INHERITANCE OF GLAUCOUSNESS IN WHEAT

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SUMMARY

It was found that all genetic variants for glaucousness over the whole plant involved genes on the chromosomes of group 2. Of three recessive nonglaucous lines which arose spontaneously in Chinese Spring background, all were found to be due to deletion or mutation of the gene for glaucousness on chromosome 2B.

Another recessively nonglaucous line which arose in the backcrossing of the inhibitor gene of C.W.S. 5075 into Chinese Spring background was found to have undergone a large terminal deletion of the short arm of chromosome 2B.

Chromosome 2D in Chinese Spring was found to bear a weaker gene homoeologous to that on chromosome 2B. It was found that chromosomes 2A bears no gene affecting glaucousness.

Two dominant ethyl methanesulfonate induced nonglaucous mutants were identified as alleles of the inhibitor gene on chromosome 2B.

The variety Mentana was found to have no fully effective group-2 genes for glaucousness. The recessive gene for nonglaucousness on chromosome 2B of Mentana was mapped as lying between 33 and 50 map units from the centromere.

A linkage study gave no recombinants between the Mentana gene and the inhibitor gene from 5075, giving a maximum distance of 8 map units between them. There is a strong case for thinking that both genes are alleles of the Chinese Spring gene for glaucousness.

Genes capable of interacting to modify the dominance relationship of the 2D inhibitor were found in the ABD XIII background Poso. It was found that an extra dose of chromosome 2B could interact with the genes of ABD XIII to give the same effect as the Poso genotype. Modifier genes in the backgrounds of Poso and 5075 also interacted to alter the dominance of the 2B inhibitor.

Group 3 chromosomes were found to bear genes specifically for glaucousness of the peduncle. Chromosome 3A bears the most effective gene, chromosome 3B a less effective one and chromosome 3D apparently bears a null allele. Both Poso and Mentana were found to have genes which could substitute for the chromosome 3A gene of Chinese Spring.

STATEMENT

Except where otherwise stated, all the work in this thesis is original and has not been previously submitted for any degree at this, or any other, university.

CONTENTS

List of Plates

GENERAL INTRODUCTION

The economic importance of wheat has resulted in many genetic and cytogenetic studies of the various types of wheat and their relatives. This has involved extensive investigations into the genetic structure and evolutionary history of wheat.

It has been shown that the domesticated forms of wheat are predominantly polyploids. All have the common basic chromosome number of seven. Two of the three diploid ancestors of the hexaploid bread wheats have been identified, but the identity of the third remains uncertain. In spite of this, it has been established that the three ancestral diploids were closely related and had probably diverged only slightly from a common ancestor at the time of origin of polyploidy.

Supporting the evidence from taxonomic and chromosome-pairing studies are investigations on the large number of triplicated or duplicated loci in hexaploid wheat. In these homoeologous gene systems, duplicated loci have been found to differ slightly. The general pattern seems to be for active loci to become hypomorphic, antimorphic or totally inactive.

This study has involved the glaucous character of wheat, and loci affecting this character show the pattern of duplicated loci mentioned above.

Glaucousness, the visible crystalline component of the waxes, is known in wheat to be composed mainly of β-diketones. This crystalline layer

is thought to have adaptive value under stress conditions such as drought, frost or high solar radiation. It is of interest in this context that most of the domestic wheats are at least partially glaucous. The main locus affecting glaucousness is on chromosome 2B. Evidence will be presented that a multiple allelic system exists at this locus.

Early in the history of genetics, it was considered that dominance was a property of the allele itself. It has now been shown for many organisms that dominance of an allele is a property of the whole genotype. This has been found to be the case with inhibitors of glaucousness. In their original background the two homoeologous inhibitors of glaucousness behave as dominants when crossed to Chinese Spring. However, in combination with other varieties this dominance is modified. The dominance of the inhibitor on chromosome 2D is shown to be affected by the background of both parents and by the dosage of the gene for glaucousness.

The distribution of glaucousness is not uniform over all plant parts. Variation of this kind is interesting because of the association of organ-specificity with the genetics of development. One homoeologous system of organ-specific genes for glaucousness has been located and there is some evidence suggesting the existence of other such genes.

SECTION 1 LITERATURE SURVEY

Taxonomy of the Triticinae

Linnaeus classified the subtribe Triticinae, in the tribe Hordeae L. (later renamed Triticae by Dumortier), on the basis of morphology. The extensive interspecific and intergeneric hybridization which has taken place in this subtribe has since resulted in considerable taxonomic difficulty.

It is evident that the Triticinae constitutes a complex of interrelated genotypes. Viable intergeneric hybrids are possible in many combinations within the existing classification; so that, as Mac Key (1968) points out, the whole group should be included in a single genus. This would severely limit the usefulness of a taxonomic system, as much known detail of relationships in the group would be obscured. On the other hand, it is improper, by taxonomic rules, to leave intergeneric hybrids (the tetraploid and hexaploid wheats) in the same genus as one of their parents (i.e. *Triticum monococcum* L.).

Bowden (1959, 1966) suggested incorporating the genus *Aegilops* L. into genus *Triticum* L. to overcome this. This is not a wholly satisfactory answer, as it is likely that *Aegilops* and *Triticum* will continue to diverge under the influence of man (Mac Key, 1968). In order to exploit all of the information available for this much-studied subtribe, Mac Key

believes that characteristics which would only be used to distinguish species in other families should be used to separate genera in this group. He therefore proposed the placing of the diploid wheats in a separate genus *Crithodium* Link., otherwise retaining the old *Aegilops* and *Triticum* boundaries. The chief disadvantage of this scheme is that it implies greater differences between the diploid and tetraploid wheats than actually exist (Bowden, 1959). However, it does minimize changes in the existing nomenclature. Mac Key's system of nomenclature will therefore be used in the remainder of this thesis.

Mac Key's (1966) proposals on the renaming of *Triticum spelta* L., *Triticum sphaerococcum* Perc., *T. macha* Dek. & Men. and *T. vavilovi* Jakubz. have been widely accepted. These species differ from *T. vulgare* (Vill.) Host. by only one or two major genes, and were renamed as subspecies of *T. aestivum* (L.) Thell. It is now known that many of the tetraploid species also differ by only a few genes. Mac Key (1966) has offered a solution similar to that proposed for the hexaploids. The tetraploids are therefore grouped as *T. timopheevi* Zhuk and *T. turgidum* (L.) Thell. and subdivided into various subspecies (see Table 1).

The Aneuploids of Wheat

Early workers in the field of wheat genetics found that hexaploid wheat was difficult to analyse by traditional genetic methods. Segregation

TABLE 1

Taxonomy of the Wheats

After Mac Key 1966, 1968

Crithodium Link.

Cr. aegilopoides Link.

Triticum L., emend Mac Key (nov. emend.)

Dicoccoidea Flaksb.

T. timopheevi Zhuk.

ssp. *araraticum* (Jakubz.) Mk.

ssp. *timopheevi*

T. turgidum (L.) Thell.

ssp. *dicoccoides* (Körn.) Thell.

ssp. *dicoccum* (Schrank.) Thell.

ssp. *paleocolchicum* (Men.) Mk.

ssp. *turgidum*

conv. *turgidum*

conv. *durum* (Desf.) Mk.

conv. *turanicum* (Jazubz.) Mk.

conv. *polonicum* (L.) Mk.

ssp. *carthlicum* (Nevski) Mk.

TABLE 1

Taxonomy of the Wheats (Cont'd)

After Mac Key, 1966, 1968

Speltoidea Flaksb.

T. zhukovskyi Men. et Er

T. aestivum (L.) Thell.

ssp. *spelta* (L.) Thell.

ssp. *vavilovi* (Tum.) Sears

ssp. *macha* (Dek. et Men.) Mk.

ssp. *vulgare* (Vill.) Mk.

ssp. *compactum* (Host) Mk.

ssp. *sphaerococcum* (Perc.) Mk.

ratios indicated a high level of duplication, and the discovery of the chromosome number as n=21 (Sakamura, 1918) predicted a high number of linkage groups.

Sears (1939) found viable and fertile plants with whole chromosome duplications or deficiencies among the progeny of a haploid, and realized that such aneuploids could be used to simplify genetic analysis.

Plants deficient for one entire chromosome, i.e. monosomics, have a complement of 20 bivalents and an unpaired chromosome or univalent (symbolized as 20" + 1') at metaphase I of meiosis. Plants which have an extra chromosome, i.e. trisomics, show a maximum pairing configuration of 20 bivalents and a trivalent (or association of three chromosomes) at metaphase I (20" + 1''').

The monosomic lines were crossed to tetraploid wheat and numbered in order of isolation. If the missing chromosome was homologous to one of the chromosomes present in tetraploid wheat, the line was assigned a number between I and XIV. Lines monosomic for chromosomes in the remaining hexaploid genome were given numbers XV to XXI (Sears, 1939). Eventually, all of the 21 possible monosomic lines were isolated for the variety Chinese Spring (Sears, 1944, 1953 and 1954).

On self-fertilization, monosomic plants give rise to approximately 73% monosomic offspring. Of the remainder, about 24% are disomic, that is,

euploid, and 3% are nullisomics. Nullisomic plants are deficient for both members of a homologous pair, and have only 20 bivalents. The frequencies of each class of offspring vary from line to line, and are dependent on the chromosome involved and environmental factors. Different monosomic lines give from 1% to 10% nullisomics. The transmission of the univalent through the female is about 25%, as the univalent often behaves abnormally and may be excluded from the nucleus. On the other hand, male transmission of the monosome may be as high as 96%, because of strong selection against the deficient pollen (Sears, 1953).

Four general methods of locating genes on chromosomes by use of monosomic lines were reported by Sears (1953). The first is by direct observation of the nullisomic, to see if the phenotypic character associated with a gene is lost. If the gene is duplicated it can only be located by this method if the lower dosage of the nullisomic gives a phenotypic change (Sears, 1954). Hemizygous-ineffective genes can be located by use of either monosomics or nullisomics. By definition, the phenotype associated with such genes is only expressed when two or more doses are present. The phenotype is therefore altered if the plant is monosomic for the critical chromosome.

Dominant genes in varieties other than Chinese Spring can be located by crossing the parent variety to each of the 21 monosomics in turn, and observing the F_2 families derived from monosomic F_1 plants. The F_2 family

for the critical chromosome will give 1% to 5% nullisomics, which will be the only recessive types observed in that family. This should deviate significantly from the frequency of recessive types in the non-critical F_2 families. Conversely, with hemizygous-ineffective genes in varieties other than Chinese Spring, it is only the disomic plants in the critical F_2 which will show the recessive phenotype.

If the F_2 cannot be scored, or if several genes are being located, the F_3 families derived from disomic F_2 plants can be used. The F_3 families should not segregate in the case of the critical chromosome.

Fourthly, minor genes or modifying genes can be located by making chromosome substitution lines. The monosomic F_1 plants are backcrossed, as male parent, to the monosomic line. This is continued until the desired percentage of the background genotype is that of the recurrent parent.

Chromosomes without a homologue are frequently lost or may misdivide at meiosis. Sears (1952b) measured the rate of misdivision of univalents, and found that as many as 30% of the monosomes transmitted through the egg were either a telocentric (a chromosome deficient for one arm), or an isochromosome (a chromosome with identical arms). The transmission of telocentrics was 18% of chromosomes. Isochromosomes were found to have been transmitted through 12% of the eggs.

Sears also studied the stability of these misdivision products (1952c). Telocentrics were normally stable at meiosis if a homologous whole chromosome was present with which they could pair. Pairing with a normal homologous chromosome was a little below that expected for a pair of entire chromosomes, presumably due to the lowered probability of chiasma formation in the shortened chromosome. An isochromosome paired with its normal homologue in about 50% of cells. The lowered pairing is here thought to be due to competition for pairing partners between the three homologous arms present in the cell. The isochromosome frequently pairs internally, forming a chiasma between its two arms. If this occurs, the isochromosome is not usually available to pair with the homologous arm of the normal chromosome.

Telocentric chromosomes were lost somatically more frequently than isochromosomes. Steinitz-Sears (1966) measured the stability of different telocentric lines which were marked with the virescent gene. This is a hemizygous-ineffective gene which shows a marked dosage effect and hence is an indicator of somatic chromosome loss. She found the lines to differ in rate of somatic loss of the chromosome. These differences in stability of the telocentrics were assumed to depend on the amount of centromere remaining after misdivision.

Telocentric chromosomes can be used to locate genes as to chromosome arm, and to map their distance from the centromere (Sears, 1963c). The variety

or line bearing the allele to be mapped is crossed to the appropriate ditelosomic line (having 20 bivalents and a pair of telocentrics, 20" + t"). If the gene under consideration is dominant to the Chinese Spring allele, then the F_2 involving the critical chromosome arm will include some recessive segregants with one or both chromosomes entire. Some of the dominant segregants will have a pair of telocentric chromosomes. Where the gene to be located is recessive to its Chinese Spring allele, the critical F_1 will exhibit the dominant phenotype, whereas the F_1 involving the opposite arm will exhibit the recessive phenotype.

To measure the distance of a dominant gene from the centromere, the F_1 involving the critical telocentric is backcrossed as male to a euploid line homozygous for the recessive allele. The number of plants with the recessive phenotype without a telocentric allows calculation of the recombination frequency. As there is selection against pollen bearing the telocentric, most of the recessive individuals in the BC_1 can be expected to possess an entire chromosome resulting from crossing-over (Sears, 1963c).

If the appropriate ditelosomic line is of low fertility, then the ditelomonotelosomic line can be used to obtain the F_1. This line has a pair of telocentrics for the selected arm and a telocentric univalent for the opposite arm (20" + t" + t'). It is used as a female parent, the telocentric of the disome being transmitted to all progeny, while the un-

paired telocentric is lost from most (about 75%) gametes. The F_1 is cytologically screened and those plants having a single telocentric selected. The remainder of the the procedure is identical to that involving the ditelosomic lines.

Driscoll (1964, 1966) used F_2 data for mapping. He made the assumption, based on Sears' (1963c) measurements, that equal transmission of the telocentric and the entire chromosome will occur through the female, and then estimated the male transmission of the telocentric and the recombination frequency by maximum likelihood methods.

The trisomic (20" + 1''') and tetrasomic (20" + 1'''') lines for all 21 chromosomes in Chinese Spring were isolated and described by Sears (1954). Like the monosomics, the trisomics were derived from asynaptic nullisomic-III plants and from haploids. Again, like the monosomics, most of the trisomic lines are close to the euploid in phenotype. The tetrasomic types which are derived from the trisomics usually show a greater degree of abnormality. Tetrasomics are produced in a frequency of about 1% to 10% on self-fertilization of trisomics. Of the remaining progeny about 45% were trisomic and the rest disomic (Sears, 1944 and 1954).

Genomic Structure of the Genera *Triticum* and *Crithodium*.

In 1918 Sakamura established that the einkorn, emmer and bread wheats have

7, 14 and 21 pairs of chromosomes, respectively. The allopolyploidy of wheat was further established by Kihara (1919, 1924). Sax (1922) confirmed the chromosome numbers for the three levels of ploidy, and analysed the pairing of hybrids between groups. It was left to Kihara (1924) however, to establish from the pairing configurations of hybrids that the genomic formulae of the three levels of ploidy were AA, AABB, and AABBDD. In other words, the genome of *Crithodium aegilopoides* Link (AA) was present, plus a new genome, symbolized B, in the tetraploid species of *Triticum.* Similarly, the two genomes of *Triticum turgidum* (AABB) in combination with a third (DD) make up the hexaploid wheats. Thus hybrids between *Crithodium aegiliopoides* and *Triticum turgidum* were found to have a maximum of 7 bivalents and 7 univalents at metaphase I of meiosis. Hybrids between *T. turgidum* and *T. aestivum* (L.) Thell were found to have a maximum of 14 bivalents and 7 univalents.

Origin of the Genomes of Wheat

Sax and Sax (1924) and Gaines and Aase (1926) found that hybrids between *Aegilops cylindrica* Host. (n=14) and hexaploid wheat had meiotic configurations with a maximum of 7 bivalents and 21 univalents. Hybrids between *Ae. cylindrica* and *T. turgidum* had no pairing. *Ae. cylindrica* therefore possessed the D and one other genome. *Ae. caudata* L. (CC) was found to have a genome in common with *Ae. cylindrica* (CCDD) but not with hexaploid wheat. When *Ae. squarrosa* L. was found to be the other parent of *Ae. cyl-*

indrica, it was deduced that *Ae. squarrosa* contained the D genome (Kihara, 1944, see Morris and Sears, 1967). Identity of *Ae. squarrosa* as the source of the D genome was definitely established when synthetic hexaploids were found to give regular pairing in hybrids with *T. aestivum* (McFadden and Sears, 1944 and 1946). Later, Riley and Chapman (1960) crossed *T. aestivum* by *Ae. squarrosa* and found that the hybrid had a mode of 7 bivalents. This was somewhat more pairing than had been obtained in hybrids between *T. aestivum* and *Cr. aegilopoides*, indicating that the D genome has diverged less in the hexaploid than has the A genome or the A genome has changed at the diploid level.

The identification of the ancestral donor of the B genome has proved to be a more difficult task than that of the D genome. *Aegilops speltoides* Tausch. was suggested as the B-genome contributor by Sarkar and Stebbins (1956), on the basis of Anderson's method of morphological extrapolation. The results of chromosome-pairing studies were ambiguous, however, as the S^S genome of *Ae. speltoides* appeared to pair more frequently with the A^m genome of *Cr. aegilopoides* ssp. *monococcum* than with the B genome of the tetraploid. The amphidiploid of this hybrid ($A^mA^mS^SS^S$) showed multivalents and poor fertility. The chromosomes of *Ae. bicornis* (Forsk.) Jaub. et Spach, showed good pairing in the hybrid with *T. turgidum* (Thell.) ssp. *dicoccoides* and poor pairing with the A^m genome (Sears, 1956a). Riley, Unrau and Chapman (1958) cited further evidence for *Ae. speltoides* as the progenitor of the B genome. They pointed out that both satellited chromo-

omes in hexaploid wheat occur in the B genome. The diploid ancestor of the genome must thus have two large-satellited metacentric chromosomes. Two such hromosomes were found only in *Ae. speltoides*. *Ae. bicornis* has one large- and ne small-satellited chromosomes but was rejected on morphological grounds.

mechanism restricting intergenome pairing has subsequently been found in the genome (Sears and Okamoto, 1958; Riley and Chapman, 1958) and *Ae. speltoides* s able to suppress this (Riley, Unrau and Chapman, 1958). Thus hybrids between *e. speltoides* and tetraploid or hexaploid wheat show intergenomic pairing, and the umber of bivalents is not necessarily an indication of the degree of homology between the S genome of *speltoides* and the other parent.

n 1966, Riley and Chapman studied the pairing affinities of group-5 telocentrics 'or one another in hybrids between double monotelocentrics of wheat and *Ae. peltoides*. They found that with the pairing inhibition thus suppressed, 5B nd 5D showed greater affinity for each other than 5B showed for 5S. They con-luded that there would be little or no pairing between the S and B genomes f the pairing inhibitor were not suppressed. Sears (1968) argues similarly 'rom Kimber's (1966) results. Kimber found that when BC_2 plants of *Ae. spel-oides* x *Ae. longissima* Schweinf. et Musehl. (with *speltoides* as the recurrent arent) were crossed to *T. aestivum* they gave two classes; high pairing (with he *speltoides* suppressor present) and low pairing (when the pairing inhibitor s active). The latter plants had an average of only 2.9 bivalents, although /8th of the chromosomes should have been from *speltoides*. This is only 1.7 ivalents more than are found in *T. aestivum* haploids.

ositive proof of *Ae. speltoides* as ancestor of the B genome is thus difficult o obtain and it seems that the actual progenitor may have differed considerably from present day *Aegilops* species. Sarkar and Stebbins (1956) suggested that he B genome might be a composite derivative from several species.

omoeology

t was realized (Sears, 1944, 1952 and 1954) that in many cases the phenotype f a nullisomic in hexaploid wheat showed marked resemblance to two other ullisomics. Further (Sears and Okamoto 1957, and Sears, 1966), it was found n synthesis of nullisomic-tetrasomic combinations (that is, plants nullisomic or one chromosome and tetrasomic for another) that such chromosomes could at east partially substitute for each other in their effect on fertility, vigour nd other characters. On this basis, Sears suggested that the 21 chromosomes ould be arranged in seven groups of three, compensation being possible between he three members of each group. Sears inferred that each group contained one hromosome from each genome.

s early as 1924, (Winge) it had been recognized that the chromosome sets of heat might be genetically very similar, though not identical. To describe the elationship of nonhomologous chromosomes derived from the same ancestral chromoome, Huskins (1931) coined the term "homoeologous".

he chromosomes belonging to the D genome had been identified by Sears (1944) ecause they did not pair with *T. durum* chromosomes. The assigning of the other

chromosomes to the A or B genomes was undertaken by Okamoto (1957a & b, 1962). He crossed ditelocentric lines of Chinese Spring to the amphidiploid of *Cr. boeticum* x *Ae. squarrosa* (i.e. AADD). Crosses involving a telocentric for an A-genome chromosome would therefore give a maximum pairing configuration of 13" + 7' + t1", while the hybrids involving a B-genome telocentric would give 14" + 6' + t'. This experiment succeeded in designating all of the chromosomes correctly except XIII and II. Both of these telocentrics participated in a low frequency of bivalents although both belong to group 2. Okamoto finally assigned XIII tentatively to the A genome and II to the B genome.

On the basis of Okamoto's work, Sears (1958) renumbered the chromosomes according to their group and genome. Chapman and Riley (1966) checked these designations by crossing the ditelocentric lines to *Crithodium aegilopoides* ssp. *thaoudar* (AA). By this means they confirmed Okamoto's findings except for chromosomes II and XIII which they showed to belong to the A and B genomes, respectively. With this modification Sears' (1958) nomenclature (shown in Table 2) will be used in the remainder of this thesis.

Pairing Control

Detailed studies on homoeologous gene sytems have been relatively few. Such as have been carried out, tend however, to support the concept of wheat being genetically an autopolyploid, but one which is evolved towards diversification of duplicated genes (Sears, 1963a & b). A number of these systems will be described, as they provide a basis for the subject of this thesis.

TABLE 2

OLD AND NEW CHROMOSOME DESIGNATION

	GENOME		
GROUP	A	B	D
1	XIV	I	XVII
2	II	XIII	XX
3	XII	III	XVI
4	IV	VIII	XV
5	IX	V	XVIII
6	VI	X	XIX
7	XI	VII	XXI

ne such detailed study has been carried out on the control of meiotic synapsis n hexaploid wheat. It was Okamoto (1957) who first proposed that chromosome B was concerned with synapsis, as 34-chromosome hybrids of monotelo 5BL x AADD ad much higher pairing than 35-chromosome hybrids. Riley and Chapman (1958) nd Sears and Okamoto (1958) suggested that the higher pairing was due to a roadening of the differential pairing affinities of the chromosomes rather han an increase in chiasma frequency in the absence of 5B. The evidence upporting this distinction comes from nulli 5B haploids which showed a marked ncrease in nonhomologous pairing and trivalents compared with euhaploids. This uggested homoeologous pairing. Nulli 5B (40 chromosomes) showed up to six hromosomes in one association, supporting this hypothesis. Riley and Kempnna (1963) showed that the translocations resulting from nonhomologous recombination in nulli5B-tetra5D plants can be arranged in homoeologous groups, nd, in the case of one family, the translocations were definitely between omoeologues, thus further supporting the hypothesis. Nulli 5B shows no increase in chiasma formation over the euploid (Riley, 1960); thus it is pairing rior to chiasma frequency which is affected by 5B. Riley, Chapman and Kimber 1960) showed by crossing all 21 monosomic lines to rye, that 5B is the only hromosome that imparts major suppression of homoeologous pairing. Riley (1960) urther located the effect to the long arm of 5B, as was suggested by Okamoto's 1957) original cross.

he evolution of the system is of considerable interest. In 1958, Riley, nrau and Chapman suggested, on the basis of hybrids of *T. aestivum* with *Ae.*

peltoides and *Ae. longissima*, that *Ae. speltoides* might carry a gene capable f suppressing the 5B effect in wheat. Their suggestion was based on the act that the *speltoides* (S) genome paired as well with the A genome as with he B genome, of which it was thought to be the progenitor. Riley, Kimber nd Chapman (1961) confirmed the presence of such a gene by crossing tetraploid heat to *Ae. speltoides* and *Ae. longissima*. The former hybrid gave trivalents, hereas the latter gave mainly univalents, despite the fact that hybrids be- ween the two *Aegilops* species gave virtually complete pairing, indicating ittle genomic difference between the two species. Crosses between monosomic 5B and the two *Aegilops* species showed that the presence or absence of 5B ade no difference to pairing in the *speltoides* hybrids, but gave low and igh pairing levels respectively with *longissima*. They concluded that the 5B effect had arisen soon after formation of the tetraploid (since hybrids etween tetraploid and hexaploid wheats give rise to no segregants for pair- ng), probably by antimorphic mutation of the dominant *Ae. speltoides* gene ather than the recessive *Ae. longissima* gene. The actual number of genes in- olved in the *speltoides* effect is unknown. Riley (1966b) however, crossed [*(T. aegilopoides* x *Ae. speltoides)* 2n = 28, amphidiploid x *(T. aegilopoides* x *Ae. bicornis)* 2n = 28, amphidiploid] F_1 x *T. aestivum*. This gave hybrids with big differences in pairing, suggesting few genes were involved. Kimber's (1966) results supported this. He crossed *Ae. longissima* x *Ae. speltoides*. This hybrid was backcrossed twice to *Ae. speltoides* to improve fertility then crossed to *T. aestivum*. Four of the progeny had high pairing, two had low pairing. This conforms with a 1:1 ratio but family size is too small to give

onclusive evidence for the presence of a single gene.

ulli-3B plants are partially asynaptic (Sears, 1944). Kempanna and Riley 1962) suggested that failure to form chiasmata (desynapsis) was the most ikely cause. They studied the interaction between chromosomes 5B and 3B by ·rossing the double monosomic (19" + 1' + 1') to *Secale montanum*. In the ·esulting hybrids they found that deficiency of 5B alone gave an increase in)airing and deficiency of 3B alone gave a decrease in pairing (relative to .he 28-chromosome hybrid with both 5B and 3B present). The hybrids with both :hromosomes absent showed lower pairing than those with 5B deficient, but much greater pairing than hybrids with the entire wheat complement present. Cells .n the doubly deficient hybrids fell into two classes, those fitting the pat- :ern for 3B deficiency (almost no pairing) and those which fitted the pattern)f 5B deficiency and showed a high level of synapsis. Kempanna and Riley (1962) also suggested that causal factors in pairing failure due to 3B deficiency vere closer to threshold when 5B was also absent, hence the bimodal distribution)f cells. The theory of a 5B-3B interaction is supported by the fact that liscontinuity occurs only when both are absent. They therefore suggested that 3B had evolved to overcome the disruptive effect of the 5B pairing inhibitor. An objection to this suggestion is the presence of weaker synapsis genes on 3A and 3D (Sears, 1966). The 3B gene might have evolved to a more powerful form but seems unlikely to have arisen *de novo* since the evolution of 5B. A further doubt on this interpretation is cast by the fact that it was the entire chromosome 5B which was missing. As will be described presently, evidence in-

icates a promoter of synapsis on the short arm of chromosome 5B. Thus it s difficult to say which arm of 5B is involved in the interaction with the B gene.

iley (1966) crossed monosomic 3B to various *Ae. speltoides* subspecies and ound that different parents gave different pairing levels, but the experi- ent was too limited to show a definite 3B effect.

eldman (1966) examined triisosomic 5BL and found that although synapsis was uch reduced, there were occasional multivalents, heteromorphic bivalents and nterlocked bivalents. This he interpreted as meaning that the effect of 5BL as not actually to reduce pairing affinity of chromosomes generally (as omoeologous pairing was higher than in euploids), but was to randomize the osition of chromosomes which would otherwise be lying already associated rior to meiosis. The effect of 5B deficiency would then be to allow the ssociation of homoeologues as well as homologues. Meiotic synapsis would hen consist merely of making the association sufficiently close to allow ecombination. Diisosomic 5BL showed similar response at extreme temperature o triiso 5BL at normal temperature. Feldman, Mello-Sampayo and Sears (1966) lso recorded mitotic association as they found homologues at metaphase to be loser than expected on a random model. Darvey and Driscoll (unpublished) ave since repeated Feldman's experiments and found no difference between the istribution of homologues and nonhomologues at mitotic metaphase. Other evi- ence (Driscoll and Darvey, 1970) supports Feldman's (1966) hypothesis on the

wo stages involved in pairing. They had observed (Driscoll, Darvey and Barber, 967) that colchicine treatment disrupts meiosis in a manner similar to extra oses of 5BL. In the more recent paper, pairing between arms of an isochromo- ome was compared with pairing between normal homologues in the presence and bsence of colchicine. It was found that colchicine had the ability to dis- upt pairing between normal homologous chromosomes, but not between homologous rms linked by a common centromere. This was interpreted as evidence for an arly association, which could be disrupted by colchicine, followed by more ntimate pairing.

eldman (1966) also observed that nulli 5D showed slight asynapsis with occas- onal multivalents and interlocking of bivalents. He interpreted this as howing that 5D carries a gene of opposite effect to 5B. Observations on ullisomic 5D-trisomic 5A and nullisomic 5D-tetrasomic 5A indicated a weaker ene, similar to the 5D gene, on chromosome 5A. Since the monoisosomic lines or 5AL and 5DL were normal, these genes were located on the long arms of the hromosomes.

iley (1966) showed nulli 5D-tetra 5B to be asynaptic at low temperatures and oncluded that 5D carries a gene necessary for pairing under these conditions. e suggested that this gene might be homoeologous to the 5BL gene, although pposite in effect.

iley, Chapman, Young and Belfield (1966) scored chiasma frequencies on group-5

neuploids under three temperature regimes. They confirmed 5D as having a ene for low temperature pairing, and 5A as having a weaker, similar gene; B could not compensate for 5D in this regard.

etraploid wheats show normal pairing at low temperatures. Either 5A is more ffective in tetraploids or the loss of the 5D gene is compensated for by the ower number of chromosomes. Riley et al. (1966) point out that Kerber (1964) eported no meiotic irregularity in the tetraploids extracted from 6X "Canhatch", supporting the latter theory. Kaltsikes, Evans and Larter (1969) lso found no asynapsis in such tetraploids at 21°C, though Feldman (1966) ad found nulli 5D to be asynaptic at glasshouse temperatures. Thus the stronger unction of the 5D gene may have been necessary only since addition of the D enome. Some further support for this theory comes from the meiotic instablity shown by synthetic hexaploids (Tabushi, 1964, see Riley, Chapman, Young nd Belfield, 1966).

n support of the alternative theory of a more powerful 5A gene in tetraploids, ayter and Riley (1967) found that the F_2 of 5D-deficient plants in the cross ono 5D x *T. turgidum* ssp. *dicoccum* segregated approximately 3:1 for low-emperature pairing. They concluded that Chinese Spring bears the recessive llele of a gene for low-temperature pairing in *T. turgidum* ssp. *dicoccum*. hus both theories of low-temperature pairing in tetraploids may hold.

In their paper, Riley et al. pointed out a discrepancy between Feldman's (1966) finding that diisomic 5BL was asynaptic at 15°C and their own finding that tetra 5B was normal. They suggested that 5BS might carry a promoter of synapsis.

Riley and Chapman confirmed this in 1967 by measuring chiasma frequencies in aneuploids having various doses of 5BL and 5BS.

Okamoto (1966), Riley, Chapman and Belfield (1966) and Riley (1968) have reported induced variations for the 5BL system which seem to indicate that a single gene is involved.

This system has been described in some detail as it is the most studied gene system in wheat and provides a comparison with the other homoeologous gene systems which will be considered.

Chlorophyll Mutants in Wheat

A second much-studied system of homoeologous genes are those mutants affecting either chlorophyll formation or chloroplast functions. None of the 21 nullisomics of wheat show lowered chlorophyll levels (Sears, 1954). The genes involved are therefore at least duplicated in Chinese Spring. Not surprisingly, as all of the nullisomics are normal, chlorophyll mutants rarely arise in irradiation experiments where most "mutants" are due to deletion of genes. Chlorophyll mutants occur spontaneously and from chemical treatment with mutagens such as ethyl methanesulfonate which cause predominantly intragenic changes.

Sears (1956b, 1957, 1959, 1963a & b) showed that Neatby's virescent, a spontaneous mutant on chromosome 3B, is a hemizygous-ineffective recessive gene. As three doses lead to albinism, it is an active allele (Sears, 1956b); that is, the mutant gene actively interferes with chlorophyll production or function. The normal allele has duplicates on chromosomes 3A and 3D (Sears, 1957). Plants simultaneously deficient for both the v_1 locus on 3B and chromosome 3D had normal chlorophyll, but plants deficient for both v_1 and 3A were virescent. The V_2 gene (normal allele) on chromosome 3A is thus more potent in promoting chlorophyll formation than the normal V_3 gene on chromosome 3D, (Sears, 1963). From studies of extra dosages however, V_3 was shown to be more effective than V_2 in reducing the expression of the virescent gene v_1 (Sears, 1959).

Chlorina-1 arose from ethyl methanesulfonate treatment (Shama Rao and Sears, 1964) and was found to be located on chromosome 7A. It also shows a dosage effect. A partially dominant allele of this gene was found by Pettigrew, Driscoll, and Rienits (1969). They also identified homoeologous normal genes on chromosomes 7B and 7D (Pettigrew and Driscoll, 1970).

A chlorophyll-deficient type, Hermsen's virescent (Sears and Sears, 1968), which was found in the F_3 of an intervarietal cross, was due to duplicate null alleles on chromosomes 3A and 3B. It is possible that these genes are alleles of V_2 and V_1, respectively. Thus null alleles and antimorphic mutations are known for some of the chlorophyll genes.

Homoeology of the *spelta* gene

The taxonomic characteristics of the *vulgare* group of hexaploid wheats include squareheaded spike (in most cases), round glumes, free-threshing seed and tough rachis. The subspecies *spelta* has a long brittle rachis, long glumes with strongly held seed and is not squareheaded. Mutations in *vulgare* types which are characterized by loss of the square head but not the other *vulgare* characteristics are known as speltoid.

This differentiating complex of effects was located on chromosome 5A by Sears (1944) and was thought to involve at least two genes (Watkins, 1940) until Mac Key (1954) showed by mutation experiments that only one, the *Q* gene, was involved. Mac Key also showed (1954 and 1966) that the speltoid mutations were due to loss of *Q* and that the differences between spelta and speltoid phenotypes were due to modifier genes. Sears (1956c, and unpub. in Muramatsu, 1963) substituted chromosome 5A from the non-squareheaded *vulgare* variety Hope into squareheaded Chinese Spring. He found the phenotype of the substitution line to be little different from that of the recurrent parent. This confirmed that the expression of *Q* with respect to squareheadedness was also affected by modifiers. *Q* thus gives the squarehead and nonspelting but is superimposed on a polygenic system with which it interacts (Mac Key, 1966).

The *Q* gene shows a dosage series. In *vulgare* varieties, nullisomics and mono-

somics for 5A are speltoid, the disomic is squarehead, the trisomic is subcompactoid, and the tetrasomic compactoid (Sears, 1954). The *spelta* gene *q*, however, gives no phenotypic change from nought to four doses (Sears unpub. in Muramatsu, 1963).

Since Kuckuck (1959) had crossed two *speltas* and found naked cultivated types in the progeny (indicating that *q* could give rise to *Q*), the *spelta* gene could not be a deficiency. Sears (1956c) suggested that *q* might be duplicated elsewhere. Alternatively, it might be an amorph, but capable of giving rise to *Q*, or it might be a weaker gene of similar effect to *Q*. In 1963, Muramatsu succeeded in showing that *q* was, in fact, a hypomorph of the *vulgare* gene. He obtained plants with five and six doses of *q*. These were squareheaded and a transitional subcompactoid type respectively. There is a threshold of expression for *q* between four and five doses. *Q* is equivalent to approximately 2.5 doses of *q* for squareheadedness and two doses for glume characters. This finding led Muramatsu to reiterate a suggestion of earlier workers (Kuckuck, 1959) that *Q* might be a duplicate of *q*, analogous to the Bar-eye gene in *Drosophila*.

This suggestion was supported by Swaminathan's (1966) findings from mutation experiments. Thus he obtained true-breeding semispeltoid types as well as complete speltoids. Both types of speltoid gave the *vulgare* phenotype when crossed to compactoid mutants. This was interpreted as supporting the model of *Q* as a

series of tandem repeats of *q*. Depending on whether all or part of *Q* was lost, speltoidy was more or less extreme. The mutants also showed complete association of *spelta* characters, verifying Mac Key's 1954 assertion that *Q* was a single locus.

Sears' suggestion that *q* might be duplicated on the homoeologues was confirmed by Muramatsu's finding that tetra *spelta* 5A nulli 5D was tougher glumed than tetra *spelta* 5A, and nulli 5B tri *spelta* 5A was tougher glumed than tri *spelta* 5A. Nulli 5B and nulli 5D are somewhat less squareheaded than euploid Chinese Spring and Sears in 1954 observed that tetrasomics 5B and 5D have somewhat shorter spikes than euploid. Thus chromosomes 5B and 5D appear to bear genes similar to *q*.

Muramatsu also detected difference in the phenotypes of tetra *spelta* 5A and a disomic *spelta* 5A having in addition an isochromosome for 5AL, although both had four doses of *q* on 5AL. The plants with only two doses of 5AS were shorter and slightly more squareheaded than the tetra *spelta* 5A. Thus the short arm appears to carry a gene of opposite effect to *q*.

The study of the *spelta* gene reveals again features observed in the study of the pairing genes and the chlorophyll genes. The duplication of loci on the homoeologues, coupled with the tendency towards loss or change of gene function

emerges as a typical pattern for wheat. A further interesting feature about the *spelta* system is the existence of different thresholds of expression for the different characters affected by a pleiotropic gene.

The Glaucous Character

Glaucousness is the greyish or whitish appearance of some plant organs. It has also been referred to as wax or waxy bloom; however, it is not the only form of epicuticular wax, and the term glaucousness is therefore to be preferred. In wheat, it was described by Watkins and Cory (1932) as a whitish bloom on leaf sheaths, glumes and stems, especially just above and below nodes, and to a lesser extent on the lamina of the leaf. Jensen and Driscoll (1962) describe it as "a waxy covering on the stems, leaf sheaths and glumes ... . Waxless wheats, on the other hand, lack this covering and have been variously described as yellowish-green, grass-green, or olive-green, but the principal visual impact is of glossy surfaces, as though dipped and polished in oil".

Many studies have been made of the physical and chemical nature of this wax layer, mainly in plants other than wheat. The first person to examine this extracuticular layer microscopically was de Bary (1871, see Daly 1964) who found needle like, rod-shaped or granular crystals. Hall, Matus, Lamberton and Barber (1965) made electronmicrographs of the surface of various glaucous and nonglaucous species. They included *Eucalyptus urnigera* from the clines studied by Barber (see below), *Poa colensoi* from clines studied by Daly (1964) and mutant lines of *Pisum* and *Brassica*. The glaucous *Eucalyptus* showed long branched rodlets forming a dense mesh over the plant surface. Flat flakes predominated in the nonglaucous types. This pattern could be partly generalized

:o the other species. *Pisum* stocks included subglaucous types which had nainly large platelets, whereas nonglaucous lines had small spherical granıles but no plates. The fully glaucous lines had both rods and plates. The ıuthors concluded that glaucousness correlated with heavy, randomly oriented leposits of crystalline wax. Nonglaucous types have wax deposits which lie flat on the cuticle and are less frequent, or may be aligned. In addition to this work, Hall et al. measured the contact angles of water droplets to the plant surfaces and concluded that contact angles in combination with visual observations give a reasonable indication of the type of wax structure. Chemical estimations by Hall et al. showed a high content of β-diketones in the glaucous *Eucalyptus* and *Poa* samples, although some of the visually glaucous *Eucalyptus* species have no β-diketones.

Horn, Kranz and Lamberton (1964) also found that wax was present on nonglaucous plants of *Eucalyptus* although glaucous plants of the same species produced more wax. Thus it is inaccurate to call the nonglaucous types nonwaxy. However, to minimize confusion, the authors' original designation will be used when discussing their papers in the remainder of this thesis, although in most cases it is purely the visual appearance of the plants which was studied.

Troughton and Hall (1967) measured contact angles, made electronmicrographs and visual observations on extracuticular wax on several varieties of wheat. They concluded that visual judgements of glaucousness were inadequate to describe

.he presence or absence of wax, but used to supplement other measurements, could :lucidate important changes in wax structure. Glaucousness was correlated with .he microscopic appearance of rods.

:he adaptive significance of glaucousness is not clear, although many functions ıave been suggested, and in some areas are supported by experimental data. As)ne might expect, the majority of theories suppose the crystal layer to have ın insulatory function. Prevention of water loss, reflection of radiation, ncreased resistance to disease have all been postulated.

)enna (1970) found that removing waxy bloom from *Brassica* leaves gave an in-·rease in cuticular transpiration. Pool and Paterson (1958) found that waxy glumes in wheat slowed drying of grain in a humid climate, although uptake of ıoisture was also slowed. It seems that the wax layers do in fact slow the)assage of moisture across the cuticle.

)aly (1964) observed glaucous clines of *Poa colensoi* were found in areas with ırid climates, nonglaucous plants were the dominant types in cool, wet areas.

Studying Tasmanian eucalypts, Barber (1955, 1956 and 1965) and Barber and ackson (1957) found glaucous types to be most common at high altitudes and in ·xposed areas. They were rare in low, sheltered regions. Barber suggested .hat the sharp change in selection coefficient might occur because the glaucous

forms were more resistant to frost due to the higher water repellency of the crystal layer. (Holloway (1969) found that the superficial wax contributed considerably to the hydrophobic nature of the leaf.) The higher light reflectivity, insect resistance and lower cuticular transpiration might also be important in determining the effect.

Given the genetic potential to produce glaucousness, many environmental factors influence the degree of expression attained. Barber (1955) found that low temperatures seemed to promote glaucousness. A maturity effect was also observed, the young stems and leaves being more glaucous than the old. Watkins and Cory (1932) also described a maturity effect in wheat, the older plants being more waxy. In addition, they noted a seasonal effect, the waxy and waxless types were easier to distinguish in spring-sown than autumn-sown material.

Juniper (1960) and Juniper and Bradley (1958) showed that peas grown in the dark lacked the surface wax projections of light-grown plants. Juniper also demonstrated that the size and number of crystals increased with increasing light intensity. Temperature was found to increase wax, as measured chemically, in the mesquite, (Hull, 1958). This kind of measure, however, probably gives only a rough correlation with glaucousness. Other factors, including day length, nutrient status and soil moisture levels were quoted by Dewey et al. (1956) as also influencing level of wax. A wide range of environmental factors are thus mentioned in the literature as affecting the quantitative development of plant waxes or glaucousness.

Inheritance of waxy bloom on the stem of *Ricinus communis* was found by White (1918) and Harland (1918) to be determined by the presence of a dominant gene B. Peat (1928) observed that bloom on the underside of the leaf in *Ricinus* was affected by an additional locus, C. This gene is manifested only in the presence of B and also intensifies the effect of B on stem, petioles and capsules. Locus D gives heavy bloom on plant parts other than the leaf, in the presence of B. Its affect may be masked if C is present. Yet another gene system, determining presence of bloom on the adaxial surface of the leaf, was noted by Peat, but not studied.

Harland (1947) showed that the bloomed types of *Ricinus* were predominant above a certain altitude, in areas where the climate was dry and sunny. The bloomless types were restricted to the rainy and foggy coastal areas.

In barley, Lundquist, von Wettstein-Knowles and von Wettstein (1968) obtained 384 mutants for wax by various mutagenic agents. The mutants involved 44 separate loci and mainly affected specific plant organs. Only one completely nonwaxy mutant was obtained. One mutant which was nonwaxy for the spike and internode showed loss of the ketone, possible β-diketone, fraction of the wax. Another mutant for leaf wax, showed loss of primary alcohols. Electronmicrographs showed in one case that a change in crystal shape was sufficient to give loss of the waxy appearance.

In wheat, Miczynski Senior, and Tschermak (both in the 1920's, see Jensen and

)riscoll, 1962) found waxless to be dominant to waxy. Biffen and Engledow (1926) crossed a waxy variety of *T. turgidum* (Rivet) to the waxy hexaploid Redfife and obtained a 15 waxy:1 nonwaxy ration in the F_2. This could either mean duplicate dominant genes in the A and B genomes or one gene in the A or B genome of Rivet and the duplicate in the D genome of Redfife.

Watkins (1928) and Watkins and Cory (1932) crossed Rivet to waxy *T. aestivum* ssp. *vulgare* var. Swedish Iron and found waxless F_2 plants always with less than 35 chromosomes. He deduced that the 4x variety had a gene in the A or B genome for which Swedish Iron was recessive. The hexaploid had a wax-producing gene in the D genome.

Miczysnki Junior (1930) obtained an F_2 segregation of 3 waxless: 1 waxy between *T. pyramidale* var. *recognitum* Perc. and *T. durum* and obtained a 13 nonwaxy to 3 waxy ratio in the F_2.

Matsumura (1951) crossed waxy *T. persicum* to waxless *T. dicoccoides* and also obtained a 13 waxless:3 waxy F_2 segregation.

Ayad (1953) crossed waxy and waxless *vulgare* wheats. Chavan, Argikor, Hattangadi, and Salanki (1955) crossed waxy and waxless *durum* wheat. Both obtained 3 waxless:1 waxy ratios in the F_2 generation.

Similarly, Deidda (1968) found waxy bloom of the stems and leaves to be monogenic and recessively inherited in several *durum* varieties when crossed to the "bloom-resistant" variety Capeiti 8.

A cross between *T. durum* varieties "N59" and Nurshit made by Patil (1968) also showed waxy bloom of the leaf sheath to be recessive and monogenic. A cross between the *durum* wheat Motiya and *T. pyramidale* reported in the same paper showed the single gene for wax to be dominant over waxlessness.

Jensen and Driscoll (1962) crossed the waxless Cornell Wheat Selection 5075 to several waxy hexaploid varieties, and found that the waxless character was inherited as a single dominant gene.

Driscoll and Jensen (1964) reported that the gene was located on chromosome 2B. Driscoll (1964, 1966) mapped the inhibitor as at least 42 map units from the centromere on the nonstandard (short) arm of chromosome 2B.

Tsunewaki (1966) located a single, dominant gene for inhibition of wax on chromosome 2D of certain waxless synthetic hexaploids. The F_2 of the Chinese Spring monosomics and the synthetics were all non-waxy. Two of the synthetics (ABD-VI and ABD-XIII) gave 3 waxless: 1 waxy ratios in the F_2 except for the monosomic-2D family. The F_2 of ABD-I segregated 17 waxy: 373 waxless, fitting a 3:61 ratio. This was interpreted as being due to inhibitor genes on chromosomes 2B and 2D, with a recessive waxless gene on 2B. ABD-VI and ABD-XIII were

considered to have a wax gene on 2B in addition to the 2D inhibitor. Kerber and Dyck (1969) mapped this inhibitor gene from *Aegilops squarrosa* at 15.1 ± 2.6 map units from the gene for threshability.

Tsunewaki (1966) also located a recessive waxless gene on chromosome 2B in the synthetic hexaploid Salmon and a wax-producing gene in the variety S615 which was later shown to be on chromosome 2D (Tsunewaki, 1968).

An examination of seven einkorns showed them all to be waxless, recessively so in the four cases testcrossed to waxy emmers. One out of four strains of *Ae. speltoides* was found to be waxy. *Ae. squarrosa* types were found to be either waxless due to the 2D inhibitor, or waxy. While few waxless hexaploid wheats were observed (1 in 220), waxless tetraploids were relatively more common (7 out of 22) (Tsunewaki, 1966).

Allan and Vogel (1960) found that Durum 396 had a hemizygous-ineffective gene for heavy wax on chromosome 2B. Muramatsu (personal communication to Driscoll and Jensen, 1964) located genes for wax on chromosomes 2B, 4B and 6B by observation on aneuploids of Chinese Spring.

The chemistry of glaucousness in wheat was studied by Barber and Netting (1968), using ditelo 2BL, which is nonglaucous due to loss of the gene for glaucousness on 2BS. The other nonglaucous lines examined were Long Kernel, a line hetero-

zygous for the inhibitor from C.W.S. 5075 back-crossed into Chinese Spring, and a line of Chinese Spring homozygous for a recessive gene for nonglaucousness on 2B. The glaucous lines used were euploid and tetrasomic 2B Chinese Spring, Bearded Yalta and the durum wheat Mindum. By thin layer chromatography and absorption spectrophotometry, they showed that all of the nonglaucous lines were lacking in β-diketones and hydroxy-β-diketones. The lines were apparently normal for hydrocarbons, esters, aldehydes and acids. There was an additional series of primary alcohols present in the line deficient for 2BS. This suggested that the new alcohol was either a precursor or a derivative of a precursor of the β-diketones.

Further work by Netting (unpublished) involved measurement of the contact angles of water droplets on different plant organs, thin layer chromatography, and measurement of β-diketone content. These measurements were correlated with visual classifications made by the present author. The lines studied were Chinese Spring, the dominant inhibitor of 5075 backcrossed into Chinese Spring for five generations (the Inhibitor-1 stock) and two lines bearing recessive genes for nonglaucousness. One of the latter was nonglaucous due to a terminal deletion involving the 2BS gene for the production of glaucousness (Mutant-1 stock*), and the other involved a recessive mutation of the same gene (Mutant-2 stock*). In addition, β-diketone estimations were made on the variety Mentana.

The organs studied were the vegetative leaves, the peduncle, flagleaf-sheath and flagleaf blade. The contact angles of the abaxial and adaxial surfaces of

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* See pages 51 and 54 respectively, for the origin of these lines.

the lamina were measured separately. All measurements were also made separately for tip and base of the abaxial surface because of a noticeable difference in the glaucousness of these regions. Since the same lines have been used in the study reported in this thesis, some of the more detailed results are shown in Table 3.

Chinese Spring has the highest contact angles for all mature organs (except adaxial leaf blade), including the nonglaucous, abaxial, leaf-blade tip.

The vegetative leaves of all varieties had high contact angles. The chemical data showed that all glaucous organs had a high β-diketone content, while nonglaucous organs with high contact angles tended to have a low β-diketone content but relatively high primary alcohol content. Here Netting's results are supported by Troughton and Hall (1967), who found platelets of wax on the vegetative and adaxial flag leaves, which in their studies also had high contact angles. Lundquist et al. (1968) found loss of wax platelets correlated with loss of primary alcohol in a barley mutant. In combination, Netting's findings suggest two types of wax metabolism, the sheath being typical of the β-diketone type and the vegetative leaves typical of the primary alcohol type. The abaxial surface of the flag leaf appeared to be a mixture of both types. The peduncle is intermediate between the two, but was measured just after anthesis, when the peduncle is not yet fully glaucous.

TABLE 3

Glaucousness, contact angles and β - diketone content of organs of five lines of wheat. After Netting (unpub.).

Line	Measurement	Flag Leaf				Peduncle	Vegetative Leaf	
		Sheath	Abaxial tip	blade base	Adaxial		Abaxial	Adaxial
Chinese Spring	1	G	NG	SG	NG	SG	NG	NG
	2	141.81°	138.34°	136.42°	141.64°	136.21°	143.98°	143.38°
	3	185	19_2	34_2	$-_2$	27	8	$-_2$
Inhibitor -1	1	NG	NG	NG	NG	NG	NG	NG
	2	120.49°	122.72°	122.84°	145.29°	125.19°	147.84°	145.04°
	3	10	11	17	-	14	10	-
Mutant-1	1	VSG	NG	NG	NG	NG	NG	NG
	2	122.88°	128.26°	124.10°	148.18°	133.55°	146.13°	144.57°
	3	71	13	16	-	21	5	-
Mutant-2	1	VSG	NG	NG	NG	NG	NG	NG
	2	121.92°	124.52°	127.90°	145.44°	135.53°	146.44°	144.40°
	3	60	22	26	-	31	11	-
Mentana	1	G-SG	VSG	SG-VSG	VSG	SG-VG	-	-
	2	-	-	-	-	-	-	-
	3	155	26	47	-	59	-	-

Key: Measurements: 1 = Appearance; 2 = contact angle; 3 = β-diketone content. A value of 300 would mean the wax was approximating pure β-diketone.

Appearance: G = glaucous; SG = slightly glaucous; VSG = very slightly glaucous; NG = nonglaucous.

Notes:

1. Contact angle - the higher the contact angle the less wettable the surface. Comparisons cannot be made between organs because of differences in surface curvature.

2. Includes adaxial as well as abaxial.

The line carrying the dominant inhibitor was consistently lowest for both contact angle and β-diketone content over all organs. This is consistent with its visual phenotype. Netting concluded that the line bearing the inhibitor gene might not form platelets on the sheath and peduncle, although it did form primary alcohols. This would result in the low contact angle observed. The two recessively nonglaucous lines gave lower contact angles than Chinese Spring for peduncle, leaf base and sheath, that is, the organs for which Chinese Spring is glaucous. Hence glaucousness is correlated with a high contact angle for these organs. The β-diketone contents are markedly reduced for these lines.

Mentana, which is visually intermediate to Chinese Spring and the Mutant-1 and -2 lines, gave intermediate β-diketone estimates.

Thus there is a correlation between degree of glaucousness determined visually and the amount of β-diketones measured chemically. Neither estimation is without error and the correlation is not perfect, but the relative rankings by either method agree. The contact angle measurements apparently do not depend solely on β-diketone content, although a high β-diketone content does give a high contact angle.

SECTION 2 MATERIALS AND METHODS

General Methods

Seed to be grown was treated to break post-harvest dormancy by moistening it and placing in petri dishes in a 4°C coldroom for one week. The seeds were then allowed to germinate for three to four days in the petri dishes at room temperature. Plants for cytological examination or for crosses were placed in 6" terracotta pots in the glasshouse. F_2 or testcross seedlings were planted either in 4" terracotta pots in the glasshouse, or in the field. As the level of glaucousness produced was generally lower in the field than in the glasshouse, it will be specified which populations were grown in the field. A small number of plants were grown, after tillering in the glasshouse, in a Sherer-Pennant controlled cabinet (conditions mentioned in text). These plants were somewhat more glaucous than the glasshouse grown material and will also be specifically mentioned as such.

Two generations a year were grown: one planted in March (the winter generation) and one in September, which gave a summer crop.

Cytology

I Meiosis. Cytological specimens were fixed in Carnoy's solution (6 parts 95% alcohol:3 parts chloroform: 1 part glacial acetic acid) for 24-48 hours. The anthers were dissected out of the head and squashed in 1% acetocarmine. Some heads were stored under refrigeration in 95% alcohol before being examined.

II Mitosis. Seed was treated to break dormancy and allowed to germinate at room temperature. The tips of primary roots were excised when the roots were 1" - 1½" long. The root-tips were placed in clean vials of tapwater at 0°C for 24 hours and fixed in Farmer's fixative for a further 24 hours. They were then hydrolysed for 12 minutes in 1N HCL at 60°C and placed immediately into Feulgen stain. After staining for 2 hours, the meristems were squashed in 1% aceto-carmine, which was found to prevent decolorization of the chromosomes. An alternative method involved fixing for 1 to 48 hours in glacial acetic acid instead of Farmer's fixative.

III Arm ratio measurements. The arm ratios of the chromosomes were measured using an ocular grid. Anaphase I measurements were made on the univalent in monosomic plants. Only cells in which the univalent was clearly lagging and had centromeric activity were used. Mitotic chromosomes were measured at metaphase in cold-water-treated cells (prepared according to the previously described method).

Derivation of nullisomics

From 50 to 150 selfed seeds from known monosomics were treated as described in order to break dormancy, and planted in 2" "Jiffy" pots. At the two- to three-leaf stage the ten seedlings with the narrowest leaves were selected from each monosomic line, except for the group-2 chromosomes where the shortest-leaved seedlings were selected (see Sears, 1956c). These seedlings were transplanted

to 6" terracotta pots and their chromosome number ascertained at meiosis.

Method of Classification

In sections 3 and 4 plants were classified for whole-plant glaucousness. This involved the general glaucousness of the entire plant but with special emphasis on the leaf sheath. The variety Chinese Spring was taken as the standard "glaucous". By comparison with this, the other categories used were "heavily glaucous" and three types less than that of Chinese Spring, viz., "slightly glaucous", "very slightly glaucous" and "nonglaucous".

In section 5, plants are classified by the different organs separately. The same five categories of "heavily glaucous" to "nonglaucous" inclusively were used, however, regard was taken of the fact that an individual had, for example, a glaucous leaf sheath and a nonglaucous peduncle. This same individual would have been classified simply as "glaucous" under the whole-plant classification scheme.

Plants were classified on the basis of their visual appearance. Although studies on contact angle of water droplets and β-diketone content (which were carried out in this Department by Mr. A. G. Netting) agree reasonably well with the visual estimations of glaucousness (see Literature Survey, p.36), the correlation is not absolute. Glaucousness is not synonymous with the presence of

crystalline β-diketones. Appearance is affected by the angle and intensity of incident light as well as form and density of the crystal layer. Shape of the organ, presence or absence of hairs, and other chemicals such as primary alcohols, influence contact angle measurements (Netting, personal communication). Chemical measurements of β-diketone content are also subject to some inaccuracy. Hence the study undertaken was restricted to the visual appearance of the plant.

Plants reached their maximum expression of glaucousness about a week after anthesis, with the exception of the variety Durum 396 which did not reach full expression until two weeks after anthesis. Classifications were made at anthesis for sheath wax and about a week later for other organs. Durum 396 was classified at the time of maximum expression.

Materials

Ten varieties were used in this study. The organs observed were the flag-leaf sheath, the peduncle, the head and in some cases, the abaxial flag-leaf blade, Glaucousness rarely developed on the leaf sheath originating from the fourth node below the head. The character was normally developed only in the last two or three weeks before emergence of the head. Mac Key (personal communication) was evidently able to classify plants in Sweden before this stage, but it is not known whether this is an environmental effect or one

particular to the varieties he used.

Description of the Varieties*

1. Chinese Spring *(T. aestivum* ssp. *vulgare)* (see plate 1).
This is the standard variety in most wheat cytogenetics studies, as it is the variety from which many of the aneuploid lines have been derived. It and the aneuploid lines were obtained from Dr. E. R. Sears of the University of Missouri. The vegetative leaves are completely nonglaucous, but when the plant enters meiosis and the young spike can be felt "in the boot", the top two leaf sheaths have started to become glaucous. The leaf blades are somewhat later and are usually markedly more glaucous at the base than at the tip. The peduncle usually emerges nonglaucous or slightly glaucous and does not reach the maximum level until after anthesis. However, peduncles which do not fully emerge from the sheath will often be found to be glaucous if the sheath is peeled back. Thus, the effect seems to be one of maturity rather than emergence. The lower parts of the sheath show a similar effect. The glumes are most glaucous at the tips and are virtually nonglaucous at the base. The rachis is also glaucous. The leaf blades, peduncle and head vary from almost nonglaucous to glaucous, depending on the environment. Like all the varieties studied, Chinese Spring tends to be more heavily glaucous

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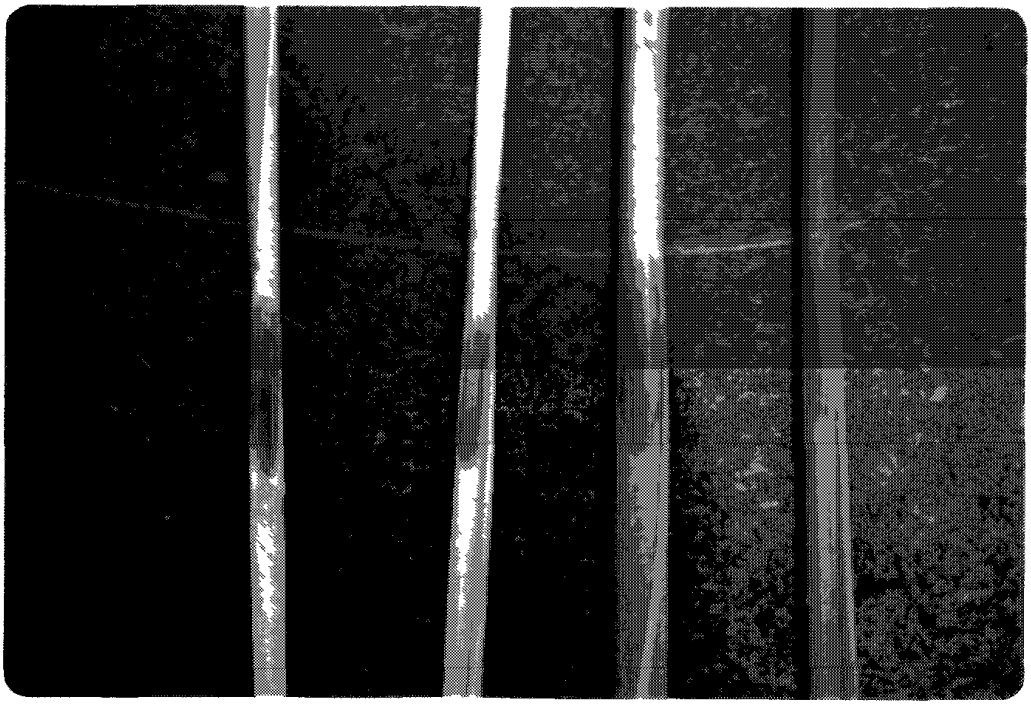
* A summary table of the phenotypes of the standard varieties is given in the appendix.

PLATE 1

Leaf sheaths of Poso (heavily glaucous), Chinese Spring (glaucous), Mentana (slightly glaucous) and Inhibitor-1 stock (nonglaucous). From left to right.



in the summer than in the winter generation.

2. Mentana (*T. aestivum* ssp. *vulgare*) (see plate 1).

This variety was obtained from Professor I. A. Watson of the University of Sydney. It is slightly glaucous on the flag-leaf sheath, that is, it is intermediate between the usual level of glaucousness of Chinese Spring and the fully nonglaucous varieties. It may be somewhat closer to Chinese Spring when the phenotype is maximal. The peduncle is similarly slightly glaucous, but the glumes are a little lower and the abaxial flag-leaf surface is usually only very slightly glaucous (that is, it shows a barely perceptible bloom).

3. Cornell Wheat Selection 5075 (*T. aestivum* ssp. *vulgare)*

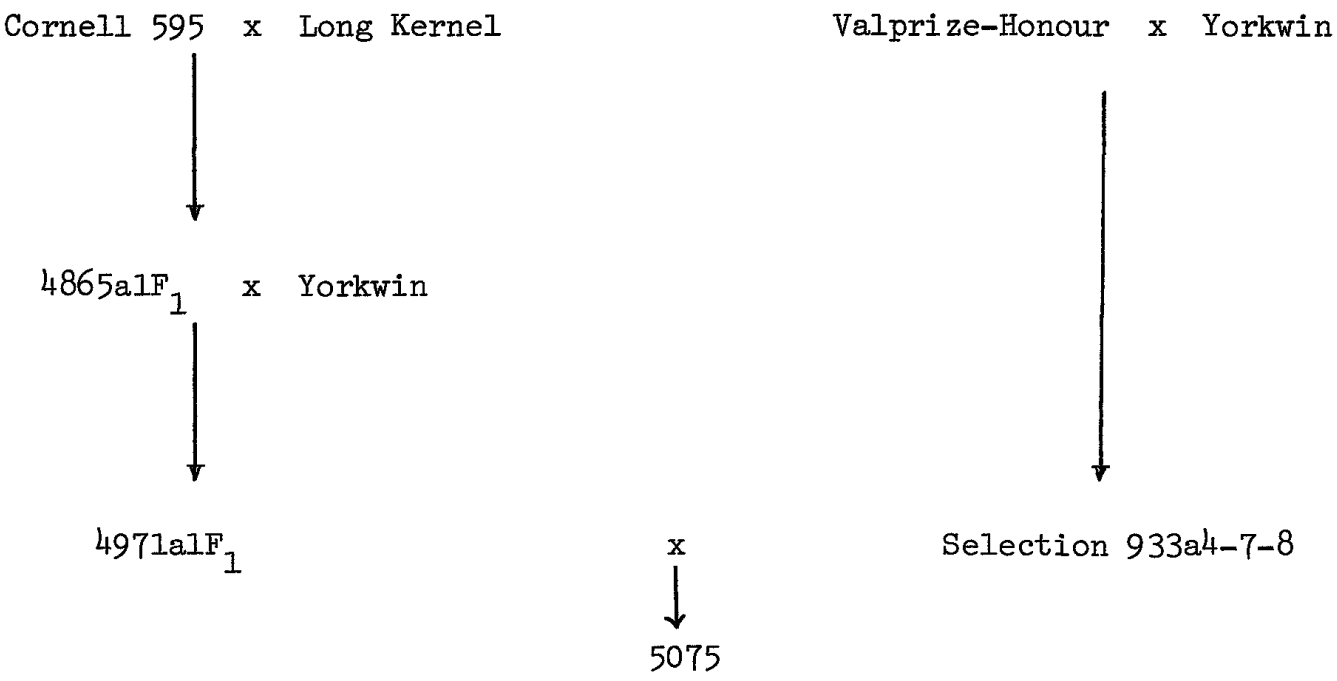
This nonglaucous variety was obtained from Professor N. F. Jensen of Cornell University. It was derived from a series of hybridizations starting with the nonglaucous tetraploid Long Kernel. Long Kernel is itself derived from a hybrid between *T. turgidum* ssp. *dicoccoides* and *T. turgidum* ssp. *turgidum* conv. *polonicum*. The hybridizations are shown in Table 4 (after Jensen and Driscoll, 1962). The nonglaucous selection was isolated as a pure line in following years. C.W.S. 5075 was found by Jensen and Driscoll (1962) to have a dominant inhibitor of glaucousness, which was later located on chromosome 2B (Driscoll and Jensen, 1964).

Under glass house conditions, 5075 is nonglaucous or very slightly glaucous for all plant parts.

TABLE 4

Hybridizations giving rise to C.W.S. 5075

(after Jensen and Driscoll, 1962)



The inhibitor was backcrossed into Chinese Spring for six generations. This backcross line has since been obtained in homozygous form and will be referred to as the Inhibitor-1 stock. It is completely nonglaucous on all plant parts (see plate 1).

4. Poso (*T. aestivum* ssp. *compactum*) (see plate 1).

This variety was obtained from Dr. C. A. Suneson, of the University of California, Davis. It is heavily glaucous on the sheath, leaf, peduncle and head. The difference between Chinese Spring and Poso for the sheath is not marked when the former achieves its maximum expression. Poso is less inclined to vary under different environments than Chinese Spring. The leaf blade, peduncle and head are considerably more glaucous in Poso than in Chinese Spring, and the abaxial leaf blade has a larger area covered. Usually the blade is glaucous to the tip in Poso.

5. Synthetic Hexaploid ABD XIII.

This is a synthetic hexaploid derived from *T. turgidum* ssp. *dicoccum* "Vernal" x *Ae. squarrosa* var. *typica* (Sears). It was obtained from Professor K. Tsunewaki of Kyoto University and is referred to by him as ABD XIII. It appears to be completely nonglaucous although it is quite hairy and thus a very low level of glaucousness would be difficult to detect. It was found by Tsunewaki (1966) to have a dominant inhibitor of

glaucousness on chromosome 2D.

A line of the inhibitor backcrossed into Chinese Spring was derived. It was ultimately backcrossed for five generations. This line will be called the Inhibitor-2 stock and the number of backcrosses will be stated, as the line was used in crosses before the fifth backcross was reached.

6. Durum 396 (*T. turgidum* ssp. *turgidum* conv. *durum* (Desf.) MK)

This variety was obtained from Dr. R. E. Allan of Washington State University. Allan and Vogel (1960) found this variety to have a hemizygous-ineffective dominant gene for glaucous culms (which can be equated to peduncle for this character) located on chromosome 2B. Under glasshouse conditions at Pullman, Washington, it was found to be heavily glaucous on the culm and glumes and possibly glaucous on the flag-leaf sheath (Allan, personal communication). Chinese Spring under these conditions was almost nonglaucous on the culm. When grown under glasshouse conditions at the University of New South Wales, Durum 396 is almost nonglaucous until anthesis, when ridges of bloom develop along the veins of the leaf sheath, giving an overall slightly glaucous appearance. The head and the peduncle slowly increase in level until the peduncle finally became glaucous and the head heavily glaucous.

7. Javelin (*T. aestivum* ssp. *vulgare*)

The glaucous and nonglaucous lines of Javelin were obtained from Dr. A. T. Pugsley, Agricultural Research Institute, Wagga Wagga. The glaucous line is similar to Chinese Spring, except that it has glaucous glumes. The "nonglaucous"

line is intermediate between very slightly glaucous and slightly glaucous. It appeared spontaneously in a field planting of the glaucous line.

8. Marfed (*T. aestivum* ssp. *vulgare*)

Marfed, and the nonglaucous-head mutant which was induced in it by ethyl methanesulfonate treatment, were obtained from Professor C. F. Konzak of Washington State University.

(a) Marfed is heavily glaucous all over in the summer generation, but is glaucous in the winter generation.

(b) Nonglaucous-head Marfed (N.G.H. Marfed) is heavily glaucous to glaucous for sheath and leaf. The peduncle is perhaps slightly less glaucous than the peduncle in Marfed. The head, however, is between very slightly glaucous and slightly glaucous, quite distinctly lower than the head of Marfed in both generations. (see plate 2).

9. Inhibitor-3 and Inhibitor-4 (*T. aestivum* ssp. *vulgare*)

These two mutants were induced by ethyl methanesulfonate treatment of Nullisomic 7B in Chinese Spring by Mr. G. D. Patil at the University of New South Wales. They are both entirely nonglaucous.

10. Salmon (Synthetic hexaploid)

This variety was obtained from Professor K. Tsunewaki of Kyoto University and

PLATE 2

Heads of normal Marfed (glaucous to heavily glaucous) and the nonglaucous-headed mutant form. Note that the peduncles of both are glaucous.

was shown by him to carry a recessive gene for glaucousness on chromosome 2B. It is very slightly glaucous.

SECTION 3 INHERITANCE OF WHOLE-PLANT GLAUCOUSNESS

I Whole-Plant Glaucousness in Chinese Spring

(a) Group-2 Chromosomes.

Chromosome 2B was first shown to bear a gene for the production of glaucousness in Chinese Spring by Muramatsu (personal communication to Driscoll and Jensen, 1964). He observed that nullisomic 2B was nonglaucous. Driscoll (1964) located this gene on the short (nonstandard) arm of chromosome 2B, by observing that the long arm ditelosomic stock (ditelo 2BL) is also nonglaucous.

It is quite common in wheat to find that a gene is duplicated on the homoeologous chromosomes. Studies were therefore undertaken to investigate the status of chromsomes 2A and 2D with respect to genes for glaucousness. The two compensating nullisomic-tetrasomic lines deficient for chromosome 2B were therefore obtained from Dr. E. R. Sears, and observed.

Nulli 2B-tetra 2A was found to be completely nonglaucous. Nulli 2B-tetra 2D was slightly glaucous (at about the level developed by the variety Mentana, see plate 1). Thus no homoeologous chromosome can compensate fully for loss of chromosome 2B with respect to glaucousness. Chromosome 2A cannot

compensate at all and therefore appears to have no gene equivalent to that on 2BS. The partial compensation by chromosome 2D indicates that it bears a hypomorph of the 2BS gene for glaucousness. It is noticeable that in the summer generation, when expression of glaucousness is maximal, the ditelosomic 2BL line has the barely perceptible level of glaucousness, referred to as "very slightly glaucous". This level is not apparent in all seasons or on all plants. It is presumably due to the activity of the 2D gene for glaucousness, when it is present in two doses, in the absence of the 2BS gene. In order to examine the phenotype given by three doses of chromosome 2D, ditelo 2BL (very slightly glaucous) was crossed to nulli 2B-tetra 2D (slightly glaucous) giving monotelosomic 2BL-trisomic 2D. These hybrid plants were phenotypically intermediate to the parents. In the absence of chromosome 2B, two doses of the 2D gene will barely exceed the threshhold of expression for glaucousness. A third dose gives a clearly distinguishable increase in glaucousness. Four doses result in approximately half the level of glaucousness of euploid Chinese Spring.

Although chromosome 2A had been shown to be unable to compensate for the absence of chromosome 2B in the nulli 2B-tetra 2A stock, the possibility that it might have a slight inhibitive effect on glaucousness had not been excluded. It was also possible that chromosome 2A bore a gene for glaucousness that is not expressed at the observed dosage of four chromosomes. As it is difficult to observe small changes in phenotype while chromosome 2B is present the hybrid of nulli 2B tetra 2A by nulli 2B tetra 2D was produced and observed

for glaucousness. This F_1 was deficient for chromosome 2B but trisomic for both 2A and 2D. If chromosome 2A has any observable effect, this hybrid should differ in phenotype from monotelosomic 2B-trisomic 2D. As it was indistinguishable from this genotype, chromosome 2A cannot be said to bear other than a null allele of the 2B gene for glaucousness.

None of the other four group-2 nullisomic-tetrasomic stocks differed phenotypically from euploid Chinese Spring. This is to be expected on the hypothesis of a very powerful gene for the production of glaucousness present on chromosome 2B, chromosome 2D having only a hypomorph and chromosome 2A a null allele. In the presence of chromosome 2B, dosage changes of 2A and 2D would therefore not be detectable. Extra dosage of chromosome 2B also gives very little phenotypic change, although the tetrasomic 2B line seems to be slightly more glaucous under field conditions than euploid Chinese Spring.

(b) The origin and identification of the Mutant-1 line.

During the backcrossing of the dominant inhibitor from 5075 into Chinese Spring (A.G. Netting), the F_2 families derived from several nonglaucous BC_5 plants included one family consisting entirely of 50 nonglaucous plants. Six of these were crossed to Chinese Spring. Of these six F_1 progenies, one consisted entirely of fourteen glaucous individuals ($\chi^2_{1:1} = 12.07$ using Yates correction, 1 d.f. $P < .01$). This indicated that some of the nonglaucous BC_5F_2 plants did not contain the dominant inhibitor. Four of these glaucous

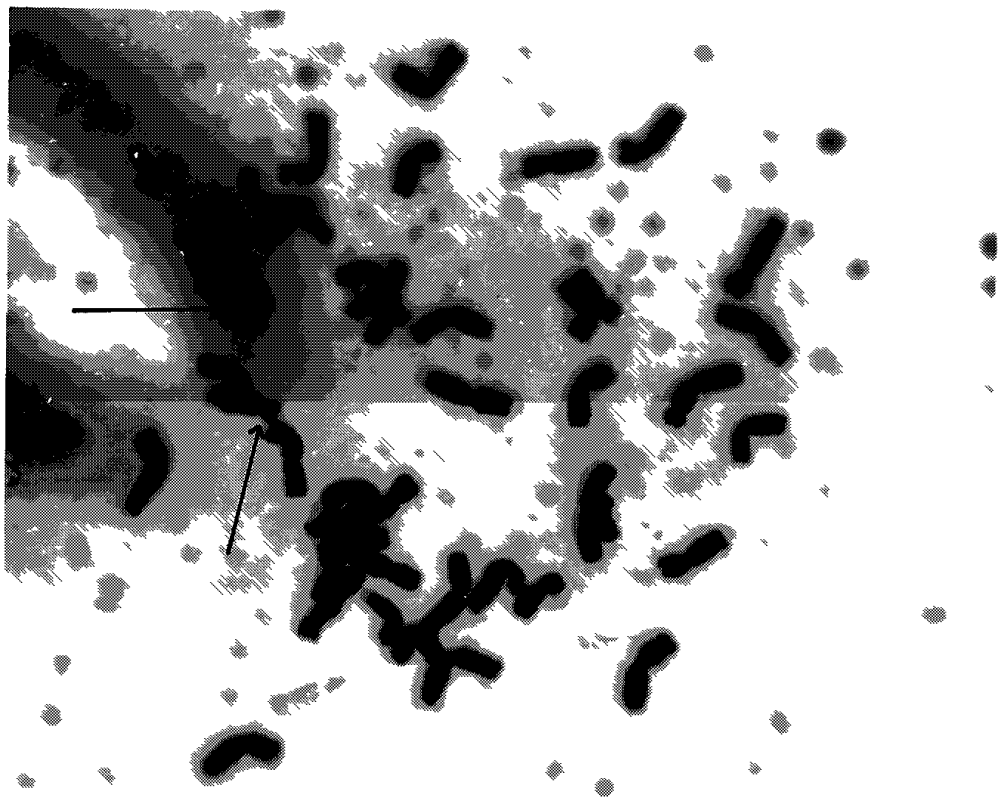
plants from the 14 glaucous:0 nonglaucous family were allowed to self, giving 18 glaucous (G):1 nonglaucous (N); 19G:1N; 16G:1N and 18G:2N, respectively. The nonglaucous types from this generation proved to be true-breeding and will henceforth be referred to as the "Mutant-1" stock.

Cytological examination of these nonglaucous types at meiosis showed a pair of markedly heterobrachial chromosomes. Measurement of this chromosome at mitotic metaphase showed the arm ratio to be 1:4.1 (arithmetic mean of 100 measurements). This chromosome is thus more heterobrachial than chromosome 5B (1:2.65 at telophase II, Sears, 1954), which is the most unequal-armed chromosome of Chinese Spring. The abnormal heterobrachial chromosome was thus readily distinguishable from normal chromosomes and telocentrics in both meiotic and mitotic cells (see Plate 3). The chromosome could not have been a satellited telocentric, as all four satellited chromosomes could be observed and the constriction was obviously centromeric in behaviour at anaphase I of meiosis.

The Mutant-1 line was crossed as male to monosomic 2B giving a nonglaucous hybrid with a chromosome complement of 20 bivalents and a heterobrachial monosome. Hence the heterobrachial chromosome was homologous to chromosome 2B. This was confirmed by a cross of Mutant-1 to ditelo 2BL, which produced a nonglaucous F_1 with 20 normal pairs and a heteromorphic bivalent. The heteromorphic bivalent consisted of the telocentric paired with the long arm of the abnormal chromosome. Pairing between the two occurred in nearly

PLATE 3

Mitotic metaphase of Mutant-1 line. The two deletion chromosomes are arrowed. All four satellited chromosomes are also visible.



100% of cells.

The Mutant-1 stock was therefore deficient for a presumably terminal portion of the short arm of chromosome 2B. Since the BC_5F_2 family derived from Chinese Spring ^{6}x 5075 segregated 50 nonglaucous : 0 glaucous, the deletion must have involved loss of the gene for glaucousness, rather than loss of the dominant inhibitor, in the BC_5 plant.

An attempt was made to map the length of the short arm of the deletion chromosome by cytological observation. The Mutant-1 stock was crossed to ditelomonotelosomic 2BS (a stock having a pair of short-arm telocentrics and a single long-arm telocentric to maintain fertility). No pairing was observed between the short-arm telocentric and the heterobrachial chromosome in 100 cells examined from the F_1 hybrids having only the short-arm telocentric from the female parent. It seems that the deletion is large enough to reduce the probability of chiasma formation in the short-arm to almost zero, although some short-arm chromatin visibly remains.

An estimate of the male transmission of the deletion chromosome compared with its normal counterpart can be obtained from the four F_2 families that segregated 18G : 1N; 19G : 1N; 16G : 1N and 18G : 2N, which when pooled constitute a ratio of 71G : 5N. If one assumes equal functioning of eggs containing either chromosome, the male transmission of the deletion chromosome approximates 2 x $^5/_{76}$, i.e., 13.2%.

(c) Chromosome 4B and the detection of "Mutant-2"

It was stated by Muramatsu that nullisomic 4B is nonglaucous (personal communication to Driscoll and Jensen, 1964). He also observed that monosomic 4B in Chinese Spring was nonglaucous. That is, the gene appeared to be a hemizygous-ineffective recessive, although this was apparently not true for other varieties. A. G. Netting (personal communication) noted that the disomics derived from the monosomic 4B line were also nonglaucous. This monosomic line was therefore crossed as female to glaucous euploid Chinese Spring. Both the monosomic and disomic F_1 plants of this cross were glaucous. The F_2 from the disomics segregated 17 glaucous:3 very slightly glaucous ($\chi^2_{3:1} = .60$, using Yates correction, 1 d.f., P = .5-.3). Selfing a monosomic F_1 gave both glaucous monosomics and a glaucous nullisomic 4B in the F_2 generation. The possibility of univalent shift was eliminated by the characteristic phenotype of the nullisomic - which was awned due to loss of the dominant hooded gene on 4B (Sears, 1954). Thus chromosome 4B does not possess a gene for glaucousness.

The disomic plants of the monosomic 4B line were crossed as male to monosomic 2B. The F_1 showed an exact correlation between chromosome number and phenotype. Four monosomic plants were nonglaucous, the single disomic plant was glaucous. Selfing the monosomics gave 24 plants, all very slightly glaucous. The F_1 of the monosomic 4B line and Salmon (which bears a recessive gene on chromosome 2B, reported by Tsunewaki, 1966) was very slightly glaucous.

It is evident that the monosomic 4B line is nonglaucous due to a recessive mutation on chromosome 2B, which is allelic to the recessive gene of Salmon. The homozygous disomic line derived from the monosomic 4B stock will henceforth be referred to as the "Mutant-2" line. A stock monosomic for chromosome 4B and homozygous for the normal allele of the 2B gene for glaucousness has been isolated.

On crossing ditelomonotelosomic 2B to the Mutant-2 line it was observed that very little pairing occurred between the short-arm telocentric and the mutant 2B chromosome. Estimates from 20 to 30 cells from each of 4 plants showed that the pairing between the telocentric and the abnormal 2B varied from a maximum of about 30% to 10% or less. This suggested that the Mutant-2 chromosome 2B had undergone some physical rearrangement, perhaps deletion or inversion. The possibility of deletion was tested by comparing the arm ratio of the Mutant-2 chromosome 2B with that of its normal counterpart at anaphase I-telophase I of meiosis. The Mutant-2 chromosome 2B was measured as the lagging univalent in the monosomic plants derived from the cross of monosomic 2B x Mutant-2. The normal chromosome 2B was identified as the lagging univalent in conventional monosomic-2B plants. The method of making the measurements is described in a previous section (Materials and Methods, p. 40).

Because of the high variation in length and arm ratio of chromosomes at this

stage of meiosis, and the low accuracy of this method of measurement, the data are presented as a frequency table of arm ratios. Arm ratio rather than length is used, as it is a more constant measure (Sears, 1954). The non-normality of the data precludes comparison of means by a "t" or similar test. The comparison is thus made by a contingency χ^2 (see Table 5).

For this distribution, χ^2 equals 13.94 with 4 d.f. The probability of obtaining a χ^2 greater than or equal to this if the distributions represent samples from the same population, lies between .01 and .005. The distribution of the Mutant-2 chromosome exhibits a bias towards the less-equal-armed values. The arithmetic mean of this distribution is 1:1.46 as against 1:1.31 for the normal chromosome 2B. A greater divergence from a 1:1 ratio could have resulted from (i) loss of a segment of the short arm (calculated from the above arithmetic means as 10.3% of the short arm), (ii) loss of a large segment of the long arm such that it became the short arm (calculated, as before, as 47.4% of the long arm), or (iii) a pericentric inversion in which the chromosome breakpoints are at unequal distances from the centromere.

As the gene for glaucousness is known to be on the short arm of chromosome 2B, alternative (i) is the only alternative that could result in nonglaucousness, reduced pairing between the Mutant-2 chromosome and telocentric 2BS, as previously discussed, and a greater divergence from a 1:1 arm ratio.

Thus the gene for glaucousness has probably been deleted in Mutant-2. If

TABLE 5

Frequency of arm-ratio measurements for normal and mutant chromosome 2B

Chromosome 2B of:	No. of chromosomes with arm-ratio measurements in the range of					
	1:1.0	1:1.1 to 1:1.29	1:1.30 to 1:1.49	1:1.50 to 1:1.69	1:>1.70	Total
Chinese Spring	23	43	10	14	10	100
Mutant-2	13	27*	21	22	17	100

*including one measurement 1:1.08

χ^2_{hom} = 13.94, 4 d.f. P = .01 to .005

this deletion is a terminal one, which is more probable than an intercalary one, the gene for glaucousness would reside in the distal 10.3% of 2BS. This conforms with the fact that this gene has been mapped to the distal portion of 2BS, as discussed later in this thesis.

The recovery of a short-arm isochromosome from the abnormal chromosome was attempted. The monosomic F_1 of monosomic 2B x Mutant-2 was crossed as female to ditelosomic 2BL. The progeny were examined in an attempt to find an isochromosome which did not pair with the 2BL telocentric. A total of 82 plants were examined without finding such an isochromosome. Hence it was not possible to raise the dosage of the abnormal chromosome above two.

As an alternative way of looking at the dosage, the Mutant-2 line was crossed to nulli 2B-tetra 2D, giving a hybrid with the mutant 2B chromosome and three doses of normal chromosome 2D. In this hybrid, a fairly small effect due to the mutant locus might be expected to give a change in phenotype. The monotelo 2BL-trisomic 2D, which has no 2B gene for glaucousness and three doses of chromosome 2D, was used as control. There was no difference detectable between these hybrids. Hence the mutant chromosome 2B cannot be shown to have a residual effect on glaucousness. This in accord with the hypothesis that nonglaucousness in Mutant-2 is due to deletion of the gene for glaucousness.

(d) Chromosome 6B and the detection of "Mutant-3".

The third chromosome indicated by Muramatsu's observation on aneuploids was chromosome 6B. During investigations at this University, however, nullisomic 6B was observed to be glaucous. In order to confirm this observation, the two ditelo stocks of chromosome 6B were observed. Ditelo 6BL was glaucous and ditelo 6BS was nonglaucous. The monoisosomic 6BS stock was also nonglaucous. This implied either a background mutation in the nonglaucous stocks, which are apparently of common origin, or an unusual dosage balance between the two arms of 6B. The rationale for the latter would be as follows: The fact that ditelo 6BS is nonglaucous would imply a gene essential for glaucousness on 6BL. However, when both 6BS and 6BL are absent, in nullisomic 6B, the phenotype is glaucous. Therefore the nonglaucousness of ditelo 6BS would be due to a gene for nonglaucousness on 6BS which is hypostatic to the gene for glaucousness on 6BL. Stocks containing the long and short arms were intercrossed to obtain different dose ratios of the 6B long and short arms in combination with different states of chromosome 2B or the homoeologues from group 6.

As may be seen from Table 6, all crosses involving glaucous stocks gave glaucous hybrids, regardless of the dosage of 6BL and 6BS. Thus there is no dosage balance between the two arms. Moreover, the F_1's involving the Mutant-2 stock and the short arm of 6B were nonglaucous, though hybrids involving the same 6BS stocks and Chinese Spring were glaucous. Since the 6BS stocks cannot complement the "recessive allele" of chromosome 2B in the

TABLE 6

Crosses involving group 6 aneuploids and phenotypes of the offspring

Cross	Dosage of chromosome			Phenotype
	6BL	6BS	Other	
Tetra 2B x Monoiso 6BS	1	2	3 x 2B	Glaucous
N6BT6A x Ditelo 6BS	0	1	3 x 6A	Glaucous
N6BT6A x Monoiso 6BS	0	2	3 x 6A	Glaucous
Ditelo 6BL x Monoiso 6BS	1	2	---	Glaucous
Ditelo 6BL x Ditelo 6BS	1	1	---	Glaucous
Diiso 6BS x Mutant-2	1	3	Null allele on 2B	Nonglaucous
Diiso 6BS x Chinese Spring	1	3	Active allele on 2B	Glaucous
Ditelo 6BS x Mutant-2	1	2	Null allele on 2B	Nonglaucous
Ditelo 6BS x Chinese Spring	1	2	Active allele on 2B	Glaucous

Mutant-2 stock, the 6BS stocks are shown to have an allelic mutation on 2B. Final proof that chromosome 6B is not involved in the production of glaucousness came from the F_2 of ditelo 6BS x Chinese Spring, which yielded awned glaucous segregants. The expression of awns is due to absence of the awn inhibitor on 6BL, indicating that these segregants are ditelosomic for 6BS but carry the normal genes for glaucousness, although possibly in heterozygous state.

Thus the nonglaucous 6B aneuploids derive their abnormal phenotype from a recessive mutation, which is allelic to mutants located on 2BS. Nonglaucous lines carrying this mutation are now referred to as "Mutant-3" lines.

(e) The nullisomics of Chinese Spring

All of the other 20 nullisomics were examined in order to observe whether any of them are nonglaucous as is nullisomic 2B. They were isolated by seedling selection from the selfed progeny of the appropriate monosomics (see description in Materials and Methods p. 40). None of the other nullisomics were nonglaucous, although the observed single plant of nulli 2D appeared to be rather less glaucous than euploid Chinese Spring. This is consistent with the evidence of a hypomorphic gene for glaucousness on chromosome 2D, although only a small effect is apparent in the presence of chromosome 2B. It does not approach the change in phenotype given by loss of 2BS. The phenotypic changes shown by plant organs rather than the whole

plant will be discussed in the section on organ-specific genes.

(f) Two induced Mutants in Chinese Spring: "Inhibitor-3" and "Inhibitor-4".

A treatment of 170 seeds of Chinese Spring nulli 7B with 0.05 M aqueous solution of ethyl methanesulfonate by Mr. G. D. Patil at this University produced two M_1 plants which were nonglaucous. M_2 progenies of these individuals segregated, indicating that the mutations to nonglaucousness in each case was dominant. The two lines were named Inhibitor-3 and Inhibitor-4. Inhibitor-3 was found to be monosomic, presumably due to outcrossing of the parent material. The Inhibitor-4 line was nullisomic. In order to obtain information as to the relationship between these mutants, M_3 plants of the two lines were crossed to several indicator lines and were intercrossed.

The F_1 of mono 2A x Inhibitor-3 plant 5 segregated for glaucousness and non-glaucousness, hence Inhibitor-3 plant 5 was heterozygous. Selfing of a non-glaucous F_1 resulted in 3N : 1G segregation (see Table 7), which implies a single dominant gene for nonglaucousness.

The F_1 of mono 2B x Inhibitor-4 plant 6 consisted entirely of 4 nonglaucous plants, and the F_1 of Inhibitor-4 plant 6 x Inhibitor-3 plant 5 consisted entirely of 14 nonglaucous plants; hence Inhibitor-4 plant 6 was homozygous for the mutation. Inhibitor-3 was subsequently isolated as a homozygote in M_4.

TABLE 7

Crosses involving Inhibitor-3 and Inhibitor-4 and their F_1 and F_2 segregations

Cross	Phenotype of		Probability of obtaining χ^2 for ratio
	F_1	F_2	
Mono 2A x Inhibitor-3-plant-5	N(seg)[a]	12N:3G	.91 (3:1)
Mono 2B x Inhibitor-4-plant-6	N[b]	14N:0G	.02* (3:1)
Inhibitor-1[c] x Inhibitor-3-plant-1	N	73N:0G	.02* (15:1)
Inhibitor-4-plant-6 x Inhibitor-3-plant-5	N	65N:0G	.02* (15:1)

N = nonglaucous; G = glaucous

a Nonglaucous 20" + 1' segregant used to give F_2

b 19" + 2' segregant used to give F_2

c Inhibitor-1 stock is 5075 inhibitor backcrossed into Chinese Spring.

* χ^2 value significant at 5% level

Six F_1 plants of Inhibitor-4 plant 6 x Inhibitor-3 plant 5 were grown out to F_2. Four families did not segregate (13, 10, 13, and 14 nonglaucous, respectively). The last-mentioned family was increased by 51 plants in the next generation, giving a total of 65 nonglaucous: 0 glaucous. This is significantly different from a 15N:1G segregation, a ratio expected on the basis of independence of the two dominant loci. The remaining two F_2 families of this cross segregated 9 nonglaucous:2 glaucous and 10 nonglaucous:2 glaucous, respectively. These were presumably segregating for the Inhibitor-4 mutation only, since the Inhibitor-4 plant 6 parent was homozygous, but the Inhibitor-3 plant 5 parent was not. The pooled ratio of these two F_2 families conforms to a 3:1 ratio (19 nonglaucous:4 glaucous, $\chi^2_{3:1} = 0.71$, 1 d.f., P = .5 to .3).

The F_2 of the cross mono 2B x Inhibitor-4 plant 6-3-1 (a direct descendant of Inhibitor-4-plant-6) consisted entirely of 14 nonglaucous plants. This is significantly different from a 3:1 segregation, indicating that the Inhibitor-4 mutant is located on chromosome 2B. The F_2 of Inhibitor-1 x Inhibitor-3-plant-1 did not segregate. The observed 73 nonglaucous:0 glaucous is again a significant deviation from a 15:1 ratio. Hence the Inhibitor-3 mutation is allelic or closely linked to the inhibitor of 5075, which Driscoll and Jensen (1964) located on chromosome 2B. The intercross of Inhibitor-3 and Inhibitor-4 did not segregate; hence, both of these mutant genes are on the short arm of chromosome 2B. It appears that Inhibitor-1, Inhibitor-3 and Inhibitor-4 all involve the same locus.

II Whole-Plant Glaucousness in Mentana

(a) Differences between Mentana and Chinese Spring.

The slightly glaucous variety Mentana was crossed to Chinese Spring mono 2B. The F_1 showed complete correlation between chromosome number and phenotype, the 2 disomics being glaucous and the 4 monosomics being slightly glaucous. The F_1 of euploid Chinese Spring x Mentana was glaucous and the F_2 consisted of 12 glaucous and 8 slightly glaucous plants ($\chi^2_{3:1} = 2.4$, 1 d.f. P = .2 to .1). Hence Mentana differs from Chinese Spring by a recessive gene on chromosome 2B. The F_1 of Chinese Spring ditelo 2BL x Mentana consisted of 3 slightly glaucous plants, all cytologically 18" + 1"" + t1". Thus the recessive gene is located on the short arm of chromosome 2B.

The cross of Mutant-2 x Mentana gave a slightly glaucous F_1. The F_2 consisted entirely of 49 nonglaucous plants; however, there was obviously segregation for the slightly glaucous type of Mentana and the very slightly glaucous type of Mutant-2. The phenotypes intergraded, however, and there was indication of an environmental effect, the plants receiving the highest light intensity appearing to be higher in level of glaucousness. The fact that no fully glaucous segregants occurred (i.e., the two lines failed to complement) indicates that the mutant allele of Mentana corresponds to the 2BS deletion of Mutant-2. This is consistent with the results of a cross to Salmon, which has a recessive gene on 2B that results in very slight glaucousness. The F_1 of Salmon x Mentana was slightly glaucous and the F_2 of

15 plants varied from slightly glaucous to very slightly glaucous types.

The above results are interpreted as Mentana possessing a mutant form of the gene for glaucousness as found on chromosome 2BS of Chinese Spring. The F_1 of nullisomic 2B-tetrasomic 2D by Mentana had the same phenotype as the nullisomic 2B-tetrasomic 2D stock. This hybrid had one dose of chromosome 2A (considered to have a null effect), two doses of chromosome 2D of Chinese Spring and one each of Mentana's chromosomes 2A, 2B and 2D. This suggests that Mentana's group-2 chromosomes collectively are as effective as two doses of Chinese Spring 2D. Several possible genotypes could give this result. It could be that 2B of Mentana carries a null allele, while 2D of Mentana is twice as effective as chromosome 2D of Chinese Spring. Alternatively, the 2B gene for glaucousness of Mentana may have some activity, while Mentana's chromosome 2D could be equivalent to 2D in Chinese Spring. An intermediate situation could exist, 2A could be involved, or the difference could be due to modifier genes.

The problem is not simplified by the fact that monosomic 2D x Mentana gives an F_1 with 19" + 1''', showing that 2D is involved in the reciprocal translocation by which Mentana and Chinese Spring differ. However, some insight into the part played by chromosome 2B can be gained by observation of the backcross of Mentana to mono 2B. This BC_1 contained seven monosomic plants; three were slightly glaucous and four were somewhat less glaucous than Mentana. This segregation, in which a 1:1 ratio is approximated, indicates that chromosome 2B does not bear the only gene for glaucousness in Mentana, but suggests that perhaps two genes are involved.

(b) Allelism of the gene for glaucousness and the inhibitor on chromosome 2B.

The map distance of the recessive gene on 2B in Mentana was estimated using a method based on the F_2 telocentric mapping method devised by Driscoll (1964, 1966). Ditelomonotelosomic 2BS (20" + tS" + tL') was crossed as female to Mentana. The F_1 plants having a monotelodisome (20" + t1") were selected and those with a trivalent involving two telocentrics were discarded. Pairing of the telocentric and the homologous whole chromosome approached 100% in all F_1 plants examined.

The F_2 plants were classified according to phenotype and chromosome complement. The recombination frequency for the gene and the centromere was estimated by maximum likelihood methods with the help of Mr. Alan Stark, Department of Human Genetics, of this University. Three factors affect the formation of this type of F_2 population. Besides the recombination frequency, the male and female transmission of the telocentric chromosome are involved. The latter two estimates will be to some extent confounded in F_2 data, and reliable estimates of them cannot be obtained by this method with family sizes of this order. Since they are of secondary interest to the recombination frequency, however, the method remains a satisfactory one for this purpose.

The expressions for the expected values of the six phenotypic classes were

derived as follows: let P be the recombination frequency, let K be the proportional transmission of the normal chromosome through the pollen where transmission of the telocentric is 1. Similarly, let L be the transmission of the normal chromosome through the eggs, where the transmission of the telocentric is 1. Drawing up a Punnet square and summing gives the theoretical expectations in terms of the three variables (Table 8). When the observed values (see Table 9) are substituted and solved by the maximum likelihood method, the following values are obtained: P = .45, standard deviation = .08; K = 30, standard deviation = 22; L = .42, standard deviation = 1. The 95% confidence limits for the recombination frequency therefore extend from 33 to 50 map units. This overlaps with the confidence limits of the map distance obtained by Driscoll (1966) for the dominant inhibitor of 5075 (42 to 50 map units) and provides evidence for the probable allelism of the two genes. The value of K obtained indicates a male transmission rate for the telocentric of about 3%. The value obtained for the female transmission indicates, however, that the telocentric was transmitted about twice as often as the normal chromosome. This is contrary to usual expectations of equal transmission of whole chromosome and telocentric through the egg (Sears, 1963). The standard error is very high, however, so that the 95% confidence limits for transmission of the entire chromosome through the egg extend from 70% to 0%. The reason for this anomalous estimate of L probably lies in the fact that estimates of K and L are not independent of each other and there is evidence to suggest that male transmission of a telocentric (the long arm of 2B) may be subject to very high

Table 8

Telocentric mapping of the Mentana allele:-

Derivation of expected frequencies of chromosome complements and phenotypes for glaucousness

Male transmission entire:telo = K:1
Female transmission entire:telo = L:1
P = recombination frequency; w = recessive Mentana allele;
W = dominant Chinese Spring allele. —o— = entire chromosome
o— = telocentric

Female Gametes \ Male Gametes	o—w $\frac{K(1-P)}{K+1}$	o—W $\frac{(1-P)}{K+1}$	—o—W $\frac{KP}{K+1}$	o—w $\frac{P}{K+1}$
—o—w $\frac{L(1-P)}{L+1}$	S.G. 21" $\frac{KL(1-P)^2}{(L+1)(K+1)}$	G 20" + t1" $\frac{L(1-P)^2}{(L+1)(K+1)}$	G 21" $\frac{KLP(1-P)}{(L+1)(K+1)}$	S.G. 20" + t1" $\frac{LP(1-P)}{(L+1)(K+1)}$
o—W $\frac{(1-P)}{L+1}$	G 20" + t1" $\frac{K(1-P)^2}{(L+1)(K+1)}$	G 20" + t" $\frac{(1-P)^2}{(L+1)(K+1)}$	G 20" + t1" $\frac{KP(1-P)}{(L+1)(K+1)}$	G 20" + t" $\frac{P(1-P)}{(L+1)(K+1)}$
—o—W $\frac{LP}{L+1}$	G 21" $\frac{KLP(1-P)}{(L+1)(K+1)}$	G 20" + t1" $\frac{LP(1-P)}{(L+1)(K+1)}$	G 21" $\frac{KLP^2}{(L+1)(K+1)}$	G 20" + t1" $\frac{LP^2}{(L+1)(K+1)}$
o—w $\frac{P}{L+1}$	S.G. 20" + t1" $\frac{KP(1-P)}{(L+1)(K+1)}$	G 20" + t" $\frac{P(1-P)}{(L+1)(K+1)}$	G 20" + t1" $\frac{KP^2}{(L+1)(K+1)}$	S.G. 20" + t" $\frac{P^2}{(L+1)(K+1)}$

Summing the phenotypes:-

Slightly glaucous 21" = $[KL(1-P)^2]/(K+1)(L+1)$
" " 20"+t1" = $[(K+L)P(1-P)]/(K+1)(L+1)$
" " 20"+t" = $P^2/(K+1)(L+1)$
Glaucous 21" = $[KLP(2-P)]/(K+1)(L+1)$
" 20"+t1" = $[(K+L)(1-P+P^2)]/(K+1)(L+1)$
" 20"+t" = $(1-P^2)/(K+1)(L+1)$

SG = slightly glaucous; G = glaucous

TABLE 9

Telocentric mapping of the Mentana allele:- Observed numbers of phenotypic classes

Phenotype	Chromosome complement			Total
	21"	20"+t1"	20"+t"	
Slightly glaucous	8	13	1	22
Glaucous	17	48	1	66
TOTAL	25	61	2	88

inter- and intra-plant variation (Stuckey, unpublished). Such variation would affect the present estimates of the female, as well as the male transmission.

The above experiment shows that the model of inheritance postulated for glaucousness by the Japanese investigators is partially incorrect. The 2B gene for glaucousness and the dominant inhibitor are not inherited independently. Confirmatory evidence was sought in a direct test for linkage of the inhibitor and the 2B gene from Mentana. The F_1 of Mentana x Inhibitor-1 was backcrossed to Mentana. On the basis of linkage of two loci, one would expect glaucous recombinants in the BC_1. Of 77 plants examined in the BC_1, none were fully glaucous, 45 were nonglaucous and 32 were slightly glaucous. This conforms with a 1:1 segregation ($\chi^2_{1:1} = 2.2$, 1 d.f. P = .2 to .1). The maximum linkage which would be expected to give only 5% probability of obtaining no recombinants in a family of this size is 7.6 map units. The gene for glaucousness and the inhibitor are therefore either linked at a maximum of 7.6 map units apart or are alleles. It should be noted that complementation tests for allelism cannot be used where one of the genes being tested is dominant. In this case, if the two genes map at the same distance from other genes or the centromere, and there is no recombination between them, in the absence of further evidence, it is accepted that they are allelic. Both of these conditions hold for the gene for glaucousness and its inhibitor. The possibility remains that they are separate loci, but the simpler assumption is that they are alleles. An

analogous situation exists for the C locus in maize (Coe, 1962), where an inhibitor is apparently allelic to an active form of the gene necessary for anthocyanin production.

The evidence against the two genes being allelic comes from Kihara (1935) and Matsumura (1951) both of whom obtained 13 nonglaucous:3 glaucous ratios from crosses between glaucous and nonglaucous tetraploids, and inferred that the inhibitor and the gene for glaucousness were independent loci. Jensen and Driscoll (1962) obtained 3 nonglaucous:1 glaucous segregations from crosses involving the nonglaucous 5075 and glaucous hexaploids. They later located both the inhibitor and the gene for glaucousness on 2BS (Driscoll and Jensen, 1964; Driscoll 1964, 1966). Tsunewaki (1966) interprets the results of Kihara and Matsumura as being due to segregation of these two genes on chromosome 2B, the inhibitor being derived in both cases from *T. turgidum* ssp. *dicoccoides*. Tsunewaki interpreted his own results similarly in the cross involving ABD I, a synthetic formed from *T. turgidum* ssp. *dicoccoides* conv. *spontaneonigrum* x nonglaucous *Ae. squarrosa*. This cross yielded an F_2 of 17 glaucous:373 nonglaucous plants. The individual family sizes were too small to locate the genes as to chromosome, but he interpreted the ratio as a 3:61 segregation indicating two inhibitor genes and a recessive nonglaucous gene in the synthetic. This is in accordance with the findings of Kihara and Matsumura. It has been pointed out by Driscoll (personal communication), however, that Tsunewaki's results also

fit a 1 glaucous:15 nonglaucous ratio ($\chi^2_{1:15}$ = 2.35, 1 d.f. P = .2 to .1) indicating that only the two inhibitors were segregating. Hence the evidence for the hypothesis of separate loci on 2B is sparse, compared with that against it. Thus it appears that the three genes on 2BS, viz., the inhibitor of 5075, the gene for glaucousness of Chinese Spring and the recessive gene of Mentana, are multiple alleles.

III Whole-Plant Glaucousness in other varieties.

(a) Poso

Poso is more heavily glaucous than Chinese Spring under most environmental regimes. The F_1 of Poso by Chinese Spring is similar to Poso in phenotype. The F_1 of Mutant-2 x Poso is also like Poso phenotypically. Backcrossing to Mutant-2 was carried out in order to determine whether or not the heavily glaucous character was due entirely to the 2B gene of Poso. The BC_1 population segregated 2 very slightly glaucous : 8 glaucous. None of the glaucous plants reached Poso level, although one plant was heavily glaucous on the late tillers. This was observed in the winter generation, when the level of glaucousness is generally lower than in the summer generation. One of the glaucous BC_1 was used to produce the BC_2 generation, which segregated 2 very slightly glaucous : 3 glaucous : 1 heavily glaucous, suggesting that the lack of heavily glaucous segregants in the BC_1 generation may have been due to environmental variation. The BC_3, which was derived

from the heavily glaucous BC_2 plant, consisted of 3 very slightly glaucous : 2 glaucous : 1 heavily glaucous plants. One of the glaucous plants was used to produce the BC_4 which segregated 3 glaucous : 2 very slightly glaucous. If the heavily glaucous phenotype of Poso were due to the activity of the 2BS gene alone, the various backcrosses would have segregated 1 heavily glaucous (as the F_1) : 1 very slightly glaucous (as Mutant-2). The much greater variation than this is evidence of the phenotype of Poso being determined by more than one gene.

Poso was also backcrossed to Chinese Spring monosomic 2B in order to provide an alternative manner of examining the 2BS gene of Poso.

The BC_1 segregated 7 heavily glaucous : 1 glaucous, all being monosomics. One of the heavily glaucous BC_1 plants was used to produce the BC_2, which segregated 1 heavily glaucous monosomic : 6 glaucous monosomics : 1 heavily glaucous disomic. One of the glaucous, monosomic BC_2 plants was used to produce the BC_3, which segregated 3 glaucous monosomics : 1 glaucous disomic. Thus Poso has a gene on chromosome 2B which has a similar effect to that of the 2B gene of Chinese Spring. The progressively lower frequency of occurrence of the heavily glaucous phenotype with successive backcrosses conforms with that observed during the backcrossing of Poso to Mutant-2, in that at least one gene in addition to the 2B gene is involved in the production of the heavily glaucous phenotype. Whether this factor (or factors) is associated with group-2 chromosomes is not known.

(b) The synthetic ABD XIII

According to Tsunewaki, (1966), this synthetic possesses a dominant inhibitor of glaucousness on chromosome 2D. He also concluded from the 3:1 ratio with Chinese Spring that it had derived a gene for glaucousness on 2B from the glaucous emmer parent (*T. turgidum* ssp. *dicoccum* variety Vernal). This finding was confirmed at this University by the first backcross generation of (monosomic 2B)^2 x ABD XIII, which contained 4 glaucous and 6 nonglaucous plants (all ten plants were monosomics). This fits the 1:1 ratio expected if the dominant inhibitor on 2D is segregating while the gene for glaucousness on 2B is always present. Since chromosome 2B of Chinese Spring is absent in this BC_1 generation, chromosome 2B of ABD XIII must have a conventional gene for glaucousness.

(c) Javelin

The very slightly to slightly glaucous line of the variety Javelin was crossed to both Mentana (using the latter as pollen parent) and the glaucous line of Javelin (using the glaucous line as female). The F_1's were respectively, slightly glaucous and glaucous. This suggested that the less glaucous phenotype of the variant line of Javelin was recessive to that of the glaucous line. The F_2 of this cross, however, segregated 17 very slightly glaucous :3 glaucous. This was in the winter generation. Residual seed of the F_2 was grown out the following summer and segregated 5 very slightly glaucous :21 slightly glaucous : 6 glaucous. Both populations showed intergrading of the

phenotypic classes; however the phenotype represented by the F_1 was in the distinct minority in the F_2.

The F_1 of the less glaucous Javelin x Mentana indicated that the variant Javelin line lacked an effective group 2 gene for glaucousness, since it failed to complement the Mentana genotype. The F_2 of this cross segregated 11 very slightly glaucous : 1 slightly glaucous : 2 glaucous plants in the winter generation. Again residual seed was grown out the following summer, giving an additional 5 slightly glaucous and 1 glaucous plants. The appearance of transgressive segregants in this F_2 seems to indicate that the variant line differs from Mentana by several genes of minor effect.

Thus, both F_2 populations indicate that the difference between the two Javelin lines involves several genes. It thus seems more likely that the less glaucous line arose as a segregant of an outcross to a nonglaucous variety, rather than from spontaneous mutation. Chromosome loss as a basis of the less glaucous phenotype seems ruled out as this would have resulted in a low proportion of nonglaucous F_2 segregants from the intercross of the two types of Javelin. Thus it is evident that the pattern of inheritance of glaucousness in the variant line of Javelin differs somewhat from that in other varieties.

IV Observations on a sectorial plant.

A field-grown plant in the F_2 generation of Inhibitor-1 x the glaucous variety C.I. 12633 was found to have about one-fourth glaucous tillers and the rest nonglaucous. Selfed seed from each type of tiller was harvested separately. The first possibility examined was loss of the inhibitor-bearing chromosome in the glaucous tillers. Offspring from each type of tiller were examined at both mitosis and meiosis. These consisted of five F_3 plants from glaucous tillers, which were themselves glaucous, and three F_3 plants from a nonglaucous tiller which were nonglaucous. All eight plants appeared to have normal chromosomes and formed 21 bivalents at metaphase I. Thus somatic loss can not account for the sectoring.

The variety C.I. 12633 can be regarded as having a normal gene for glaucousness, as an F_2 of C.I. 12633 x Chinese Spring did not segregate for glaucousness. One possibility is that the sectorial F_2 plant was heterozygous for the 2B inhibitor and the C.I. 12633 gene for glaucousness on 2B, and that the inhibitor mutated to either a gene for glaucousness or to a recessive gene for nonglaucousness. To distinguish between these two alternatives an additional 20 plants were grown out from a glaucous tiller. All were glaucous; thus, if the inhibitor mutated, it did so to a gene for glaucousness, rather than to a recessive allele for nonglaucousness.

Another possibility is that the F_2 plant was homozygous for the gene for

glaucousness on 2B and one of these genes mutated to a dominant inhibitor. This possibility cannot be distinguished from the inhibitor mutating to a gene for glaucousness, but it might be expected that there would have been more glaucous than nonglaucous tillers in this case.

A further possibility is somatic loss of one chromosome 2B, bearing the inhibitor, and an increase to two doses, by nondisjunction, of the homologue bearing the gene for glaucousness. This possibility and the possibility of somatic crossing over are regarded as being less likely than spontaneous mutation of an inhibitor to a gene for glaucousness or vice versa.

General Discussion of Whole-Plant Glaucousness

Genes affecting whole-plant glaucousness have been found on both chromosomes 2B and 2D in Chinese Spring. If the hypothesis of a common progenitor for the ancestoral genomes of hexaploid wheat is correct, then the A genome may have included a homoeologous gene for glaucousness at some time. It appears that no such gene exists in the variety Chinese Spring. Tsunewaki (1966) found no indication of genes for glaucousness in the *Crithodium aegilopoides* subspecies which he observed. Mochizuki (personal communication to Dr. Driscoll) has found that monosomic 2A of Stewart Durum is more glaucous than euploid Stewart. This suggests a mild dosage-sensitive inhibition of glaucousness associated with chromosome 2A in this variety.

The hypomorphic gene for glaucousness on chromosome 2D in Chinese Spring appears to give a linear increase in glaucousness between two and four doses, in the absence of chromosome 2B. However, dosage response is not necessarily additive in wheat. The *spelta* gene, for instance, does not give rise to any phenotype for the squarehead character until five doses are reached. Above five doses it gives a linear response. Hemizygous-ineffective genes do not show a change in phenotype from nought to one dose (or from four to five doses, if homoeologous genes are involved) but do give a change in phenotype from one to two doses. In these cases there are threshold levels which must be exceeded for the expression of the phenotype. This may be the case with chromosome 2D below two doses.

In the presence of 2B a plateau is reached which gives little or no increase in the level of glaucousness from one to four doses of 2B. Perhaps one dose of the gene gives a dense enough crystal layer to reflect almost all of the incident light; hence, additional doses of the gene give little apparent increase in phenotype. However, this fails to explain the fact that some varieties appear considerably more glaucous than does Chinese Spring and may have up to twice as much β-diketone as Chinese Spring, whereas tetrasomic 2B has only slightly more β-diketones than the euploid (Barber and Netting, 1968). The heavy glaucousness of some other varieties (such as Poso) is determined by genes in addition to the 2B gene. Overall, it appears that increasing the dosage of chromosome 2B from nought to one gives a marked change in phenotype,

but a ceiling effect operates at doses greater than one.

The most common type of mutation that results in whole-plant nonglaucousness is mutation of the 2BS gene. This was found to be the case in Mutant-1, Mutant-2 and Mutant-3. At least two of these three recessive mutants are deletions of a segment of 2BS; the first involves most of the arm and the second a smaller segment.

In addition to the 5075 inhibitor, which was known to be located on chromosome 2BS, two additional inhibitors were also located on this arm. These were the E.M.S.-produced mutants, referred to as Inhibitor-3 and Inhibitor-4. There is some indication that the 5075 inhibitor may have mutated back to a gene for glaucousness.

The only exception to a 2BS location of a mutant effecting whole-plant glaucousness in this study was the inhibitor in synthetic ABD XIII, which was known to be located on a homoeologue, 2D.

The various mutants located on 2BS appear to be allelic to one another, the locus being distally located on 2BS.

SECTION 4 MODIFICATION OF THE DOMINANCE OF INHIBITORS OF GLAUCOUSNESS

I Detection

It was stated in Section 3 that genes for wholeplant glaucousness and inhibitors of these genes, have been located on chromosomes 2B and 2D. Evidence has been obtained that the expression of the dominant inhibitors of glaucousness of ABD XIII and C.W.S.5075 is subject to modification by genes in the background of these varieties and in the variety Poso.

This phenomenon was first observed in the F_1 of ABD XIII x Poso, which was fully glaucous. This is in contrast to the fact that the F_1 of ABD XIII x Chinese is nonglaucous. The dominance of the 2D inhibitor of ABD XIII thus depends on the genotype of the glaucous variety to which it is crossed.

A similar situation was detected with the inhibitor on chromosome 2B. The F_1 of Poso x C.W.S. 5075 is slightly glaucous. However the F_1 of Inhibitor-1 (the inhibitor of 5075 backcrossed into Chinese Spring) x Poso was nonglaucous. Hence, the genotypes of Chinese Spring and 5075, other than the 2BS locus involved in glaucousness, differ in their effect on the inhibitor in crosses to Poso.

II Analysis of the modification of the 2D inhibitor

(a) Complementary action of Poso and ABD XIII genes

The modification of the 2D inhibitor mentioned above was analysed as follows.

It is known from the above that the Poso genotype is involved in this modification. It has also been determined that the background genotype of ABD XIII is also involved in this phenomenon. This was demonstrated by the progeny of a nonglaucous segregant from the BC_1 (Chinese Spring^2 x ABD XIII). This nonglaucous plant can be considered heterozygous for the 2D inhibitor. If the dominance-modifying effect mentioned above was exerted purely by the Poso genotype, this BC_1 plant would be expected to give an all-glaucous progeny when crossed to Poso. The progeny of this cross, however, consisted of three nonglaucous and one glaucous plants. Thus the 2D inhibitor is now being expressed in crosses to Poso.

It appears that the nonglaucous BC_1 plant had, by virtue of the crossing, lost a gene or genes of ABD XIII that are essential to the dominance-modifying effect. As ABD XIII itself is nonglaucous, the dominance-modifying effect must be a complementary system involving at least one gene from each of ABD XIII and Poso.

ABD XIII was also crossed to tetrasomic 2B. This F_1 was fully glaucous. This result is of considerable importance, as the difference in phenotype between this hybrid and that of ABD XIII x Chinese Spring can only be due to the extra dose of chromosome 2B in the former.

The observation that an extra dose of chromosome 2B can change the dominance of the inhibitor suggested that a dosage effect of the 2B gene for glaucousness

was involved. This in turn suggested that the complementary modifier genes of Poso and ABD XIII might also be genes for glaucousness. Both varieties are known to have genes for glaucousness on chromosome 2B. The expression of the inhibitor would thus depend on the dosage balance between it and the genes for glaucousness.

(b) The Poso contribution

In order to examine the possible role of chromosome 2B of Poso in this complementary dominance modification, chromosome 2B of Poso was progressively substituted into Chinese Spring by backcrossing to mono 2B. The 2B substitution line was testcrossed as male to ABD XIII. The rationale for doing this was that almost all of the pollen would bear the Poso 2B chromosome, so that if 2B was the only chromosome involved in the modifying effect, all the progeny would be glaucous.

The first testcross generation of ABD XIII x a monosomic plant of the BC_1 [$(Mono\ 2B)^2$ x Poso] consisted of one nonglaucous and five glaucous plants. This indicated that the modifying effect (due to one or more genes) was still present in the substitution line but was segregating. Thus, if chromosome 2B is involved, it is not the only chromosome involved in the modification of dominance.

The second testcross of ABD XIII x two monosomic BC_2 [$(Mono\ 2B)^3$ x Poso] plants, gave 20 nonglaucous plants but no glaucous segregants. The modifier

effect has been lost between the first and second backcross generations (the same BC_1 plant was used in the testcross and as parent of the BC_2 generation). This suggests that besides the possible role of 2B, either a single gene is involved or a system of complementary genes. The five glaucous to one nonglaucous segregation in the testcross of the BC_1 plant suggests that a single gene is most likely. A complementary-gene system would be expected to give a higher proportion of nonglaucous plants in this testcross. For instance, 3/4 nonglaucous plants would be expected for a two-gene complementary system not involving chromosome 2B.

Similar observations have been made on a population in which the Poso 2B gene is also segregating. The F_1 of Poso x Chinese Spring was testcrossed to ABD XIII. This produced five nonglaucous plants and no glaucous plants. The lack of glaucous segregants in this cross, when 50% would be expected on the basis of a single modifier, indicates that the 2B gene of Poso is involved in the dominance modification. The probability of obtaining five nonglaucous plants on the expectation of 1/2 glaucous is 1/32, or less than 5%. The expectation for two complementary genes is 3/4 nonglaucous, giving a probability of 24%. Also, if neither of the two postulated genes is located on chromosome 2B, the high proportion of glaucous plants in the testcross of the BC_1 [$(\text{Mono 2B})^2$ x Poso] has only 0.4% probability of occurrence. Thus the most plausible hypothesis is that the modifying effect of the Poso genome is due to complementary interaction between a gene on chromosome 2B and a second gene in the background of Poso. This could of course be a homoeologue of 2B; however, this has not been tested.

General Discussion on the Mode of Action of Genes Affecting Glaucousness

Various phenotypes of whole-plant glaucousness have been observed in this study. These range from "nonglaucous", through a series of intermediates, to "heavily glaucous". Various types of alleles affecting the whole-plant glaucousness, such as the gene for "glaucousness" in Chinese Spring, a deletion in Mutant-2, an apparent recessive allele for "slight glaucousness" in Mentana and the inhibitors of glaucousness in 5075 and ABD XIII, have also been characterized.

All mutants affecting whole-plant glaucousness have been located on chromosome 2B or 2D. Some phenotypes, such as "heavily glaucous" as seen in Poso, are not entirely due to the major gene on 2B. However, all observations in this study are consistent with all of the various whole-plant glaucousness phenotypes being governed by a series of homoeoalleles on the chromosomes of homoeologous group 2.

On this basis the question arises as to how these various homoeoalleles interact to give rise to the various phenotypes. The defective-monomer hypothesis as discussed by Sears (1969) in connection with chlorophyll mutants of hexaploid wheat may apply to the observed variation in glaucousness. This hypothesis states that gene products, monomers, combine to give enzymatically-active multimers. Furthermore if more than one type of monomer is produced by two alleles, or a series of homoeoalleles, these various types of monomers may combine, perhaps at random, to produce a family of similar multimers.

A mutant gene is looked upon as producing a defective monomer which, when incorporated into a multimer with other defective monomers, or with unchanged monomers renders that multimer enzymatically ineffective or of low effectivity. If the pool of multimers is altered such that the amount of functional enzyme is below the threshold value, a change in phenotype occurs.

In the simplest case, the F_1 of Chinese Spring x C.W.S. 5075 is nonglaucous. As the inhibitor of 5075 and the 2B gene for glaucousness of Chinese Spring are regarded as alleles, the first allele is producing a defective monomer and the second a normal monomer. Three types of dimers, to take the simplest case, should be produced: 1/4 normal-normal; 1/2 normal-defective; 1/4 defective-defective. Thus only 1/4 of the dimers formed are capable of enzymic activity. In the case of the Mutant-2 deletion the heterozygote would form only half the amount of dimer formed in Chinese Spring, but all the dimers would be normal-normal.

This simple version of the model can be extended to include the homoeoalleles on 2D and perhaps on 2A. (Chinese Spring appears to have a null allele on 2A; however, 2A of 5075 may contain an active allele for glaucousness). In this case monomers would be produced by all active homoeoalleles. In some genotypes all six homoeoalleles may produce monomers.

The most direct evidence for the interaction of homoeoalleles is the contrast of the F_1's of ABD XIII x Chinese Spring and of tetrasomic 2B of Chinese Spring x ABD XIII. The former is nonglaucous and the latter glaucous.

The inhibitor of ABD XIII is on chromosome 2D, and the difference between the two F_1's is the dosage of chromosome 2B of Chinese Spring; thus the inhibitor on 2D is interacting with the gene for glaucousness on 2B.

The various phenotypes observed in this study, for example those resulting from the recessive allele of Mentana, and the complementary modification of dominance of the inhibitor of ABD XIII, can be explained by this hypothesis, particularly if one invokes varying strengths of alleles for production of glaucousness. This could involve variation in the amount of monomer produced, and the degree of defectiveness of the monomer, which could in turn variously alter its ability to combine with other monomers and its effect on the enzymic activity of multimers.

SECTION 5 ORGAN-SPECIFIC GENES FOR GLAUCOUSNESS

I Organ-specific genes in Chinese Spring

Although observations on the 21 nullisomics revealed that only nullisomic 2B is totally nonglaucous, there was nevertheless variation in phenotype amongst the other nullisomics (see Table 10).

Euploid Chinese Spring shows more variability of glaucousness of the peduncle, head and leaf blade than it does for the leaf sheath. Hence some variation for the former organs among the nullisomics is ascribable to environmental factors. There is some evidence that less vigorous plants may also show a lower level of glaucousness generally. The phenotypes of the nullisomics have therefore been interpreted with this caution .

Five plants of nullisomic 3A were observed initially in the winter generation. All were completely nonglaucous for the leaf, peduncle and head, but glaucous for the sheath of the primary tillers. A few of the plants did develop a slight general glaucousness on late tillers. The nullisomics were growing among normally glaucous monosomic 3A plants, making it unlikely that the effect was purely one of environment. Since all five of the nullisomics were nonglaucous for these organs it was concluded that a genotypic effect is involved.

Nullisomic 3B appeared to be less glaucous for the peduncle and leaf blade

TABLE 10

Phenotypes of the nullisomics of Chinese Spring

Genotype	No. of Plants		Leaf Sheath	Leaf Blade	Peduncle	Head
	Summer	Winter				
Nulli 1A		1	G	N	G	N
Nulli 1B		2	G	N	G	S
Nulli 1D		2	G	N	N	-
Nulli 2A		1	G	G	G	-
Ditelo 2BL			V	N	N	N
Nulli 2D	1		G+	N	S	-
Nulli 3A	6	5	G	N	N	N
Nulli 3B	2	1	G	G	S	S
Nulli 3D	4	1	G	G to N	H to G	S to N
Nulli 4A		2	G	G	G	S
Nulli 4B	1	1	G	G	G	S
Nulli 4D		3	G	G	N	-
Nulli 5A		3	G	S	S	S
Nulli 5B		1	G	G	G	-
Nulli 5D		4	G	S	S	S
Nulli 6A		1*	G	G	G	S
Nulli 6B		2	G	G	V	S
Nulli 6D		2	G	S to N	G to S	S
Nulli 7A		3	G	G	G	S
Nulli 7B		3	G	G	G	S
Nulli 7D		3	G	G	G	S
Chinese Spring Euploid			G	G	G to S	S

\+ lower than normal. * identified phenotypically, not checked cytologically.

G = glaucous

S = slightly glaucous

V = very slightly glaucous

N = nonglaucous

H = heavily glaucous

than euploid Chinese Spring or the monosomic 3B plants next to it on the bench. A further two plants observed in the summer generation have tended to confirm this observation.

The single nullisomic-3D plant observed in the winter generation was as glaucous as Chinese Spring for all organs. This observation was also confirmed by examination of four other nullisomic-3D plants in the summer generation.

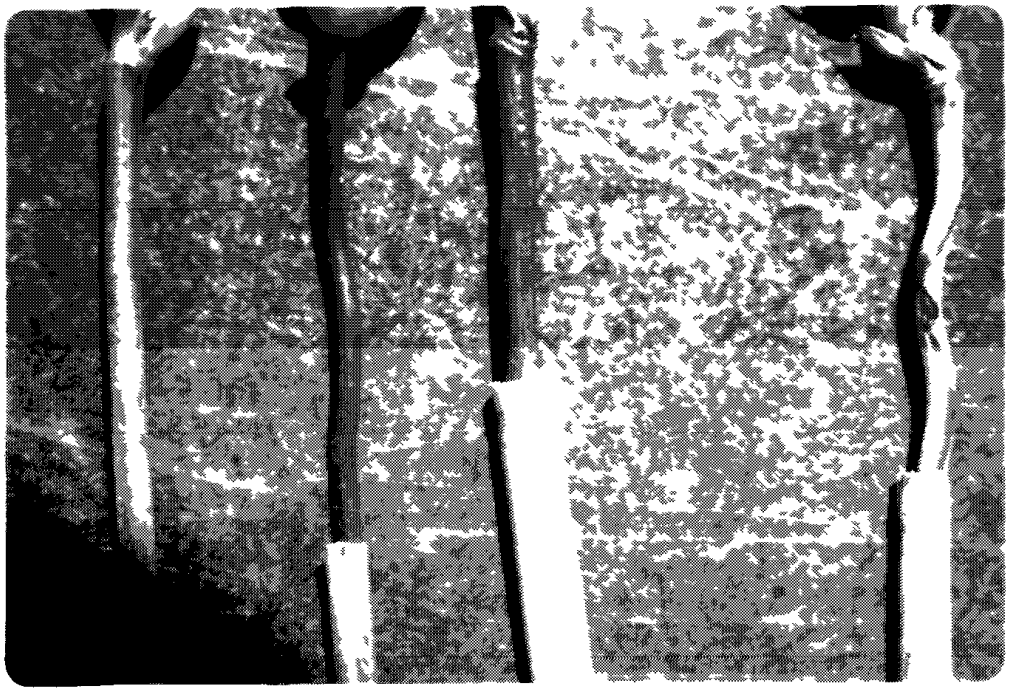
It seemed that chromosome 3A might carry a gene specific for glaucousness on the peduncle, leaf blade and head, while chromosome 3B might carry a hypomorphic homoeologous gene. Chromosome 3D appeared to have no effect on phenotype for these organs. (See Plate 4)

Some of the group-5 and -6 nullisomics also showed lower phenotypes for peduncle. Group-1 nullisomics had nonglaucous leaves.

Confirmation of some of these results was sought by observing the appropriate nullisomic-tetrasomic combinations for groups-3 and -6. The group-6 nulli-tetras were consistently like the euploid. This could only be so if any genes affecting glaucousness on the homoeologues were of equal effectiveness. But if this were so, all of the group-6 nullisomics would show an equal reduction in glaucousness. In actual fact, nulli 6A was glaucous

PLATE 4

Peduncles of Chinese Spring (glaucous to slightly glaucous), nullisomic 3A (nonglaucous), nullisomic 3B (slightly glaucous) and nullisomic 3D (glaucous).



for the peduncle, nulli 6B was very slightly glaucous and nulli 6D was between glaucous and slightly glaucous. It seems probable therefore, that the differences between the nullisomics were due to variation in the environment.

Observations of group 3 nulli-tetras, however, gave confirmation of the hypotheses formed from observations on the nullisomics. Nulli 3A-tetra 3D has the phenotype of Nulli 3A; that is, it is nonglaucous for the peduncle, leaf and head. Chromosome 3D thus cannot compensate for absence of 3A. Chromosome 3B, on the other hand, does have some ability to compensate for loss of chromosome 3A; nulli 3A-tetra 3B is slightly glaucous for the peduncle, while the leaf and head are nonglaucous. This observation, taken in conjunction with later observations on nulli-3D plants which, unlike the original observation, are often glaucous for peduncle but nonglaucous for leaf and head, suggests that there is not necessarily correlation for glaucousness of the three organs. Group-3 chromosomes appear to affect the peduncle most consistently. (See Plate 5)

Nullisomic-tetrasomic combinations having greater than normal dosage of chromosome 3A (that is, nulli 3D-tetra 3A and nulli 3B-tetra 3A) are heavily glaucous for peduncle. The remaining two combinations, nulli 3D-tetra 3B and nulli 3B-tetra 3D are both within the phenotypic range of normal Chinese Spring for all organs.

PLATE 5

Peduncles of nulli 3D tetra 3A (heavily glaucous), nulli 3A tetra 3D (nonglaucous), euploid Chinese Spring (glaucous), nulli 3A tetra 3B (slightly glaucous) and nulli 3B tetra 3A (heavily glaucous).

Since the phenotypes of the nullisomics and nulli-tetras differed somewhat between various environments, the phenotypes of the latter were checked under several different regimes. The glasshouse-grown material previously referred to has been compared with that grown in the field, where expression of glaucousness is low, and material grown in the controlled environment cabinet at the settings indicated. Under these conditions the level of glaucousness is maximal (see Table 11). Although the phenotypes do differ under varying conditions, the ranking is the same as that observed in the glasshouse. Environments differ in their suitability for distinguishing between particular genotypes.

The hybrid of nulli 3A-tetra 3B and nulli 3A-tetra 3D was examined in order to observe the phenotype of nulli 3A-trisomic 3B-trisomic 3D. The peduncle of this hybrid was, however, still within the range of the nulli 3A-tetra 3B phenotype, and no conclusion about the dosage relationships of 3B and 3D could be reached because of the insensitivity of the test.

The examination of tetrasomic 2B under field and glasshouse conditions reveals that although sheath glaucousness may be enhanced by addition of extra doses of the 2B gene, the peduncle of tetrasomic 2B is less glaucous under field conditions than that of Chinese Spring. This means that although the 2B gene is epistatic to the group-3 genes in the sense that the latter are not expressed in the absence of the 2B gene, higher dosage of 2B cannot

TABLE 11

Phenotypic Variation of Aneuploid Lines of Chinese Spring under different Environmental Conditions.

Genotype	Environment	Phenotype			
		Sheath	Leaf	Peduncle	Head
Euploid)	Field	G - S	N	G - V	-
Chinese)	Glasshouse	G	G - N	G - S	S - N
Spring)	C.E. Cabinet	H	H	H	G - S
	Field	G	N	S	-
N3AT3B	Glasshouse	G	N	S	N
	C.E. Cabinet	H	G	G - S	S
	Field	G - S	N	V - N	-
N3AT3D	Glasshouse	G	N	N	N
	C.E. Cabinet	H	G	S	S
	Field	H	N	H - G	-
N3BT3A	Glasshouse	H - G	S	H - G	G
	C.E. Cabinet	H	H	H	S
	Field	H - G	N	G	-
N3DT3A	Glasshouse	H - G	G	H - G	S
	C.E. Cabinet	H	H	H	S
Tetra 2B	Field	H - G	N	V - H	-
Poso	Field	H	H	H	-

The heads of plants in the field were not examined.

C.E. Cabinet - 14 hours full light, 6 hours dark. 80% humidity. Light temperature 70°F. Dark temperature 60°F.

raise the level of glaucousness of the peduncle.

Thus the major organ-specific genes in Chinese Spring are in homoeologous group 3. Lack of chromosome 3A results in a nonglaucous peduncle. Chromosome 3B bears a hypomorphic homoeoallele to that on 3A, whereas 3D appears to have a null allele.

II Organ-specific genes in other varieties

(a) Poso

Poso is heavily glaucous all over. The F_1 of Poso x Chinese Spring is similar to Poso with respect to sheath and peduncle, but intermediate for the leaf blade and head. The leaf blade is, however, more like Poso in that more of the abaxial surface is glaucous. The F_2 of Poso x Chinese Spring segregated for leaf glaucousness as 7 glaucous : 10 intermediate : 3 heavily glaucous. The glaucousness of the peduncles of the same plants segregated differently as 18 heavily glaucous : 2 glaucous. The correlation between the phenotypes is shown in Table 12.

It appears from the occurrence of plants heavily glaucous for peduncle but only glaucous for leaf, that glaucousness for these two organs could be inherited separately. This is consonant with the evidence from the group-3 nullisomic-tetrasomics in Chinese Spring, where the increased glaucousness of the peduncle in nulli 3D-tetra 3A and nulli 3B-tetra 3A is not accompanied

TABLE 12

Correlation between glaucousness for leaf blade and peduncle in the F_2 population of Poso x Chinese Spring

Phenotype of peduncle	Phenotype of leaf			Total
	Heavily Glaucous	Intermediate	Glaucous	
Heavily glaucous	3	10	5	18
Glaucous	0	0	2	2
Total	3	10	7	20

by a corresponding increase in leaf glaucousness. The leaf is greatly affected by environmental factors, however, and it is difficult to be certain of the genetic relationship between the two organs.

The F_1 of Poso and the Mutant-2 line was, again, similar to Poso for the sheath and peduncle. The F_2 of this cross showed a complex segregation with much intergrading. The correlation of the organ phenotypes are shown in Table 13.

These tables indicate that the glaucousness of the sheath and peduncle may be correlated, but there is little to indicate that the glaucousness of the leaf blade is correlated with that of the peduncle or sheath. The inheritance of the glaucous head was not specifically studied, but it was noticeable that no segregants heavily glaucous for the head, as Poso is, appeared in the F_2 of either Chinese Spring x Poso or Mutant-2 x Poso.

Further data on the inheritance of glaucousness of the peduncle were sought in the early backcrosses of Poso into monosomics 2B, 3A and 3D. Up to the BC_2 of monosomic 2B x Poso, the plants were still heavily glaucous for peduncle, but by the BC_3 most of the plants in the substitution line were back to Chinese Spring level for this organ. This indicates that the intensity of peduncle glaucousness is not a function of the 2B allele derived from Poso. This conforms with the evidence that the extra dosage of 2B in Chinese Spring tetrasomic 2B cannot raise the level of glaucousness of the

TABLE 13

The correlation for glaucousness between sheath, peduncle and leaf blade in the F_2 of Mutant 2 x Poso.

Classification of peduncle	Classification of Sheath			
	H	G	S	Total
H	3	0	0	3
G	1	10	0	11
S	0	3	2	5
Total	4	13	2	19

Classification of leaf	Classification of Sheath			
	H	G	S	Total
G	2	4	0	6
S	1	5	1	7
N	1	4	1	6
Total	4	13	2	19

Classification of leaf	Classification of peduncle			
	H	G	S	Total
G	1	4	1	6
S	1	4	2	7
N	1	3	2	6
Total	3	11	5	19

H = heavily glaucous, G = glaucous, S = slightly glaucous, N = nonglaucous.

peduncle.

The evaluation of the substitution lines involving group-3 chromosomes are shown in Table 14. These data show that Poso has a gene which is equivalent to the chromosome-3A gene of Chinese Spring. If this were not so, the 3A-substitution line would be nonglaucous for the peduncle. No slightly glaucous or nonglaucous peduncles were found among the 10 BC_2 plants of the 3A substitution line. Since, on the average, 87.5% of the background genotype would be from Chinese Spring in these plants, it seems probable that a Poso gene for glaucousness of the peduncle is located on chromosome 3A. However, as this BC_2 generation segregated 1 heavily glaucous : 9 glaucous, the heavily glaucous peduncle of Poso is not simply derived from the 3A locus alone. The BC_2 and BC_3 generations of the 3D substitution line indicate that the genetic basis of the heavily glaucous peduncle is not entirely due to a locus or loci on 3D either. The possibility still remains that the combination of the group-3 genes of Poso is responsible for the heavily glaucous peduncle of that variety.

(b) Mentana

The variety Mentana is slightly glaucous in phenotype due to lack of a fully active group-2 gene. Although nullisomic 3A in Chinese Spring is nonglaucous for peduncle, the monosomic F_1 plants of mono 3A x Mentana are of normal Chinese Spring level for the peduncle. Mentana therefore has a

TABLE 14

Peduncle phenotypes of group-3-substitution lines of Poso into Chinese Spring

Line	Peduncle phenotype for monosomic substitution plants of Group-3 monos x Poso			
	Heavily Glaucous	Glaucous	Slightly Glaucous	Total
BC_1M3A	5			5
BC_2M3A	1	9		10
BC_1M3D	3			3
BC_2M3D		1		1
BC_3M3D		4		4

gene(s) which is equivalent in effect to the gene on chromosome 3A of Chinese Spring. This confirms the epistatic interaction of group-2 over group-3 genes, as Mentana carries this gene(s) for peduncle glaucousness which is not expressed in the absence of an active group-2 gene for whole-plant glaucousness. The BC_1 of the substitution of chromosome 3A of Mentana into Chinese Spring consisted of two plants glaucous, two slightly glaucous, and one very slightly glaucous for the peduncle (all plants are monosomics). Thus if chromosome 3A of Mentana is involved in the glaucousness of the peduncle, it is not solely responsible, for if this were the case all BC_1 plants would have been glaucous for the peduncle. Once again, the possibility remains that a combination of genes for peduncle glaucousness resides on two or three of the group-3 chromosomes.

(c) Durum 396 and a Marfed mutant

The results obtained by Allan and Vogel (1960) at Pullman, Washington, using this variety are reviewed in the literature survey of this thesis (page 35). Briefly, these authors found that the difference between the culms (which can be equated to peduncle for this character) of Durum 396 (heavily glaucous) and Chinese Spring (almost nonglaucous) was due to a single hemizygous-ineffective gene on chromosome 2B. Chinese Spring is glaucous for peduncle under University of N.S.W. conditions. Presumably this variation in phenotype is due to a difference(s) in the environment between the two locations. The phenotype of Durum 396 under University of N.S.W. conditions is described fully in the Materials and Methods section (p. 46). This variety is peculiar in that of all the varieties studied at University of N.S.W., it was the only

one to be less glaucous for the sheath than it was for the peduncle and head. This is of interest, because taken in conjunction with Allan and Vogel's findings it suggests that there may be separate organ-specific genes for the leaf sheath. The characteristic of the sheath being lower in glaucousness than the peduncle is affected by the gene for glaucousness on chromosome 2B of Chinese Spring, as the F_1's of Mono 2B x Durum 396 (13" + 8') and Mutant-2 x Durum 396 were slightly glaucous for sheath but glaucous for peduncle (Table 15). As the eupentaploid F_1 was glaucous for the sheath as well as the peduncle, the 2B gene itself may be determining this organ specificity, or alternatively, the 2B gene of Chinese Spring, but not Durum 396, may be epistatic to another genetic system which directly determines this variation.

Thus populations segregating for types of organ variation could actually be segregating primarily for epistatic and non-epistatic alleles on chromosome 2B. This may have been the case in Allan and Vogel's F_2 populations, which seemed to associate organ specificity with group 2.

The 3A-deficient F_1 was similar to the eupentaploid hybrid in that the sheath was not less glaucous than the peduncle. These plants would however, be heterozygous for the 2B locus, which on the above hypothesis would result in epistasis of the potential organ specificity. It does mean, nevertheless, that Durum 396 has a gene on chromosome 3A approximately equivalent to that on 3A of Chinese Spring.

TABLE 15

Phenotypes observed for crosses involving Durum 396 at the University of New South Wales.

Genotype	Generation	Number of plants	Phenotypes Sheath	Peduncle	Head
Durum 396	Parent		V - S	G	H
Chinese Spring	Parent		G	G	N - S
Chinese Spring × Durum 396	F_1	2	G	G	G
Mutant 2 × Durum 396	F_1	4	S	G	N
Mono 2B × Durum 396 (13"+8')	F_1	3	S	G	N
(14"+7')	F_1	2	G	G	G
Mono 3A × Durum 396 (13"+8')*	F_1	2	H	H	H
(13"+8')*	F_1	1	G	G	G

N = nonglaucous

V = very slightly glaucous

S = slightly glaucous

G = glaucous

H = heavily glaucous

* This variation probably reflects environmental differences.

The specificity regarding the head is also affected by the 2B gene of Chinese Spring, as removal of this chromosome results in a change from glaucous to nonglaucous. The same type of epistatic action of the Chinese Spring 2B allele may be involved; however, the shift is in the other direction in this case, i.e., even further away from the Durum 396 phenotype. Thus the glaucousness of the sheath and the head appear to be under different genetic controls.

Thus this glaucousness pattern of Durum 396, which is different from all other patterns observed, appears to have a rather complex genetic control. It is possible that this control resides on the two homoeologues of group 3, and the 2B allele of Chinese Spring, but not of Durum 396, is epistatic to this control of organ specificity. Production of substitution lines of chromosomes 3A and 3B of Chinese Spring into Durum 396 would provide further data on this question.

The suggestion that the glaucousness of the head and the sheath are under separate genetic control is strengthened by the phenotype of the nonglaucous-head Marfed mutant. The sheath (as well as the peduncle and leaf blade) is identical in mutant and parent variety; however, the head is distinctly different. Allelism of the one locus has not, however, been excluded in this case.

General Discussion on Organ-Specific Genes for Glaucousness

The 21 nullisomics of Chinese Spring were isolated and examined for glaucousness. Although nulli 2B was the only one that showed a reduction in glaucousness in all plant parts, nullisomics of group 3 displayed non-glaucousness for specific organs. Chromosome 3A plays the major role in this regard, nulli 3A being nonglaucous except for the sheath, which is identical to that of euploid. Nulli 3B had a similar but less obvious effect, whereas nulli 3D is similar to euploid. These results were confirmed with observations on the group-3 nullisomic-tetrasomics under field conditions (low level of glaucousness), glasshouse conditions (intermediate level), and controlled environment of high temperature and high light (high level of glaucousness).

Deficiency of the gene for whole-plant glaucousness is epistatic to those for organ specificity.

Organ-specific genes are also present in the varieties Poso, Mentana Durum 396 and a mutant of Marfed. In the cases examined by means of back-crossing to monosomic 3A, viz., Poso and Mentana, it was determined that both varieties have genes that can substitute for chromosome 3A of Chinese Spring; however, these genes are not all located on 3A. Hence, the inheritance of organ-specific glaucousness can be regarded as being complex; however, it is not known whether genes outside of homoeologous group 3 are involved.

SECTION 6 CONCLUSIONS

A gene for the production of glaucousness homoeologous to that on chromosome 2B was found on chromosome 2D of Chinese Spring. The 2D gene was considerably weaker in effect than that on chromosome 2B. Chromosome 2A of Chinese Spring was also examined, but no gene affecting glaucousness was found.

It was shown that chromosome 4B, which had been reported to carry a gene for glaucousness, had no effect on the phenotype for glaucousness. The nonglaucous phenotype of the monosomic 4B line was found to be due to homozygosity for a recessive allele of the 2B gene for glaucousness. A homozygous disomic line of this mutant was isolated and named the Mutant-2 line. The pairing of chromosome 2B of this line with the short-arm telocentric of 2B was found to be low. The possibility of deletion of the 2B gene of Chinese Spring was tested indirectly by a comparison of the arm ratio of the mutant and normal chromosome 2B. A significant difference was found between the two arm ratios, the mutant chromosome being the more heterobrachial of the two. This apparently resulted from loss of a portion of the short arm of 2B. The mutant "gene" also behaved as a null allele in a dosage test. The evidence is thus compatible with the mutation being a deletion of a segment of 2BS which includes the gene for glaucousness.

The recessive nonglaucous plants which appeared in the progeny of the BC_5 of (Chinese^6 x 5075) were found to be due to the deletion of most of the short arm of chromosome 2B including the locus for glaucousness. The arm ratio of the deletion chromosome in the line homozygous for the abnormality (the Mutant-1 line) was found to be 1:4.1 at metaphase of mitosis. In addition, the short arm was mapped cytologically. Since no pairing was observed between the short arm and the normal 2BS telocentric, the length of the short arm was zero map units, although the short arm was cytologically observable.

Nullisomic 6B, which had been reported as nonglaucous, was found to be glaucous when produced at this University. The nonglaucous phenotype of ditelosomic 6BS and monoisosomic 6BS was found to be due to homozygosity for a recessive allele of the Chinese Spring gene for glaucousness on 2BS. Nonglaucous lines carrying this mutation are referred to as Mutant-3 lines.

The 20 nullisomics of Chinese Spring, excluding nullisomic 2B, were observed to be glaucous for the leaf sheath, although the single nulli-2D plant observed appeared to be somewhat less glaucous than Chinese Spring.

The ethyl methanesulfonate-induced mutants, both behaving as dominant inhibitors of glaucousness, have been located on chromosome 2B and behave as alleles of the inhibitor derived from 5075.

The slightly glaucous variety Mentana was found to lack an effective 2B gene. The 2B allele of Mentana was mapped by the F_2 telocentric method and was found to lie between 33 and 50 map units from the centromere. Driscoll (1964 & 1966) found that the 5075 inhibitor mapped at 42 to 50 units from the centromere. A linkage test of the recessive Mentana gene and this inhibitor gave no recombinants. This indicated a maximum distance of 7.6 map units between the inhibitor and the Mentana allele. Although positive proof of allelism cannot be obtained when one of the alleles is dominant, this constitutes reasonable evidence that the two genes are allelic to each other and to the gene for glaucousness. The 2B locus is thus a multiple allelic system, with the inhibitor dominant to the gene for glaucousness and the latter dominant to the recessive alleles for nonglaucousness.

The heavily glaucous variety Poso was found to have a gene for glaucousness on chromosome 2B; however, this gene alone is not responsible for the heavy glaucousness of Poso.

The less glaucous variant of variety Javelin was found to have an ineffective group-2 gene for glaucousness, but one or more other genes are also involved in the low glaucousness of this variant. It seems probable that this type arose from segregation following outcrossing rather than by mutation within Javelin.

Spontaneous mutation is thought to have been involved, however, in a sectorial F_2 plant of the cross Inhibitor-1 x C.I. 12633.

It was found that the effect of the dominant inhibitors from both ABD XIII and 5075 can be altered by the background genotype of the cross. Thus the hybrids of both varieties with Chinese Spring were nonglaucous, but the hybrids with Poso were glaucous and slightly glaucous, respectively. The inhibitor on chromosome 2D from ABD XIII was also modified by an extra dose of chromosome 2B from Chinese Spring. In crosses to Poso, the genotypes of Poso and the background genotype of ABD XIII were both necessary to modify the dominance of the inhibitor. The contribution of Poso to this complementary interaction appears to involve the 2B gene and one other.

The mode of action of the various alleles and homoeoalleles affecting glaucousness, including the inhibitors is consistent with a "defective-monomer" hypothesis of gene interaction.

Chromosome 3A of Chinese Spring was found from observation of the nullisomics and the group-3 nullisomic-tetrasomic combinations, to bear a gene for glaucousness of the peduncle. Chromosome 3B was found to bear a less effective homoeologous gene, while chromosome 3D appeared to have no gene with this effect. Absence of the 2B gene for whole-plant glaucousness is epistatic to these genes in the varieties studied, since the peduncle has not been found to be glaucous in the absence of an effective 2B allele.

Evidence from Mentana supports this. Mentana was found to have a gene(s) able to substitute for chromosome 3A in Chinese Spring with respect to peduncle. This gene(s) was not expressed in the presence of Mentana's ineffective group-2 genes. Poso was also found to have a gene(s) able to substitute for chromosome 3A of Chinese Spring in its effect on peduncle. In both cases, however, compensation is not due to chromosome 3A alone.

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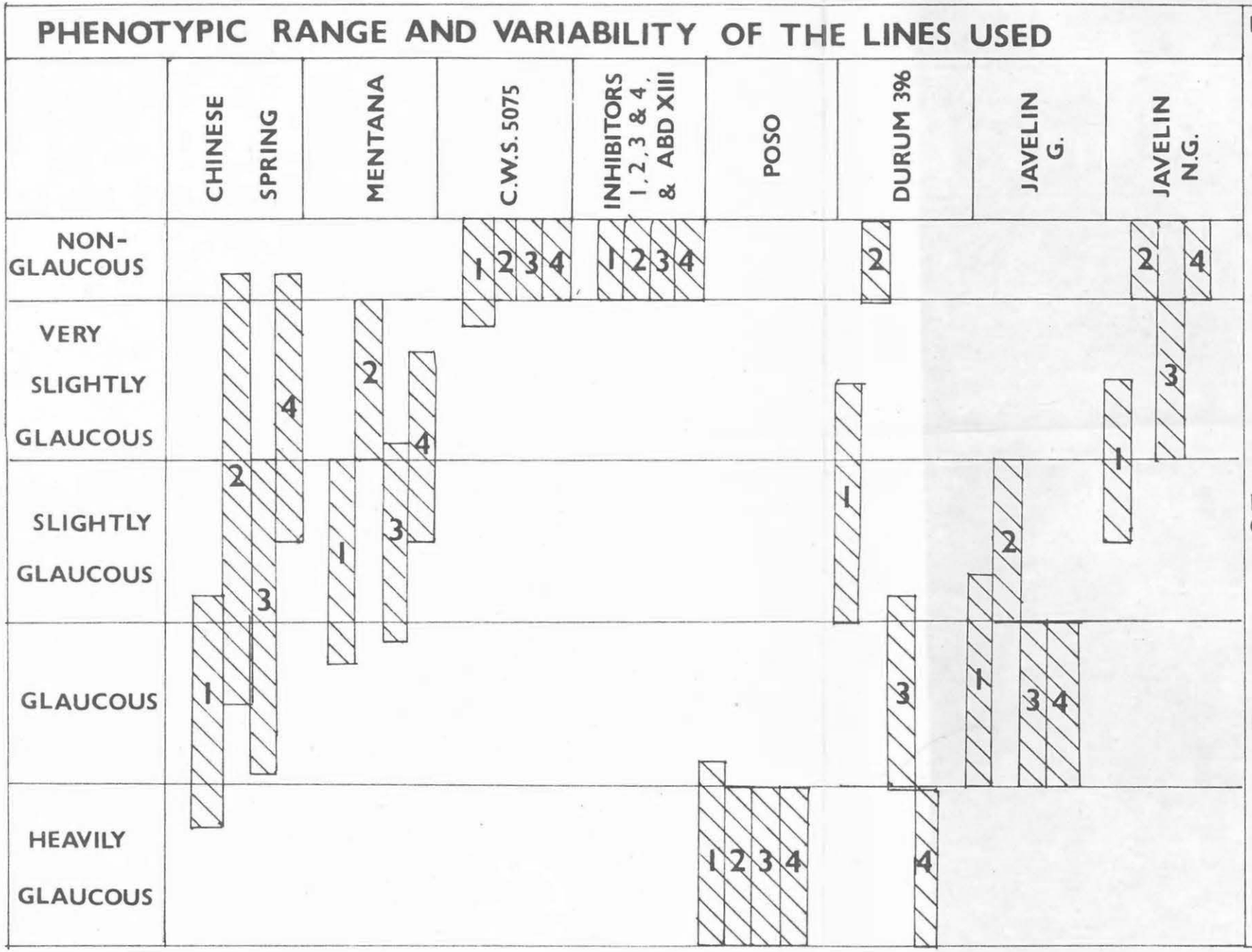
PHENOTYPIC RANGE AND VARIABILITY OF THE LINES USED



N.B. 1. THE PROPORTION OF A BAR IN A PHENOTYPIC CLASS IS EQUIVALENT TO THE PERCENTAGE OF PLANTS WHICH WERE OBSERVED TO SHOW THAT PHENOTYPE.

2. ALBEIT CLASS INTERVALS ARE SHOWN AS EQUAL, THIS DOES NOT IMPLY QUANTIFICATION OF THE PHENOTYPIC CLASSES - SEE P. 41

KEY:

1. SHEATH
2. BLADE BASE
3. PEDUNCLE
4. HEAD