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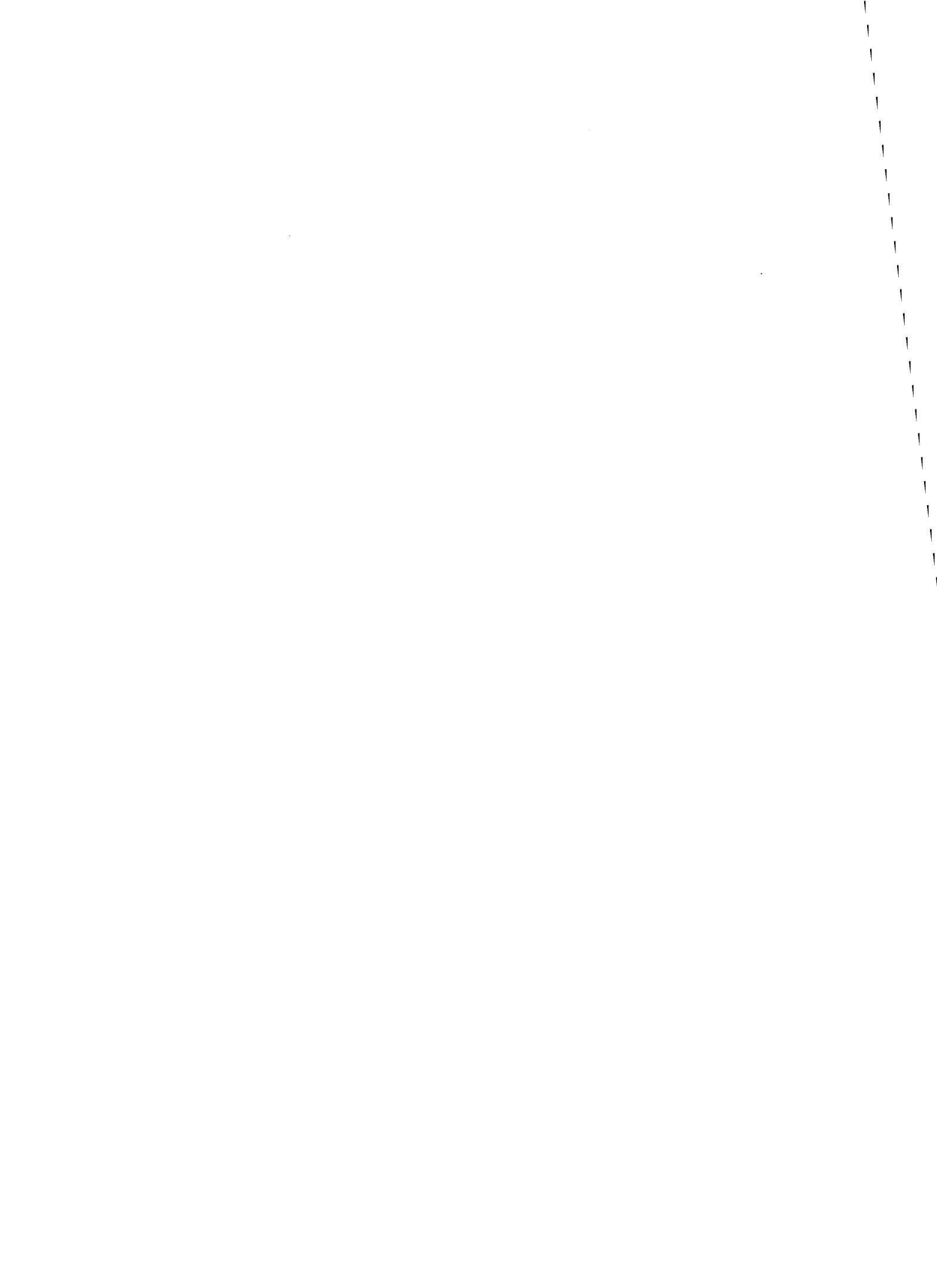
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Characterization of the crumpled-mottled mutation in *Gossypium hirsutum* L.

Settle, Carroll Eugene, Ph.D.
The University of Arizona, 1993

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CHARACTERIZATION OF THE CRUMPLED-MOTTLED MUTATION
IN *GOSSYPIUM HIRSUTUM* L.

by

Carroll Eugene Settle

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A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PLANT SCIENCES
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1993

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Carroll Eugene Settle

entitled Characterization of the Crumpled-Mottled Mutation
in *Gossypium hirsutum* L.

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SIGNED: Carroll Eugene Felton

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DEDICATION

To those who mean the very most
and with whom I share an intellectual and genetic heritage

PJ and Jaime, Chad and Amber

and

To my parents

J. C. and Florence Settle *of blessed memory*

Also,

To my teachers

in the sciences and in the humanities
in appreciation of their dedication, germane
scholarship, and quiet guidance.

Be very careful in teaching, for an error in teaching
is tantamount to a willful transgression.

Perke Avot, 4:13

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ABSTRACT

Of the 62 chromosome translocations in *Gossypium hirsutum* that have been isolated, made homozygous, identified as to the chromosomes involved, and maintained as homozygous tester lines, only T8-12 is phenotypically abnormal. Homozygotes are dwarf with a mutant "*crumpled-mottled*" phenotype that includes negligible fertility in field-grown plants; heterozygotes have a more normal morphology and variable fertility. The mutation has been reported to be tightly linked with the translocation and has never been separated from it.

Comparative pollen viability, quantitative morphological measurements, and segregation data were used to dissociate the mutant phenotype from the T8-12 breakpoint- and from the mimic mutant, *Crp*. The T8-12 breakpoint was also demonstrated to be independent of the semilethal locus *Lf* on chromosome 12.

Homozygous T8-12 and *Crp* individuals were shown to be morphological, but not functional, equivalents. Heterozygous and homozygous *Crp* plants produced essentially normal pollen; T8-12 heterozygotes had significantly reduced mean pollen viability scores. Mean bracteole measures were significantly different between T8-12 and *Crp* heterozygotes.

The fluorescein diacetate-based fluorochrome reaction identified both chromosomal and genic effects on pollen viability in this study; the potential usefulness of the FCR method was thus extended to the level of single mutant gene effects.

CHAPTER 1

INTRODUCTION

Overview of Cotton Genetics Research

Gossypium was one of the early crop genera to which Mendelian principles of genetic analysis were applied. The inheritance of lint color in *G. barbadense* L. was reported in 1906; other studies describing the inheritance of leaf shape and leaf color traits in *G. hirsutum* L. followed within two years. As cotton genetics research gained momentum, later investigators discovered that the cultivated species differed in ploidy level (Endrizzi et al., 1985). The Asiatic species, *G. arboreum* L. and *G. herbaceum* L., were shown to be diploids ($2n=2x=26$), whereas the New World species, *G. barbadense* and *G. hirsutum*, proved to be allotetraploids ($2n=4x=52$). These unexpected cytological findings, combined with changes in cotton cultural practices, profoundly affected the direction of cotton genetics research after 1945. Qualitative genetic research on the Asiatic diploids effectively halted as worldwide use of the allotetraploid species, especially upland cotton (*G. hirsutum*), increased and eventually dominated world production. As interest in the Asiatic species waned, so did efforts to preserve Asiatic germplasm; the result has been the loss of significant amounts of genetic information especially that pertaining to mutant alleles and stocks (Endrizzi et al., 1984).

The widespread shift in cultural practice to cultivars of *G. hirsutum* did not increase interest in basic genetic research on any of the allotetraploid species. Research emphasis shifted toward the discovery and understanding of genomic relationships among the cultivated tetraploids and their wild relatives (J. E. Endrizzi, personal

communication). Significant interest in the cytology and cytogenetics of *G. hirsutum* continued to develop after 1950; however, the emphasis remained on studies of genome manipulation with considerable effort expended on understanding the evolution of the species. Subsequent investigations produced a wealth of information concerning the cytological and morphological relationships among the species of *Gossypium* (Fryxell, 1979; Endrizzi et al., 1985) but produced only limited information concerning the genetic effects of individual chromosomes on agronomic traits or on the basic problems of growth and development.

Failure to develop sustained research efforts directed toward these basic areas can be traced to at least three factors: (1) the prevailing research orientation among cytogeneticists was strongly focused on further exploration of the modern synthesis of evolution (Huxley, 1942; Dobzhansky, 1970; Lewontin, 1974b); (2) agricultural cytogenetics, although recognized as an important approach to long-term genetic improvement, was not viewed as an effective tool to increase short-term economic returns (Niles and Feaster, 1984; Lee, 1987); and (3) to increase yields, producers relied almost exclusively upon the traditional (and normally profitable) approach of refining standard techniques in breeding, selection, and testing methods (Fehr, 1987; Lee, 1987).

In 1990, more than 12 million acres of upland cotton were grown in the United States, yielding an aggregate (fiber plus seed) market value of nearly \$5.5 billion (Anonymous, 1990). Germplasm supporting the majority of cotton cultivars in the United States is descended from about twelve separate introductions (Lee, 1987). Recent estimates revealing unexpectedly low genetic diversity in *G. hirsutum* (Wendel et al.,

1992) coupled with reevaluation of the United States National Cotton Germplasm Collection (Percival and Kohel, 1990) and the severity of the current whitefly epidemic (Faust, 1992; Perring et al., 1993) suggest that an intense, focused study of basic cotton genetics and cytogenetics may be expedient in the immediate future. As Endrizzi et al. (1985, p. 273) have pointed out

In view of the present status of varietal improvement, it is agreed generally that fundamental knowledge of the specific genes each chromosome carries, of the linkage relationships of these genes, and of the genetic interactions affecting agronomic traits is of paramount importance for further improvement of cotton cultivars.

Paradoxically, *G. hirsutum* is one of the most important agronomic crops in the United States, but it is not thoroughly characterized genetically. Of approximately 100 identified loci, some 65 genes have been mapped to 17 of the 26 linkage groups (Endrizzi et al., 1985; Price et al., 1990). However, only 13 of these linkage groups have been associated with their respective chromosomes. Differential staining techniques for identification of cotton chromosomes have yet to be developed, and a definitive banding karyotype of any *Gossypium* species has never been published. Molecular mapping is nascent with the first biochemical and initial molecular markers recently reported (Saha, 1989; Price et al., 1990). Genome organization and repetitive DNA studies have been carried out for a few species of *Gossypium* and preliminary work toward understanding developmental genetics and embryogenesis in *G. hirsutum* began recently (Galau and Hughes, 1987; Hughes and Galau, 1989). Mobile genetic elements have yet to be reported for any species of *Gossypium*.

Given the promise inherent in genetic engineering techniques and their potential for improving the commercial varieties of *Gossypium*, basic genetic analyses will find continuing application in the future. Successful application of genetic engineering is greatly enhanced by knowledge about the fundamental genetic organization of chromosomes gained through the classical genetic and cytogenetic approaches (Adolph, 1991; Meese and Trent, 1991; Endrizzi et al., 1985). Cytogenetic study remains a very important first step in pinpointing the general location of genes that may subsequently be more precisely analyzed by molecular genetic techniques. While RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) maps are both novel and powerful tools for genome mapping, they provide only an empty grid unless (and until) traits of agronomic value can also be located within their grid definition. As Devine (1992) cogently noted

Lest we lose sight of the obvious, it needs to be said that the very reason for funding genome mapping of crop plants is precisely because they are crops and the maps are intended to be useful for crop improvement by breeders. In the past, often the principal forum for discussion of genome mapping has been outside the historic agricultural community. For the development of *useful* maps, it is most important that the genome cartographer maintain a continuing dialog with crop breeders and geneticists.

Application of RFLPs to mapping polyploids has lagged behind that of diploids due to several complications: (1) large number of segregating genotypes; (2) comigration of fragments; (3) poorly characterized genome constitution; (4) poorly characterized chromosome pairing behavior; and (5) complicated genotype characterization secondary

to multiple fragments (Sorrells, 1992). Such complications can be largely overcome, but not without an adequate database of fundamental genetic information (Lande and Thompson, 1990; Hillel et al., 1990; Allard, 1988). Molecular mapping of transgressive variation theoretically could open a window of opportunity for utilization of wild and exotic germplasm, since wild relatives of *G. hirsutum* represent a potentially significant source of new alleles for genetic improvement (Sorrells, 1992).

Gossypium appears to be a genus in which molecular mapping and marker-assisted breeding techniques could be profitably applied (Galau et al., 1988; Wendel et al., 1991). With a continuing accumulation of data from classical cytogenetic studies on *G. hirsutum*, the prospects look promising for a productive partnership between cotton molecular geneticists and cytogeneticists.

Role of Translocations in Cotton Cytogenetics

G. hirsutum is an allotetraploid ($2n=4x=52$) with A and D subgenomes (Beasley, 1942; Skovsted, 1934). Significant diploidization appears to have occurred during its evolution (Endrizzi, 1962) and it regularly forms 26 bivalents at metaphase I. Chromosomes are designated by Arabic numerals and, within each subgenome, were sequentially ordered according to discovery of some distinguishing feature as determined from tests among chromosomal translocation lines and between translocations involving A and D genome diploid species (Menzel and Brown, 1954a, 1978a; Brown, 1980; Menzel et al., 1982). In the standardized cotton nomenclature (Kohel, 1973a), chromosomes 1-13 have been assigned to the A subgenome, chromosomes 14-26 to the

D subgenome until such time as the homeologous chromosome pairs can be identified. The A subgenome chromosomes are approximately twice as large as those of the D subgenome; unfortunately, there is a size overlap between the largest D and the smallest A chromosomes. As yet, no definitive banding karyotype has appeared for *G. hirsutum* (Nie and Li, 1985).

Meiotic evaluations have been the primary method for obtaining cytogenetic analyses in *Gossypium*. Numerous cytogenetic stocks, including monosomics, monotelodisomics, reciprocal translocations, and segmental duplication-deficiencies, have been developed through several cooperative research projects and provide a powerful array of tools for genetic analyses (Endrizzi et al., 1985; Anonymous, 1981). Translocations are especially valuable in that they may be maintained as true-breeding homozygous lines from which heterozygotes and duplication-deficiencies can be regenerated as needed. Voucher seed for the various stocks and tester lines are maintained by several land grant institutions (including The University of Arizona) and are centrally deposited at Texas A&M University as part of the United States National Cotton Germplasm Collection (Percival and Kohel, 1990).

Multivalent configurations in *Gossypium* pollen mother cells were noted by cotton cytogeneticists prior to 1950. Beasley (1942) ascribed the multivalents to translocations but did not determine the number involved. Gerstel (1953) reported that chromosomal interchanges were responsible for multivalents observed in hybrids of *G. herbaceum*, *G. arboreum*, *G. anomalum*, and the allotetraploids. Workers at the Beasley Cotton Genetics Laboratory, College Station, Texas noted a high frequency of metaphase I

multivalents in trispecies hybrids between *G. hirsutum* x Asiatic-New World diploid F₁. Upon reanalysis of the hybrids involved, they were able to work out the chromosome end arrangements and to transfer three of the interchanges to *Gossypium hirsutum*, thereby providing the first set of chromosomally identified translocations in *Gossypium* (Menzel and Brown, 1954a; Brown, 1980).

The initial induced translocations reported for *Gossypium* were from *G. hirsutum* seed exposed in the first atomic test at Bikini Island (Brown, 1950). Following analysis of these induced interchanges, additional seed were exposed to radiation to acquire other translocations for identification in *G. hirsutum* (Menzel and Brown, 1954b; Brown, 1980). At present, 62 translocations have been isolated, made homozygous, identified as to the chromosomes involved, and are maintained as homozygous tester lines (Brown, 1980). Of the 62 translocations, 53 were radiation induced; four of the remaining nine were derived from the *G. hirsutum* x Asiatic species hybrids, and five were spontaneous in origin. Fifty-eight of the 62 are simple reciprocal translocations involving only two nonhomologous chromosomes; the other four involve multiple interchanges among three or four chromosomes. A tester set developed from the 62 translocations can be used to identify any one of the 26 *G. hirsutum* chromosomes (Ray, 1981; Ray and Endrizzi, 1982). Menzel (1955) outlined the cytological procedure for assigning interchanged chromosomes to their respective genomes. Chromosome identity in each of the 62 interchanges was, in most cases, established by intercrossing the homozygous translocations and ascertaining whether the same or different chromosomes were involved (Brown, 1980).

The breakpoints in the interchanged chromosomes have been determined and assigned for many of the 62 translocations (Menzel and Brown, 1978a, 1978b; Brown et al., 1981). Endrizzi et al. (1985) summarized the published data relating to arm location, predominate heterozygote metaphase I configuration, frequency of duplication-deficiencies recovered, and the marker genes associated with each translocation line. For those interchanges in which assignment of breakpoint has yet to be determined, position was tentatively assigned to a right or left arm (instead of long or short) with right arbitrarily designated as the arm involved in the larger number of breaks (Endrizzi et al., 1985).

Translocations have been used for several purposes during the development of cotton genetics (Ray, 1981). Since *G. hirsutum* is an allotetraploid, viable duplication-deficiencies for various chromosomal segments can be recovered as a result of adjacent disjunction (Brown, 1950; Brown et al., 1981; Menzel and Brown, 1952; 1954b; 1978b). In theory, duplication-deficiencies can be recovered for any given interchange in *G. hirsutum*; if so, then translocations can be used to assign genes to chromosomes (Menzel and Brown, 1954b; Endrizzi et al., 1985). Translocations have also been used to study "incipient genome differentiation" (Menzel et al., 1978; 1982; Hasenkampf and Menzel, 1980), and to generate aneuploids from heterozygous translocations that disjoin unequally at the first meiotic division (Menzel and Brown, 1954b; Endrizzi, 1963; Endrizzi et al., 1985).

Specific translocations produced by *Gossypium spp.* have proven to be especially informative in studies of the alternate chromosome orientations at metaphase I.

Reciprocal translocations involve the exchange of non-homologous chromosome segments (Sybenga, 1975; Wagner et al., 1993). If the breakpoints are very close to the centromeres, the usual configuration observed at metaphase I is a ring-of-four chromosomes which can present with either an alternate ("zigzag") or an adjacent ("open") orientation. During the 1930's, the adjacent orientation was shown to be of two types: adjacent-1 and adjacent-2 (Burnham, 1962; Brown, 1972). In theory, the alternate orientation should also be of two types; until quite recently, however, the alternate-2 orientation had not been demonstrated. Using favorable material from *Gossypium*, Endrizzi and his co-workers were able to demonstrate that both alternate-2 and a completely random orientation of centromeres occurs (Endrizzi, 1974; Endrizzi et al., 1983).

The various translocations have also been used to identify specific chromosomes in studies of interspecific hybrids between *G. hirsutum* and diploid species having the A or D genomes (Endrizzi et al., 1985). Such studies have shown a relatively low level of chromosome differentiation separating the genomes of *G. hirsutum* from the genomes of their A and D genome congeners. The tetraploid species of *Gossypium* also exhibit a low level of chromosome differentiation particularly between *G. hirsutum* and *G. mustelinum*.

Although the majority of reciprocal translocations presently in use were induced, spontaneous chromosome alterations are not rare in the genomes of *Gossypium* (Brown, 1980; Endrizzi, 1966; Hasenkampf and Menzel, 1980; Barrow and Dunford, 1974). Endrizzi et al. (1985) reported results of a four-year study in which 20 of 45 "off-type"

plants analyzed exhibited 21 translocations. Fifteen of the 21 translocations observed were felt to represent interchanges between A and D chromosomes suggesting that limited intergenomic pairing and exchange are presently occurring in *Gossypium*.

The T8-12 Translocation

Of the 62 chromosome translocations in *Gossypium hirsutum* that are maintained as homozygous lines by self-pollination, all but one are phenotypically normal, indicating that the breaks involved are not accompanied by visible mutations. The single exception is T8-12 ("2778"), an AA translocation, which was first recovered in 1961 from *G. hirsutum* Deltapine 15 seeds subjected to 12 Kr of ^{60}Co irradiation (Brown, 1980). Plants heterozygous for the T8-12 interchange have essentially normal morphology and slightly reduced fertility in field-grown plants. Homozygous individuals, however, are dwarf with a mutant phenotype ("crumpled-mottled") that includes misshapen leaves, very short internodes, pollen abortion, and negligible fertility in field-grown plants. The mutation is reported to be tightly linked to the translocation and has never been dissociated from it (Anonymous, 1981; Brown, 1980; Endrizzi et al., 1985; J. E. Endrizzi, personal communication).

Menzel et al. (1985), using arbitrarily assigned "Left" (L) and "Right" (R) designations for chromosome arms, demonstrated that in line 2778 the translocation is T8R;12L (hereafter referred to as T8-12). The L arm of 12 is thought to be the same as the Long arm of 12 as defined with a telosome by Endrizzi and Ramsay (1979).

The T8-12 interchange is very unequal with approximately 96% of 8R having been

exchanged for the distal 1.2% of 12L (as measured in Cm). The modal configuration at metaphase I in the heterozygote is 24 bivalents and one quadrivalent ("frying-pan") with 8 and 8¹² forming the "handle" and 12 plus 12⁸ comprising the "pan" (Menzel et al., 1985).

Two types of adjacent-1 duplication-deficiencies in backcross progenies from such heterozygotes can be recognized cytologically: dp8R;df12L and dp12L;df8R (Brown, 1950; Menzel and Brown, 1952; Anonymous, 1981). At present, only dp8R;df12L has been recovered; the complementary duplication-deficiency, which would be monosegmental for most of 8R, has not yet been reported (Menzel et al., 1986).

Plants homozygous for T8-12 are dwarfs that, under summer field conditions in Arizona, appear to be near death. They flower very late in the season, if at all, and any flowers that form are small and frequently fail to fully open; pollen is poorly shed, and fertility is negligible. When ratooned and moved to a winter greenhouse, growth may improve; flowering and fertility improve somewhat and, with close attention to the timing of anthesis, a sufficient number of crosses can be made to maintain the line by selfing (J. E. Endrizzi, personal communication).

The recessive mutant phenotype ("crumpled-mottled") has never been separated from the translocation. Critical experiments to determine whether the mutant is the expression of chromosome damage at a break-point or whether it is a separate locus closely linked to the T8-12 translocation have not been reported. Significantly, the mutation has never been tested for allelism with other mutations. Of direct interest are observations that (1) the homozygous "crumpled-mottled" phenotype is virtually identical

with the phenotype of homozygous *Crp* ("crumpled") plants in *G. hirsutum* (Kohel, 1973b; D. T. Ray, personal communication); and that (2) the "crumpled-mottled" phenotype has several similarities to the intermediate (heterozygote) and extreme mutant (homozygote) phenotypes described for *Lf* ("leaf fleck") plants (Kohel et al., 1977). The *Lf* locus is part of Linkage Group V in *G. hirsutum* and is located in the Long arm of chromosome 12 (Kohel et al., 1977; Endrizzi et al., 1985; J. E. Endrizzi, unpublished data); the chromosomal location of *Crp* within the *G. hirsutum* genome is unknown at present.

Research Objectives

In this research, I focused on four questions related to the genomic effects of translocation T8-12 in *G. hirsutum*: (1) Is the phenotypic expression of T8-12 ("crumpled-mottled") caused by the breakpoint in chromosome 12? (2) Does the mutant trait "crumpled-mottled" segregate independently of the translocation? (3) Is the T8-12 breakpoint "allelic" with the Crumpled (*Crp*) locus? (4) Is the T8-12 breakpoint "allelic" with the semilethal locus Leaf fleck (*Lf*) on chromosome 12L?

At least two plausible hypotheses may be advanced to explain the chromosomal and phenotypic observations seen in the T8-12 interchange. First, the nearly complete overlap in phenotype between homozygous T8-12 plants and *Crp* homozygotes suggests that the T8-12 mutant genotype might be identical with the *Crp* genotype (Kohel, 1973b). Second, the mutant phenotype that is closely linked with the T8-12 translocation might be due to a position effect resulting from formation of the 12⁸ chromosome (Wilson et

al., 1990).

The simplest, most heuristic hypothesis to test is the first: if the overlap in phenotype between T8-12 and *Crp* plants extends to the level of the genotype, then the mutant T8-12 locus should be identical to the *Crp* locus (H_0 : T8-12 = *Crp*). By extension, similar logic can be applied to the *Lf* trait to test for allelism between the T8-12 breakpoint and the *Lf* locus. If H_0 can be invalidated for any given parameter, then the suite of phenotypic effects linked with T8-12 can be separated from the translocation.

CHAPTER 2
MATERIALS AND METHODS

Cytogenetic Stocks

Nine marker lines, selected from those maintained at The University of Arizona by D. T. Ray, were used to construct parents and crosses for five field experiments conducted at The University of Arizona Campus Agricultural Center between May 1990 and October 1992. The lines utilized, field numbers assigned, and seed source are given in Table 1.

Texas Marker-1 (TM-1) is an highly inbred, genetically stable, homozygous tester stock developed at Texas A & M University during the period 1947-1956. TM-1 is the current genetic and cytogenetic standard of reference for American Upland cotton, *Gossypium hirsutum* L. (Kohel et al., 1970). TM-1 has qualitative characters similar to those observed in commercial varieties of Upland cotton and contains none of the mutant genes responsible for leaf abnormalities, male sterility, deformed stigmas, and related conditions (Kohel et al., 1970; Endrizzi et al., 1985). Characters encoded in the TM-1 genome that are relevant to the present research include: green plant body (r_1r_1), normal bracts ($Bw_1Bw_1Bw_2Bw_2$), glanded plant parts ($Gl_1Gl_1Gl_2Gl_2$), nectaries ($Ne_1Ne_1Ne_2Ne_2$), and fuzzy seeds (n_1n_1).

The utilization of Telo 1L and Telo 3L lines was coincidental to the research reported here and resulted from the shared use of plant genetic materials by multiple researchers. The Telo 1L plants (deficient for the short arm of chromosome 1) were selected from a tester line that had been backcrossed to TM-1 10 times, then crossed with

Table 1. The marker lines used for field experiments involving the T8-12 translocation in *Gossypium hirsutum*.

Line	Field No.	Source ¹
<u>Backcross Experiment (1991):</u>		
Telo 1L (TM-1)	A7-3-90 A7-6-90 A7-7-90	A1-6-83 BC10 x TM-1 S/38
TM-1 / T8-12 (2778) Heterozygote	G7-90	F ₁ (J13-10-78 x OPGH 78-79)
<u>Testcross Experiment (1991):</u>		
Telo 3L (TM-1)	A11-5-90 A11-6-90 A11-9-90 A11-10-90	A5-2-83 BC5 x TM-1 S/38
Crp (G257) / T8-12 (2778) Heterozygote	F7-90 G18B-90	F ₁ (E28C-82 x E2A-82)
<u>F₂ Population Experiment (1991):</u>		
TM-1 / T8-12 (2778) Heterozygote	G7-90	F ₁ (J13-10-78 x OPGH 78-79) Bulk Selfed
<u>Controls (1991):</u>		
TM-1 Homozygote	E9-91	E21-3-90 S/46
Crp (G257) Homozygote	D11A-91	P5B-88 S/2 Bulk
T8-12 (2778) Homozygote	D11B-91	DPL-15 GH#35 OPGH 75-76 S/3 Bulk

Table 1. --Continued

Line	Field No.	Source
<u>Chromosome 12L Linkage Crosses (1991):</u>		
bw gl ne (AG 195) Homozygote	B16/17-91	F15/16-82 S/3
Lf (AZ 1050) Heterozygote	A2B-1-88	D5B-2-87
bw gl ne N ₁ (AG 262) Homozygote	A3A-88	R2S21H2N 82 ²
Lf bw gl ne N ₁ Tester Plant Heterozygote	A4-24-90 ³	A4-24-89 (Selfed)
T8-12 (AZ 962) Homozygote	E23A-2-90 (G18A-90)	C1-7-81 OPGH 81-82
Lf/T8-12 Heterozygote Tester Line	B14-91	A4-24-90 x E23A-2-90 (GH 90-91)
bw gl ne N ₁ (AG 262) Homozygote	E23B-5a-90	A3A-88 (Selfed) Bulk
T8-12 (AZ 962) Homozygote	E23A-2a-90	C1-7-81 OPGH 81-82
lf/f/T8-12 Heterozygote Tester Line	B15-91	E23B-5a-90 x E23A-2a-90 (GH 90-91)
<u>Leaf fleck/T8-12 Allelism Test (1992):</u>		
(bw gl ne) / (Lf/f/T8-12) Heterozygote	A1-92 through A4-1-92	B17-91 x B14-6/11a-91
(bw gl ne) / (lf/f/T8-12) Heterozygote	A4-5-92 through B1-10a-92	B16/17-91 x B14-1/1a/4-91

Table 1. --Continued

Line	Field No.	Source
<u>Control (1992):</u>		
lflf/T8-12	B2-92	B16/17-91 x
Heterozygote	through	B15-2/6/8-91
Tester Line	B9-92	

¹ Symbols and field numbers are those of various cotton geneticists at The University of Arizona.

² Obtained as seed in September 1982 from Dr. C. L. Rhyne by Dr. J. E. Endrizzi

³ Use of tester plant kindly granted by Dr. J. E. Endrizzi, Department of Plant Sciences, University of Arizona

TM-1 S/38 (self-pollinated for 38 generations) plants, and finally examined cytologically to verify that all plants used in crossing exhibited 26 bivalents at metaphase I. Telo 1L plants have a very distinctive phenotype: smaller, narrower leaf; narrow or twisted bracteole; and lighter green plant color (Endrizzi et al., 1985). Phenotypic contrast permitted field scoring into Telo 1L and TM-1 classes; plants were later verified cytologically in the laboratory. The Telo 3L plants (deficient for the short arm of chromosome 3) used in this research were handled in an analogous manner.

The T8-12 reciprocal translocation originated from seed irradiated with ^{60}Co at Oak Ridge National Laboratory. Brown and her co-workers at Texas A & M University isolated the heterozygote between 1961-1971; the homozygote was isolated in 1972, and verified in 1973 (Brown, 1980). Verified homozygous seed, obtained from Brown's laboratory in 1975, was multiplied in the greenhouse during 1975-1976 by J. E. Endrizzi. Subsequently, he assigned a portion of this seed as The University of Arizona Cotton Cytogenetics line number AZ962 (J. E. Endrizzi, personal communication). The T8-12 translocation line was employed in the present study as a tester (heterozygous) and as a control (homozygous). The T8-12/TM-1 heterozygotes (Field No. G7-90) had narrower, non-mottled leaves that were trilobed and shallow based. These heterozygotes were similar to TM-1 plants in size but exhibited greater amounts of anthocyanin deposition, some leaf crumpling, and variably shortened internodes. Homozygous T8-12 plants (Field No. D11B-91) showed the characteristic deformities associated with the translocation: dwarf size, very short internodes, markedly misshapen and crumpled leaves with red veins, distinct irregular mottling distributed throughout both new and old

leaves, flowering abnormalities, and significant pollen abortion in plants grown under both field and greenhouse conditions.

The Crumpled (G257) marker line used for this project was developed by R. J. Kohel from seeds of a mutant line discovered at the USDA Cotton Research Center, Phoenix, Arizona. When the mutant seed was grown out, the resulting plants were dwarfed, developed chlorotic leaf areas, produced irregularly shaped leaves with puckered laminal areas and wavy edges, and exhibited narrow bracteoles that frequently withered prior to boll maturity (Kohel, 1973b). Hybrids between the Crumpled mutant and normal (TM-1) plants developed a less severe phenotype: hybrids were intermediate in size, initial leaves appeared normal, later leaves developed mottling and irregular shape, wavy margins produced a cupped leaf shape, and some bracteoles were normal. Kohel (1973b) determined that the phenotype was conditioned by a dominant allele at a single locus and assigned the symbols *Crp Crp* to the homozygous mutant genotype. Homozygous seed obtained from Kohel's laboratory in 1981 was assigned Arizona Cotton Cytogenetics line number AG257. Subsequently, AG257 was reassigned line number G257 (J. E. Endrizzi, personal communication) and note was made of the marked similarity in phenotype between *Crp* homozygotes and T8-12 homozygotes. The G257 Crumpled line was used in the present study to construct the critical heterozygous tester in the testcross experiment (Field Nos. F7-90, G18B-90) and as a homozygous control (Field No. D11A-91).

Arizona AG195 is a multiple recessive marker line containing the following duplicate loci: withering bracts ($bw_1bw_1bw_2bw_2$) from chromosomes 12 and 26;

glandless plant ($gl_2gl_2gl_3gl_3$) from chromosomes 12 and 26; and nectariless ($ne_1ne_1ne_2ne_2$) from chromosomes 12 and 26. These plants are short (but not dwarf) with a decumbent growth form at maturity. AG195 plants produce glandless leaves, bracteoles, stipules, and stems; develop bracteoles that wither prior to boll maturity; lack floral and extrafloral nectaries; and are characterized by very high boll loss. The AG195 line was verified in 1974 by J. E. Endrizzi using seed received from R. J. Kohel (J. E. Endrizzi, personal communication). AG195 was used as the female parent in the critical linkage crosses B16/17-91 x B14/15-91.

Arizona AG262 is a marker line containing the dominant gene Naked seed (N_1N_1) from chromosome 12, in addition to the recessive loci withering bracts, glandless plant, and nectariless found in Arizona AG195. The Arizona AG262 line is based on R2S21H2N-82, a tester plant developed by C. L. Rhyne. Selfed seed of R2S21H2N, obtained from Rhyne in 1982, was used by J. E. Endrizzi as the male parent during development of the important Leaf fleck tester plant A4-24-90 (J. E. Endrizzi, personal communication). In the present study, A4-24-90 was used as the female parent in constructing the Leaf fleck/T8-12 heterozygote tester B14-91.

The Arizona AZ1050 marker line contains dominant genes for leaf fleck ($Lflf$), normal bracts ($Bw_1Bw_1bw_2bw_2$), nectaries ($Ne_1Ne_1ne_2ne_2$), and naked seed (N_1n_1). AZ1050 has, in addition, recessive genes for glandless plant ($gl_2gl_2gl_3gl_3$), open bud (ob), and the independent locus mottled leaf (mt). AZ1050 was used as the female parent during development of the Leaf fleck tester plant A4-24-90 (J. E. Endrizzi, personal communication).

Arizona AZ962 is a marker line containing the T8-12 translocation in addition to dominant genes for normal bracts, glanded plant, and nectaries. Plants grown from seed of this marker line are dwarf, have very small crumpled leaves with red veins, produce red mottling in the leaves, and exhibit the abnormalities characteristic of plants carrying the T8-12 translocation. AZ962 was used as the male parent in constructing the Lf/T8-12 tester line B14-91; B14-91 subsequently served as the pollen parent in setting up a critical cross (B16/17-91 x B14-91) used to evaluate chromosome 12L.

Field Methods

All seed materials for this study were germinated in expandable peat pellets--one or two seed per pellet--at The University of Arizona Campus Agricultural Center greenhouses. After three weeks, surviving seedlings were scored, sun-hardened for 3 to 5 hours daily over a three day period, and then transplanted to cotton plots at the Campus Agricultural Center. In the field, seedlings were planted two per hill, 20 to 30 plants per row. Hills were spaced 45 cm apart within each row; rows were placed at 91 cm intervals. Routine cultural practices of the Campus Agricultural Center were followed throughout the duration of this project.

In each of the three years of this study, seedlings were flood irrigated immediately after transplantation. For the following 4 to 6 weeks seedlings were irrigated at weekly intervals; thereafter, plants were placed on a bi-weekly maintenance irrigation schedule. Unusually heavy rainfall during July 1990 and July-August 1991 produced a luxuriant plant growth in both years, and resulted in some plants being subjected to periods of standing water. Rainfall amounts were more normal in Summer 1992. Low precipitation

during June 1992, coupled with an adverse plot location, induced selective plant losses among certain genotypes due to predation by native herbivores--notably *Sylvilagus audubonii*, *Spermophilus tereticaudus*, and *Sigmodon arizonae*.

Crossing Program

The crossing program was designed to answer four questions concerning the effects of translocation T8-12 on the genome of *Gossypium hirsutum*: (1) Is the mutant phenotype "crumpled-mottled" caused by the breakpoint in chromosome 12? (2) Does the "crumpled-mottled" trait segregate independently of the translocation? (3) Is the *Crp* locus "allelic" with the translocation breakpoint? (4) Is the T8-12 breakpoint "allelic" with the mutant locus leaf fleck (*Lf*) in chromosome 12L? The experimental design addressing these four interrelated questions incorporated four separate field experiments carried out between May 1990 and October 1992. Table 2 lists these four pivotal aspects of the experimental problem, the parental phenotypes involved in each critical cross, the null hypotheses, and the research question addressed by each experiment.

Pollination was controlled using standard methods (Lee, 1980; Niles and Feaster, 1984) with minor variations employed for certain tester lines. Cultivated cottons normally have complete flowers and are self-pollinated. Since cotton pollen is relatively heavy and sticky, wind-aided pollen transfer is usually not a serious problem in field experiments. Natural out-crossing may, however, account for 50% or more of fertilizations under conditions of open pollination in some fields (Niles and Feaster, 1984). Open-pollination under winter greenhouse conditions is accepted as the equivalent

Table 2. Critical field experiments to assess the genomic effects of translocation T8-12 in *Gossypium hirsutum*.

Experiment	Parents		H ₀	Insight and Questions Addressed
	Female	Male		
<u>Summer 1991</u>				
Backcross (T8-12)	TM-1 (Telo 1L)	TM-1/ T8-12	TM-1 P.A. = T8-12 P.A.	Separate T8-12 from TM-1; Question 1 & 2
Testcross (<i>Crp</i>)	TM-1 (Telo 3L)	<i>Crp</i> / T8-12	<i>Crp</i> = T8-12 (P.A. and Index)	Test allelism of <i>Crp</i> and T8-12; Question 3
F ₂ (T8-12)	TM-1	T8-12	F ₂ Ratio = 1:2:1	Assess variance of T8-12 at MI; Question 1 & 2
<u>Summer 1992</u>				
Testcross (Lf)	AG195	<i>Lf</i> / T8-12	<i>Lf</i> = T8-12 (P.A. and Index)	Test allelism of <i>Lf</i> and T8-12; Question 4

Symbols: P.A. read "viable pollen %"
 = read "not significantly different from"
 Index read "morphologic index"

of a self-pollination (D. T. Ray, personal communication).

Given a favorable temperature regime, floral development in *Gossypium hirsutum* follows a rigid, predictable schedule (Lee, 1980; Oosterhuis, 1990). Flowers approaching maturity were commonly at the whitebud stage (ready for emasculation) by 1000 hours on the day prior to anthesis. Once identified, females for crossing were prepared by emasculation later that same day. Cutting the petals away with a single-edge razor blade, followed by stripping of the anthers with recurved forceps, proved least injurious to flowers and improved fruit set. The majority of all normal (TM-1) plants emasculated in this manner, and carefully pollinated, yielded from 20 to 26 seed per boll; however, the rate of fruit set varied widely among the several tester lines utilized during the study. Late afternoon emasculations proved to be the most effective during all years of the study. Stigmas of emasculated flowers were protected by slipping a 3 cm length of paper soda straw over the prepared stigma; folding over the terminal 0.5 cm segment of the straw effectively excluded pollinators.

Flowers used as pollen sources were prepared the day before anthesis by sealing the corolla with a cellulose acetate selfing solution applied by means of specially designed glass selfing tubes. The selfing solution was prepared by combining 300 ml (by volume) of flake cellulose acetate with 2000 ml of acetone and adding 3 ml ethanol in which 0.1 g of crystal violet stain had been dissolved. All reagents were transferred to a 4 liter container, placed on a mechanical shaker, and mixed for 12 hours. The resulting solution was stored in an airtight bottle, at 25°C, for the duration of the field season. Portions were removed at 2 to 4 day intervals for field use. Only those flowers found

to be shedding abundant pollen at the time of use were employed as male parents. Pollination was effected by gently tapping the pollen onto the prepared stigma within minutes of male parent collection.

When required by experimental design, or to multiply voucher seed, selfing of certain experimental plants was carried out in a manner similar to that employed for obtaining the pollen parent. Buds thus sealed were identified with selfing tags, left to mature undisturbed, and harvested in early October.

Field Scoring of Cytotypes

Of the four critical field experiments done to assess the genomic effects of translocation T8-12 (Table 2), three were completed during Summer 1991. All plants from these experiments were scored in situ under field conditions, independent of pollen analysis and bracteole morphologic data, on three separate occasions (early/mid/late) during the season. Plants were evaluated for deviations from normal phenotype in 0.5 (unitless) increments using a semi-quantitative "Morphologic Index" (Appendix A) developed from the original phenotypic descriptions in the primary literature. Morphologic Index parameters included relative bracteole width, petal length, leaf shape (including leaf margin and leaf surface texture), depth of leaf base, sympodial branch length, and internode distance/plant height.

Cytological Verification

Buds for cytological verification of each cytotype and the TM-1 control were

collected between 0630 and 1100 hours at weekly intervals from early July through early November 1991 and 1992. Buds were held in a portable cooler containing artificial ice packs for varying times (not exceeding two hours) until removal of the calyx and corolla ("peeling") could be accomplished. Peeled buds, with staminal columns exposed, were placed in plastic tissue embedding capsules and fixed in a fresh mixture of seven parts 95% ethanol to three parts glacial acetic acid. After 24 hours, buds were transferred to fresh fixative and refrigerated at 4°C until cytological analysis was completed. Pollen mother cells (PMCs) were prepared for cytological evaluation by a modified iron-propriocarmine squash technique (Berlyn and Miksche, 1976; Sharma and Sharma, 1980). Slides were sealed with paraffin and examined with a Zeiss Axioskop microscope equipped with achrostigmatic objectives (Slayter and Slayter, 1992). Metaphase I chromosomal configurations were scored until the modal chromosome number could be established.

Pollen Viability Analysis

Flowers used in pollen viability studies were identified on the day prior to anthesis and prepared by sealing the corolla tip with cellulose acetate solution. Flowers were harvested between 1000 and 1200 hours the following morning, sealed in individual coin envelopes, and transported to the laboratory in an ice chest cooled by artificial ice packs. For all cytotypes examined, only flowers shedding abundant mature pollen--as evidenced by firm, swollen corollas--were harvested and subsequently examined in the laboratory (Heslop-Harrison et al., 1984). Pollen obtained from Texas Marker-1 (TM-1) plants

served as the control for all cytotypes examined.

The fluorochrome reaction (FCR) method, originally introduced by Heslop-Harrison and Heslop-Harrison (1970) as an assay for pollen quality and recently modified by Gwyn and Stelly (1989) to examine translocations in *G. hirsutum*, was used to assess differences in pollen viability among cytotypes. The fluorochrome reaction method infers pollen viability by evaluating the integrity of the vegetative cell plasmalemma. When pollen grains are mounted in a fluorescein diacetate (FDA) solution, the non-polar, non-fluorescent FDA readily enters the pollen cytoplasm. Active esterases hydrolyze FDA releasing fluorescein, which is polar and fluorescent, and which passes sparingly through an intact membrane. Accumulation of fluorescein in the cytoplasm of viable pollen grains imparts a bright- to yellowish-green fluorescence when examined in ultra-violet light (Heslop-Harrison and Heslop-Harrison, 1970; Shivanna and Heslop-Harrison, 1981; Heslop-Harrison et al., 1984; Shivanna and Rangaswamy, 1992). If the esterases that hydrolyze FDA are lacking, pollen grains do not fluoresce; if the plasmalemma is not intact, fluorescein readily escapes from the cell into the mounting medium and pollen grains fail to fluoresce brightly (Heslop-Harrison et al., 1984; Russell, 1991). The FCR test for fluorochromasia thus assesses two properties of pollen cells in *G. hirsutum*: the integrity of the plasma membrane, and the activity of esterases capable of hydrolyzing the fluorescein ester (Shivanna and Johri, 1985; Shivanna and Rangaswamy, 1992). The specific protocol employed during this study and the formulation for all solutions used are described in Appendix B.

Specimens were prepared by generously dusting mature pollen onto a slide, adding

three to five drops of fresh working solution, and gently applying a coverslip. Slides were left undisturbed for five minutes to permit sufficient time for fluorochrome uptake and enzymatic reaction to occur. Pollen counts of at least 300 grains per flower from 3 to 5 flowers per plant were made at 200X magnification using an American Optical Fluorolume microscope equipped with epifluorescence (Taylor and Salmon, 1989; Ploem, 1989). Criteria used to define pollen grain semi-sterility and non-viability were those adopted by Gwyn and Stelly (1989) and applied successfully in other recent studies of *G. hirsutum* pollen (Stelly et al., 1990; Endrizzi and Mamood, unpublished data).

Evaluation of Bracteole Characters

Plant development in *G. hirsutum* proceeds through five main growth stages: germination and emergence, seedling establishment, development of leaf area and canopy, flowering and boll development, and maturation (Mauney, 1984; Benedict, 1984; Oosterhuis, 1990). Reproductive growth begins four to five weeks after planting with formation of floral buds in the apical areas of the plant; flowering and initiation of boll development follow within a few weeks. Floral buds appear first as small, green, pyramidal structures ("squares"); each square is composed of three (rarely, four or more) broad, foliar, cordate involucral bracteoles ("bracts") which completely enclose and protect the growing floral parts (Fryxell, 1979). In addition, bracts contribute about 10% of all boll photosynthetic requirements (Oosterhuis, 1990; Benedict, 1984). Appreciable genetic variance has been observed in screening genotypes for several bract traits including bract surface area, teeth per bract, and bract flare (Meredith, 1984;

Anonymous, 1981).

Preliminary field observations, made during summer 1990, of empirical differences in bract morphology between T8-12 and TM-1 flowers suggested that selected bract characteristics might be reliable indicators of chromosomal constitution. Consequently, plants of each cytotype recovered during the testcross experiment (1991-1992) were assessed for selected bract characteristics--and compared with the genetic standard (Texas Marker-1)--to provide an analysis independent of the concurrent pollen viability studies. The selected bracteole characteristics, divided into primary and derived categories, are listed in Table 3. The derivations of the bract surface area formulae used are shown in Appendix C.

Statistical Analysis

The experimental design focused on three principal areas: (1) conformity of data sets to expected backcross, testcross, and F_2 ratios; (2) analysis of variance; and (3) examination of significant differences among multiple means. Analyses were run using the CoStat statistical package (CoHort Software, 1990). Data were initially characterized using standard descriptive statistical procedures (Sokal and Rohlf, 1981; Zar, 1984). Frequency analysis and goodness-of-fit testing were done using the Kolmogorov-Smirnov procedure and the G-test. Sources of variation observed in some data--the 1991 testcross results in particular--were analyzed employing a model I one-way completely randomized analysis of variance (ANOVA). Multiple means testing was done with the Student-Newman-Keuls (SNK) and Duncan's Multiple Range tests. Association between

Table 3. Primary and derived characteristics used to evaluate bracteole morphology among testcross [TM-1 x (Crp/T8-12)] cytotypes.

Name	Acronym	Description
<u>Primary Bracteole Characteristics:</u>		
Number of Teeth	Teeth	Total number of teeth on bract
Total Length	L_{max}	Length (cm) of bract from base to tip of longest tooth
Basal Length	L_{basal}	Length (cm) of bract from base to bottom of longest tooth
Tooth Length	L_{tooth}	Length (cm) of longest tooth from bottom to tip
Maximum Width	W_{max}	Width (cm) of bract at widest horizontal distance across the base
Greatest Dimensions (Theoretical)	D_{max}	Bract Area (cm ²) defined by the expression ($L_{max} * W_{max}$)
<u>Derived Bracteole Characteristics:</u>		
Basal Surface Area	BSA	Bract Area (cm ²) defined by the expression ($L_{basal} * W_{max}$) * 0.80 ^a
Tooth Surface Area	TSA	Bract Area (cm ²) defined by the expression ($L_{tooth} * W_{max}$) * 0.25 ^a
Bract Surface Area	BrSA	Bract Area (cm ²) defined by the expression (BSA + TSA) ^a
Bract Ratio	BrRat	Ratio (%) defined by the expression (BSA / BrSA) * 100
Filling Ratio	FillRat	Ratio (%) defined by the expression (BrSA / D_{max}) * 100

^a See Appendix C for derivation.

significant variables within each approach to cytotype identification (pollen viability, bract characteristics, and morphologic index) were analyzed using the Pearson product moment correlation coefficient.

Data from the Summer 1991 T8-12 testcross were analyzed using a model I one-way completely randomized ANOVA procedure. ANOVA is a powerful statistical technique for gaining insight into the nature of variation embedded within natural events (Sokal and Rohlf, 1981; Zar, 1984; Bell, 1989). As with other strong models in science, ANOVA can foster potentially misleading constructions or lead to unproductive conclusions (Peters, 1991; Harvey and Pagel, 1991). Lewontin (1974a) gives an illuminating discussion of the pitfalls inherent in ANOVA procedures. The purpose in carrying out an analysis of variance is to estimate the true differences among the group means.

The basic assumption of a model I ANOVA is that differences among group means are due to fixed treatment effects determined by the experimenter (Eisenhart, 1947; Sokal and Rohlf, 1981). The one-way ANOVA procedure applied to the 1991 T8-12 testcross experiment had a single factor ("Cytotype") which received five treatments: "Standard" (*TM-1 TM-1 tt*); "Normal" (*crp crp tt*); "Gene" (*Crp crp tt*); "Chromosome" (*crp crp Tt*); and "Combined" (*Crp crp Tt*). Variables measured were: [1] "Morphologic Index" (Appendix A); [2] "Pollen Viability" (Appendix B); and [3] "Derived Bracteole Characteristics" (Table 3; Appendix C). The experimental units were genotypes; replicates were individual plants. ANOVA procedures for the 1991 backcross and F₂ experiments were devised in an analogous way.

CHAPTER 3

RESULTS

Vayase... y no ande buscando cinco pies al gato.

--Cervantes

Homozygous Control Populations

Preliminary Investigation

In *Gossypium hirsutum*, homozygous T8-12 plants can be identified by a distinctive phenotype ("*crumpled-mottled*") that includes dwarf size, very short internodes, misshapen and crumpled leaves, red leaf venation, distinct irregularly distributed leaf mottling, and significant pollen abortion. In heterozygotes, the T8-12 interchange is identified cytogenetically by a characteristic "frying-pan" quadrivalent plus 24 bivalents. In the homozygous state, homologous pairing is normal and regularly produces 26 bivalents.

Homozygous Crumpled (*Crp*) plants are similarly dwarfed, exhibit variably shortened internodes, show leaf shape abnormalities, develop leaf mottling and chlorosis, and have narrower bracteoles. Genetically, *Crp* acts as a dominant allele involving a single locus. Pollen viability studies have not been reported for the *Crp* line.

The heterozygous phenotypes of both mutants--T8-12 and *Crp*--are intermediate in severity between that of normal *Gossypium hirsutum* plants (e.g., Texas Marker-1, the cytogenetic and genetic standard) and that observed in either homozygote.

Pollen semisterility is a useful criterion for detecting both cytogenetic

deficiencies and heterozygosity of chromosomal aberrations in diploid crop species. The fluorescein diacetate-based fluorochrome reaction procedure developed by Heslop-Harrison and Heslop-Harrison (1970) was modified by Gwyn (Gwyn and Stelly, 1989) and used successfully (Gwyn and Stelly, 1989; Stelly et al., 1990) to evaluate cytogenetically induced semisterility in the allotetraploid *G. hirsutum*. The dwarf mutant phenotype occurring in the homozygous T8-12 line (and exhibited also by homozygous *Crp* plants) has never been separated from the T8-12 interchange. However, critical experiments have not been reported and allelism between the T8-12 breakpoint and the *Crp* locus has never been tested. Results presented here are the first to separate the "crumpled-mottled" phenotype from the T8-12 translocation; to evaluate pollen viability in the *Crp* line; and to test for allelism between (a) T8-12 translocation breakpoint and the *Crp* locus, and (b) T8-12 translocation breakpoint and the semilethal Leaf fleck (*Lf*) locus.

The fundamental H_0 tested was {T8-12 breakpoint = *Crp* locus} or, during the allelism experiments, {T8-12 breakpoint = *Lf* locus}. Thirteen pollen and morphologic variables were operationally defined and used to characterize the three homozygous control lines--Texas Marker-1 (TM-1), T8-12, and *Crp*--and the heterozygous T8-12 testcross population utilized in this study. Two of the thirteen variables, morphologic index and viable pollen %, were used to analyze the T8-12 backcross and the F_2 T8-12 populations. The observed cumulative frequency distribution for each variable from each population was tested against its expected normal distribution using the Kolmogorov-Smirnov procedure--and the result was verified with a log likelihood ratio test. Results

of the Kolmogorov-Smirnov goodness-of-fit comparisons (Table 4) indicate that observed distributions did not differ significantly from expected normal distributions.

Analysis of Morphologic Index Data

The morphologic index ("index") variable (Appendix A) was used to score the whole plant phenotype of each control plant ($n = 30$) and all experimental plants ($n = 722$). The index, based on deviations from TM-1 standards, was utilized as an initial procedure to evaluate populations and to identify plants for subsequent pollen analysis and bracteole evaluation. Morphologic index values for each control line are given in Table 5. The TM-1 mean index was significantly different ($P < 0.01$) from those of the *Crp* and T8-12 lines (Appendix D, Table D1). T8-12 and *Crp* index means were not significantly different--confirming the field observation that T8-12 and *Crp* phenotypes exhibit essentially complete overlap. The null hypothesis was accepted for morphologic index.

Evaluation of Pollen Analysis Data

Viable pollen percentage scores were obtained experimentally for nine of eleven populations sampled. Pollen values for the two homozygous T8-12 populations were derived from data in the literature (Gwyn and Stelly, 1989); this minor variation in experimental design was necessitated by failure of both homozygous T8-12 populations to produce--under either field or winter greenhouse conditions--flowers adequate for fluorochrome pollen analysis. Homozygous T8-12 plants were very late in blooming and developed few flowers in either environment; the few buds produced were diminutive, exhibited erratic anthesis patterns, and were nearly devoid of pollen. Theoretically,

Table 4. Kolmogorov-Smirnov values for goodness-of-fit of thirteen characteristics in three homozygous control populations of *Gossypium hirsutum*.

Characteristic	TM-1 controls			<i>Crp</i> controls			T8-12 controls		
	n ¹	D _{obs}	D _(.01) ²	n	D _{obs}	D _(.01)	n	D _{obs}	D _(.01)
Index ³	18	0.166	0.371	6	0.089	0.364	6	0.089	0.364
Viable pollen ³	18	0.167	0.371	6	0.029	0.364	6	0.028 ⁴	0.364
No. of teeth	52	0.036	0.143	47	0.040	0.150	47	0.029	0.150
Total length	52	0.033	0.143	47	0.047	0.150	47	0.092	0.150
Basal length	52	0.049	0.143	47	0.041	0.150	47	0.029	0.150
Tooth length	52	0.063	0.143	47	0.034	0.150	47	0.106	0.150
Max. width	52	0.064	0.143	47	0.031	0.150	47	0.051	0.150
Max. dimensions	52	0.049	0.143	47	0.062	0.150	47	0.018	0.150
Basal area	83	0.032	0.113	47	0.043	0.150	47	0.016	0.150
Tooth area	83	0.026	0.113	47	0.036	0.150	47	0.041	0.150
Bract area	83	0.035	0.113	47	0.013	0.150	47	0.011	0.150
Bract ratio	83	0.015	0.113	47	0.044	0.150	47	0.057	0.150
Filling ratio	83	0.022	0.113	47	0.095	0.150	47	0.058	0.150

¹ n = number of plants (Index; Viable pollen) or bracts (all others) sampled.

² Critical value of the test-statistic *D* at ($P < .01$).

³ Observation fitted to an extrinsic hypothesis.

⁴ Theoretical value derived from data in Gwyn and Stelly (1989).

pairing of homologous chromosomes in homozygous reciprocal translocation plants should be essentially normal; pairing does not, however, ensure viable pollen production. Abnormalities in pollen production by homozygous T8-12 plants have been reported previously and significant pollen abortion was not an unexpected finding.

Results of fluorescein diacetate-based fluorochrome pollen analyses for each homozygous control line are shown in Table 5. As expected, mean viable pollen values for TM-1 plants did not differ appreciably from mean values reported for other TM-1 populations (Gwyn and Stelly, 1989) or from reported values for homozygous T8-12 populations (Gwyn and Stelly, 1989; Stelly et al., 1990). Mean viable pollen values for the *Crp* population were significantly different ($P < 0.01$) from both TM-1 and T8-12 lines (Table D2, Appendix D) leading to rejection of the null hypothesis (H_0 : TM-1 = T8-12 = *Crp*). Discovery of these small, but significant, differences between the *Crp* and T8-12 lines suggested that pollen analysis could be used to separate the "*crumpled-mottled*" phenotype from the T8-12 translocation in heterozygous populations.

Analysis of Bracteole Characteristics

Six of eleven bracteole characteristics developed for this study proved useful in differentiating the homozygous control populations: total length, basal length, tooth length, tooth surface area, bract ratio, and filling ratio. Mean values for each of these bracteole variables are compared in Table 6; for all six, the TM-1 mean was significantly different from the *Crp* and T8-12 means (Appendix D, Tables D3-D8). Three variables--tooth length, bract ratio, and filling ratio--were sufficiently robust to differentiate all three control lines and thus invalidate the null hypothesis (H_0 : TM-1 = T8-12 = *Crp*).

Table 5. Morphologic index and viable pollen values for three homozygous *Gossypium hirsutum* control plant lines.

Line	No. of plants sampled	Morphologic index ¹			Viable pollen (%)		
		Mean	C.V. ²	Range	Mean %	C.V.	Range
TM-1	18	9.56 a ³ (0.291) ⁴	3.1	9-10	98.6 a (0.88)	0.9	97-100
<i>Crp</i>	6	5.58 b (0.859)	15.4	4.5-7	96.0 b (1.42)	1.5	94-98
T8-12	6	4.92 b (0.861)	17.5	3.5-6	98.0 a ⁵ (1.41)	1.4	96-100

¹ Morphologic index is unitless (see Appendix I).

² C.V. = Coefficient of Variation

³ Means within columns with the same letter are not significantly different ($P < 0.01$).

⁴ Values in parentheses are standard errors.

⁵ Theoretical value derived from data in Gwyn and Stelly (1989).

Table 6. Mean bracteole characteristics for three homozygous *Gossypium hirsutum* control plant lines.

Line	No. of bracts sampled	Total length (cm)	Basal length (cm)	Tooth length (cm)	Tooth surface area (cm ²)	Bract ratio (%)	Filling ratio (%)
TM-1	52	4.32 a ¹ (0.391) ²	2.49 a (0.267)	1.84 a (0.261)	1.51 a (0.219)	80.5 a (2.46)	56.2 a (2.29)
<i>Crp</i>	47	2.48 b (0.199)	1.72 b (0.149)	0.75 b (0.046)	0.32 b (0.010)	87.9 b (1.99)	63.4 b (2.33)
T8-12	47	2.38 b (0.224)	1.82 b (0.153)	0.56 c (0.110)	0.23 b (0.057)	91.2 c (2.66)	67.3 c (3.25)

¹ Means within columns with the same letter are not significantly different ($P < 0.01$).

² Values in parentheses are standard errors.

Correlation and Regression Analysis

Pearson's product moment correlation coefficients were calculated for five covarying pairs of variables; each pair of variables displayed a bivariate normal distribution. Correlation coefficients and independence test results are in Appendix E (Table E1). The null hypothesis (independence of variables) was tested using two R x C independence measures: chi-square and a *G*-test. Highly significant correlations were found for all pairs of variables except for Index and Viable Pollen in the *Crp* line ($.05 > P > .01$), and Index with Viable Pollen in the T8-12 line. The non-significant negative correlation observed for the T8-12 homozygotes was most likely an artifact of the random method used to assign pollen values within this line. The inverse relationships observed for tooth length/bract ratio and tooth length/filling ratio provide important insights into the differential bracteole morphology encountered in the control lines.

Least squares regression was used to evaluate the predictive power of each pair of variables. Coefficients of multiple determination--resulting from second order polynomial analysis--are presented in Appendix F (Table F1). As expected, each R^2 value--an indicator of the proportion of total variation of each Y variable explained by the regression--was very close to its corresponding coefficient of determination (r^2) indicating that no dormant non-linear interactions were embedded in the regression model. Analyses of the correlation and regression data support the conclusion that the selected variable pairs were significantly correlated and robust.

T8-12 Backcross Population

The T8-12 backcross population (n = 175) was scored for morphologic index in the field; a randomly chosen subset (n = 89) was independently scored for viable pollen in the laboratory. The backcross population was not evaluated for bracteole characteristics. Selected plants from both phenotypic classes recovered (TM-1; T8-12) were verified cytologically. The observed frequency distribution for each class was checked for normality using a Kolmogorov-Smirnov test--and the result confirmed by a log likelihood ratio test. Results of the Kolmogorov-Smirnov testing (Table 7) indicate that both variables--index and viable pollen--were normally distributed.

Results of index and viable pollen scoring are shown in Table 8. The backcross TM-1 mean index did not differ appreciably from those of other TM-1 populations (Appendix D, Table D9); it did differ significantly ($t_{0.001(2),173} = 10.728$; $P < 0.001$) from the backcross T8-12 mean index. The backcross T8-12 mean index and the control T8-12 mean index also differed significantly ($t_{0.001(2),96} = 7.759$; $P < 0.001$).

Results of fluorochrome reaction pollen analyses (n = 89) done on the backcross pollen subset are shown in Table 8. The mean viable pollen value for the TM-1 class was not significantly different from other TM-1 pollen means. Differences between mean viable pollen values for the TM-1 and T8-12 classes were highly significant ($t_{0.001(2),87} = 17.696$; $P < 0.001$) and confirmed published reports that the fluorescein diacetate fluorochrome reaction is capable of detecting cytogenetically induced semisterility in *Gossypium hirsutum*.

Pearson's product moment correlation coefficients were computed to test the

Table 7. Kolmogorov-Smirnov values for goodness-of-fit of morphologic index and viable pollen % in the T8-12 backcross and T8-12 F₂ populations.

Population	Characteristic					
	Morphologic index			Viable pollen %		
	n ¹	D _{obs}	D _(.01) ²	n	D _{obs}	D _(.01)
BC TM-1	83	0.027	0.113	45	0.054	0.153
BC T8-12 Heterozygote	92	0.028	0.107	44	0.067	0.155
F ₂ TM-1	61	0.034	0.132	30	0.058	0.188
F ₂ T8-12 Heterozygote	137	0.026	0.088	60	0.035	0.133
F ₂ T8-12 Homozygote	61	0.045	0.132	35	0.021 ³	0.174

¹ n = number of plants sampled.

² Critical value of the test-statistic *D* at (P < .01).

³ Theoretical value derived from data in Gwyn and Stelly (1989).

Table 8. Morphologic index and viable pollen values for nine *Gossypium hirsutum* experimental plant lines.

Line	Morphologic index ¹				Viable pollen (%)			
	n ²	Mean	C.V. ³	Range	n	Mean	C.V.	Range
BC ⁴ TM-1	83	9.17a ⁵ (0.526) ⁶	5.74	8-10	45	97.42a (1.469)	1.51	94-100
BC TM-1/ T8-12	92	7.93b (0.926)	11.68	6-9.5	44	78.02b (7.203)	9.23	63-89
F ₂ ⁷ TM-1	61	9.16a (0.583)	6.36	8-10	30	97.20a (1.541)	1.58	94-100
F ₂ TM-1/ T8-12	137	7.71b (0.804)	10.42	6-9.5	60	77.35b (6.519)	8.43	62-89
F ₂ T8-12/ T8-12	61	5.14d (0.719)	14.01	3.5-7	35	98.37a ⁹ (1.003)	1.02	96-100
TC ⁸ TM-1	67	9.31a (0.442)	4.75	8.5-10	31	97.42a (1.177)	1.21	95-99
TC TM-1/ <i>Crp</i>	66	8.34ab (0.691)	8.29	7-9.5	31	95.61a (1.687)	1.76	92-99
TC TM-1/ T8-12	75	7.33b (0.932)	12.72	5.5-9	34	78.26b (7.391)	9.44	65-89
TC <i>Crp</i> / T8-12	80	6.51c (1.381)	21.23	4-9	35	72.74c (8.237)	11.32	58-89

Table 8. --Continued

¹ Morphologic index is unitless (see Appendix I).

² n = number of plants sampled.

³ C.V. = Coefficient of Variation.

⁴ Backcross [TM-1 x (TM-1 x T8-12)]; field numbers A7-90 x G7-90.

⁵ Means within columns with the same letter are not significantly different ($P < 0.01$).

⁶ Values in parentheses are standard errors.

⁷ Progeny from selfing G7-90 [F_2 (J13-10-78 TM-1 x OPGH 78-79 T8-12)].

⁸ Testcross [TM-1 x (*Crp* x T8-12)]; field numbers A11-90 x F7-90.

⁹ Theoretical value derived from data in Gwyn and Stelly (1989).

association of index and viable pollen; the bivariate distribution was normal. The null hypothesis (independence of index and viable pollen) was tested using both chi-square and a G-test. Correlation coefficients and independence test results (Appendix E, Table E2) indicate significant covariance for both variables in both backcross classes (TM-1; T8-12). Least squares polynomial regression analysis was used to test the predictive ability of viable pollen regressed on morphologic index. Coefficients of multiple determination are listed in Appendix F (Table F2); each R^2 value was in close agreement with its corresponding r^2 --indicating that no covert non-linear interactions were detected within the regression model. Although the overall R^2 values were lower than those found in the homozygous control populations, the results were significant ($P < .001$). Correlation and regression data from the backcross population support the conclusion that morphologic index and viable pollen % are significantly correlated and that index is a robust predictor of viable pollen.

The data obtained from morphologic index and pollen scoring were tested for fit to a 1:1 ratio (extrinsic hypothesis) using a chi-square goodness-of-fit test. The results for all backcross plants ($n = 175$) shown in Table 9 indicate that the observed ratio of 83 TM-1 to 92 T8-12 plants was not significantly different from the expected 87.5 to 87.5 ratio. The observed ratio for the pollen analysis subset was 45 TM-1 to 44 T8-12. The backcross data demonstrate that T8-12 can be separated from TM-1 by use of the morphologic index and viable pollen analysis.

Table 9. Segregation data and goodness-of-fit test results for three experimental *Gossypium hirsutum* populations: T8-12 backcross [TM-1 x F₁(TM-1 x T8-12)], T8-12 F₂ [F₂(TM-1 x T8-12)], and T8-12 testcross [TM-1 x F₁(Crp x T8-12)].

Population	Observed phenotypic class and class size					Expected Ratio	Chi-square test	
	TM-1/ TM-1	TM-1/ T8-12	T8-12/ T8-12	TM-1/ Crp	T8-12/ Crp		X ² value	P value
Backcross T8-12	83	92				1:1	0.4628	.4963 ns ¹
F ₂ T8-12	61	137	61			1:2:1	0.8687	.6477 ns
						<i>Original H₀</i> : ²		
Testcross T8-12	67	75		66	80	1:1 ³	66.13	.0000 *** ⁴
						<i>Revised hypothesis</i> : ⁵		
	67	75		66	80	1:1:1:1 ⁶	1.861	.6017 ns

¹ Indicates a nonsignificant value (P > .05 that null hypothesis is correct).

² Original H₀: {T8-12 = Crp}.

³ Expected ratio: 144 TM-1/TM-1 : 144 TM-1/T8-12 (extrinsic hypothesis).

⁴ Significant at the P < .001 level.

⁵ Revised hypothesis: {T8-12 and Crp are separate genetic characters}.

⁶ Expected ratio: 72 TM-1/TM-1 : 72 TM-1/T8-12 : 72 TM-1/Crp : 72 T8-12/Crp.

T8-12 F₂ Population

The T8-12 F₂ population (n = 259) was scored under field conditions for morphologic index; a randomly selected subset (n = 125) was drawn from two classes (TM-1; T8-12 heterozygote) and independently scored for viable pollen % in the laboratory. Plants from the homozygous T8-12 class were not scored for viable pollen as these plants failed--under both field and winter greenhouse conditions--to produce flowers adequate for fluorochrome reaction analysis. Viable pollen values for these T8-12 homozygotes were derived from data in the literature (Gwyn and Stelly, 1989) and randomly assigned to individuals (n = 35) within the population. Selected plants from all three phenotypic classes recovered (TM-1; T8-12 heterozygotes; T8-12 homozygotes) were cytologically verified. The F₂ population was not scored for bracteole variables.

The Kolmogorov-Smirnov procedure followed by a log likelihood ratio test was used to assess the observed frequency distribution of each class for normality. The results (Table 7) indicate that both index and viable pollen were normally distributed.

Index and viable pollen scoring results are summarized in Table 8. The TM-1 mean index did not differ significantly from those of other TM-1 populations (Appendix D, Table D9). However, it did differ significantly from mean index values for F₂ heterozygotes and homozygotes (Table D11, Appendix D). The F₂ T8-12 heterozygote mean index was not significantly different from other heterozygous TM-1/T8-12 mean indices--but did differ significantly from the F₂ homozygote mean index--and from the control T8-12 homozygote index (Appendix D, Table D12). The F₂ homozygote mean

index did not differ significantly from the control T8-12 mean index (Appendix D, Table 13). Importantly, F_2 morphologic index data analysis establishes that the T8-12 heterozygote mean index differs significantly from that of the T8-12 homozygote.

The TM-1 mean viable pollen % for the F_2 population was not significantly different from other TM-1 pollen means (Appendix D, Table D10). The F_2 TM-1 mean differed from the T8-12 heterozygote mean but not from the mean value derived for F_2 T8-12 homozygotes (Table D14, Appendix D). The heterozygous F_2 T8-12 viable pollen mean did not differ significantly from those of other TM-1/T8-12 heterozygotes--but was very different from the homozygous control T8-12 mean pollen value (Appendix D, Table D15). The derived mean viable pollen % for the F_2 T8-12 homozygote class was not significantly different from the control T8-12 homozygote mean--nor did it differ significantly from the TM-1 pollen means (Table D16, Appendix D). Importantly, F_2 population data indicates that pollen analysis can be used to separate T8-12 heterozygotes from homozygotes--as well as separating T8-12 heterozygotes from normal plants as was demonstrated in the backcross population.

Product moment correlation coefficients were computed to test the association of index and viable pollen in the F_2 population. The bivariate distribution was normal. Independence of index and viable pollen (H_0) was tested with both chi-square and a G-test. Correlation coefficients and independence test results (Appendix E, Table E2) demonstrated significant covariance in all classes except the T8-12 homozygotes.

Least squares regression was again used to test the predictive power of viable

pollen regressed on morphologic index. Coefficients of multiple determination are listed in Appendix F (Table F2); each R^2 value closely agreed with the corresponding r^2 --indicating that no dormant non-linear interactions were detected within the regression model. The overall R^2 values were lower than those found in either the homozygous control or the backcross populations but were significant ($P < .001$). Morphologic index and viable pollen are significantly correlated and index is a robust predictor of viable pollen % among F_2 plants.

The data obtained from morphologic index and pollen scoring were tested for fit to a 1:2:1 ratio (extrinsic hypothesis) using a chi-square goodness-of-fit test. The results for all F_2 plants ($n = 259$) are shown in Table 9. The observed ratio (61 TM-1 : 137 T8-12 heterozygotes : 61 T8-12 homozygotes) did not differ significantly from the expected ratio of 64.75 : 129.5 : 64.75. The observed ratio for the viable pollen subset was 30 TM-1 : 60 T8-12 heterozygotes : 35 T8-12 homozygotes. The F_2 data demonstrate that T8-12 heterozygotes can be effectively separated from T8-12 homozygotes, and from TM-1 plants, using the morphologic index and fluorochrome pollen analysis.

T8-12 Testcross Population

Evaluation procedures developed for the homozygous control lines were extended *in toto* to the heterozygous testcross plants with one exception: the *Crp*/T8-12 class--for which no control line existed--was treated under H_0 as equivalent to the

homozygous T8-12 class.

The T8-12 testcross population ($n = 288$) was scored in the field for morphologic index; a randomly selected subset ($n = 131$) was scored for viable pollen. Bracteole measurements were made on a subset ($n = 28$) randomly selected from the viable pollen sample. Selected plants from each testcross class were verified cytologically.

Data obtained from morphologic index and pollen scoring were tested for fit to a 1:1 ratio (extrinsic hypothesis) using a chi-square goodness-of-fit procedure. The results (Table 9) were so significantly different--four phenotypic classes rather than the expected two--as to merit rejection of H_0 and revision of the alternative null hypothesis. When retested with the revised H_0 (1:1:1:1), the data fit improved dramatically.

Morphologic index was used to evaluate whole plant phenotypes; mean morphologic index values for each testcross class are shown in Table 8. The testcross TM-1 mean index did not differ markedly from other TM-1 mean indices (Appendix D, Table D9) but it was significantly different from all other testcross index means except that of the *Crp* class (Table D17, Appendix D). The *Crp* mean index value was differentiated--but not unambiguously--from the TM-1 and T8-12 mean indices by ANOVA on index values alone. The testcross *Crp* mean differed significantly from the control *Crp* mean index, the control T8-12 mean index, and the testcross *Crp*/T8-12 mean index (Table D18, Appendix D). The T8-12 mean index did not significantly differ from other heterozygous T8-12 index means with the exception of the *Crp*/T8-12

mean (Appendix D, Table 18). The *Crp*/T8-12 mean index was significantly different from all other mean indices including the T8-12 homozygous control mean index. The morphologic index data indicate that all testcross heterozygotes can be separated from each other with the exception of the *Crp* class.

Results of pollen analyses on the testcross classes are shown in Table 8. One paramount discovery of this study was that the mean viable pollen % values for TM-1 homozygotes and TM-1/*Crp* heterozygotes do not differ significantly (Table D19, Appendix D). However, the mean pollen % values for TM-1/*Crp* and TM-1/T8-12 are significantly different--and the mean pollen value for the *Crp*/T8-12 class is significantly different from the mean for the T8-12 homozygote control group. When fluorochrome reaction (FCR) pollen analysis is coupled with morphologic index evaluation, TM-1/T8-12 and TM-1/*Crp* heterozygotes are separated and observed to be morphological--but not functional--equivalents. Demonstration of this unusual situation in the testcross heterozygotes confirms the suggested relationship seen earlier in the comparison between homozygous *Crp* and homozygous T8-12 controls.

Bracteole evaluation on the heterozygous testcross population was done using the same set of six variables previously applied to the homozygous control lines; a sample (n = 241) of bracteoles representing all four testcross classes in approximately equal proportions was examined. The observed cumulative frequency distribution for each variable from each testcross group was compared with its expected normal distribution using the Kolmogorov-Smirnov procedure; each result was then verified with a likelihood

ratio test. Results of the Kolmogorov-Smirnov goodness-of-fit comparisons (Table 10) indicate that observed distributions did not differ significantly from expected normal distributions. However, in some classes containing the T8-12 interchange chromosome, some distributions were statistically "noisy": tooth length, in the *Crp*/T8-12 class for example, showed departures from normality that were significant ($.05 > P > .01$) when checked with the independent--but less rigorous--likelihood ratio procedure (Sokal and Rohlf, 1981).

Mean values for each of the six bracteole variables are compared in Table 11. Total length was capable of distinguishing plants producing normal pollen from those exhibiting pollen with reduced viability. Tooth length, tooth surface area, and filling ratio were robust and separated all four testcross classes.

Pearson's product moment correlation coefficients were calculated for five covarying variable pairs; each pair of variables adhered to a bivariate normal distribution. Correlation coefficients and independence test results are given in Appendix E (Table E3). The null hypothesis (independence of variables) was tested using two measures of R x C independence: chi-square and the G-test. Highly significant correlations were found for all pairs in all testcross groups. The inverse relationships between tooth length/bract ratio and tooth length/filling ratio were again informative of differential bracteole morphology.

Table 10. Kolmogorov-Smirnov values for goodness-of-fit of thirteen characteristics in four *Gossypium hirsutum* testcross populations.

Characteristic ¹	TM-1/TM-1 ("Normals")			TM-1/Crp ("Crumpled only")		
	n ²	D _{obs}	D _(.01) ³	n	D _{obs}	D _(.01)
Index	67	0.040	0.126	66	0.039	0.126
Viable pollen	31	0.043	0.185	31	0.043	0.185
No. of teeth	49	0.035	0.147	51	0.025	0.144
Total length	49	0.091	0.147	51	0.076	0.144
Basal length	49	0.071	0.147	51	0.056	0.144
Tooth length	49	0.054	0.147	51	0.078	0.144
Maximum width	49	0.024	0.147	51	0.055	0.144
Greatest dimensions	49	0.043	0.147	51	0.038	0.144
Basal surface area	49	0.046	0.147	51	0.031	0.144
Tooth surface area	49	0.059	0.147	51	0.065	0.144
Bract surface area	49	0.067	0.147	51	0.066	0.144
Bract ratio	49	0.031	0.147	51	0.081	0.144
Filling ratio	49	0.063	0.147	51	0.056	0.144

Table 10. --Continued.

Characteristic ¹	TM-1/T8-12 ("Translocation only")			T8-12/Crp ("Both")		
	n ²	D _{obs}	D _(.01) ³	n	D _{obs}	D _(.01)
Index	75	0.041	0.119	80	0.023	0.115
Viable pollen	34	0.099	0.177	35	0.067	0.174
Number of teeth	61	0.027	0.132	80	0.019	0.115
Total length	61	0.024	0.132	80	0.047	0.115
Basal length	61	0.049 ⁴	0.132	80	0.034	0.115
Tooth length	61	0.048 ⁵	0.132	80	0.080 ⁶	0.115
Maximum width	61	0.047	0.132	80	0.044 ⁷	0.115
Greatest dimensions	61	0.051	0.132	80	0.033	0.115
Basal surface area	61	0.037	0.132	80	0.043	0.115
Tooth surface area	61	0.059	0.132	80	0.021	0.115
Bract surface area	61	0.063	0.132	80	0.027	0.115
Bract ratio	61	0.031	0.132	80	0.083	0.115
Filling ratio	61	0.039	0.132	80	0.050	0.115

¹ Characteristics are described in Table 3.

² n = number of plants (Index; VP) or bracts (all other characteristics) sampled.

Table 10. --Continued

- ³ Critical value of the test-statistic D at ($P < .01$) level.
- ⁴ Population sample was leptokurtic ($g_2 = 1.056$; $P = 0.0427$)
- ⁵ Frequency distribution had one empty class interval. Likelihood ratio, Chi-square, and g values were not significant ($P < .05$).
- ⁶ Population sample was platykurtic ($g_2 = -1.1065$; $P = 0.2030$) for this variable. The frequency distribution showed multiple empty class intervals that were partially rectified by regrouping with wider class intervals. Likelihood ratio ($G_{0.02(1)} = 5.277$) and chi-square ($X^2_{0.02(1)} = 5.195$) values were significant in the range ($.05 > P > .01$).
- ⁷ Population sample was platykurtic ($g_2 = -0.9765$; $P = .035$) with respect to this characteristic. Likelihood ratio and chi-square test values were not significant.

Least squares regression was used to evaluate the predictive ability of viable pollen regressed on morphologic index. Coefficients of multiple determination--deriving from second order polynomial analysis--are given in Appendix F (Table F2). As predicted, each R^2 value--an indicator of the proportion of total variation of each Y variable explained by the regression--was very close to the corresponding coefficient of determination (r^2) indicating that the regression model was free of embedded non-linear interactions. The most divergent R^2/r^2 pair was that of the F_2 T8-12 heterozygote. Analysis of the testcross correlation and regression data support the conclusion that morphologic index and viable pollen were significantly correlated and robust. In summary, the testcross data invalidate H_0 and confirm that T8-12 and *Crp* are separate genetic entities--as characterized by morphologic index, viable pollen %, and selected bracteole variables.

Tests of Allelism Between *Lf* and T8-12

The potential allelism of *Lf* and the T8-12 breakpoint was field tested in 1992 by planting out 69 seed from the 1991 cross

AG195 (*lflf gl₂gl₂gl₃gl₃ bw₁bw₁bw₂bw₂ ne₁ne₁ne₂ne₂ n₁n₁)*

X

F_1 (*Lflf gl₂gl₂gl₃gl₃ bw₁bw₁bw₂bw₂ ne₁ne₁ne₂ne₂ N₁N₁ x T8-12)*

and determining their segregation ratio for normal and abnormal leaf pattern. Of 69 plants transplanted on 7 May 1992, 47 survived until 23 July 1992--the completion

Table 11. Mean bracteole characteristics for the homozygous TM-1 control line and four *Gossypium hirsutum* testcross plant lines.

Line	No. of bracts sampled	Total length (cm)	Basal length (cm)	Tooth length (cm)	Tooth surface area (cm ²)	Bract ratio (%)	Filling ratio (%)
Control TM-1	52	4.32 a ¹ (0.391) ²	2.49 a (0.267)	1.84 a (0.261)	1.51 a (0.219)	80.5 a (2.46)	56.2 a (2.29)
TC TM-1	49	4.25 a (0.248)	2.50 a (0.198)	1.75 a (0.196)	1.37 b (0.165)	81.9 a (2.19)	57.4 a (1.99)
TC <i>Crp</i>	51	4.17 a (0.269)	2.67 a (0.251)	1.51 b (0.189)	1.16 c (0.158)	84.9 b (2.26)	60.2 b (2.26)
TC T8-12	61	3.79 b (0.365)	2.52 a (0.257)	1.27 c (0.263)	0.72 d (0.185)	86.3 b (2.74)	61.7 c (2.82)
TC <i>Crp</i> / T8-12	80	3.42 b (0.414)	2.57 a (0.332)	0.86 d (0.187)	0.39 e (0.074)	90.5 c (1.97)	66.3 d (2.38)

¹ Means within columns with the same letter are not significantly different ($P < 0.01$).

² Values in parentheses are standard errors.

date for the first round of field scoring. The steep survivorship curve was attributed to severe rodent and lagomorph predation--not to the semilethality of the *Lf* locus. Pollen analysis was done on a randomly selected sample ($n = 29$) of the survivors. The combined leaf pattern and viable pollen data are given in Appendix G; the results are summarized in Table 12. The null hypothesis (H_0 : T8-12 breakpoint = *Lf* locus) was rejected based on the observed behavior of *Lf* in both experimental classes--T8-12 and AG195.

Genomic Effects of *Crp* and T8-12 Mutants

After separating T8-12 and *Crp*, three theoretical questions concerning genomic effect were formulated. First, I asked if the interaction of mutant gene with mutant chromosome is similar across all variable classes--whole plant morphology, reproductive function, and bracteole development (H_0 : $\text{significance}_{\text{index}} = \text{significance}_{\text{pollen}} = \text{significance}_{\text{filling ratio}}$)? I used two-way, completely randomized analysis of variance--run on the testcross index/pollen/bract subset data--followed by Duncan's multiple range test to test this hypothesis. The results, summarized in Table 13 (and Appendix D, Tables D20-D22), indicate that the chromosome x gene interaction was statistically significant only for the mean filling ratio (FRat) value ($P = 0.0409$). Chromosome effects and gene effects were highly significant for morphologic index and for filling ratio; only chromosome effects were significant for viable pollen ($P_{\text{gene}} = 0.1558$).

Table 12. Summary of phenotype and viable pollen % data from an allelism test between the T8-12 breakpoint and Leaf fleck (*Lf*) locus in *Gossypium hirsutum*.

Line and Leaf class	Phenotypes		Viable pollen %			
	Expected number (H_0) ¹	Observed number	n ²	Range	Mean	C.V. ⁴
T8-12 Normal	0	11	10	84-56	72.11 (9.433) ³	13.08
T8-12 <i>Lf</i>	33	22	11	79-64	72.36 (4.611)	6.37
AG 195 Normal	14	13	8	96-89	92.63 (2.615)	2.82
AG 195 <i>Lf</i>	0	1	1	[90]		
Total	{47}	47	29			

¹ H_0 : T8-12 breakpoint = *Lf* locus.

² Number of plants examined.

³ Values in parentheses are standard errors.

⁴ Coefficient of variation.

The second question asked if the mutant gene/mutant chromosome interaction was similar in *Crp* homozygotes and *Crp*/T8-12 heterozygotes (H_0 : $\text{significance}_{\text{index}} = \text{significance}_{\text{pollen}} = \text{significance}_{\text{filling ratio}}$). The results of the ANOVA and Duncan's test (Table 13) of this hypothesis indicate that the chromosome x gene interaction was significant for mean filling ratio and for morphologic index. Chromosome effects and gene effects were significant for morphologic index and filling ratio; again, only chromosome effects were significant for viable pollen ($P_{\text{gene}} = 0.6524$).

The third question addressed the comparative interaction between mutation type and dosage level for all variable classes among all plant groups--experimental and normal (H_0 : $\text{significance}_{\text{index}} = \text{significance}_{\text{pollen}} = \text{significance}_{\text{filling ratio}}$). I tested this hypothesis using two-way ANOVA with mutation type (chromosome/gene) and dosage level (none/one/two) as factors; means were separated by Duncan's multiple range test. Results (Table 13) indicate that dosage and mutation type--the main effects--were significant for all classes of variable. Interaction (dose x type) was significant for index and viable pollen but not for filling ratio ($P_{\text{FRat}} = 0.0702$). In summary, data on comparative genomic effects support the conclusion that T8-12 and *Crp* are separate genetic entities.

Table 13. Mean morphologic index, percent viable pollen, and filling ratio values from three theoretical type x dosage interaction tests.

Effect	Treatment number	Morphologic index	Viable pollen %	Filling ratio
<i>Among testcross groups:</i>				
Chromosome	1	9.33 a ¹	96.58 a	63.84 a
	2	7.04 b	72.58 b	58.83 b
Gene	1	8.46 a	85.67 a	63.11 a
	2	7.92 b	83.51 a	59.56 b
Interaction ²		ns ³	ns	*
<i>Between Crp heterozygotes and homozygotes:</i>				
Chromosome	1	7.58 a	97.08 a	63.84 a
	2	7.04 a	72.58 b	60.69 b
Gene	1	8.42 a	85.92 a	65.27 a
	2	6.21 b	83.75 a	59.25 b
Interaction ⁴		***	ns	**
<i>Among all plant groups:</i>				
Dosage	1	9.61 a	98.29 a	65.95 a
	2	8.13 b	84.25 b	60.81 b
	3	5.25 c	75.17 c	57.22 c
Type	1	8.13 a	96.58 a	61.91 a
	2	7.39 b	76.53 b	60.31 b
Interaction ⁵		**	***	ns

¹ Within effects, means within columns with the same letter are n.s.d.

² Significance level of interaction tested; see Appendix D, Table D20

³ Significance levels: n.s. = $P > .05$; * = .05; ** = .01; *** = .001

⁴ Significance level of interaction tested; see Appendix D, Table D21

⁵ Significance level of interaction tested; see Appendix D, Table D22

CHAPTER 4

DISCUSSION

The distinctive "*crumpled-mottled*" mutant phenotype observed in T8-12 homozygotes, and associated with a characteristic "frying-pan" cytogenetic marker in T8-12 heterozygotes, was previously reported in the *Gossypium hirsutum* literature to be tightly bound to the T8-12 translocation--and had never been separated from the interchange. Recognition of a similar, essentially overlapping phenotype among homozygous Crumpled (*Crp*) plants suggested two testable hypotheses about the homozygous T8-12 mutant phenotype: (1) the homozygous phenotype was identical with the homozygous *Crp* genotype; and (2) the homozygous T8-12 phenotype could be a position effect activated during formation of the interchange chromosome. The simplest, most heuristic hypothesis to test is the first: if the overlap in phenotype between homozygous T8-12 and homozygous *Crp* plants extends to the level of the genotype, then the mutant T8-12 locus should be identical with the *Crp* locus ($H_0: T8-12 = Crp$).

The focus of this project was on gathering critical information about comparative male reproductive ability in *Crp* and T8-12 plants--data that would permit characterization of the "*crumpled-mottled*" phenotype. Use of the Gwyn/Stelly (1989) modification of Heslop-Harrison and Heslop-Harrison's (1970) fluorescein diacetate fluorochrome reaction (FCR) greatly facilitated investigation of the T8-12/*Crp* relationship by obviating knowledge of the *Crp* map location. Although removing much of the pleasure associated with examining hundreds of metaphase I pollen mother cells,

use of the FCR technique also reduces significantly the amount of time involved in a project of this magnitude--and generates informative reproducible data (Stelly et al., 1990).

In this research, I specifically addressed four questions related to genomic effects of the T8-12 translocation: (1) Is the mutant phenotype "*crumpled-mottled*" caused by the breakpoint in chromosome 12? (2a) Does the "*crumpled-mottled*" trait segregate independently of the translocation? (2b) Are T8-12 heterozygotes variable in phenotype? (3) Is the T8-12 breakpoint allelic with the *Crp* locus? (4) Is the T8-12 breakpoint allelic with the semilethal locus *Lf* (Leaf fleck) on chromosome 12L? The following discussion is organized around these four questions.

Preliminary investigation involving the homozygous control lines established that each of the thirteen assessment variables was normally distributed (Table 4); subsequent testing of each experimental population was also done to establish normality of distribution (Tables 7 and 10). Demonstration of normality was a significant finding, biologically and biostatistically, because of the limiting fundamental assumptions in the analysis of variance (ANOVA) procedure: independence, homogeneity of variance, normality, and additivity.

The morphologic index ("Index") variable was used to score all plants ($n = 821$) involved in the various experiments; randomly selected plants ($n_{\text{total}} = 404$), including representatives from each control and experimental population, were examined by FCR to assess viable pollen status. The early discovery of reciprocal differences in index and

viable pollen between the homozygous T8-12 and *Crp* control lines was important. Fortuitously, small sample size for the T8-12 and *Crp* lines did not obscure the limited--but significant--differences in viable pollen production (Table 5) that proved to be crucial for separating the T8-12 and *Crp* classes in the testcross experiment (Table 8).

Development of bracteole variables provided a significant, largely independent group of measurements that proved useful in distinguishing among the homozygous controls and heterozygous testcross populations (Tables 6 and 11). Tooth length (L_{tooth}), tooth surface area (TSA), and filling ratio (FRat) variables yielded especially important insights into differential bracteole morphology. While assessment of viable pollen status proved to be the test of choice in nearly every experimental situation, the coupling of pollen assessment with filling ratio provided a very robust approach when handling small samples drawn from the testcross populations (e.g., testcross index/pollen/bracteole subset).

The T8-12 backcross population yielded no experimental surprises and corroborated published reports that FCR is capable of detecting cytogenetically induced semisterility in *G. hirsutum*. The backcross data unequivocally demonstrated that the heterozygous T8-12 phenotype can be separated from that of TM-1 by use of either morphologic index or viable pollen analysis. The scoring data fit easily to the expected 1:1 backcross ratio, clearly invalidated H_0 , and provided information to answer--in part--the first experimental question ("Is the mutant phenotype "*crumpled-mottled*" caused by the breakpoint in chromosome 12?"). Testcross plants scored into two phenotypic

classes: "normal" (TM-1 phenotype) and "translocation" (T8-12 heterozygote phenotype); no "*crumpled-mottled*" phenotypes appeared in the population ($n = 175$). If the "*crumpled-mottled*" phenotype were attributable to the T8-12 breakpoint, then either (a) a third phenotypic class should have appeared in the population *or* (b) all T8-12 heterozygotes should have been "*crumpled-mottled*" as well as "translocation". The answer to research question one appears to be--from the backcross data--no.

The T8-12 F_2 population provided substantiating data for the answer to question one--and a single surprising finding. The extensive failure of T8-12 homozygotes to produce, under both field and winter greenhouse conditions, flowers adequate for FCR analysis was not fully expected. Previous reports in the literature (e.g., Gwyn and Stelly, 1989; Brown, 1980) have commented on the late-blooming and pollen abortion traits of T8-12 heterozygotes and homozygotes, but none has described the extensive failure noted in this study.

The F_2 data demonstrated that the TM-1, T8-12 heterozygote, and T8-12 homozygote phenotypes can easily be separated using morphologic index. On an assumption that the mean viable pollen value (98.37%) for the F_2 T8-12 homozygotes is biologically valid, the F_2 data indicate that T8-12 heterozygotes can be separated from TM-1 homozygotes and T8-12 homozygotes--but neither F_2 homozygote can be distinguished solely on the basis of viable pollen. The scoring data obtained from the T8-12 F_2 population fit easily to the anticipated 1:2:1 phenotypic ratio, clearly invalidated H_0 , and provided additional information to answer the first experimental question ("Is the

mutant phenotype "*crumpled-mottled*" caused by the breakpoint in chromosome 12?"). The F₂ plants scored into three phenotypic classes: "normal" (TM-1 phenotype), "translocation" (T8-12 heterozygote phenotype), and a "dwarf" (T8-12 homozygote phenotype) class that was not significantly different from the control T8-12 homozygotes in either mean morphologic index or mean viable pollen. If the "*crumpled-mottled*" phenotype were attributable solely to the T8-12 breakpoint, then either (a) a fourth phenotypic class should have appeared in the population *or* (b) all plants carrying the T8-12 interchange should have been "*crumpled-mottled*". The answer to research question one appears to be no--based on data obtained from both the F₂ population and the backcross population.

The backcross data, when coupled with the F₂ data, also provide answers to both parts of the second research question: (a) Does the "*crumpled-mottled*" trait segregate independently of the translocation? (b) Are T8-12 heterozygotes variable in phenotype? Part (a) is addressed by the data discussed for question one: If "*crumpled-mottled*" were due to the T8-12 breakpoint, then both backcross and F₂ populations should have scored for an additional phenotypic class--or else the observed phenotypic ratios should have been significantly different. Part (b) is addressed in the following observation: While T8-12 heterozygotes vary in type and severity of morphologic deformity (secondary to the amount of duplication / deficiency present in the genome), all T8-12 heterozygotes have clearly identifiable translocation chromosome configurations--and none exhibit the "*crumpled-mottled*" phenotype associated with T8-12 (and *Crp*) homozygotes. The

testcross experiment was designed to address the third research question: Is the T8-12 breakpoint allelic with the *Crp* locus? The fundamental null hypothesis tested in this project (H_0 : T8-12 = *Crp*) also addresses this third question and constitutes the critical test missing from prior studies in the literature.

The unexpected discovery of four phenotypic classes--rather than two--among the testcross plants argues strongly for the independence of *Crp* and T8-12. The data indicate that *Crp* and the T8-12 breakpoint are not allelic--nor are they located sufficiently close to each other so as to function as pseudoalleles. If this interpretation is correct, then the mutant phenotype ("*crumpled-mottled*") associated with T8-12 homozygotes is not likely to result from a position effect activation.

The testcross data demonstrated that TM-1, T8-12, and T8-12/*Crp* classes could be distinguished on the basis of mean morphologic index alone but that the *Crp* class was not fully separable when only mean index was considered. Viable pollen % was successful in discriminating among the *Crp*, T8-12 heterozygote, and *Crp*/T8-12 heterozygote classes. Distinction among the four testcross classes--TM-1, *Crp*, T8-12, and *Crp*/T8-12--was complete when index and viable pollen measures were jointly considered. The data obtained from the bracteole measurements--especially that data generated by interclass comparison of tooth length, tooth surface area, and filling ratio--was particularly robust and provided independent evaluation of the relationships demonstrated by the index and viable pollen variables. The segregation data obtained

from the testcross population--data that initially displayed a very poor fit to the expected ratio of 1:1 (Table 9)--fit easily to a revised expected ratio of 1:1:1:1 (Table 9). The fit to a 1:1:1:1 ratio provides additional strong evidence for the independence of *Crp* and the T8-12 breakpoint. If *Crp* were allelic with (or linked to) the T8-12 breakpoint--or if the breakpoint activated the *Crp* locus via a position effect phenomenon--then all testcross plants should have scored into two classes with approximately equal frequencies: (1) T8-12 heterozygote with *Crp*; and (2) TM-1 without *Crp*.

The last research question addressed a possible allelic relationship between the T8-12 breakpoint and the semilethal locus Leaf fleck (*Lf*) on the long arm of chromosome 12. The results obtained during this portion of the experiment (Table 12) indicate unequivocally that the *Lf* locus is not allelic with the T8-12 breakpoint: *Lf* segregates independently of T8-12 and, as demonstrated by the AG195 results, is capable of crossing-over.

Theoretical constructs of the genomic effects of *Crp* and the T8-12 breakpoint (Table 13) indicate that for the testcross subset analyzed ("index/viable pollen/bracteoles") both main effects--chromosome and gene--were significant influences on mean index and mean filling ratio (FRat) but only chromosome effect significantly influenced mean viable pollen. Such an outcome is consistent with the overall results gained from this project. Multiplicative interaction of chromosome x gene effects were limited to influence on mean filling ratio. The interactive effects of chromosome x gene appear to increase with increasing dosages of both chromosome (T8-12) and gene (*Crp*)--

an outcome consistent with the interpretation that *Crp* and T8-12 are separate genetic entities. Interaction can be due to a variety of causes; most frequently it results from synergism or interference--an inherent property of a given natural system (in this instance, the genome of *G. hirsutum* T8-12/*Crp* heterozygotes).

CHAPTER 5

CONCLUSIONS

Thirteen morphologic and reproductive variables were investigated in nine experimental populations and three homozygous control lines. The data obtained from backcross, F_2 , and testcross experiments frequently invalidated the null hypothesis of equality between the *Crp* locus and the T8-12 breakpoint.

Segregation data--and their fit to expected phenotypic ratios--presented strong arguments for the independence of T8-12 and the *Crp* locus. The T8-12 breakpoint was shown to be independent of both the *Crp* and *Lf* loci.

Assessment of viable pollen status among the experimental classes proved to be a valid predictor of cytogenetically-induced semisterility. The validity of the fluorescein diacetate-based fluorochrome reaction was confirmed for allotetraploid *Gossypium hirsutum*--and was extended for use in discerning depressed pollen viability among mutant single gene lines.

APPENDIX A

**MORPHOLOGIC INDEX
USED TO SCORE BACKCROSS, TESTCROSS, AND F₂ PLANTS
UNDER FIELD CONDITIONS**

Morphologic Parameter (Relative) ¹	Phenotype Description	Maximum Normal Value ²
Bract Width	Normal or Narrowed	2
Petal Length	Normal or Attenuated	2
Leaf Shape Margins and Surface Texture	Normal/Deep Lobes/Smooth Narrow/Shallow Lobes/ Crumpled	1
Leaf Base Depth	Normal or Shallow	1
Sympodial Branch Length	Normal or Shortened	2
Internode Distance/ Plant Height	Normal or Shortened/Dwarfed	2

¹ Relative to the parameter in Texas Marker-1 (TM-1)

² Total MNV for TM-1 = 10

APPENDIX B

FLUOROCHROME REACTION METHOD
USED TO ASSESS POLLEN GRAIN VIABILITY
IN EXPERIMENTAL CYTOTYPES

REAGENTS

Stock Solution No. 1

1.75 M Sucrose	$C_{12}H_{22}O_{11}$	F.W. = 342.3
3.23 mM Boric Acid	H_3BO_3	F.W. = 61.83
3.05 mM Calcium Nitrate	$Ca(NO_3)_2$	F.W. = 236.2
3.33 mM Magnesium Sulfate	$MgSO_4$	F.W. = 120.4
1.98 mM Potassium Nitrate	KNO_3	F.W. = 101.1
Distilled Water q.s. to volume	H_2O	

Stock Solution No. 2

7.21 mM Fluorescein Diacetate	$C_{24}H_{16}O_7$	F.W. = 416.4
Acetone q.s. to volume	$(CH_3)_2CO$	

Working Solution

Using a disposable Pasteur pipette, add 12-15 drops of Stock Solution No. 2 to 10 ml of Stock Solution No. 1. The composite solution will turn slightly, but persistently, cloudy in appearance. Bring the cold Working Solution quickly to room temperature by rotating the graduated cylinder between the palms. Mix the Working Solution thoroughly prior to use. For optimal results, fresh Working Solution should be prepared every 30 minutes. Do not cross-contaminate the Stock Solutions!

PROTOCOL

1. Harvest a properly prepared *Gossypium hirsutum* bud.
2. Examine the bud for evidence of pollinator attack; discard any bud whose cellulose acetate seal is not intact.
3. Place the bud into a coin envelope which has been previously labelled.
4. Immediately place the sealed envelope into a portable ice chest containing artificial ice.
5. Transport the harvested buds to the laboratory as soon as possible.
6. At the laboratory bench, remove the cellulose acetate bound corolla tip and approximately 60% of the petals.
7. Dust a generous amount of mature pollen onto a glass slide. Using a disposable glass Pasteur pipette, gently add 3-5 drops of Working Solution to the pollen and then carefully apply a glass coverslip.
8. Allow the prepared slide to remain undisturbed at room temperature for five minutes prior to examination.
9. Examine the slide under ultra-violet light (460 nm), at 200X magnification, for evidence of pollen viability. Observe appropriate safety precautions! Normal, viable pollen grains will fluoresce brightly. Abnormal grains will exhibit greatly reduced fluorescence and varying amounts of structural abnormality.
10. Count at least 300 pollen grains from each flower examined; examine a minimum of 3-5 flowers per plant.

APPENDIX C

DERIVATION OF BRACTEOLE SURFACE AREA FORMULAE

PRELIMINARY OBSERVATIONS

Initial observations, made during the Summer 1990 field season, indicated that demonstrable differences in bracteole ("bract") width and petal height existed between the genetic standard (TM-1) and the T8-12 lines used in this project (Table 1). TM-1 bracts were consistently found to be longer and to develop greater basal widths than T8-12 bracts. Petals from TM-1 flowers were tall, showy, and extended well above the bract teeth at anthesis. Bracts obtained from T8-12 flowers at anthesis had shorter, narrower teeth; maximum width was narrowed in comparison with TM-1 bracts. Petals from T8-12 flowers were frequently attenuated in height, often extending only slightly beyond the bract teeth. The anticipated usefulness of a robust supplemental indicator for chromosomal cytotype warranted the effort to develop bract measures. Accordingly, data on bract morphology were collected during Summer 1990, empirical formulae were derived from this material, and the formulae were applied to the bract data collected during 1991.

During Summer 1990 field season, bracteoles from 100 TM-1 flowers were collected, pressed, and measured for five primary characteristics: number of teeth, total length, basal length, tooth length, maximum width, and greatest dimensions. Each bract was traced, and individual tracings were combined into a single composite outline.

Bracts from 100 T8-12 flowers were assessed using identical procedures. The composites were used to develop the bracteole characteristics given in Table 3. Six characteristics proved to be informative: three primary measurements (Total Length, Basal Length, Tooth Length), one derived surface area measurement (Tooth Surface Area), and two derived ratios (Bract Ratio, Filling Ratio).

BRACTEOLE SURFACE AREA FORMULAE

On the assumption that the TM-1 line represented the normal bract condition, the TM-1 composite was used as the basis for developing the derived bracteole characteristics (Table 3). Any deviation from the TM-1 values for a given derivative characteristic should be a function of the cytotype of the deviant plant.

The formulae were derived in the following manner. First, the composite bract was partitioned into regions as shown in Figure C-1.

Composite Bract	Bract Region	Surface Area Formula	Total Bract Surface Area
	1	$TSA = (L_{tooth} * W_{max}) * 0.25$	BrSA = (TSA + BSA)
	2a	$BSA = (L_{basal} * W_{max}) * 0.80$	
	2b		

Figure C-1. Method of partitioning TM-1 composite bract into anatomic regions for derivation of surface area formulae.

Second, Region 1 ("Tooth Surface Area") occupancy was determined as follows:

(1) the theoretical rectangle was divided into halves as shown in the composite bract diagram; (2) one half was found to be entirely empty; (3) the other half was approximately 50% filled; therefore, (4) occupancy was calculated to be about equal to $[(L_{\text{tooth}} * W_{\text{max}}) * 0.25]$ since the filled area of Region 1 was estimated to be $(0.5 * 0.5)$.

Third, Region 2 ("Basal Surface Area") occupancy was assessed as follows: (1) the rectangle defined by $(L_{\text{basal}} * W_{\text{max}})$ was divided into equal parts ("Region 2a" and "Region 2b") as shown in Figure AIII-1; (2) Region 2a was then divided horizontally into quarters; (3) quarters two and three were filled, quarters one and four approximately 50% filled; therefore, (4) Region 2a occupancy was calculated as $[(L_{\text{basal}} * W_{\text{max}}) / 2] * 0.75]$ since the filled area was estimated to be $(0.125 + 0.25 + 0.25 + 0.125) = 0.75$.

Next, Region 2b was (1) divided horizontally into eight equal sectors (eighths); (2) sectors four and five were each subdivided vertically into four equal subsectors (thirty-seconds); (3) sectors two, three, six, and seven were filled $\{4/8 = 0.5\}$; (4) sectors one and eight were approximately 50% filled $\{1/8 = 0.125\}$; (5) of the eight subsectors (thirty-seconds) in sectors four and five, six were fully occupied and two were approximately 50% filled $\{7/32 = 0.21875\}$; therefore, (6) Region 2b occupancy was calculated as $[(L_{\text{basal}} * W_{\text{max}}) / 2] * 0.84375]$ since the filled area was estimated to be $(0.5 + 0.125 + 0.21875) = 0.84375$.

Finally, the total occupancy ("Basal Surface Area") for Region 2a plus Region 2b

was calculated:

$$\begin{aligned} \text{BSA} &= [\{ (L_{\text{basal}} * W_{\text{max}}) / 2 \} * 0.75] + \\ &\quad [\{ (L_{\text{basal}} * W_{\text{max}}) / 2 \} * 0.84375] \\ &= [(L_{\text{basal}} * W_{\text{max}}) * \{ (0.75 + 0.84375) / 2 \}] \\ &= [(L_{\text{basal}} * W_{\text{max}}) * 0.796875] \end{aligned}$$

For ease in computation, 0.796875 was rounded up to 0.80:

$$\text{BSA} = [(L_{\text{basal}} * W_{\text{max}}) * 0.80]$$

APPENDIX D

ANALYSIS OF VARIANCE TABLES

Table D1. Analysis of variance for morphologic index in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cytype	2	134.8056	67.4028	205.38	0.0000 ***
Error	27	8.8611	0.3282		
Total	29	143.6667			

Table D2. Analysis of variance for viable pollen % in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cytype	2	29.4222	14.7111	12.24	.0002 ***
Error	27	32.4444	1.2016		
Total	29	61.8667			

Table D3. Analysis of variance for bracteole total length in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cyotype	2	12.6884	6.3442	270.94	0.0000 ***
Error	14	0.3278	0.0234		
Total	16	13.0162			

Table D4. Analysis of variance for bracteole basal length in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean squares	F value	P > F Level
Cyotype	2	1.8659	0.9329	153.40	0.0000 ***
Error	14	0.0851	0.0061		
Total	16	1.9511			

Table D5. Analysis of variance for bracteole tooth length in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cyotype	2	5.0298	2.5149	322.61	0.0000 ***
Error	14	0.1091	0.0078		
Total	16	5.1389			

Table D6. Analysis of variance for bracteole tooth surface area in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cyotype	2	7.8564	3.9282	439.91	0.0000 ***
Error	18	0.1607	0.0089		
Total	20	8.0171			

Table D7. Analysis of variance for bracteole bract ratio in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cyotype	2	454.5895	227.2948	181.63	0.0000 ***
Error	18	22.5260	1.2514		
Total	20	477.1155			

Table D8. Analysis of variance for bracteole filling ratio in homozygous control lines of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cyotype	2	477.1027	238.5513	190.73	0.0000 ***
Error	18	22.5128	1.2507		
Total	20	499.6155			

Table D9. Analysis of variance for morphologic index in homozygous TM-1 experimental populations of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Population	3	2.8592	0.9531	3.73	0.0120 ns
Error	225	57.4399	0.2553		
Total	228	60.2991			

Table D10. Analysis of variance for viable pollen % in homozygous TM-1 experimental populations of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Population	3	12.9386	4.3129	2.26	0.0851 ns
Error	111	211.5483	1.9058		
Total	114	224.4869			

Table D11. Analysis of variance for morphologic index in the T8-12 F₂ experimental population of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Copy number	3	589.0243	196.3414	380.87	0.0000 ***
Error	273	140.7319	0.5155		
Total	276	729.7562			

Table D12. Analysis of variance for morphologic index among heterozygous and homozygous T8-12 experimental *Gossypium hirsutum* populations.

Source	df	Sum of squares	Mean square	F value	P > F Level
Copy number	5	160.4297	32.0859	32.22	0.0000 ***
Error	390	388.3802	0.9958		
Total	395	548.8099			

Table D13. Analysis of variance for morphologic index in homozygous T8-12 experimental populations of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Population	1	0.2708	0.2708	0.5063	0.4793 ns
Error	65	34.7739	0.5349		
Total	66	35.0447			

Table D14. Analysis of variance for viable pollen % in the T8-12 F₂ experimental population of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Copy number	3	14044.8056	4681.6018	232.57	0.0000 ***
Error	130	2616.8436	20.1295		
Total	133	16661.6492			

Table D15. Analysis of variance for viable pollen % among heterozygous and homozygous T8-12 experimental *Gossypium hirsutum* populations.

Source	df	Sum of squares	Mean square	F value	P > F Level
Copy number	5	5337.8531	1067.5706	21.55	0.0000 ***
Error	179	8867.9306	49.5415		
Total	184	14205.78			

Table D16. Analysis of variance for viable pollen % in homozygous T8-12 experimental populations of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Population	1	0.7066	0.7066	0.6238	0.4344 ns
Error	39	44.1714	1.1326		
Total	40	44.8780			

Table D17. Analysis of variance for morphologic index in the heterozygous T8-12 testcross population of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cytype	4	376.0719	94.0179	108.66	0.0000 ***
Error	301	260.4354	0.8652		
Total	305	636.5073			

Table D18. Analysis of variance for morphologic index among heterozygous and homozygous *Crp* populations in *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cytype	6	122.6792	20.4465	26.9429	0.0000 ***
Error	244	185.1674	0.7588		
Total	250	307.8466			

Table D19. Analysis of variance for viable pollen % in the heterozygous T8-12 testcross population of *Gossypium hirsutum*.

Source	df	Sum of squares	Mean square	F value	P > F Level
Cyotype	4	16501.2569	4125.3142	131.27	0.0000 ***
Error	135	4242.4288	31.4254		
Total	139	20743.6857			

Table D20. Analysis of variance for chromosome x gene interaction among testcross groups.

Source	df	Sum of squares	Mean square	F value	P > F value
Chromosome	1	31.5104	31.5104	155.93	0.0000 ***
Gene	1	1.7604	1.7604	8.71	0.0079 **
Chromosome x Gene	1	0.0938	0.0938	0.46	0.5036 ns
Error	20	4.0417	0.2021		
Total	23	37.4063			

Matrix for Table D20:

		Chromosome effect	
		0	1
Gene Effect	0	1,1 TM-1/TM-1 TC "Normals" Normal phenotype Normal viable pollen	1,2 TM-1/T8-12 TC "T8-12" Abnormal phenotype Abnormal viable pollen
	1	2,1 <i>Crp</i> /TM-1 TC "Crumpled" Abnormal phenotype Normal viable pollen	2,2 <i>Crp</i> /T8-12 TC " <i>Crp</i> + T8-12" Abnormal phenotype Abnormal viable pollen

Table D21. Analysis of variance for chromosome x gene interaction among testcross groups: % viable pollen.

Source	df	Sum of squares	Mean square	F value	P > F value
Chromosome	1	3456	3456	266.87	0.0000 ***
Gene	1	28.1667	28.1667	2.17	0.1558 ns
Chromosome x Gene	1	2.6667	2.6667	0.206	0.6549 ns
Error	20	259	12.95		
Total	23	3745.8334			

Table D22. Analysis of variance for chromosome x gene interaction among testcross groups: filling ratio.

Source	df	Sum of squares	Mean square	F value	P > F value
Chromosome	1	150.2392	150.2392	107.5525	0.0000 ***
Gene	1	75.0585	75.0585	53.7325	0.0000 ****
Chromosome x Gene	1	6.6705	6.6705	4.7753	0.0409 *
Error	20	27.9379	1.3969		
Total	23	259.9061			

Table D23. Analysis of variance for chromosome x gene interaction among *Crp* homozygotes, *Crp*/T8-12 heterozygotes, T8-12/TM-1 heterozygotes, and TM-1 homozygotes: morphologic index.

Source	df	Sum of squares	Mean square	F value	P > F value
Chromosome	1	1.7604	1.7604	4.62	0.0441 *
Gene	1	29.2604	29.2604	76.75	0.0000 ***
Chromosome x Gene	1	19.2604	19.2604	50.52	0.0000 ***
Error	20	7.625	0.3813		
Total	23				

Table D24. Analysis of variance for chromosome x gene interaction among *Crp* homozygotes, *Crp*/T8-12 heterozygotes, T8-12/TM-1 heterozygotes, and TM-1 homozygotes: filling ratio.

Source	df	Sum of squares	Mean square	F value	P > F value
Chromosome	1	59.5563	59.5563	68.07	0.0000 ***
Gene	1	217.3945	217.3945	248.47	0.0000 ***
Chromosome x Gene	1	12.2355	12.2355	13.98	0.0013 **
Error	20	17.4989	0.8749		
Total	23	306.6852			

Table D25. Analysis of variance for dosage level x mutation type interaction among TM-1 homozygotes, *Crp*/TM-1 heterozygotes, T8-12/TM-1 heterozygotes, *Crp* homozygotes, and T8-12 homozygotes: morphologic index.

Source	df	Sum of squares	Mean square	F value	P > F value
Dosage	2	124.9666	62.4833	192.94	0.0000 ***
Type	1	5.1579	5.1579	15.93	0.0004 ***
Dosage x Type	2	5.3808	2.6904	8.31	0.0012 **
Error	32	10.3631	0.3238		
Total	37	145.8684			

Table D26. Analysis of variance for dosage level x mutation type interaction among TM-1 homozygotes, *Crp*/TM-1 heterozygotes, T8-12/TM-1 heterozygotes, *Crp* homozygotes, and T8-12 homozygotes: % viable pollen.

Source	df	Sum of squares	Mean square	F value	P > F value
Dosage	2	3546.6209	1773.3105	294.71	0.0000 ***
Type	1	3820.0263	3820.0263	634.86	0.0000 ***
Dosage x Type	2	2654.1999	1327.0999	220.55	0.0000 ***
Error	32	192.5476	6.0171		
Total	37	10213.3947			

APPENDIX E
CORRELATION COEFFICIENTS

Table E1. Product moment correlation coefficients and independence test values for covarying pairs of variables in three homozygous *Gossypium hirsutum* control plant lines.

Line	Variable ¹ pairs	Correlation test			Independence tests				
		n-2	r value	P ⁶	df	X ² value	P	G value	P
TM-1	Index ² VP ³	16	0.81 (0.146) ⁴	4.2E-5 *** ⁵	6	27.0	1.5E-4 ***	21.56	0.0015 **
	L _{tooth} TSA	50	0.87 (0.069)	8.7E-7 ***	45	109.9	2.3E-5 ***	94.66	2.2E-5 ***
	L _{tooth} Brat	50	-0.77 (0.089)	1.7E-9 ***	50	74.46	0.0142 *	73.10	0.018 *
	L _{tooth} Frat	50	-0.72 (0.098)	2.4E-9 ***	30	55.62	0.0031 **	56.59	0.0023 **
	BRat FRat	50	0.97 (0.033)	3.4E-8 ***	60	237.1	6.7E-9 ***	147.1	2.8E-9 ***
C _{rp}	Index VP	4	0.91 (0.215)	0.0136 *	20	24.0	0.2414 ns ⁵	18.73	0.5395 ns
	L _{tooth} TSA	45	0.75 (0.099)	1.5E-9 ***	42	99.05	1.6E-6 ***	44.39	0.3714 ns
	L _{tooth} BRat	45	-0.88 (0.069)	2.4E-6 ***	56	131.1	5.9E-8 ***	81.44	0.0148 *
	L _{tooth} FRat	45	-0.83 (0.084)	6.8E-5 ***	70	123.9	7.6E-5 ***	72.21	0.4046 ns
	BRat FRat	45	0.92 (0.058)	2.3E-9 ***	40	143.8	1.3E-9 ***	108.6	2.9E-8 ***

Table E1. --Continued

Line	Variable ¹ pairs	Correlation test			Independence tests				
		n-2	r value	P ⁶	df	X ² value	P	G value	P
T8-12	Index ² VP ³	4	-0.25 (0.485) ⁴	0.6379 ns ⁵	20	19.5	0.4896 ns	15.96	0.7194 ns
	L _{tooth} TSA	45	0.96 (0.041)	1.6E-7 ***	35	92.87	3.9E-7 ***	80.24	2.1E-5 ***
	L _{tooth} BRat	45	-0.96 (0.042)	7.8E-8 ***	49	137.3	2.6E-9 ***	93.32	1.4E-4 ***
	L _{tooth} FRat	45	-0.95 (0.045)	6.7E-8 ***	35	105.5	5.3E-9 ***	83.14	8.6E-6 ***
	BRat FRat	45	0.98 (0.021)	1.2E-9 ***	35	172.8	3.8E-9 ***	106.6	3.6E-9 ***

¹ Bracteole variables are identified by their respective acronyms (see Table 3).

² Index = morphologic index (see Appendix I).

³ VP = viable pollen % (see Appendix II).

⁴ Values in parentheses are standard errors.

⁵ Significance: ns = $P > .05$; * = $.05 > P > .01$; ** = $.01 > P > .001$; *** = $.001 > P > .0001$

⁶ E indicates "exponent" in the probability values.

Table E2. Product moment correlation coefficients and independence test values for covarying pairs of variables in the T8-12 backcross and F₂ populations.

Group	Pairs	Correlation test			Independence tests				
		n-2	r value	P	df	X ² value	P	G value	P
BC TM-1	Index ¹ VP ²	43	0.56 (0.13) ³	7.1E-5 ⁴ **** ⁵	12	19.17	0.084 ns ⁶	23.15	0.026 *
BC T8-12	Index VP	42	0.72 (0.108)	4.3E-8 ***	35	60.55	0.004 **	60.16	0.005 **
F ₂ TM-1	Index VP	30	0.63 (0.147)	2.1E-4 ***	24	37.56	0.038 *	38.09	0.034 *
F ₂ TM-1/ T8-12	Index VP	60	0.59 (0.106)	9.1E-7 ***	35	55.63	0.015 *	51.49	0.036 *
F ₂ T8-12/ T8-12	Index VP	35	0.29 (0.167)	0.096 ns	28	23.02	0.732 ns	20.93	0.828 ns

¹ Index = morphologic index (see Appendix I).

² VP = viable pollen % (see Appendix II).

³ Values in parentheses are standard errors.

⁴ E indicates "exponent" in the probability values.

⁵ Significance levels: * = .05 > P > .01; ** = .01 > P > .001; *** = .001 > P > .0001

⁶ Indicates a nonsignificant value (P > .05 that null hypothesis is correct).

Table E3. Product moment correlation coefficients and independence test values for covarying pairs of variables in four heterozygous *Gossypium hirsutum* testcross groups.

Group	Pairs ¹	Correlation test			Independence tests				
		n-2	r value	P	df	X ² value	P	G value	P
TM-1/ TM-1	Index ² VP ³	29	0.75 (0.123) ⁴	1.1E-6 ⁵ *** ⁶	12	38.67	1.2E-4 ***	43.29	2.1E-5 ***
	Ltooth TSA	47	0.82 (0.083)	4.7E-9 ***	35	79.49	2.6E-5 ***	62.59	0.003 **
	Ltooth Brat	47	-0.85 (0.076)	6.8E-10 ***	45	133.4	1.1E-9 ***	83.78	3.9E-4 ***
	Ltooth Frat	47	-0.85 (0.076)	7.1E-9 ***	45	120.8	7.4E-9 ***	74.05	0.004 **
	Brat Frat	47	0.99 (0.008)	3.4E-18 ***	81	291.6	0.0000 ***	154.2	1.7E-6 ***
TM-1/ <i>Crp</i>	Index VP	29	0.52 (0.159)	0.0029 **	14	29.38	0.0093 **	29.02	0.0104 *
	Ltooth TSA	49	0.88 (0.069)	9.2E-18 ***	35	81.41	1.5E-5 ***	63.79	0.0021 **
	Ltooth BRat	49	-0.86 (0.073)	8.3E-16 ***	64	121.3	2.1E-5 ***	90.49	0.0163 *
	Ltooth FRat	49	-0.86 (0.073)	8.5E-16 ***	72	138.6	4.1E-6 ***	98.17	0.0219 *
	BRat FRat	49	0.99 (0.015)	7.3E-18 ***	72	351.2	0.0000 ***	193.9	3.9E-9 ***

Table E3. --Continued

Group	Pairs ¹	Correlation test			Independence tests				
		n-2	r value	P	df	X ² value	P	G	P
TM-1/ T8-12	Index ² VP ³	32	0.73 (0.121) ⁴	9.5E-7 ⁵ *** ⁶	12	45.91	7.2E-6 ***	44.91	1.1E-5 ***
	Ltooth TSA	59	0.84 (0.069)	1.8E-17 ***	48	81.31	0.0019 **	78.48	0.0036 **
	Ltooth BRat	59	-0.89 (0.058)	1.9E-17 ***	25	91.75	1.5E-9 ***	87.47	7.4E-9 ***
	Ltooth FRat	59	-0.89 (0.057)	4.1E-18 ***	30	119.2	1.4E-9 ***	94.25	1.5E-8 ***
	BRat FRat	59	0.99 (0.008)	2.2E-18 ***	30	175.9	1.4E-9 ***	141.5	2.1E-9 ***
<i>Crp</i> / T8-12	Index VP	33	0.69 (0.126)	4.9E-6 ***	10	25.57	0.0044 **	23.48	0.0091 **
	Ltooth TSA	78	0.71 (0.081)	2.6E-13 ***	30	49.31	0.0146 *	54.79	0.0037 **
	Ltooth BRat	78	-0.84 (0.062)	2.4E-17 ***	72	275.1	0.0000 ***	129.1	4.3E-5 ***
	Ltooth FRat	78	-0.84 (0.062)	7.2E-17 ***	81	146.7	1.1E-5 ***	119.4	0.0036 **
	BRat FRat	78	0.99 (0.010)	3.9E-17 ***	72	382.5	0.0000 ***	252.7	6.5E-9 ***

Table E3. --Continued

¹ Bracteole variables are identified by their respective acronyms (see Table 3).

² Index = morphologic index (see Appendix I).

³ VP = viable pollen % (see Appendix II).

⁴ Values in parentheses are standard errors.

⁵ E indicates "exponent" in the probability values.

⁶ Significance levels: ns = $P > .05$ that null hypothesis is true;
* = $.05 > P > .01$; ** = $.01 > P > .001$; *** = $.001 > P > .0001$

APPENDIX F
REGRESSION TABLES

Table F1. Coefficients of multiple determination (R^2), coefficients of determination (r^2), polynomial regression equations, and F-values with their associated P-values for significantly covarying pairs of variables in three homozygous *Gossypium hirsutum* control lines.

Line	Variables ¹ (x,y)	R^2 value	r^2 value	Regression equation ⁵	F value	P
TM-1	Index, ² VP ³	0.6785	0.6561	$y = -15.5 + 21.5*x^1 + (-1*x^2)$	15.83	0.0002 *** ⁶
	Ltooth, TSA	0.7594	0.7569	$y = -0.29 + 1.15*x^1 + (-0.11*x^2)$	77.35	0.00003 ***
	Ltooth, BRat	0.6072	0.5929	$y = 84.57 + 3.59*x^1 + (-2.89*x^2)$	37.87	0.00001 ***
	Ltooth, FRat	0.5198	0.5184	$y = 59.44 + 3.35*x^1 + (-2.56*x^2)$	26.52	0.00004 ***
	BRat, FRat	0.9491	0.9409	$y = 103.44 + (-2.07*x^1) + 0.02*x^2$	456.35	0.00000 ***
Crp	Index, VP	0.7001	0.8281	$y = 83.6 + 3.3*x^1 + (-0.2*x^2)$	3.51	0.1643 ns ⁴
	Ltooth, TSA	0.5633	0.5625	$y = 0.16 + .09*x^1 + 0.15*x^2$	28.38	0.00001 ***
	Ltooth, BRat	0.7873	0.7744	$y = 102.7 + (-25.6*x^1) + 7.68*x^2$	81.43	0.00004 ***
	Ltooth, FRat	0.6951	0.6889	$y = 80.33 + (-30.13*x^1) + 9.75*x^2$	50.16	0.00002 ***
	BRat, FRat	0.8484	0.8464	$y = -13.65 + 0.68*x^1 + 0.002*x^2$	123.15	0.00001 ***

Table F1. --Continued

Line	Variables ¹ (x,y)	R ² value	r ² value	Regression equation ⁵	F value	P
T8-12	Index, ² VP ³	0.0609	0.0625	$y = 99.31 + (-0.11*x^1) + (-0.03*x^2)$	0.62	0.5431 ns ⁴
	Ltooth, TSA	0.9256	0.9216	$y = -0.05 + 0.49*x^1 + (-0.002*x^2)$	273.58	0.00001 **** ⁶
	Ltooth, BRat	0.9262	0.9216	$y = 98.91 + (-12.52*x^1) + (-1.92*x^2)$	276.01	0.00001 ***
	Ltooth, FRat	0.9071	0.9025	$y = 77.65 + (-18.99*x^1) + 1.01*x^2$	214.85	0.00002 ***
	BRat, FRat	0.9824	0.9604	$y = 90.86 + (-1.75*x^1) + 0.02*x^2$	1226.9	0.00000 ***

¹ Bracteole variables are identified by their respective acronyms (Table 3).

² Index = morphologic index (Appendix I).

³ VP = viable pollen % (Appendix II).

⁴ Indicates a nonsignificant value ($P < 0.05$).

⁵ In the regression equations the symbol ^ indicates "raised to the power of".

⁶ Significance levels are indicated as follows: * = probability that null hypothesis is correct is $0.05 > P > 0.01$; ** = $0.01 > P > 0.001$; *** = $0.001 > P > 0.0001$; **** = $P < 0.0000$

Table F2. Coefficients of multiple determination (R^2), coefficients of determination (r^2), polynomial regression equations, and F-values with their associated P-values for the covarying characteristics morphologic index and viable pollen % in nine *Gossypium hirsutum* experimental lines.

Line	R^2	r^2	Regression equation	F-value	P-value
BC TM-1 Homozygous	0.3381	0.3102	$y = 139.58 + (-10.87*x^1) + 0.68*x^2$	10.73	.0002 *** ¹
BC T8-12 Heterozygous	0.5323	0.5143	$y = -16.89 + 19.09*x^1 + (-0.89*x^2)$	23.33	.0000 ***
F ₂ TM-1 Homozygous	0.3935	0.3924	$y = 92.97 + (-0.8*x^1) + 0.13*x^2$	8.76	.0012 ***
F ₂ T8-12 Heterozygous	0.4082	0.3424	$y = -74.12 + 35.76*x^1 + (-2.06*x^2)$	19.66	.0000 ***
F ₂ T8-12 Homozygous	0.0818	0.0817	$y = 96.73 + 0.25*x^1 + 0.01*x^2$	1.42	.2554 ns ²
TC TM-1 Homozygous	0.5672	0.5647	$y = 61.42 + 6.07*x^1 + (-0.235*x^2)$	18.35	.0000 ***
TC TM-1/ <i>Crp</i> Heterozygous	0.2771	0.2677	$y = 53.26 + 8.52*x^1 + (-0.42*x^2)$	5.37	.0106 *
TC TM-1/T8-12 Heterozygous	0.5525	0.5331	$y = 103.64 + (-14.37*x^1) + 1.43*x^2$	19.14	.0000 ***
TC <i>Crp</i> /T8-12 Heterozygous	0.4843	0.4734	$y = 70.91 + (-5.14*x^1) + 0.76*x^2$	15.03	.0000 ***

¹ Significance levels: * = .05 > P > .01; ** = .01 > P > .001; *** = .001 > P > .0001

² Indicates a nonsignificant value (P > .05 that null hypothesis is correct).

APPENDIX G

FIELD DATA FROM T8-12/*Lf* ALLELISM TEST

Plant ID	Phenotype				Viable pollen %
	T8-12		AG195		
	Normal leaf	Leaf fleck	Normal leaf	Leaf fleck	
A1-3-92			+		95
A1-3a			+		
A1-4	+				74
A1-4a	+				80
A1-5 ¹		+			
A1-6		+			75
A1-6a			+		90 ²
A1-7			+		94
A1-7a ¹		+			
A1-8		+			69
A1-8a		+			64
A1-9		+			74 ²
A1-9a	+				76
A1-10 ¹		+			
A1-10a	+				84
A2-1-92 ¹		+			
A2-1a ¹		+			
A2-2			+		90 ²
A2-2a	+				70
A2-3 ¹		+			
A2-3a ¹		+			
A2-4	+				68

Phenotype

Plant ID	T8-12		AG195		Viable pollen %
	Normal leaf	Leaf fleck	Normal leaf	Leaf fleck	
A2-5-92	+				56
A2-6			+		96
A2-6a			+		
A2-7		+			71 ²
A2-8		+			78
A2-8a ¹		+			
A2-9		+			67
A2-9a		+			79
A2-10		+			76
A3-1-92			+		
A3-2			+		94
A3-2a	+				58
A3-3				+	90 ²
A3-3a	+				73
A3-4	+				
A3-4a			+		
A3-5			+		
A3-6	+				82
A3-7		+			72
A3-8		+			71
A3-8a ¹		+			
A3-9 ¹		+			
A3-10			+		93
A3-10a			+		89 ²
A4-1-92 ¹		+			

¹ Dwarf plant with marked leaf crumpling; resembles the T8-12/*Crp* testcross phenotype.

² "Hemibract" plant; Bw^h is a possible new allele of the bw locus.

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