions of many ovules might show aberrant chromosome segregation. However, meiosis of Haplo 9 appears normal in every respect like that in TM1.

As described earlier, G. hirsutum, like most angiosperms, forms an eight nuclei or seven-cell megagametophyte which ultimately becomes a five-nuclei or four-cell megagametophyte. In the formation of the megagametophyte from a megaspore, there is an increase in the number of nuclei or cells because of the three mitotic division, after which the number of nuclei or cells decrease because of the degeneration of the antipodals. These changes in the number of nuclei or cells of the megagametophyte within ovules of TM1 and Haplo 9 plants were related to bud size. The results are given in Table 12.

Bud size 7.2 - 7.5mm was chosen as a starting point because this was the size that Haplo 9 began to show differences in gametophyte development. As the bud size increases, the percentage of ovules containing one cell decreases in TM1 more rapidly than in Haplo 9 plants. Also, from bud size 8.1 to flowering, the number of nuclei per gametophyte in Haplo 9 plants is more variable than in TM1 plants. At 8.3 - 8.5mm it can be seen that there are four ovules or 7% with less than

eight nuclei per ovule in TM1 plants whereas there were 35 ovules or 22% with less than eight nuclei per ovule in Haplo 9 plants. The most prominent difference was observed when the bud size was 8.5 to flowering in that TM1 only had one type of ovule with five nuclei whereas Haplo 9 plants had five different classes of ovules which had one, two, four, five and nine or more nuclei (Table 12). These data demonstrate that in Haplo 9 plants there was a decrease in frequency of nuclear division, i.e., the nuclear division appears to occur at a slower rate. For example, at the 8.3 - 8.5mm bud size, TM1 has 2 or 4% of its ovules in the four-nuclei stage and Haplo 9 has 23 or 14%. At this stage, 52 or 93% of TM1 plants have ovules at the eight-nuclei stage, whereas Haplo 9 plants have only 127 or 78% at this stage of development (Table 12).

Thus the data in Table 12 on the number of cells in the developing gametophyte at bud size 7.2 and greater in TM1 and Haplo 9 show that Haplo 9 does not show the synchrony of cell division like that in TM1. These differences seen during maturation of the female gametophyte in Haplo 9 plants are believed to be due to aberrant chrommsomal segregation. However, it may be due to the physiological effects of the monosomic condition of the plant.

At the time of flowering, the ovules contain mature gametophytes. The sectioning of ovaries in this stage of development revealed in Haplo 9, that 15 ovules were judged to be abnormal in comparison to TM1 (Table 13). The data were taken from flowers that were freshly opened or on the day prior to flowering. In TM1, only five nuclei are observed in

Table 12. Comparison of TM1 and Haplo 9 Plants With Respect to the Relationship of Bud Size and the Number of Ovules with 1 to 9 or More Nuclei.

Cytotype	Bud Size	No. of Ovules	Number of nuclei (1,2,3, ... 9 & above) present per ovule								
			1	2	3	4	5	6	7	8	9[a]
TM1	7.2 - 7.5	122	88	34							
H9		188	128	60							
TM1	7.5 - 7.8	53	19	34							
H9		85	36	49							
TM1	7.8 - 8.1	102	5	6	1	3					
H9		104	8	73		23					
TM1	8.1 - 8.3	105	1	2		49		1		52	
H9		203	4	13	4	93		2	2	85	
TM1	8.3 - 8.5	56	1	1		2				52	
H9		162	3	5	2	23		2		127	
TM1	8.5 - Flower	133				1	133				
H9		271	3	3		6	256				3
Total		1,584									

a = Ovule contains nine or more nuclei.

the mature gametophyte which consists of two synergids, one egg nucleus and two polar nuclei. The antipodals are not normally present in the mature gametophyte. In mature gametophytes of Haplo 9 plants, descrepancies in the number of nuclei per gametophyte were observed. Here it was noted (Table 13) that three ovules had only one nucleus at the chalazal end of the gametophyte; three ovules contained two nuclei, both of which were at the chalazal end of the gametophyte; six ovules had four nuclei, all of which were in a cluster in the center of the gametophyte; two ovules had 14 nuclei of which nine were at the chalazal end, and one ovule had 17 nuclei of which 12 were at the chalazal end of the gametophyte. The latter three ovules had multiple nuclear divisions that probably occurred among the antipodals because the egg apparatus and the central cell were normal. The abnormalities seen here were not observed in any of the ovules of TM1 plants. These 15 abnormal ovules constitute 11% of the ovules observed in the flowering stage which is far from the expected 50%. Nevertheless, it is assumed that the nuclear abnormalities observed in the gametophytes of Haplo 9 may be the effect of abnormal chromosome complements which resulted from nondisjunction in the divisions of megaspores that were deficient for chromosome 9.

Table 13. Number of Abnormal Ovules Among 135 Ovules At The Flowering Stage in Haplo 9.

Number of Ovules	Number of Nuclei Per Ovule
3	1
3	2
6	4
2	14
1	17

Abnormality consists of the number and the position of nuclei in each ovule. Normal ovules have five nuclei per ovule positioned at the micropylar end at the time of flowering.

SUMMARY

The progeny of plants which are monosomic for chromosome 9 consist of a very low frequency of monosomics for this chromosome. But more importantly, the progeny resulting from either self-pollination or outcrossing Haplo 9 plants show a high incidence of other aneuploid types. Cytological analysis was conducted to determine the stage and mechanism in Haplo 9 plants that would account for the production of the different kinds of aneuploid progeny.

During the investigation, two new cytotypes were isolated, monotelodisome 9S and monoisodisome 9S. The chromosome involved in these two cytotypes was identified by crossing the two to two translocations, T_{3-9} and T_{9-25}, and in both crosses the telosome and isosome were associated with the interchange chromosomes, establishing that they were deficiencies involving chromosome 9. Analysis of seed set was determined in plants that were monosomic for chromosome 9 or were deficient for one or the other arm of chromosome 9. Tests were also conducted with marker genes to determine whether one or more of the genes were located on chromosome 9.

The average amount of seed set per boll was determined for TM1, Haplo 9, Telo 9L, Telo 9S, and 2n sibs of the latter four cytotypes. There was no significant difference in seed set for TM1 and the 2n sibs

both of which averaged approximately 30 seeds per boll. All four aneuploids involving chromosome 9 differed significantly from TM1 and their 2n sibs in amount of seed set per boll. The average amount of seeds per boll in Haplo 9, Telo 9L, Telo 9S and Iso 9S were 15.5, 25.6, 22.9 and 24.2 respectively. There was no significant difference between Iso 9S and Telo 9S; however, the two other cytotypes, Telo 9L and Haplo 9 plants did differ significantly from each other as well as from Iso 9S and Telo 9S plants. These results on seed set per boll indicate that normally when an n gamete became the functional megaspore, nuclear division and gamete formation was normal, and that when a deficient gamete became the functional megaspore, nuclear division and gamete formation may be abnormal which would affect ovule viability.

As pointed out above, Haplo 9 plants have a significantly lower seed set than Telo 9L, Telo 9S and Iso 9S plants, but that Telo 9L plants have a significantly higher seed set than Telo 9S and Iso 9S plants. These results show that ovule abortion is higher in plants deficient for chromosome 9 than in plants deficient for only one arm of chromosome 9; and that plants which are deficient for the long arm have a higher frequency of ovule abortion than plants which are deficient for the short arm of chromosome 9.

Marker genes, consisting of cup leaf (cu), glandless boll and stem (gl_1), frego bract (fg), virescent plant (v_1), Crumpled leaf (Crp) Brown lint ($Lc_y Lc_z$), Pollen color ($P_1 P_2$), and Yellow petal (Y_1)

were used in genetic tests to determine if they were on chromosome 9. The results of analysis of the F_1, F_2 and testcross progenies revealed that none were on chromosome 9.

Haplo 9, Telo 9L, Telo 9S, and Iso 9S were crossed as female parents with the standard TM1, and all except Telo 9L produced aneuploid progeny of various types. The aneuploid cytotypes produced by the three cytotypes consisted of telosomics, isosomics, monosomics, trisomics, multiple monosomics and different monosomic-trisomic combinations. Statistical analysis of the progenies produced by Haplo 9, Telo 9S and Iso 9S show that there was no significant difference between the latter two, but that there was a significant difference between these two and Haplo 9. This difference may occur because of differences in population size. In Haplo 9, 1226 plants were analyzed, whereas in Telo 9S and Iso 9S, 281 and 151 plants were analyzed, respectively.

Haplo 9, Telo 9S and Iso 9S showed no significant difference in the chromosomes subtracted or added to the chromosome complement of their respective aneuploid progenies. All three cytotypes behaved very similarly in that more chromosomes are eliminated, particularly the larger A subgenome chromosomes, than added to the chromosome complement. These data indicate that the gene(s) on the long arm of chromosome 9 not only exerts control over chromosome disjunction during gametogenesis, but has a mode of action that results in the elimination of mostly the

A subgenome chromosomes. Since G. hirsutum is an allotetraploid and since chromosome 9 is an A subgenome chromosome, the high frequency of elimination of the A subgenome chromosomes might be accounted for by assuming that the gene(s) on the long arm of chromosome 9 controls primarily the regular division and/or segregation of the A subgenome chromosomes and that they have only limited control over the D subgenome chromosome.

Development of the female gametophyte was cytologically analyzed in 1170 ovules of TM1 plants. This study served two purposes: (1) to determine the relationship of bud size and nuclear division in the female gametophyte and (2) to establish a control so that abnormalities in Haplo 9 plants could be detected. The mitotic division prior to meiosis occurred when the bud size was 6.0 - 6.6mm, meiosis occurred when the bud size was 6.3 - 7.2 mm, the first mitotic division of the megaspore occurred when the bud size was 6.9 - 7.8, and the second and third mitotic divisions of the megaspore occurred when the bud size was 7.8 - 8.5 mm.

Development of the female gametophyte was cytologically analyzed in 2061 ovules of Haplo 9 plants. Of the 17 ovules observed in metaphase I, none were abnormal; however, upon analysis of ovules from monotelodisome 9S, five ovules were found at metaphase I of meiosis of which one ovule showed a bivalent off the metaphase plate. An accurate chromosome count could not be made because of the serial section being perpendicular to the spindle axis.

The analysis of bud size with respect to the number of nuclei per ovule was compared in TM1 and Haplo 9 plants and the results showed that nuclear divisions in Haplo 9 plants were slower in that more ovules were found during earlier stage of development. The largest difference was found when the bud size was 8.1 to flowering in that in Haplo 9 there were five different types of ovules present at the flowering stage with respect to the number of nuclei. In TM1 plants, only one type of ovule was present during this stage of bud development. The variable types of ovules in Haplo 9 could be due to the absence of chromosome 9 and/or the presence or absence of other chromosomes.

Analysis of 135 mature female gametophytes of Haplo 9 plants revealed 15 abnormal ovules. Twelve showed fewer than the normal five nuclei which were as follows: three ovules with one nucleus which was at the chalazal end; three ovules with two nuclei, both of which were at the chalazal end; and six ovules with four nuclei, all of which were in a cluster in the center of the gametophyte. The remaining three ovules had more than the normal five nuclei. Two of the three ovules had 14 nuclei and the other ovule had 17 nuclei. In all three ovules, the egg nucleus, synergids, and polar nuclei were normal whereas the extra nuclei were found at the chalazal end. It appeared that the antipodals may not have degenerated but continued to divide. The cause may have been an abnormal chromosome complement.

At the beginning of the cytological analyses of the ovules, it was assumed that I could observe irregularities of chromosome segregation at the meiotic or the post-meiotic divisions. It was also assumed that if chromosome nondisjunction was missed due to chance, I should observe some ovules with nuclei of various sizes because of lagging or abnormal disjunction of chromosomes during meiotic or post-meiotic division. However, except for one minor case, these kinds of irregularities were not observed.

There was no significant number of abnormalities that were found in Haplo 9 which would indicate the exact stage during the development of the female gametophyte that chromosomal nondisjunction occurred. However, I did find a rather distinct difference between Haplo 9 and TM1 in the latter stages of development of the female gametophyte of Haplo 9, particularly in the mature gametophyte. These ovules had variable numbers of nuclei. This was not observed in mature gametophytes of the control, TM1.

It is concluded that most likely nondisjunction occurs throughout female gametogenesis in Haplo 9, but that most of it probably occurs during the three mitotic divisions following meiosis.

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