LINKAGE STUDIES INVOLVING TWO CHROMOSOMAL MALE-STERILITY MUTANTS IN HEXAPLOID WHEAT[1]

K. K. BARLOW[2] AND C. J. DRISCOLL[3]

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ABSTRACT

The factors for Cornerstone and Probus male sterility are allelic on chromosome arm *4Aα*. They map independently of the centromere, but show linkage with a rye segment located 1 crossover unit from the centromere on the *β* arm. The alien segment causes asynapsis and some precocious terminalization of chiasmata when in repulsion with the mutants. The mutants, presumed to be terminal deletions, cause some desynapsis, but not asynapsis.

THE chromosomal male-sterility mutants referred to as "Pugsley's male-sterile" and the "Probus" male-sterile, reported by PUGSLEY and ORAM (1959) and FOSSATI and INGOLD (1970), respectively, were examined cytologically by DRISCOLL (1975). The two mutants were found to be allelic in that the intercross was male sterile and they were both located on chromosome *4A*; they now bear the symbols *msla* and *mslb*, respectively. A third male-sterility mutant referred to as the "Cornerstone" mutant (DRISCOLL and BARLOW 1976; DRISCOLL 1977) has been examined with regard to its relationship to the two described previously. Further, the Cornerstone and Probus mutants have been subjected to linkage tests with the *4A* centromere and with a rye segment that had been translocated to the opposite arm of chromosome *4A*. The effects of the Cornerstone and Probus mutants, presumed to be terminal deletions, on precocious terminalization of chiasmata and the effects of a translocated alien segment on asynapsis and precocious terminalization have been measured.

MATERIALS AND METHODS

The two male-sterility mutants of hexaploid wheat (*Triticum aestivum* L.) involved in this study are "Cornerstone" (DRISCOLL 1977) and "Probus" (FOSSATI and INGOLD 1970), both of which were induced by ionizing radiation. The two mutants were crossed with normal male-fertile wheat and examined for segregation in the F_2. One homozygous mutant was pollinated by the other mutant as a heterozygote in order to test for allelism. Both mutants were crossed as heterozygotes to Chinese Spring monosomics *4A*, *4B* and *4D* in order to confirm the specific chromosome location of the Probus mutant and to establish that of the Cornerstone mutant. The mutants were crossed to telocentric stocks of chromosome *4A* in order to locate the mutants to specific chromosome arm. Homozygous mutants were crossed as females to ditelocentric *4Aα* and heterozygous mutants were crossed as males to monoditelocentric *4Aβ*. Ditelocentric *4Aα*

[1] This study was carried out in the School of Botany, University of New South Wales.
[2] Present address: Comonwealth Institute of Health, Sydney, New South Wales, Australia, 2006.
[3] Present address: Department of Agronomy, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, South Australia, 5064.

was maintained as such; however, ditelocentric *4Aβ* is sterile, so that monoditelocentric *4Aβ* (with one dose of *4Aα* and two doses of *4Aβ*) was used. Approximately three-fourths of the eggs of this aneuploid contain only the *β* telocentric. The F_1's involving *4Aα* were backcrossed to their respective homozygous mutant parents in order to map the mutants with respect to the centromere, as outlined by SEARS (1966a).

The mutants were also crossed with 'Transec' in a Federation background. This is a wheat-rye translocation line that involves translocation to wheat chromosome arm *4Aβ* of a segment of rye chromosome *2R*, bearing a gene conferring resistance to wheat leaf rust. The proximal end of this rye segment maps one crossover unit from the centromere (DRISCOLL and BIELIG 1968). Linkage between the male-sterility mutants and the rye segment was determined in backcross populations, the rye segment being recognized by means of the resistance gene.

RESULTS

The mutants: The Cornerstone and Probus male-sterile plants do not differ from their fertile counterparts in vegetative vigor or female fertility. The mutants are phenotypically indistinguishable from each other and differ from normal in that the anthers are arrow-shaped, a deeper yellow color, do not dehisce and are thinner than anthers of fertile plants. The filaments of the sterile anthers rarely elongate. All pollen grains of the mutants are essentially without cytoplasm.

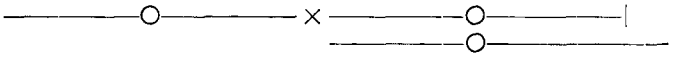
Heterozygotes of each, on selfing, segregated in conformity with a single recessive mutant in each case (477 fertile : 137 sterile in the case of Cornerstone and 399 : 130 in the case of Probus).

The progeny from the intercross between male-sterile Cornerstone and heterozygous Probus segregated 23 fertile : 17 male-sterile plants; thus, the mutants fail to complement each other.

Chromosome and chromosome arm location: The segregation patterns of the F_1 populations of monosomics *4A*, *4B* and *4D* by the heterozygous mutants are shown in Table 1. Each mutant involves a single recessive mutation; therefore,

TABLE 1

Segregation for fertility/sterility in F_1 progeny from crosses of monosomic 4A, 4B *and* 4D × *Cornerstone and Probus heterozygotes*



Monosomic	Cornerstone		Probus	
	Fertile	Sterile	Fertile	Sterile
4A	5	4	3	2
			4‡	4
4B	6*	0	4	0
			12‡	0
4D	4	0	5	1†

See Figure 1 for drawings of bivalents.

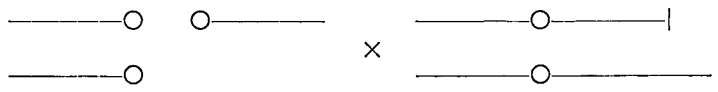
* Includes two partially male fertile plants.

† This individual also had abnormal stigmas; its male sterility is regarded as being due to extraneous factors.

‡ From DRISCOLL (1975).

TABLE 2

Segregation for fertility/sterility in F_1 progeny of crosses of monoditelocentric 4Aβ × Cornerstone and Probus heterozygotes



Mutant	Chromosome Complement in Root Tips 41 + 2t		41 + 1t	
	Fertile	Sterile	Fertile	Sterile
Cornerstone	7	0	3	4
Probus	5	0	6	5

alteration in only one chromosome is involved in each case. This is chromosome 4A in both cases. Segregations of 4:0, 5:0 and 6:0, as occurred with chromosomes *4B* and *4D*, have probabilities of 1/16, 1/32 and 1/64, if *4B* or *4D* were the critical chromosome. Although the highest probability in itself is inconclusive, the data as a whole, including those of DRISCOLL (1975), are consistent with both mutants being located on chromosome *4A*.

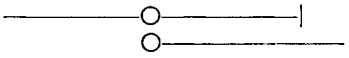
The F_1 progenies from crosses between the two male-sterile mutants and ditelocentric *4Aα* consisted entirely of fertile plants (9 in the case of Cornerstone and 7 in the case of Probus). However, both F_1 progenies resulting from the cross of monoditelocentric *4Aβ* × the heterozygous mutants segregated among plants with one telocentric chromosome, as shown in Table 2.

Telocentric mapping: The F_1's of ditelocentric *4Aα* by each of the heterozygous mutants were analyzed for pairing of the telocentric at metaphase I, as shown in Table 3. Figure 1 depicts the critical bivalent. These F_1 plants were backcrossed as males to the respective male steriles, and the BC_1 populations were scored for both mitotic chromosome complement and fertility/sterility, as shown in Table 4.

Linkage of mutants with the Transec translocation: The male-sterile mutants were crossed to Transec, and the F_1 was cytologically analyzed at metaphase I, as shown in Table 5. Figure 1 depicts the critical bivalent. The F_1 plants were backcrossed to the respective male-sterility mutants, and the segregation of resistance/susceptibility and fertility-sterility was observed, as shown in Table 6.

TABLE 3

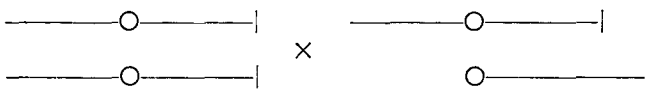
Number of cells with telocentric paired/unpaired at metaphase I in F_1 progeny in crosses of male-sterile homozygotes × ditelocentric 4Aα



Telocentric	Cornerstone	Probus
Paired	15	25
Unpaired	65	35

TABLE 4

Segregation for chromsome complement and fertility/sterility in BC_1 generations of mutant × (mutant × ditelocentric 4Aα)

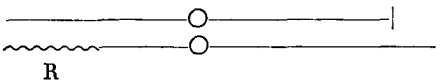


Mutant	Chromosome complement*	Fertile	Sterile
Cornerstone	42	23	29
	41 + *t*	5	0
Probus	42	22	20
	41 + *t*	3	0

* 42 = 42 entire chromosomes.
41 + *t* = 41 entire chromosomes plus one telocentric chromosome.

TABLE 5

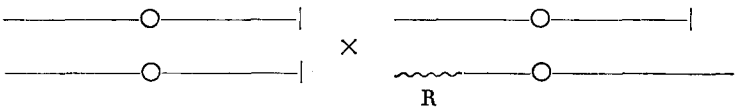
Pairing patterns at metaphase I in F_1 progeny of each male-sterility mutant × Transec.



Mutant	No. cells observed	% Cells at MI with			
		21″	19″ + 1‴ + 1′	20″ + 2′	19″ + 4′
Cornerstone	200	3	0	74	23
Probus	80	10	3	70	17

TABLE 6

Segregation for resistance/susceptibility and fertility/sterility in BC_1 populations of male sterile × (male sterile × Transec)



BC_1	Cornerstone	Probus
Fertile, resistant	26	23
Sterile, susceptible	22	19
Fertile, susceptible	6	8
Sterile, resistant	7	16

DISCUSSION

The mutants exhibit monofactorial inheritance and fail to complement each other, in that approximately 50% of intercross plants, when the mutant used as male parent is heterozygous, are male sterile. Thus the mutants are either allelic or homoeoallelic, *i.e.*, forms of corresponding genes on homoeologous chromosomes. Allelism, rather than homoeoallelism, was established when both mutants were located on chromosome *4A* by monosomic analysis (Table 1). The absence of authentic steriles in the populations involving monosomic *4B* and *4D* excludes those two chromosomes from bearing the mutants. Thus, the Cornerstone mutant is a member of the same allelic series and takes the symbol *mslc*. The fact that these three male-sterile mutants (Pugsley, Probus and Cornerstone) are allelic is significant. Most of the genes in hexaploid wheat are duplicated or triplicated; however, chromosome *4A* possesses a gene for male fertility that is not present on homoeologues *4B* and *4D*. This is reflected in nullisomic *4A*, tetrasomic *4B* (or *4D*) being male sterile and the other nullisomic-tetrasomics of homoeologous group *4* being fertile (SEARS, 1966b).

This unduplicated locus is available for mutation to male sterility and subsequent monofactorial behavior. Among the genes for male fertility, this *4A* gene may be unique in being unrepresented in homoeologues. There does, however, appear to be a second gene for male fertility on chromosome *4A*. This was argued by DRISCOLL (1975) on the basis of the occurrence of partially fertile plants in the F_1 of monosomic *4B* × Probus heterozygote, but not Pugsley heterozygotes. The same intermediate type occurred in this study with Cornerstone. The intermediate phenotype is regarded as involving heterozygosity of *4A* and hemizygosity of *4B*. Hence, a second gene for male fertility on *4A*, that is also on *4B*, is involved.

Ionizing radiation often induces deletions (STADLER and ROMAN 1948) and both induced mutants *mslb* and *mslc* are regarded as involving deletions of both genes for male fertility on *4A*; whereas, the spontaneous mutant *msla* is thought to involve change in the unique gene only. It is likely that *mslb* and *mslc* involve terminal deletion and healing of the broken end, as this requires only one induced chromosome break. Healing of broken ends of chromosomes has been described in Tradescantia by SAX (1940) and in maize by MCCLINTOCK (1941). The study by SAX involved X-ray induced breaks, some of which resulted in terminal deletions.

Both *mslb* and *mslc* are located on the α arm of chromosome 4A, as would be expected on the basis of ditelocentric 4Aα being fertile and ditelocentric 4Aβ being sterile. In each of the two F_1's of Table 2, approximately half of the monotelocentric offspring, which lack *4A*α, were male sterile. This is the proportion expected with crosses involving heterozygous male parents.

The F_1 plants of homozygous mutant × ditelocentric *4A*α showed considerably less than 100% pairing of the telocentric chromosome at metaphase I (Table 3). This behavior is consistent with the mutants being terminal deletions and could involve failure of *4A* pairing in some meiocytes (*i.e.*, asynapsis) or pairing, crossing over and precocious terminalization of chiasmata in other meiocytes (de-

synapsis). Heterozygous deletions are known to reduce map distances (BRIDGES 1919). The distinction between asynapsis and desynapsis is gleaned from the backcross data (Table 4). Crossing over in the F_1 produces two types of telocentrics that are available for incorporation into male gametes; the parental telocentric and the crossover product telocentric. The latter involves deletion of part of the α arm (the mutant), as well as loss of the complete β arm and is thus particularly vulnerable to adverse selection pressure. Therefore the eight plants in Table 4 that contain a telocentric chromosome are ignored because their inclusion in the calculation would certainly introduce a bias. The fact that all eight telocentric-bearing BC_1 plants are fertile supports this concept.

Of the BC_1 plants with 42 entire chromosomes segregation for fertility/sterility conforms to a 1:1 ratio in each case (23:29, $P = 0.4$; 22:20, $P = 0.75$). Thus, the fertility/sterility alleles assort independently of the presence/absence of the β arm. Hence, the genetic distance between centromere and the deletion is 50 crossover units in each case. Meiotic pairing must occur in this region in all meiocytes; thus, bivalent absence at metaphase I must involve desynapsis rather than asynapsis. Reduction in chiasma frequency has been observed between diakinesis and metaphase I in a heteromorphic bivalent in wheat involving a translocation chromosome and a telocentric chromosome (FU and SEARS 1973). The greater desynapsis involving Cornerstone (81.25%) as compared with Probus (58.33%) probably reflects loss of a longer segment in the Cornerstone mutant than in the Probus mutant.

In F_1's of Cornerstone or Probus × Transec, the *4A* chromosomes have not been distinguished from each other; thus, it has been assumed that where two or more univalents are observed at metaphase I, chromosome *4A* univalents are involved. On that basis, a *4A* bivalent occurs as a minimum in 3% and 13% of metaphase I meiocytes involving Cornerstone and Probus, respectively (Table 5). Heterozygous translocations are known to lower map distances (BURNHAM 1934). The contributions of asynapsis and desynapsis to the remaining 97% and 87%, respectively, are obtained from the BC_1 data (Table 6).

In the Cornerstone backcross, a 1:1 ratio is observed for each character pair; however, 48 sampled gametes were parental types and 13 were recombinant types, which constitutes a significant departure from a 1:1 ratio ($P < 0.01$). Similarly, with the Probus backcross, 42 parental : 24 recombinant types constitutes a departure from a 1:1 ratio ($P < 0.05$). Thus, linkage is involved in each case, and not all meiocytes incur an exchange in the section being mapped. This failure results in asynapsis, as no crossing over is possible in other regions of this potential bivalent: outside the synaptic region on the α side there is hemizygosity caused by the deletion, and outside of it on the β side there is heterozygosity for nonhomologous wheat and rye segments. Thus, a proportion of the cases involving absence of a bivalent at metaphase I result from asynapsis. As to whether a contribution from desynapsis is also involved is determined after the linkage distance is calculated. The consequences of synapsis, desynapsis and asynapsis have

to be taken into account in determining map distances in cases such as this. The formula for determining this, derived by DRISCOLL (1978), is as follows:

$$\text{Map distance} = \frac{6R - 5\theta\ (P-R)}{6G} \times 100\ ,$$

where G = total number of gametes involved in the testcross = $P+R$, P = number of parental gametes involved in the testcross, R = number of recombinant gametes involved in the testcross, and θ = proportion of meiocytes that involve a bivalent at metaphase I for the critical chromosome.

The calculated map distances between Cornerstone and Probus mutants and the Transec segment are 20 and 34 crossover units, respectively. Using approximate methods (see Chapter 10, KENDALL and STEWART 1969) 95% confidence intervals for the two distances are 10 to 30 map units and 25 to 43 map units, respectively.

As mentioned above, the values of θ entered in the equation are minimal values. Because of this, the calculated map distances are possible overestimates. These distances are shorter than those of the mutants to the centromere, despite the fact that some homologous *4Aβ* chromatin exists in Transec cases. The map distances 20 and 34 are just significantly different at the 5% level (SND = 2.0), indicating that the Cornerstone mutant may involve a longer deletion than the Probus mutant.

A genetic distance of 20 map units reflects exchange in 40% of meiocytes; thus, asynapsis occurs in 60% of meiocytes. Of the 40% that incur an exchange, only 3% are seen at metaphase I; thus, 37% result in desynapsis. These and the comparable figures for the Probus mutant are shown in Table 7. It is noteworthy that both asynapsis and desynapsis are involved in this case; whereas, only desynapsis is involved with the deletion-bearing chromosomes and the α telocentric. Hence, the translocation β arm, but not the absence of the β arm, induces some asynapsis in the α arm. This may occur only when a deletion is present on the α arm; certainly there is an interaction, as the amount of asynapsis when Cornerstone is involved is almost double that when Probus is involved (Table 7). Induced asynapsis in the opposite arm, in these circumstances, almost certainly means considerable asynapsis in the arm bearing the translocation. The fact that this segment maps only 1 crossover unit from the centromere (DRISCOLL and BIELIG 1968) may mean a very short interstitial segment of *4Aβ* in the functional, but not the physical, sense. The asynaptic influence of the rye segment, perhaps only when a deletion occurs at the opposite end of the chromosome, could result from a repulsion force between nonhomologous segments that is manifested in this "weakened" condition, *viz.*, deletion heterozygosity in the opposite arm.

The deletion-induced precocious terminalization of chiasmata may stem from the deletions being terminal and the bivalent not having a homologous pair of telomeres and, thus, not a strong attachment point. This assumes that homologous pairs of telomeres play an important role in preventing precocious terminalization of chiasmata. It has been suggested that homologous pairs of telomeres play a

TABLE 7

Synapsis (S), desynapsis (D) and asynapsis (A) and linkage distances of Cornerstone or Probus mutants and the Transec alien segment

	Cornerstone		Probus	
	Centromere	Transec	Centromere	Transec
S	19	3	42	13
D	81	37	58	55
A	0	60	0	32
$\frac{D}{S+D}$ %	81	92	58	80
Maximum map units (95% interval)	50	20 (10–30)	50	34 (25–43)

role in initiating pairing (SVED 1966). Considerable desynapsis also occurs in the Cornerstone (or Probus) × Transec *4A* bivalent. In this case the α telemeres do not have homologous telomeres and the the same argument can be advanced. The proportion of chiasmata that precociously terminalize in this case is: $\frac{\text{desynapsis}}{\text{synapsis + desynapsis}}\left(\frac{D}{S+D}\right)$ is equal to 92% and 80% with Cornerstone and Probus, respectively (Table 7). These proportions, compared with 81% and 58% for Cornerstone and Probus, respectively, with the telocentric are roughly in the same ratio (1.2:1 with Transec and 1.4:1 with the telocentric), but considerably higher with Transec. This is interpreted as the alien segment exaggerating the desynapsis that takes place mainly in the α arm (presumably there is very little chiasma formation in the β arm). The degree of exaggeration is 14% (92/81 = 1.14) in the case of the Cornerstone deletion and 38% (80/58 = 1.38) in the case of the Probus deletion.

Thus, the Cornerstone and Probus mutants, presumed to be terminal deletions of *4A*α, induce 81% and 58%, respectively, desynapsis of the bivalent involving telocentric *4A*α; however, they induce no asynapsis.

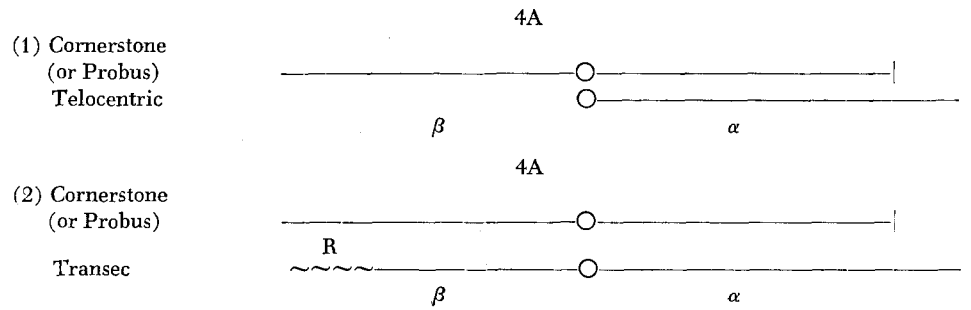


FIGURE 1.—The bivalent *4A* involving (1) a Cornerstone (or Probus) chromosome and a telocentric chromosome, and (2) Cornerstone (or Probus) chromosome and a Transec chromosome. (————| represents a deletion and $\overset{R}{\sim\sim\sim\sim}$ represents an alien segment bearing a resistance gene).

The Transec alien segment attached to *4Aβ*, presumably in a terminal position, exaggerates the desynapsis mentioned above by 14% and 38%, respectively, and induces 60% and 32% asynapsis, respectively, in Cornerstone or Probus and Transec *4A* bivalents.

LITERATURE CITED

BRIDGES, C. B., 1919 Vermilion deficiency. J. Gen. Physiol. **1**: 645–656.

BURNHAM, C. R., 1934 Chromosomal interchanges in maize: reduction of crossing over and the association of non-homologous parts. Am. Naturalist **68**: 81–82.

DRISCOLL, C. J., 1975 Cytogenetic analysis of two chromosomal male-sterility mutants in hexaploid wheat. Austr. J. Biol. Sci. **28**: 413–16. ——, 1977 Registration of Cornerstone male-sterile wheat germplasm. Crop. Science **17**: 190. ——, 1978 Mapping alien segments. Intern. Sympos. on Cytogenetics and Crop Improvements, Varanasi, India. (In press.)

DRISCOLL, C. J. and K. K. BARLOW, 1976 Male sterility in plants: Induction, isolation and utilization. pp. 123–131. In: *Induced Mutation in Cross-Breeding*, IAEA, Vienna, Austria.

DRISCOLL, C. J. and L. M. BIELIG, 1968 Mapping of Transec wheat-rye translocation. Can. J. Genet. Cytol. **10**: 421–425.

FOSSATI, A. and M. INGOLD, 1970 A male sterile mutant in *Triticum aestivum* Wheat Information Service (Kyoto) **30**: 3–10.

FU, J. K. and E. R. SEARS, 1973 The relationship between chiasmata and crossing over in *Triticum aestivum*. Genetics **75**: 231–246.

KENDALL, M. G. and A. STEWART, 1969 *The Advanced Theory of Statistics*, 3rd ed. Griffin, London.

MCCLINTOCK, B., 1941 The stability of broken ends of chromosomes in *Zea mays*. Genetics **26**: 234–282.

PUGSLEY, A. T. and R. N. ORAM, 1959 Genic male sterility in wheat. Aust. Pl. Breed. and Genet. Newsletter **14**: 10–11.

SAX, K., 1940 An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. Genetics **25**: 41–68.

SEARS, E. R., 1966a Chromosome mapping with the aid of telocentrics. Proc. 2nd Intern. Wheat Genetics Sympos. Lund, Sweden. Hereditas (Suppl.) **2**: 370–381. ——, 1966b Nullisomic-tetrasomic combinations in hexaploid wheat. pp. 29–45. In *Chromosome Manipulations and Plant Genetics*. Edited by R. RILEY and K. R. LEWIS. Plenum Press, New York.

STADLER, L. J. and H. ROMAN, 1948 The effect of X-rays upon mutation of the gene *A* in maize. Genetics **33**: 273–303.

SVED, J. A., 1966 Telomere attachment of chromosomes. Some genetical and cytological consequences. Genetics **53**: 747–756.

Corresponding editor: S. WOLFF