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CONTROLLED INTROGRESSION OF ALIEN CHROMATIN INTO WHEAT

by

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Abstract

Attempts to transfer alien genetic material to wheat have in the past involved addition or substitution of whole chromosomes or chromosome arms from an alien related species to the genome of wheat. Generally, such lines have suffered from loss of yield or quality compared to the normal wheat parent. The amount of alien chromatin present in these lines can be reduced by induction of meiotic recombination between alien and wheat chromosomes through suppression or deletion of the *Ph1* gene on the long arm of wheat chromosome 5B. Although induced allosyndetic pairing has been frequently observed, few successful alien gene transfers have been achieved in this way. The possible mechanisms of action of *Ph1*, the experience with alien introgression and the known effects of alien chromatin on wheat are reviewed.

The work reported in this thesis demonstrates for the first time that the chromosomes of wheat and cereal rye can be recombined by induction of homoeologous pairing by means of nullisomy for chromosome 5B and by the utilization of the *ph1b* mutant. The frequency of recombination is low, but by developing rapid and reliable techniques using established biochemical and other markers, it was possible to screen for these recombinants relatively easily.

Two different wheat-rye translocation lines were used as starting points for the induction of allosyndetic recombination. The translocation chromosome involving the short arm of rye chromosome 1R carries a useful gene for resistance to stem rust, but lines with this rye segment translocated to the long arms of either 1D or 1B are characterised by a dough quality defect. By testcrossing a homozygous *ph1b* plant heterozygous for the 1DL-1RS translocation, one recombinant involving 1RS and 1DS was isolated out of 394 progeny, while progeny derived by self-fertilisation of nullisomic 5B plants heterozygous for the same translocation produced a further three wheat-rye recombinants in 531 progeny. The rye segment present in the 1BL-1RS translocation has also been recombined with wheat. The recombinants were selected on the basis of their

endosperm storage protein phenotype (two independent loci on 1DS, one on 1RS and on 1BS) and their reaction to stem rust. Recombinant lines were further characterised by analysis of phenotype for two isozymes, which have structural genes on the short arms of the homoeologous group 1 chromosomes. Twelve independent plants with an altered chromosome 1DS were also obtained; eleven of these possessed the proximal but not the distal endosperm storage protein locus, while one possessed the distal without the proximal locus. These lines will prove useful in the elucidation of the contribution of the gene products of these two protein loci to dough quality.

The long arm of rye chromosome 1R, which is marked both by a heterochromatic telomere and at the *Glu-R1* locus, closely linked to the centromere, was induced to pair with wheat in a *ph1bph1b* background and 17 recombined chromosomes were recovered among 731 progeny derived by self-fertilisation. Due to self-sterility of some plants, some suspected recombinants could not be subjected to a progeny test for verification and an estimate of the total number of recombinants obtained was made, giving a gametic recombination frequency of 1.4%. Control populations, where homoeologous pairing was suppressed, did not produce any confirmed recombinants.

Chromosome 1U from *Aegilops umbellulata* was also used in a study of wheat-alien recombination. It was expected that the allosyndetic recombination frequency would be higher in this case than in rye, given that *Aegilops* and wheat are more closely related than wheat and rye. This chromosome was chosen as it possesses three easily scorable marker loci. Over a segment of the short arm of the *Aegilops* chromosome between the prolamin locus *Gli-U1* and a structural gene for the isozyme *Gpi-U1*, a gametic recombination frequency of 8.0% was estimated within a population derived from a *ph1bph1b* parent, a third of the value for homologous recombination within wheat. Some double homoeologous cross-overs in the interval *Glu-U1 - Gli-U1* were also recovered. When both the alien chromosome and a wheat homoeologue were present as monosomes, the rate of recombination was approximately double that recorded in

populations derived from a monosomic addition of chromosome 1U. No cross-overs were found in a control population derived from a *Ph1bph1b* parent.

Since codominant genetic markers allow the classification of both gametes in a single progeny, F_2 populations were employed in most of this work, rather than the more conventional backcross techniques used in previous work with alien introgression. These populations are both simple to produce and are more efficient than test-cross populations as two gametes are screened simultaneously in a single individual. A comparison of the efficiency of induction of allosyndetic recombination showed that 5B nullisomy was at least as effective as the *ph1b* mutant. The availability of a urea soluble endosperm protein controlled by a gene on chromosome 5BL made selection of 5B deficient plants simpler than those homozygous for *ph1b*, which required time-consuming cytological analysis and a progeny test to verify the identification.

STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any other university. To the best of my knowledge and belief, it contains no material previously published or written by any other person, except where due reference is made in the text.

Robert M.D. Koebner

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Chapter 1: General introduction

Modern agricultural practices lay great stress on genetic homogeneity in crop plants so that the physical and biological characteristics of the crop are both predictable and uniform. For some decades, scientifically planned breeding methods have been applied to cereal improvement and have succeeded in achieving steady increases in crop yields (Silvey, 1979; Austin *et al.*, 1980). However a major effect of these practices has been the erosion in genetic variability of crop plants, as new varieties consisting of a single genotype supplant the heterogeneous mixtures which constitute the traditional land races. Despite extensive efforts to conserve stocks of these primitive varieties, much of the variation which they possessed has now been lost, and it is a commonly held view that the genetic material of the cultivated wheats has already been almost wholly exploited (Feldman and Sears, 1981).

The genus *Triticum* , and more widely the subtribe Triticinae to which wheat belongs, contain a number of both wild and cultivated species which can be hybridised with wheat, thereby offering the opportunity to introgress into wheat much novel genetic variability. Some of this variability can be transferred by simple homologous recombination as one or more of the three genomes which constitute hexaploid wheat are present in some of these wild and cultivated relatives. Where no genomic homology exists, alien chromosomes can only be maintained in a wheat background if they are present as an homologous pair. In synthetic amphiploids the entire alien genome is doubled so that each alien chromosome is represented twice, while in alien addition lines just one alien pair is added to the wheat genome. In substitution lines an alien disome replaces one of wheat. Amphiploids, addition and substitution lines involving a number of alien species within the Triticinae and some from the more distantly related subtribe the Hordeinae, have been produced, but of the amphiploids, only triticale, the wheat x rye hybrid, has as yet found a use in commercial agriculture; no addition line has proven superior to wheat or even sufficiently stable to be used directly, but some spontaneous substitution lines are in use as commercial

varieties.

The transfer of whole alien chromosomes to wheat may succeed in introducing genes of value to wheat breeders, but genes which have deleterious effects on yield and/or quality are almost invariably transferred along with the target gene. Such genes are more likely to be excluded if only a segment of alien chromosome can be introgressed, as occurs in wheat-alien translocations. These translocations can occur spontaneously or be induced by irradiation. However the discovery that the deletion of a single gene on wheat chromosome 5B allows related non-homologous (homoeologous) chromosomes to synapse and therefore meiotically recombine, presents a more controlled way of transferring small alien chromosome segments into wheat. Chromosomes from *Aegilops* spp. and from *Agropyron* spp. have been recombined with wheat chromosomes in this way, but despite much interest in the possibility, no proven recombinants have yet been obtained from cereal rye chromosomes.

Where the alien segments can be genetically marked, not only by the target gene, but also by one or more other genes, then the extent and nature of the incorporation of alien chromatin into the wheat genome can be followed with more precision than is possible by using conventional cytological techniques. Biochemical markers are ideal for this purpose, although to be applicable, single seed or plant assay must be possible and they must be reliable, rapid and inexpensive, as the frequency of allosyndetic pairing and hence the rate of recombination between homoeologues is low.

In this thesis such genetic markers have been exploited and this has enabled the recombination of two different rye chromosomes arms with wheat to be achieved; one of these arms is of major interest to wheat breeders as it is involved in translocations associated with high yields over a diversity of environments, while the results using the other arm give an expectation of the maximum frequency with which rye chromatin can be recombined with wheat. This is compared to a much greater frequency obtained when using an homoeologous chromosome from *Aegilops umbellulata.*

Chapter 2: Review of literature

2.1 Genomic structure of wheat and the concept of homoeology

It is well established that bread wheat (*Triticum aestivum* (L.) em Thell) is an allopolyploid species composed of three distinct but related genomes (for a review, see Morris and Sears, 1967). These genomes, referred to as A, B and D are all assumed to have originated from a common progenitor within the Gramineae. While *Triticum monococcum* L. and *Aegilops squarrosa* L. [= *Triticum tauschii* (Coss.) Schmal.]* are now generally accepted as being the diploid species which donated the A and D genomes respectively (Morris and Sears, 1967), the identity of the B genome donor remains unresolved, although it is assumed that, if a modern B genome diploid exists, it will be found within the Sitopsis section of the genus *Aegilops* (Riley *et al.*, 1958); this conclusion, based on chromosomal homology and morphology, has been supported by the results of DNA hybridisation studies (Nath *et al.*, 1984).

A major consequence of the common ancestry of the three genomes is that there exists substantial triplication of genetic material within hexaploid wheat. The demonstration by Sears (1952a, 1966) that the abnormal phenotypes caused by nullisomy for any particular chromosome can usually be corrected, at least partially, by tetrasomy for certain other chromosomes provided ample evidence of gene duplication. Phenotypic resemblances between some nullisomic lines led to their being arranged into seven groups of three each, a classification confirmed by the successful compensation of nullisomy for any one

* The genus *Aegilops* was revised and incorporated into *Triticum* by Bowden (1959). However the old designations are still widely used in the literature, and therefore these will be used in this thesis, with the new designation, following Bowden as amended by Morris and Sears (1967), appearing in parentheses when a species is first mentioned.

chromosome by tetrasomy for only two other chromosome pairs. It thus became possible to divide the haploid complement of wheat into seven groups, numbered 1 to 7, each consisting of three related chromosomes, or homoeologues. The development of the 21 possible monosomic lines in cv. Chinese Spring (Sears, 1954) permitted further manipulation which allowed each chromosome to be assigned to a particular genome. This permitted the 21 wheat chromosomes to be labelled 1A, 1B, ..., 7B, and 7D (Sears, 1958).

The development of the monosomic, and the majority of the possible ditelocentric (Sears and Sears, 1978) lines of wheat in cv. Chinese Spring has made possible the determination of the chromosomal location of many genes affecting physiological, morphological and biochemical characters (for a current listing of known wheat genes and their chromosomal location, see McIntosh, 1983). Gene triplication is difficult to demonstrate unless gene products can be unequivocally identified; thus the most striking illustration of homoeology between genomes is shown by studies on proteins, where electrophoretic techniques can readily separate protein molecules which differ in minor ways from each other. Hart (1979a) has reviewed the chromosomal location of 57 isozyme structural genes in cv. Chinese Spring, comprising 15 triplicate and one duplicate series. Since this review many more duplicated and triplicated series have been described, including isozymes and other proteins. The triplicate genes are generally found on each of the three chromosomes of a given homoeologous group.

Despite this genetic similarity, and by inference, also similarity in DNA content of the homoeologous chromosomes, meiotic pairing in euploid wheat is strictly diploid-like, showing almost exclusively 21 bivalents at metaphase I; allosyndesis is even substantially suppressed in euhaploids where homologues are represented only singly, although three homoeologues are present. Up to three bivalents (McGinnis and Unrau, 1952) - presumably involving homoeologues - and very rare trivalents are observed in euhaploids (Riley and Chapman, 1958a). McGinnis and Unrau (1952) produced progeny from a cross of haploid x euploid wheat and observed as many as three trivalents or quadrivalents

in the pollen mother cells (pmcs) of derivatives of these plants; these multivalents were thought to have resulted from reciprocal translocations following homoeologous pairing in the haploid. Okamoto and Sears (1962) produced and analysed a number of translocations from a similar cross and showed that most involved homoeologous chromosomes. However the simultaneous isolation of a few non-homoeologous exchanges led Sears (1972a) to later propose centric fusion as a more plausible mechanism for these transfers (see section 2.5).

2.2 Genetic control of the suppression of allosyndesis

Only the absence of chromosome V (later designated as 5B) in haploid wheat causes a radical departure from the meiotic pairing behaviour of the euhaploid (Okamoto, 1957; Riley and Chapman, 1958a; Sears and Okamoto, 1958). In nulli-5B haploids a mean of 10.95±0.33 (with a range of 4 to 19) chromosomes is seen to be conjugated at metaphase I, compared with 2.86±0.23 (0 to 9) in euhaploids. While trivalents are rare in euhaploids, there is a mean of almost one per pmc in haploids lacking 5B. Quadrivalents and higher associations are seldom seen in these aneuhaploids, and are never seen in euhaploids (Riley and Chapman, 1958a). Mello-Sampayo (1971a) has demonstrated that the absence of chromosome 3D also increases allosyndesis in interspecific hybrids, while chromosomes 3A (Driscoll, 1972), 3B (Miller *et al.*, 1983) and 4D (Driscoll, 1973) have been found to possess loci having a significant, though weaker, suppressive effect on homoeologous pairing. The chromosome 3D effect is much weaker than that of 5B (Mello-Sampayo, 1971a), although the simultaneous absence of 3D and 3A results in a level of pairing almost as high as in plants deficient for 5B (Mello-Sampayo and Canas, 1973). Sears (1976) states that plants simultaneously deficient for both 3D and 5B show a level of pairing no greater than that observed in the absence of 5B alone. The location of the 5B gene(s) to the long arm of the chromosome was determined by showing that the

presence of the 5BL telosome alone suppressed allosyndetic pairing in haploids (Riley, 1960a), while the reciprocal 5BS telosome was unable to prevent this pairing (Riley and Chapman,1964). A series of weak pairing promoters (that is, having an antagonistic effect to the 5BL gene(s) are known to be present on the short arms of the homoeologous group 5 chromosomes as well as on various chromosomes in homoeologous groups 2 and 3. The effect on chromosome pairing of all of these genes, along with their chromosomal arm location has been reviewed by Sears (1976).

The occurrence of frequent trivalents (up to 5 per pmc) but only rare higher multivalents in nulli-5B haploid meiocytes led Riley (1960a) to suggest that the pairing observed was predominantly between homoeologues. Genetic evidence for this hypothesis was provided by Riley and Kempanna (1963) who showed that the translocations induced in progeny obtained from a nullisomic 5B - tetrasomic 5D plant always involved homoeologous pairs. Riley and Chapman (1966) used cytologically marked chromosomes to show that the pairing induced in wheat x *Aegilops speltoides* Tausch [= *Triticum speltoides* (Tausch) Gren. ex Richter] hybrids (see section 2.3) similarly always involved homoeologues.

Following the demonstration of the importance of the 5BL gene(s) in the control of meiotic chromosome pairing, a substantial effort was undertaken to mutate these gene/s. The effect of the loss of chromosome 5B is highly visible in haploids, and as a similar level of allosyndesis to that observed in euhaploid wheat is observed in amphihaploids of wheat x rye (Riley *et al.*, 1959), rye was commonly used as the pollen parent onto wheat plants that had been subjected to mutagenesis. Thus any mutation of the 5BL gene(s) would cause the amphihaploid to show higher than normal pairing. Okamoto (1963) irradiated wheat with X-rays and detected individual wheat x rye hybrid progeny with more than 4 bivalents per pmc. However he was unable to recover these mutants as the amphihaploids were sterile and could not be doubled by colchicine. Riley*et al.* (1966) applied ethylmethansulphonate (EMS) to mutate ditelosomic 5BL of cv. Chinese Spring, and screened over 200 wheat x rye amphihaploid progeny for abnormally high pairing in

the presence of the cytologically marked 5BL telosome. Three such individuals were identified; however the mutation appeared not to be transmitted to the sib progeny obtained by self-fertilising the mutated wheat parent. The first report of the successful isolation of a mutant affecting meiotic chromosome pairing in wheat was by Wall *et al.* (1971a), who used derivatives of the material produced by Riley *et al.* (1966). This mutant, termed "10/13", in crosses to rye appeared to give similar levels of allosyndesis as nulli-5B wheat x rye hybrids. Wall *et al.* (1971b) named the locus *Ph* (pairing homoeologous) and were able to map the mutation to a position independent of the centromere on chromosome 5BL. The 10/13 mutant, later named *ph1a* (McIntosh, 1979) has since, however, been shown to contain a mutation at the *Ph2* locus on chromosome 3DS rather than on 5BL (Sears, cited in Ceoloni *et al.*, 1984 and in Dvorak *et al.*, 1984), and thus it has been suggested to rename the mutant *ph2a* (McIntosh, pers. comm.). A true *Ph1* variant, named *ph1b*, was finally obtained by Sears (1977); the material from which this mutation was obtained also gave rise to a low-pairing mutant - *ph2b* - at the *Ph2* locus (Sears, 1982). Recently, Giorgi and Cuozzo (1980) obtained a radiation induced mutant in durum (*Triticum turgidum* L. var. *durum*) (tetraploid) wheat which appeared to involve a *Ph1* deletion. The mutated durum 5B has an abnormal Giemsa C-banding pattern (Dvorak *et al.*, 1984), which suggests that the *Ph1* locus lies in the middle section of the chromosome arm. This is consistent with the conclusion of Mello-Sampayo (1972), who showed that a line involving a large segment of 5DL translocated distally onto 5BL of hexaploid wheat still possessed the *Ph1* locus, thereby excluding a distal location for the *Ph1* gene.

2.3 Interaction of *Ph1* with genes from other species

Hybrids of wheat with three *Aegilops* species - *Ae. speltoides* (Riley *et al.*, 1961), *Ae. mutica* Boiss. [= *Triticum tripsacoides* (Jaub. et Spach.) Bowden] (Riley, 1963), and *Ae. longissima* Schweinf. et Muschl. in Muschl. [= *Triticum longissimum* (Schweinf. et Muschl. in Muschl.) Bowden] (Mello-Sampayo, 1971b) - depart from the expected pattern of meiotic pairing of most interspecific hybrids which include *Ph1* (Riley and Law, 1965). The original strains of *Ae. speltoides* and *Ae. mutica* hybridised to wheat induced a high chiasma frequency (approximately 16 per pmc) in the interspecific F_1 progeny despite the presence of chromosome 5B. *Ae. longissima* suppressed *Ph1* to a lesser degree, inducing at most six chiasmata per pmc (Sears, 1976). Genetic variation for the ability to suppress *Ph1* has been demonstrated in all three species, so that some strains of *Ae. speltoides* are not capable of any significant suppression (Dvorak, 1972; Kimber and Athwal, 1972), while the 'low-pairing' strain of *Ae. mutica* induces a mean of only 1.51 chiasmata per pmc (Dover and Riley, 1972a). Initially it was thought that the *Ae. speltoides* suppression of allosyndesis was independent of the presence or absence of *Ph1* (Riley and Law, 1965), but Feldman and Mello-Sampayo (1967) were able to show that pairing was significantly enhanced in nulli-5B hybrids of wheat x *Ae. speltoides* ; a similar result was obtained with *Ae. mutica* by Dover and Riley (1972b). Chen and Dvorak (1984) have suggested, however, that the major genes suppressing *Ph1* in *Ae. speltoides* are only operative in the presence of *Ph1*, so that the higher pairing of nulli-5B amphihaploids observed by Feldman and Mello-Sampayo (1967) may be due rather to interactions of the minor pairing suppressors in *Ae. speltoides* with *Ph1*. Allosyndetic pairing in nulli-5B amphihaploids of wheat x *Ae. longissima* is usually very high and the species was therefore commonly used as a pollen parent to examine the effect of chromosome 5B deficiency on chromosome pairing (e.g. Riley *et al.*, 1959).

The accessory, or B, chromosomes of both *Ae. speltoides* and *Ae. mutica* , where present, can compensate for the loss of *Ph1* in 5B deficient wheat x *Aegilops* spp.

amphihaploids. Chromosome pairing in these 27 chromosome (nulli-5B) hybrids is restricted, when B chromosomes are present, to the level observed in the respective 28 chromosome amphihaploid (Vardi and Dover, 1972). However, in both 28 chromosome wheat x *Ae. speltoides* and wheat x *Ae. mutica* hybrids, the level of allosyndesis is unaffected by the presence or absence of the B chromosomes.

Variation for the degree of *Ph1* suppression within the *Aegilops* spp. mentioned has been observed to create four distinct classes of chiasma frequency - ranging from 1.5 to 12.9 chiasmata per pmc - in wheat x *Aegilops* hybrids. This observation led Dover and Riley (1972a) to suggest a two locus-four allele system of pairing control in both species. Cytological confirmation of the existence of two separate loci came from Dover's (1973) seven addition lines of *Ae. mutica* chromosomes onto wheat. Six of these lines were not meiotically disturbed, but the seventh, carrying a satellited chromosome 'M' showed homoeologous pairing. Hybrids of this line to rye segregated for the level of allosyndesis from high (2n = 29 = 21 wheat + 7 rye + M) through intermediate (2n = 28 + Mt [Mt was a truncated version of M]) to low (2n = 28). The intermediate level of pairing in the hybrid involving the shortened *mutica* chromosome was thought to have been the result of loss of one of the two pairing control loci. As a preponderance of the wheat x *Aegilops* spp. hybrids fell into the two intermediate pairing classes (as opposed to either the very high or the very low), Dover (loc. cit.) suggested that the two loci are linked with an estimated recombination frequency of 28.1% in *Ae. speltoides* and 23.8% in *Ae. mutica.* The presence of two genes in *Ae. speltoides* which have a major suppressive effect on *Ph1* was recently confirmed in a comprehensive study of the variation for the level of suppression within this species (Chen and Dvorak, 1984). However the extent of their linkage was questioned on the basis that the population size used by Dover (1973) was too small to make meaningful inferences, and because it was believed that the data of Dover (1973) were probably confounded by segregation for minor genes within *Ae. speltoides* which were proposed to extensively modify the major gene effects.

Early work on wheat x rye hybrids showed that a single genome of rye has no measurable effect in inducing allosyndesis (Riley *et al.*, 1959). Indeed, Riley and Law

(1965), in comparing the levels of allosyndesis in nulli-5B haploids and nulli-5B wheat x rye hybrids suggested that the rye genome in fact is responsible for a slight reduction in allosyndetic pairing. In contrast, Dvorak (1977a) found continuous variation within rye for the ability to suppress *Ph1* ; however the highest pairing genotype induced a mean of only 3.6 chiasmata per pmc when hybridised to wheat. Lelley (1976a) produced wheat x rye hybrids having two doses of each of the rye chromosomes in turn, along with the other six rye chromosomes represented singly. One particular rye chromosome (7R) was suspected to possess a strong *Ph1* suppressor gene; however this conclusion was based on the scoring of only a few pmcs, as the chromosomal configurations in the majority of cells in this genotype could not be analysed as the chromosomes were clumped together.

Increasing the relative proportion of rye to wheat chromosomes, as in hybrids of tetraploid rye (as male) x wheat - giving an ABDRR genome constitution, x octoploid triticale - ABDRRR - and x hexaploid triticale - ABRRR - led to higher levels of pairing than were expected from the behaviour of wheat x diploid rye hybrids (Miller and Riley, 1972). The ability of higher dosages of the rye genome to suppress *Ph1* confirms the existence of weak pairing suppressors in rye, and suggests that these suppressors can act additively. Naranjo and co-workers were able to extend the results of Miller and Riley (loc. cit.) by producing plants having genomic constitutions ABRRR (Naranjo *et al.*, 1979) and ABRR (Naranjo *et al.*, 1979; Jouve *et al.*, 1980; Naranjo and Palla, 1982); when the number of wheat chromosomes was further reduced to 6 - 8 A and/or B genome chromosomes by crossing ABRR genotypes to tetraploid rye, somewhat higher levels of wheat-rye and wheat-wheat homoeologous pairing were observed (Naranjo and Lacadena, 1980; Naranjo, 1982). However, these authors did not consider whether their plants included chromosome 5B. Differential staining of the chromosomes using Giemsa C-banding allowed a more refined analysis of the nature of the pairing in these genotypes. Nonetheless, the maximal suppression of *Ph1* obtained induced only about one multivalent per pmc (Naranjo *et al.*, 1979; Naranjo and Lacadena, 1980). Sears (1976) has suggested that the effect of the rye genome appears to depend on a balance between the two arms of chromosome 5R, with 5RL having a suppressive effect, while 5RS has a

stronger promotive effect on allosyndesis. The accessory chromosomes of rye have also been reported to have an effect on chromosome pairing. In 28 chromosome wheat x rye hybrids their presence is reported to enhance pairing (Romero and Lacadena, 1982), while in nulli-5B hybrids they appear to have the opposite effect (Romero and Lacadena, 1980; Viegas, 1980), similar to the effect of the B chromosomes of *Aegilops* spp.

Weak suppression of *Ph1* has also been reported in *Haynaldia villosa* (L.) Schur. (Halloran, 1966; Chen *et al.*, 1982; Blanco *et al.*, 1983) by analysis of chromosome pairing in hybrids with wheat, and Mochizuki (1963) found that a particular pair of *Agropyron elongatum* (Host) Beauv. chromosomes, when added to durum wheat, induced a noticeable amount of allosyndesis; however, no equivalent effect was observed when individual chromosomes of either *A. elongatum* (Dvorak and Knott, 1974) or *A. intermedium* (Host) Beauv. (Cauderon *et al.*, 1973) were added to hexaploid wheat.

2.4 Mechanism and action of the *Ph1* gene

Early hypotheses regarding the mechanism of *Ph1* proved unsatisfactory (for a review, see Sears, 1976). Some weight of evidence, however, now supports the suggestion of Feldman (1968) that the *Ph1* gene product strengthens the tendency of homologous chromosomes to be associated in the pre-meiotic nucleus. In a number of studies, using squashes of mitotic metaphases taken from meristematic root tips, it has been shown that cytologically marked homologues are not randomly distributed in the cell, but rather that they tend to lie closer to each other than would be predicted by a model proposing a non-ordering of the chromosomes in the nucleus (Feldman *et al.*, 1966; Feldman and Avivi, 1973; Mello-Sampayo, 1973). The heterochromatic telomeres of rye chromosomes can be easily identified by Giemsa C-banding, and this allowed the somatic association of the telomeres of homologous rye chromosomes present in wheat-rye addition lines to be demonstrated in interphase cells, while non-homologous telomeres were randomly

distributed in the interphase nucleus (Singh *et al.*, 1976). Somatic association is suggested to extend to two telosomes which share only an homologous centromere and, less strongly, to homoeologues (Feldman *et al.*, 1972). Other workers have been unable to repeat these observations (Darvey and Driscoll, 1972b; Dvorak and Knott, 1973), and their failure to do so has been ascribed by Avivi *et al.* (1982a) to experimental error due to inappropriate choice of cells for measurement and/or to artifacts resulting from prefixative treatment of root tips. Avivi *et al* . (1982b) have since considered that homoeologues may not be associated any more strongly than unrelated chromosomes of different genomes. Other contrary evidence regarding the existence of somatic association in wheat was provided by Darvey and Driscoll (1972a) who showed that the non-homologous nucleoli associated with the satellites of chromosomes 1B and 6B had a tendency to fuse as often as did homologous nucleoli, despite the observation that nucleolar fusion is a regular phenomenon as cells pass into interphase. Sears (1976) has however questioned the value of extrapolating from the behaviour of the satellited chromosomes to the whole genome, as these chromosomes possess a number of unusual properties.

A contrasting model of the arrangement of chromosomes in somatic cells was provided by Bennett *et al.* (1983), who made serial sections of unsquashed, unpretreated root tip cell nuclei in order to develop a three-dimensional reconstruction of the nucleus *in vivo* . Somatic association was not detected in 39 reconstructed cells of barley where all the somatic chromosomes could be recognised, nor among the few wheat homologues that could be identified using this technique. A similar result was obtained from the reconstruction of 12 metaphase cells from *Ae. umbellulata* Zhuk. [= *T. umbellulatum* (Zhuk.) Bowden] (Heslop-Harrison and Bennett, 1983b). Rather, a spatial ordering of chromosomes was proposed based on the separation of haploid genomes and the association of pairs of most similar long and pairs of most similar short chromosome arms (Bennett, 1981; Heslop-Harrison and Bennett, 1983a). Recently, however, the statistical basis of this model has been questioned (Callow, 1985).

Somatic pairing has been noted extensively in both the plant and animal kingdoms (Brown, 1972; Avivi and Feldman, 1980); in some organisms, premeiotic pairing first

occurs shortly after the division of the zygote and so occurs throughout the somatic tissue (*Diptera*, *Yucca*), while in others (*Happlopappus*, lice) it first occurs a few divisions before the onset of meiosis. In wheat, an association between homoeologous bivalents at meiotic metaphase I was observed using cytologically marked bivalents possessing one telosome and one normal chromosome (Riley, 1960b; Kempanna and Riley, 1964; Yacobi *et al.*, 1985a, b); however the use of such altered bivalents to infer the spatial distribution of normal bivalents has been questioned by Heslop-Harrison (cited in Bennett, 1984). There is general agreement that somatic association exists in wheat, but the stage at which it first occurs remains unresolved.

Measurement of the separation between homologues and between homoeologues in mitotic metaphases in the absence of *Ph1* has shown that the somatic association of homologues is relaxed sufficiently to allow homoeologues to lie as close to each other as homologues (Feldman and Avivi, 1973). The effect of the deficiency of *Ph1* on meiosis in haploids has been discussed above (see section 2.2); in nulli-5B aneuploids or *ph1b* mutant euploids, metaphase I chromosome configurations are irregular, but they are generally characterised by a reduction in chiasma frequency compared to those seen in pmcs of normal genotypes. This reduction is manifested by the appearance of univalents, a greater proportion of rods over rings among the bivalents, and some multivalents, quadrivalents being the commonest of these, although associations of up to six chromosomes in hexavalents have been observed (Riley, 1960a; Driscoll *et al.*, 1979; Yacobi *et al.*, 1982). While multivalents are direct evidence of allosyndesis, the presence of often large numbers of univalents in the pmcs of these genotypes, and of an unusually high proportion of rod to ring bivalents, is taken to indicate that many of the bivalents observed in the pmcs of *Ph1* deficient plants are the result of homoeologous rather than homologous synapsis, with the remaining homologous partners left unpaired (Koebner and Shepherd, 1985).

The effect on meiosis of higher than normal doses of *Ph1* was investigated by Feldman (1966). Four doses of chromosome 5BL led to some reduction in chiasma frequency, but a more dramatic effect was caused by the presence of three

isochromosomes of 5BL, or six doses of the arm. These plants gave consistently high levels of asynapsis, many univalents being observed even during late prophase, suggesting the origin of the univalents to have been asynaptic rather than desynaptic. There was an average of 0.48 trivalents per pmc, rare higher multivalents, some heteromorphic bivalents, and frequent interlocking of ring bivalents, effects mimicked by the administering of colchicine to premeiotic sporogenous tissue (Driscoll *et al.*, 1967; Dover and Riley, 1973). Colchicine, a mitotic and meiotic spindle inhibitor, was also shown to suppress somatic association in normal wheat root tip cells (Avivi *et al.*, 1969), and to interact with dosage of *Ph1* in the control of somatic association (Avivi *et al.*, 1970; Ceoloni *et al.*, 1984).

A unifying hypothesis to account for the variation in meiotic pairing resulting from changes in the dosage of *Ph1* was provided by Feldman (1966, 1968). In normal euploid wheat, homologous chromosomes are more closely associated in premeiotic tissue than homoeologues. Thus in prophase I of meiosis, autosyndesis is favoured as the homologues are more intimately coaligned. In the absence of *Ph1*, both homologues and homoeologues have a chance to become coaligned in premeiotic tissue and therefore allosyndesis can occur at meiosis. Homoeologous pairing is much more pronounced in *Ph1* deficient haploids as no homologues are present to compete with the homoeologues for pairing partners, whereas in euhaploids the degree of association of homoeologues is insufficient to induce more than a low level of allosyndesis. When the dose of *Ph1* rises to four, and particularly to six, somatic association is suppressed to a degree that both homologues and homoeologues are widely separated at prophase I; thus pairing failure is common, and pairing is randomly auto- and allosyndetic (as inferred by the presence of trivalents and heteromorphic bivalents). The frequent interlocking of up to 7 bivalents in one chain observed in triisosomic 5BL results when bivalents are formed by chromosome pairs which are not collinearly aligned along their whole length (Yacobi*et al.*, 1982).

A contrasting model of the effect of *Ph1* has been proposed by Hobolth (1981), who applied the methodology and models of the mechanics of meiosis developed by Rasmussen and Holm (1980) in a range of other organisms. Electron micrographs of serial sections of

nuclei from meiocytes of both euploid and *ph1b* mutant plants were used to construct a three dimensional representation of chromosomal arrangement at various stages of prophase I. Multivalents and interlocking bivalents were common in zygotene of normal wheat, but these were corrected in early pachytene to give strict bivalent pairing by the end of pachytene. True crossing-over, as evidenced by the formation of the synaptonemal complex did not occur until after pairing correction. In the *Ph1* deficient stock, the appearance of the synaptonemal complex was found to be brought forward into late zygotene, so that chiasmata were formed before pairing correction could occur. In this way multivalents persisted into metaphase. This model of the action of *Ph1* supports the conclusion of Driscoll *et al.* (1979) that the gene affects the process of chiasma formation rather than the association of chromosomes. Yacobi *et al.* (1982), in confirming that the frequency of interlocking bivalents is positively correlated with the dosage of *Ph1* between two and six, argue that Hobolth's model is inadequate as it fails to predict that six doses of the gene would cause multivalents and interlocking bivalents; rather it would suggest that the high gene dosage would be expected to delay crossing-over until the optimal conditions for its occurrence had passed, thus leading to total asynapsis at metaphase I. The model also fails to account for high univalent frequency in *Ph1* deficient genotypes.

Baker *et al.* (1978) have reported that, in *Drosophila melanogaster*, six out of seven meiotic mutations studied also affected mitotic chromosome stability. Thus, although it has not been ruled out that *Ph1* and the gene(s) controlling somatic association are not identical, the similarity between the *ph1b* mutant and nulli-5B genotypes in both the disturbance of meiotic pairing and the disruption of somatic association makes this hypothesis increasingly unlikely (Ceoloni *et al.*, 1984). Assuming the identity of the genes controlling both somatic association and suppression of allosyndesis, some indications of the mode of action of *Ph1* have been proposed using results of studies of the effects of various spindle inhibitors on somatic association in dividing root tip cells. Colchicine suppresses homologous associations in mitosis (Avivi *et al.*, 1969), while an increased dosage of *Ph1* lessens this sensitivity (Avivi *et al.*, 1970). Thus the product of *Ph1* appears to be antagonistic to the action of colchicine, which is thought to bind specifically

to the tubulin subunits of microtubules (reviewed in Dustin, 1978), thereby disrupting microtubule organisation and hence the mitotic spindle. Studies of the effect on somatic association of other antitubulins (Avivi and Feldman, 1973) and of spindle inhibitors which have different targets within the mitotic spindle apparatus (Gualandi *et al.*, 1984) have shown that spindle sensitivity is enhanced in *Ph1* deficient individuals only by drugs which affect tubulin-microtubule equilibrium, and not by those which disrupt subcellular structures involved in the regulation of microtubule organisation and orientation. Thus it is now postulated that the *Ph1* gene could code for a modified tubulin or for a protein able to modify tubulin conformation (Gualandi *et al.*, 1984).

2.5 Introgression of alien chromatin into wheat

The wild and cultivated relatives of wheat present wheat breeders with a large resource of potentially useful characters (see Zeller and Hsam, 1983 for variation associated with specific chromosomes of cereal rye). Introgression of alien chromatin is therefore an important goal in wheat improvement. At present, the introduction of this genetic material requires first that a viable hybrid can be made between wheat and the donor alien species. Many successful hybridisations have been made both within the *Triticum -Aegilops* genus and more widely between wheat and species within the genera *Agropyron* , *Secale* , *Haynaldia* , *Hordeum* and *Elymus*. The current status of wide hybridisation in wheat has recently been reviewed by Sharma and Gill (1983). Interspecific and intergeneric hybrids are almost invariably at least male sterile, and the alien chromosomes can therefore only be maintained in wheat by either induction of chromosome doubling to give a fertile amphiploid, or by backcrossing the hybrid as female to wheat and recovering rare progeny resulting from fertilisation of the occasional unreduced gamete in the hybrid.

Introgression from relatives of wheat which share one or more genomes with wheat can be achieved simply, following synapsis and crossing-over between homologous chromosomes in the hybrid. The D genome of hexaploid wheat is present in the diploid

Aegilops squarrosa and in the allopolyploids *Ae. ventricosa* Tausch [= *Triticum ventricosum* Ces.], *Ae. crassa* Boiss. subsp.*vavilovi* Zhuk. [= *T. syriacum* Bowden], *Ae. juvenalis* (Thell.) Eig [= *T. juvenale* Thell.] and *Ae. cylindrica* Host [= *T. cylindricum* Ces.] as listed by Morris and Sears (1967); similarly, the A genome occurs in a number of diploid species, now grouped as a single species *T. monococcum*, and in the allopolyploids *T. turgidum* and *T. timopheevii* ; while the B genome is found only in *T. turgidum*, with only slight homology with the genomes of some diploids in the Sitopsis section of *Aegilops* (Riley *et al.*, 1958). Thus, for example, genes for disease resistance have been transferred to hexaploid wheat by homologous recombination from *T. monococcum* (Kerber and Dyck, 1973), *T. turgidum* (Athwal and Watson, 1956), *T. timopheevii* (Allard and Shands, 1954), *Ae. ventricosa* (Kimber, 1967a; Maïa, 1967) and *Ae. squarrosa* (Kerber and Dyck, 1969).

In the absence of homology between alien and wheat chromosomes, other techniques are necessary to reduce the amount of alien chromatin present in the amphiploid. O' Mara (1940) back-crossed a wheat x rye amphiploid to wheat and, after self-fertilisation, was able to produce three lines, each consisting of a different rye bivalent added to the euploid complement of 21 wheat bivalents. Addition lines equivalent to those involving the chromosomes of rye have now been produced from a number of interspecific and intergeneric crosses (reviewed in Law, 1981). These stocks have proven useful in the assignment of alien genes to particular alien chromosomes (O' Mara, 1940), and also as an important starting point for the transfer of alien genes to wheat. Alien chromosomes present as additions have been shown in many cases to be meiotically unstable (Riley, 1960c): pairing failure of the alien disome results in the production of some gametes with 22 chromosomes (that is, including the addition chromosome), and some with just the 21 wheat chromosomes. Euhaploid gametes have a competitive advantage in fertilisation over those with 22 chromosomes, and hence 44 chromosome plants tend to revert to a somatic chromosome number of first 43, and subsequently 42 chromosomes. Alien chromosomes in addition lines are therefore often lost, and the lines tend to return to the euploid condition unless they are periodically monitored.

Substitution of an alien disome for one of the wheat disomes was considered likely to improve the stability of alien chromosomes; pairing failure of the alien disome in substitution lines gives rise to 20 and 21 (20 wheat + 1 alien) chromosome gametes, and, as pointed out by Riley (1960c), any pollen grain deficient for the alien chromosome is also deficient relative to the euploid condition, and will therefore compete less well than the substitution gamete, provided that the alien chromosome adequately compensates for the missing wheat chromosome. Thus, in compensating substitution lines, the alien chromosome tends to be retained. An alien substitution line can be produced by crossing a wheat, monosomic for any particular chromosome, with an alien addition line. A proportion of the progeny from this cross will be doubly monosomic for a wheat and for the addition chromosome, and self-fertilisation of these plants will give rise to progeny which will include monosomic substitutions of the initial monosome by the alien chromosome. Disomic substitutions can then be selected among the progeny of these plants. The viability of substitution lines is variable, depending on the extent to which the alien disome can compensate for the removal of a native chromosome pair (Riley and Kimber, 1966). This feature of variable compensation has made it possible to extend the concept of homoeology to a number of alien chromosomes. Substitution experiments of alien for specific wheat chromosomes have shown that, with few exceptions (e.g. Koller and Zeller, 1976), only chromosomes from a single homoeologous group could be successfully substituted by a given alien chromosome (Riley and Kimber, 1966). Where substitution across homoeologous groups was observed, it was suggested that translocation differences between the alien chromosome and its ancestral homologue were responsible for the breakdown of strict homoeology with wheat chromosomes (Koller and Zeller, 1976). The assignment of homoeologous groups to alien chromosomes was further supported by studies on the location of alien protein genes using addition lines. There are many examples in which the same linkage relationships have been found on alien chromosomes as on their wheat homoeologues (e.g. Hart, 1979a), leading Hart *et al.* (1980) to propose the notion of gene synteny and its conservation, as a result of the common origin of the species and genera of the Triticinae. These authors suggested

further that protein homoeology itself could be used as a criterion for the assignment of homoeologous group to alien chromosomes. A comprehensive review of the homoeology between rye and wheat chromosomes based on these criteria has been assembled by Miller (1984).

The amount of alien chromatin present in a substitution line can be reduced, while simultaneously restoring some of the lost native chromatin, by taking advantage of the tendency of newly formed meiotic misdivision products to fuse around a single centromere in the process termed centric fusion by Sears (1972a). It has been suggested that these centric fusion translocations are formed with some certainty whenever two simultaneous misdivision products occur within a single meiocyte, whether the two original monosomes are homoeologues or not (Sears, loc. cit.). Large numbers of this type of translocation involving wheat-rye combinations have been reported (Shepherd, 1973; Lawrence and Shepherd, 1981; May and Appels, 1980,1982; Lukaszewski and Gustafson, 1983; Merker, 1984). Karyotypic changes of a similar nature have been observed in regenerants from embryo scutellar calli maintained in culture for prolonged periods (Lapitan *et al.*, 1984).

A current listing of characterised alien addition, substitution and translocation lines can be found in Driscoll (1983).

The earliest reports of the use of irradiation to introgress small segments of alien chromatin into wheat described the transfer of a gene from *Ae. umbellulata* conferring resistance to leaf rust (*Puccinia recondita* f. sp. *tritici* Rob. ex Desm.) (Sears, 1956) and several genes from *A. elongatum* for resistance to stem rust (*P. graminis* Pers. f. sp. Eriks. et E. Henn.) (Elliot, 1957; Knott, 1961). The mechanism of these transfers was supposed to have been radiation-induced chromosome breakage and subsequent repair. Subsequently, genes conferring disease resistance have been transferred to wheat by means of various forms of radiation from a range of alien species (reviewed in Knott, 1971; Knott and Dvorak, 1976). The chromosome break points induced by radiation are thought to be randomly distributed and the rejoining of the many fragments produced

similarly random. Knott (1968) has noted that the majority of successful transfers involve homoeologous segments, and argues that this is because these suffer the least adverse selection during gametogenesis and fertilisation. Such homoeologous interchanges may be relatively common if somatic association operates to coalign homoeologues in the interphase nucleus (Dvorak and Knott, 1977). Since irradiation damages DNA non-specifically, most radiation-induced transfers (16 out of 17 produced by Sears, 1956) are likely to be deleterious, and thus even a favourable transfer of alien chromatin may be lost due to lethality induced by changes elsewhere in the genome. Very few of these transfers have been incorporated into breeding programmes, although Knott's (1961) 9A-1 chromosome has been used in a number of Australian cultivars (listed in Sharma and Gill, 1983) to confer resistance to stem rust.

The discovery that homoeologous pairing was induced in the absence of *Ph1* allowed the possibility of a more controlled method for alien introgression. In *Ph1* deficient plants, synapsis of homoeologues should give rise to recombination between related wheat and alien chromosomes, just as occurs between wheat homoeologues (Riley and Kempanna, 1963). Such homoeologous synapsis has been observed repeatedly, involving hexaploid wheat chromosomes with those of *Aegilops* spp. (Riley *et al.*, 1959; Muramatsu, 1959; Riley *et al.*, 1961; Riley and Kimber, 1966; Lacadena, 1967; Driscoll, 1968), *Agropyron* spp. (Johnson and Kimber, 1967; Dvorak and Knott, 1972; Sears, 1972b; Cauderon *et al.*, 1973; Wang*et al.*, 1977; Yasumuro *et al.*, 1981), *H. villosa* (Halloran, 1966), *S. cereale* (Riley *et al.* 1959; Okamoto, 1963; Lacadena, 1967; Bielig and Driscoll, 1970 and others) and other *Secale* spp. (Riley and Kimber, 1966; Schlegel and Weryszko, 1979); and between durum wheat chromosomes and those of various *Aegilops* spp. (Giorgi and Barbera, 1981) and rye (Giorgi and Cuozzo, 1980). *Ph1* deficiency was not however able to induce homoeologous pairing between the chromosomes of hexaploid wheat and *Hordeum chilense* Brongr. var. *muticum* (Presl.) Hauman (Martin and Sanchez-Monge Laguna, 1980).

Despite these numerous observations of the induction of allosyndetic pairing involving wheat and alien chromosomes, documented examples of wheat-alien recombination are scarce. In part this results from the poor fertility of the nullisomic 5B amphihaploids in which the cytological observations of wheat-alien chromosome pairing have usually been made, so that sufficient progeny cannot be obtained. Riley and Kimber (1966) described introgression from *Ae. bicornis* (Forsk.) Jaub. et Spach. [= *T. bicorne* Forsk.], achieved by crossing and backcrossing a nulli-5B amphiploid to euploid wheat until the euploid wheat chromosome number was restored. These euploid progeny differed from the recurrent wheat parent in a number of phenotypic characters, including qualitative changes such as disease resistance and grain colour, and quantitative changes in characters such as height, maturity and grain size. A gene conferring resistance to leaf rust was transferred from *Ae. umbellulata* to wheat in a similar fashion by Riley *et al.* (1967). Riley *et al.* (1968a, b) restricted the initial amount of alien chromatin present to the single chromosome present in an addition line, and succeeded in introgressing a gene conferring resistance to stripe rust (*Puccinia striiformis* West.) from chromosome 2M of *Ae. comosa* Sibth. et Sm. [= *T. comosum* (Sibth. et Sm.) Richter] by *Ae. speltoides* suppression of *Ph1*. The 29 chromosome (21 wheat + 2M + 7 *speltoides*) hybrid was crossed and backcrossed to euploid wheat until a 2n=42 rust resistant plant which formed 21" at meiosis was obtained. This line was named Compair. Cytological investigation of diagnostic crosses involving Compair revealed that chromosome 2D had been recombined with 2M such that the left arm of 2D (= 2DL - Sears and Sears, 1978) and the proximal segment of the right arm had been replaced by the homoeologous segments of 2M, with only the distal portion of the right arm of 2D remaining intact, indicating that a single cross-over had occurred between 2M and 2D. The phenotype of Compair largely resembled the parental addition line, having the target *Yr8* gene, and longer florets, glumes and grains than the wheat parent.

Dvorak (1977b) transferred a gene from *Ae. speltoides* conferring resistance to leaf rust to wheat by inducing allosyndesis in the amphihaploid and backcrossing it to euploid

wheat. Segments from two *A. elongatum* chromosomes were transferred to wheat employing both nulli-5B amphiploids (Sears, 1972a, b; 1973) and the *ph1b* mutant (Sears, 1981). The *A. elongatum* transfer lines have been analysed cytologically as was Compair, and have been found to contain differing lengths of *Agropyron* chromatin, indicating a series of different cross-over points (Sears, 1973; 1978; 1981). Kibirige-Sebunya and Knott (1983) have also been successful in inducing recombination between wheat and *A. elongatum* chromosomes. A gene located on the short arm of the homoeologous group 4 chromosome of *Agropyron intermedium* which confers resistance to wheat streak mosaic virus (*Marmor virgatum*) (Wang and Liang, 1977) was transferred to wheat using the *ph1b* mutant by Liang *et al.* (1979). Wang *et al.* (1980) observed that 4BS and the transfer chromosome almost never paired in plants carrying both these chromosomes, whereas pairing between 4BL and the transfer chromosome was frequent; it was concluded from the heteromorphic appearance of the bivalent comprising a normal 4B and the translocated $4B/4E^i$ that a terminal segment of the alien chromosome had been translocated terminally onto 4BS, replacing little, if any native chromatin. The evidence for this suggestion is inconclusive however, and it is possible that the transfer chromosome is simply a $4BL\text{-}4E^i$ centric fusion translocation.

As Law (1981) has pointed out, the choice of alien genes suitable for transfer to wheat is restricted at present to those whose effects are readily identifiable. In wheat, only genes conferring disease resistance and electrophoretically distinguishable proteins fall into this category. The positive correlation which has been proposed between the breadmaking quality of flour and certain high-molecular-weight glutenin subunits (Payne *et al.*, 1979; 1981; Burnouf and Bouriquet, 1980; Moonen *et al.*, 1982; 1983) and the ease of their identification on polyacrylamide gels made a gene from *Ae. umbellulata* controlling a very high molecular weight subunit of this type an potential candidate for transfer to wheat. This was achieved by making a chromosome 1U substitution line nullisomic for chromosome 5B and screening for recombinants among its progeny; while some recombinants were obtained, they have not proven useful for wheat improvement (Law,

1984).

Cereal rye possesses a wide spectrum of genes potentially useful to wheat breeders and it has the advantage of itself being a crop plant with a history of selection for agronomic characters. The species is therefore a desirable source of new germplasm in wheat. The feasibility of transferring rye chromatin by homoeologous recombination has been doubted (Riley and Kimber, 1966; Knott, 1971; Sears, 1974; Law, 1981) on the grounds that both conventional cytological staining (Riley *et al.*, 1959; Bielig and Driscoll, 1970; Lelley, 1976b; Shnaider and Prilinn, 1984) and Giemsa C-banding (Mettin *et al.*, 1976; Dhaliwal *et al.*, 1977; Schlegel and Weryszko, 1979) of the meiocytes of *Ph1* deficient wheat-rye derivatives show very little wheat-rye synapsis. Joshi and Singh (1978) crossed a high-pairing wheat x rye amphihaploid to wheat and succeeded in isolating euploid wheat-like plants with recognisable rye characters. They suggested that these lines had arisen from homoeologous wheat-rye pairing in the original hybrid. However no evidence was provided to show that these lines were not further examples of centric fusions, known to occur frequently when univalents are abundant in the meiocyte (Lukaszewski and Gustafson, 1983; Merker, 1984). Furthermore, no confirmatory follow-up work has been published to support their original conclusions.

2.6 Effect of alien chromatin on wheat phenotype

Alien chromatin has been introduced into wheat in varying amounts, ranging from the complete genome in amphiploids to the translocation of relatively small segments, as discussed above in section 2.5. As expected, the greater the proportion of alien genetic material present, the greater the phenotypic changes to the recipient wheat plant. Although many different amphiploids have been produced, only one - triticale (x *Triticosecale* Wittmack) - has found direct application in agriculture (Driscoll, 1981). This is not surprising, as the alien donor has in all other cases been a wild, often weedy species which has never undergone man-made selection for agronomic traits. The phenotypes of some

wheat - *Aegilops* spp. amphiploids have been described by Riley and Kimber (1966). Many undesirable *Aegilops* characters were found to be epistatic to those of wheat, and thus the hybrids were not considered to be directly utilisable as crop plants. Triticale has undergone intensive selection for improved characteristics in the past 30 years. Even so it has been difficult to overcome the deleterious effects of meiotic instability and kernel shrivelling associated with the presence of rye chromosomes. These problems have been recently discussed in two reviews (Müntzing, 1979; Gupta and Priyadarshan, 1982). The kernel shrivelling phenomenon, correlated with the occurrence of mitotic abnormalities in the early endosperm (Kaltsikes *et al.*, 1975; Gustafson and Bennett, 1982), has also been noted in rye addition and substitution lines (Darvey, 1973; Kaltsikes and Roupakias, 1975).

The effects on wheat phenotype of an individual alien chromosome can be assessed by studying addition lines. The meiotic instability of some of these lines has been alluded to earlier. The phenotypic effects of the different individual additions is varied; some have little discernible effect, while others cause changes sufficiently pronounced to be diagnostic for the presence of the alien chromosome. Many alien chromosome addition lines are characterised by depressed fertility (e.g. Riley, 1960c). Some effects of individual chromosome additions have been detailed for chromosomes of rye (Riley and Chapman 1958b; Evans and Jenkins, 1960; Miller, 1984), *Agropyron elongatum* (Dvorak and Knott, 1974; Dvorak and Sosulski, 1974), *A. intermedium* (Cauderon *et al.*, 1973), barley (Islam *et al.*, 1981), *Hordeum chilense* (Miller *et al.*, 1982), *Ae. umbellulata* (Kimber, 1967b), *Ae. variabilis* Eig [= *Triticum kotschyi* (Boiss.) Bowden] (Jewell and Driscoll, 1983), *Ae. longissima* (Levy *et al.*, 1985), and *H. villosa* (Hyde, 1953).

Alien genes are not invariably fully or even partially expressed in a hexaploid wheat background. For example, Kerber and Dyck (1973) found that *Sr22* , a gene from diploid einkorn wheat, progressively lost its effectiveness as it was transferred to tetraploid and finally to hexaploid wheat, and Quinones *et al.* (1972) observed that some triticales derived from durums with a low level of resistance to leaf rust and ryes which were strongly

resistant, expressed only the resistance of the wheat parent. Chromosome 7D is thought to carry suppressor gene(s) which prevent the expression of genes for resistance to both stem and leaf rust derived from the lower ploidy levels of wheat (Kerber and Green, 1980; Kerber, 1983). The phenomenon of intergenomic gene suppression has now been shown at the gene product level by Galili and Feldman (1984), who compared the endosperm protein profiles of durum wheat and durum x *Ae. squarrosa* hybrids. In the hybrids, not only were new proteins introduced as expected, but other proteins present in the durum parent were either expressed weakly or were totally absent.

While addition lines generally do express the major genes conferring disease resistance present on the alien chromosome, undesirable genes on the same chromosomes are also frequently expressed; for example lines carrying chromosome $7el_1$ or $7el_2$ from *A. elongatum* (Knott, 1968b; Knott *et al.*, 1977) carry *Lr19* and *Sr25* respectively, but the flour derived from them is coloured yellow. This yellow pigment problem has since been overcome by EMS-mediated mutagenesis (Knott, 1980; 1984). A loss in yield is the commonest effect of alien additions; like lines of wheat tetrasomic for a particular chromosome, addition lines may suffer from genetic imbalance and hence loss of fertility. A contribution to the yield depression may also arise both directly from alien deleterious genes or indirectly through the interaction of alien and wheat genes.

The phenotypic effects of alien substitutions are sometimes more severe than in alien additions, as the loss of wheat chromatin compounds the presence of foreign chromatin (Riley and Kimber, 1966). A successful alien substitution chromosome must be able to fully compensate for the loss of the wheat disome, and to date no directed alien substitution line has achieved the status of a commercial variety (Law, 1981), although spontaneous (1B) 1R substitutions are common among European winter wheats (Mettin *et al.*, 1973; Zeller, 1973). As with additions, the substitution of a complete alien chromosome increases the likelihood of introgression of undesirable genes along with the target ones. These adverse effects on phenotype have been noted in all examples of substitution lines analysed for yield and other characters by Law (1981).

While substitutions involve the loss of an entire wheat and its replacement by an entire alien chromosome, translocations represent the exchange of less than an entire chromosome. Radiation-induced translocations, as pointed out by Sears (1972a), are only likely to be successful if the target alien gene to be introduced is near the end of the alien chromosome, as otherwise long alien segments will need to be transferred, increasing the probability of deleterious gene transfer. Centric fusion chromosomes reduce the length of alien chromatin present to one chromosome arm, as the other arm of the translocation chromosome is unaltered wheat chromatin. Thus the effect of introgression is lessened both because the loss of wheat and the gain of alien chromatin are reduced. While some wheat-alien translocations produced by irradiation or by centric fusion have been found to depress yield (Wienhues, 1973; Driscoll, 1981; Zeller and Hsam, 1983) or quality (Sharma and Knott, 1966; Knott, 1971; Zeller *et al.*, 1972; Koebner *et al.*, 1984), the 'Veery' lines which have been shown to contain a 1BL-1RS translocation (Merker, 1982) and many West German winter wheats involving the same translocation (Zeller, 1973) have been associated with high yield over a range of environments (Rajaram *et al.*, 1983; Zeller and Hsam, 1983). A 2AL-2RS line is reported to yield better than its euploid control under water stress (Lahsaiezadah *et al.*, 1983), while a 5RL-4AS translocation is known to improve yields in soils deficient in copper (Graham, 1984).

There is little doubt that the optimal form of alien introgression will involve the minimum amount of alien chromatin translocated along with the desired alien gene(s). Homoeologous recombination offers the best means to reduce the size of the introgressed segment, either by the induction of a double cross-over between the alien chromosome and a wheat homoeologue, or by the manipulation of recombinant chromosomes involving different break-points in the scheme proposed by Sears (1981). The production of such chromosomes involving rye and *Ae. umbellulata* transfers and the characterisation of these recombinants using biochemical techniques are the subject of this thesis.

Chapter 3: Methodology

3.1. Electrophoresis

3.1.1. Single dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was employed to monitor the presence in unreduced total protein extracts of seed endosperm of the prolamins Gli-D1 and Sec-1, and of Tri-1, one of the "triplet" proteins (Singh and Shepherd, 1985), in progenies segregating for 1D entire and 1DL-1RS, and Gli-B1 and Sec-1 in those segregating for 1B entire and 1BL-1RS. Aneuploid analysis has shown that the structural genes *Gli-D1* and *Tri-D1* are located on chromosome 1DS, *Gli-B1* on 1BS and *Sec-1* on 1RS (Shepherd, 1968; 1973; Singh and Shepherd, 1985). The gel technique is largely that of Lawrence and Shepherd (1980) as modified by Singh and Shepherd (1985) for one-dimensional separation of endosperm proteins.

A small distal part of the endosperm was removed and crushed with a hammer and the proteins extracted by a 2 hour incubation at 60°C in a 2 ml Kartell Dispolab disposable centrifuge tube by addition of 100μl of a buffer consisting of 0.06M tris(hydroxymethyl)-aminomethane (tris), 4% w/v SDS, 15% v/v glycerol and a trace of bromophenol blue, brought to pH 6.8 with HCl. To obtain a reduced protein extract, 2-mercaptoethanol (2ME) was added to the buffer to give a final concentration of 1% v/v. When both unreduced and reduced proteins needed to be electrophoresed separately, the unreduced samples were run first and the samples kept overnight at 4-6°C. The following day 10μl of 25% v/v 2ME was added to the samples, which were then incubated at 60°C for about one hour. Prior to electrophoresis, the samples were briefly centrifuged to obtain a clear supernatant. Initially 15 samples were accommodated per gel by loading 15μl of supernatant (12μl for reduced samples) into 4 mm wide slots spaced 3 mm apart, but the

combs used to form the slots were later modified so that 33 slots, 3 mm wide and spaced 2 mm apart could be formed in the same gel; in these narrower slots, 12µl of sample (8µl for reduced extracts) was loaded. A 1.5 cm deep stacking gel, made up of 3% w/v acrylamide, 0.08% w/v bisacrylamide (bis), 0.1% w/v SDS in 0.125M tris-HCl, pH 6.8 overlaid a 7.5 cm deep separating gel made up of 10% w/v acrylamide, 0.08% w/v bis, 0.1% w/v SDS in 0.375M tris-HCl, pH 8.8. Both gels were polymerised by adding 2.5µl/ml N,N,N,N' - tetramethyl - ethylenediamine (TEMED) and 0.25 mg/ml ammonium persulphate (APS). The overall gel dimensions were 1.2 mm thick by 20 cm wide by 12.5 cm long, and the gels were poured in pairs between two glass plates on either side of a vertical perspex stand. The electrode buffer for both upper and lower tanks contained 0.1% w/v SDS in 0.025M tris brought to pH 8.3-8.4 with glycine. Each batch of electrode buffer was used for two runs before discarding. The gels were electrophoresed at a constant current of 40 mA per gel for 1-1.5 hours (fresh buffer) and at 1.5 to 2 hours at 50 mA per gel using 'old' buffer. Electrophoresis was normally carried out at 4-6°C. Gels were stained overnight in a solution of 1% w/v Coomassie Brilliant Blue R mixed with 40 parts of 6% w/v trichloroacetic acid in water, methanol and glacial acetic acid (80:20:7), and destained in tapwater for several hours before storing in plastic bags. Neither variation in ambient temperature nor strength of current affected the relative separation of the protein species; however, in general, short runs gave sharper bands, and the best results were obtained when the dye front had penetrated about 5 cm into the separating gel.

3.1.2. Two dimensional SDS-PAGE (2D SDS-PAGE)

2D SDS-PAGE was used to separate LMW glutelins. The technique followed that described by Singh and Shepherd (1985). In the first dimension, gel rods of 3 mm internal diameter were made to the same recipes as for single dimensional SDS-PAGE. A space of 1 cm was left at the top of the stacking gel to accommodate 50µl of sample

extract. The gels were run, up to 20 at a time, in a disc gel apparatus at a constant current of 2 mA per rod. When the dye front had travelled 5 cm into the separating gel, the gels were removed from the glass tubes and incubated for 1 hour at 60°C in a 1% v/v solution of 2ME containing 16.3 g glycerol, 1.4 g tris, 3.8 g SDS and 100 ml water made to pH 6.8 with HCl. This reducing buffer fully disrupts the disulphide bonds which cause protein subunits to aggregate (Brown *et al.*, 1979). A 0.5 cm length of the separating gel was then cut from close to the boundary of stacking gel to separating gel and loaded on top of an SDS-PAGE slab gel identical to single dimensional gels (3.1.1) except that the acrylamide and bis concentrations of the separating gel were increased by 10% and the gel thickness was increased to 1.8 mm. The second dimension was run at 50mA per slab until the light brown dye front reached the bottom of the gel. Staining and destaining were identical to single dimensional gels.

3.1.3. Acid PAGE

Acid PAGE, based on the methods of Bushuk and Zillman (1978) was employed to score for the presence of a fast migrating urea soluble endosperm protein controlled by a gene on chromosome arm 5BL (Shepherd, unpubl., and this thesis, Chapter 4). The protein was extracted by the procedure of Shepherd (1968). Endosperm fragments were crushed and incubated overnight at 4°C in 100μl of 2M urea and 5μl of 1% w/v methyl green. Prior to electrophoresis the samples were briefly centrifuged and 10μl of the clear supernatant was loaded into a 5 mm slot formed in a gel made up of 11.2% w/v acrylamide, 0.45% w/v bis, 0.08% w/v ascorbic acid, 0.2% v/v of a saturated solution of $FeS0_4$, 0.24% w/v aluminium lactate made to pH 3.1 with lactic acid and polymerised by addition of 1μl/ml 3% H_20_2. Overall gel dimensions were similar to those of the SDS-PAGE gels, with the exception that acid PAGE gels were 1 mm thick. The same stands were used as above and the electrode buffer, which could be reused repeatedly, in both upper and lower tanks was 0.25% w/v Al lactate pH 3.1. Electrophoresis was carried out at a constant voltage of

150V until the methyl green had travelled 7.5 cm (about 1.5 hours). Gels were stained identically to SDS-PAGE gels, though staining was more rapid and the gels were transferred to distilled water after 2 hours.

3.1.4. Isoelectric focussing (IEF)

The isozymes of two enzyme systems - glucose phosphate isomerase (E.C. 5.3.1.9) (gpi) and leaf peroxidase (E.C. 1.11.1.7) (per) - were separated by flat bed IEF. The gels were 12 cm wide and 0.5 mm thick, and were poured between two glass plates. In order to allow the gels to be handled one glass plate was either covered with a sheet of Gelbond PAG Film (FMC), or was silanized by dipping into 0.2% v/v of Silane A174 (Pharmacia) in chloroform, followed by air drying. Gels were poured by the capillary suction technique (Righetti, 1983). Gels comprised 5% w/v acrylamide, 0.13% w/v bis and 5% w/v carrier ampholytes (Servalyte 3-10) in 16% v/v glycerol. The pH gradient was flattened around pH 7 in some gpi gels by the addition of 25 mg/ml β-alanine to the gel (Righetti, 1983). The gels were polymerised by addition of 1μl/ml TEMED and 0.5 mg/ml APS and left for at least 16 hours at 4-6°C before use. Per gels were run on LKB 3.5-9.5 PAG plates. For electrophoresis, the gels were laid on the cooling platen of an LKB Multiphor, excluding air bubbles between the gel and the platen with a film of kerosene or water. Ice water was pumped through the cooling platen to maintain a gel temperature of 1-2°C throughout the run. The electrode wicks consisted of a lamination of three layers of Whatman 3MM chromatography paper 0.5 cm wide and cut to the length of the gel. The catholyte was 0.5M NaOH and the anolyte 0.5M acetic acid. Gels were prefocussed for at least 500Vh at 0.75W/cm of gel length. Following sample application, focussing was resumed at the same wattage until the potential difference across the gel reached 2000V, whereafter focussing continued at this voltage.

Crude endosperm enzyme extracts for gpi gels were obtained by incubating a crushed fragment of dry endosperm overnight at 4-6°C in 50μl of distilled water, centrifuging

briefly and transferring 15µl of supernatant onto a 0.4 cm x 1 cm sample wick made of Whatman 3MM. The wicks were loaded near the cathodic end of the gel, leaving a 1mm space between samples. In this way 47 samples could be accommodated on a 25 cm long gel. Leaf extracts for per gels were obtained by grinding in a glass mortar 1-2 cm of mature flag leaf in 100-200µl of 0.01M dithiotheitol in the presence of insoluble polyvinylpyrrolidine (Calbiochem) and a few grains of clean sand which aided in grinding the fibrous tissue. The slurry was transferred to a 2 ml disposable centrifuge tube and centrifuged for three minutes at 10000 rpm in a Beckman Microfuge 11. 30µl of supernatant was transferred on to two sample wicks (dimensions and material as described above) which were loaded one on top of the other near the anodic end of the gel. The samples were focussed for 1000-1500 Vh before removal of the sample wicks, and the run was complete after a total of 3500 Vh.

Gpi gels were initially stained according to the recipe of Chojecki and Gale (1982). However this was later replaced by an overlay technique, whereby a length of Cellogel 250 (Chemetron) was soaked in a solution consisting of 1 ml 0.3M tris-HCl pH 8, 0.2 ml 0.3M $MgCl_2$, 0.2 ml 0.2% w/v 3 - (4,5 - dimethyl - thiazol - 2 - yl) -2,5 diphenyltetrazolium bromide (MTT), 0.2 ml 0.05% w/v phenazine methosulphate (PMS), 3 mg D - fructose -6 - phosphate, 2 mg β-nicotinamide adenine dinucleotide phosphate (β-NADP) and 5µl of glucose - 6 - phosphate dehydrogenase (263 U/ml) (Sigma). The excess liquid was drained from the overlay film, which was then laid over the focussing gel. Development time for the bands was 10 minutes at room temperature, compared to at least 1 hour at 37°C for directly stained gels. When the required staining intensity was achieved, the overlay was peeled off the gel, bathed in 7% v/v glacial acetic acid, and left for at least 20 minutes in a 20% v/v glycerol solution containing a trace of formaldehyde. The overlay film was blotted and left to air dry at room temperature. When dry the gels could be photographed, if required, using transmitted light. Further advantages of the overlay technique over the conventional direct staining technique are the economy of expensive stain chemicals and the possibility of sequential staining of multiple isozyme

systems as long as the pH gradient of the gel is appropriate for each enzyme system. The staining protocol for peroxidase followed Kobrehel and Feillet (1975). The gels were incubated at room temperature for 10 minutes in 5% w/v aluminium lactate, washed in distilled water and dipped in a solution containing 2.5 g catechol, 1.96 g tris, 0.15 g boric acid, 0.19 g EDTA and 1.5 g $CaCl_2.2H_2O$ in 100 ml of water. After incubation for 30 minutes at 30°C, the gels were immersed in 0.01M H_2O_2 and fixed in 3% v/v glacial acetic acid.

3.1.5. Cellulose acetate gel electrophoresis

Cellogel 250 was used as a medium for separation of the isozymes of gpi controlled by a structural gene on chromosome 1BS (Hart, 1979b) Sample preparation was as for IEF. A 9 x 10 cm strip of gel was removed from storage in 30% v/v methanol, blotted dry and bathed in distilled water for at least 15 minutes to remove traces of methanol. The gel was then equilibrated for a further 15 minutes in the electrode buffer, consisting of 0.025M tris and 0.2M glycine. The gel was blotted dry and loaded with approximately 2µl of sample supernatant with a Super Z Applicator (Helena Laboratories). Twenty four samples were accommodated on each gel in two rows of 12. The gels were run in a refrigerator at 4-6°C for 45 minutes at a constant voltage of 350V. The staining and fixing protocols were as for overlays on IEF (3.1.4) except that the gels were left face down in the staining solution until sufficient staining intensity had been achieved.

3.2 Cytology

3.2.1. Mitosis

In order to ascertain the somatic chromosome number of a particular plant, the seed was germinated on moist filter paper in the dark at circa 28°C for 40-48 hours, when two roots were removed from the seedling and prefixed in distilled water maintained at 0-1°C for up to 24 hours in order to increase the mitotic index within the root tip. After fixation in 3:1 ethanol:glacial acetic acid for at least 1 hour the root tips were subjected to the standard Feulgen squash procedure and somatic counts of at least 5 mitotic cells per seedling made.

3.2.2. Giemsa staining of root tip meristem interphase cells

Seeds were germinated as above, but the excised root tip was neither prefixed nor fixed. Rather, the meristematic cells were dissected directly into a drop of 45% v/v glacial acetic acid on a microscope slide, the debris removed and the cells squashed under a coverslip. Interphase preparations from three separate seedlings were accommodated on a single slide. After squashing, the slides were immersed in liquid nitrogen for 5 seconds to allow removal of the coverslip, then air dried over a heater for 10 to 15 minutes. The slides were stored overnight in a desiccator at room temperature. The C-banding procedure is adapted from Hossain (1983). The slides were treated in saturated $Ba(OH)_2$ at 60°C for 6 minutes, washed in tapwater, incubated for 15 minutes in 2xSSC at 60°C, washed in distilled water and finally stained for 10 minutes in 0.05% w/v Giemsa (Sigma) in phosphate buffer pH 7. The slides were air dried under hot air and analysed without permanent mounting.

3.2.3. Meiosis

Anthers for meiotic analysis were selected so that the chromosomes were at metaphase I. The chromosomes were either stained by the standard Feulgen technique or by C-banding. For the latter procedure, the anthers were briefly (<10 minutes) fixed in 3:1 ethanol:glacial acetic acid and transferred to a drop of 45% v/v glacial acetic acid on a microscope slide. The ends of the anther were removed and the pmcs were expressed into the liquid drop. The anther tissue was removed from the liquid and the cells were squashed under a coverslip. The staining procedure followed the protocol of 3.2.2, except that the barium treatment time was more critical, and needed to be standardised from batch to batch of chemical; in general meiotic cells required a less harsh barium treatment than somatic cells. Feulgen stained slides were made permanent by removing the coverslip as in 3.2.1, immersing in absolute ethanol for at least 15 minutes, air drying and finally replacing a coverslip over a drop of Euparal. C-banded preparations required only the final step of this procedure.

3.3. Stem rust inoculation

Embryo halves of seed previously analysed by SDS-PAGE were sown directly into soil in 58 x 40 x 10 cm boxes, which accommodated up to 9 rows of 12 seedlings each. To ensure a regular stand of seedlings, the holes into which the seed was placed were plugged with vermiculite, so that the seedling nearly always emerged in the same position as it was sown; this was critical in order to maintain the identity of each plant. At the two leaf stage the seedlings were inoculated with stem rust (mixture of races 21 and 343, virulent on both cv. Chinese Spring and cv. Gabo) by puffing a mixture of urediospores, either freshly collected from infected susceptible plants, or maintained under vacuum in the refrigerator for some months, in talcum powder (approximately 1 part rust spores to 40 parts talcum

powder) over the previously moistened foliage. Inoculation was performed in the evenings to avoid high daytime temperatures and the boxes were kept under high humidity overnight; this was best achieved by containing the boxes in heavy polythene bags containing about 1 litre of water. The rust pustules took between 14 and 21 days to develop depending on prevailing temperatures and the plants were scored for resistance or susceptibility at this time.

Chapter 4: Wheat-rye recombination

I. The short arm of rye chromosome 1R

4.1 Introduction

The homoeology of chromosome 1R of cereal rye with wheat chromosomes belonging to homoeologous group 1 has been demonstrated by the successful production of substitution lines of chromosome V of 'King II' rye for chromosomes 1B and 1D in a mixed Chinese Spring / Holdfast background (Lawrence, 1969) and for chromosome 1A of Holdfast (Miller, 1984); and of chromosome E of 'Imperial' rye for 1B, 1D (Shepherd, 1973) and 1A (N.K. Singh and K.W. Shepherd, unpubl.) of Chinese Spring. Many undirected (1B) 1R substitutions have been reported among European winter wheats (Mettin *et al.*, 1973; Zeller, 1973). Spontaneous translocations between 1R and its wheat homoeologues are commonplace, and it is thought that they have arisen from centric fusion, following simultaneous misdivision of 1R and one of its wheat homoeologues. All six possible reciprocal translocations, involving 1RS with the long arm of the wheat homoeologous group 1 chromosomes and 1RL with the complementary wheat short arms have now been isolated (Shepherd, 1973; Lawrence and Shepherd, 1981; N.K. Singh and K.W. Shepherd, unpubl.) by selecting among the progeny of a self-fertilised plant monosomic for both 1R and a homoeologous group 1 wheat chromosome. These lines are all vigorous and highly fertile. The yield of a 1DL-1RS translocation in a Chinese Spring background was shown by Shepherd (1977) to be greater than that of ditelosomic 1DL, but less than that of the euploid. In certain other wheat backgrounds lines carrying this translocation did not suffer any yield disadvantage (Shepherd, pers. comm.).

A number of useful genes are known to be located on the short arm of rye chromosome 1R. The wheat cultivar Amigo, produced by irradiation of a triticale-wheat backcross derivative (Sebesta and Wood, 1978), has been shown by Zeller and Fuchs (1983) to possess a 1AL-1RS translocation; the rye arm carries a gene(s) for resistance to

the greenbug *Schizaphis graminum* Rond. (Wood *et al.*, 1974), and probably is responsible for both the powdery mildew (*Erysiphe graminis* em. Marchal f. sp. *tritici*) resistance (Lowry *et al.*, 1981) and the leaf and stem rust resistances of this cultivar (McIntosh, cited in Zeller and Fuchs, 1983).

The origin of the 1BL-1RS translocation present in many European winter wheats has been traced by Mettin *et al.* (1973) to two independent wheat breeding programmes in Germany which utilised triticale as a source of germplasm in the 1930's. It is now widespread among spring and winter wheats in both Europe and Mexico. The translocation is associated with high yielding performance across a wide range of environments (Rajaram *et al.*, 1983; Blackman, pers. comm.) and it carries genes for resistance to several foliar diseases (*Pm8* , *Yr9* , *Sr31* , *Lr3* and *Lr26* - McIntosh, 1983); however breeding lines and cultivars carrying this translocation have been found to produce flour with a pronounced dough quality defect (Zeller *et al.*, 1982; D.J. Martin, pers. comm.) which has prevented the use of this translocation line in the development of high yielding breadmaking varieties both in the United Kingdom (Blackman, pers. comm.) and in Australia (Martin and Stewart, 1984).

The 1DL-1RS translocation was originally isolated in cv. Chinese Spring and was found to confer resistance to stem rust (Shepherd, 1977). Since the resistance gene is located on the same chromosome arm in 'Imperial' rye as *Sr 31* from 'Petkus' rye, it is likely that the two genes are either identical or allelic. The 'Imperial' gene will be referred to in this work as *SrR* as no formal number has yet been assigned to it and its relationship to *Sr31* has not been elucidated. The translocation was transferred into a number of locally adapted genotypes by backcrossing and it was found that, in some genetic backgrounds, its presence regularly induced a depression in yield compared to that of the recurrent parent, although in other backgrounds there was no evidence of significant yield loss. A common property of all backgrounds was the pronounced weakness in the dough made from flour of the translocation homozygote, and this defect made the translocation unsuitable for use in breeding programmes for breadmaking wheats (Shepherd, 1977;

Koebner *et al.*, 1984). A spontaneous 1BL-1RS translocation chromosome arose in the course of an attempt to substitute entire 1R for 1B in cv. Gabo; this translocation also carried the gene for resistance to stem rust and was high yielding, but the translocation lines produced a dough which, although superior to that from the 1DL-1RS lines, was still poorer than that from Gabo itself (Koebner *et al.*, 1984).

The short arms of homoeologous group 1 chromosomes of wheat carry genes coding for the non-aggregating gliadin storage proteins (Shepherd, 1968; Wrigley and Shepherd, 1973), for the aggregating low-molecular-weight (LMW) glutenin subunits (Jackson *et al.*, 1983; Payne *et al.*, 1984b; Singh and Shepherd, 1985) now given the gene designation *Glu3* (Singh, 1985) and for a new class of aggregating proteins given the provisional designation 'triplet' (*Tri*) (Singh and Shepherd, 1985). The quantity and quality of protein in the endosperm is thought to have a major influence on the breadmaking quality of dough (Finney and Barmore, 1948; Bushuk *et al.*, 1969). An association between four quality attributes and gliadin phenotype was observed by Wrigley *et al.* (1982a), and the presence of certain gliadin bands was found to be closely correlated with particle size index and dough strength by Wrigley *et al.* (1982b). Although these correlations were not claimed to be causal, the involvement of many protein species in the gliadin phenotypes correlated with higher quality suggests that this is at least possible. A strong correlation between dough strength and the presence of a single gliadin band on electrophoregrams of total protein extracts of durum wheat has been observed by Damideaux *et al.* (1978). The loci controlling the LMW glutenin subunits and gliadins are genetically very tightly linked (Payne *et al.*, 1984b; Singh and Shepherd, 1985), and therefore the products of the former genes, rather than the gliadin proteins themselves, may be responsible for the major contributions to dough quality noted above (Payne *et al.*, 1984a). Correlations between particular high-molecular-weight (HMW) glutenin subunits and breadmaking quality has also been reported in some European wheats (Payne *et al.*, 1979; 1981; Burnouf and Bouriquet, 1980; Moonen *et al.*, 1982, 1983). The genes controlling these proteins are genetically independent of the gliadins and are located on the long arms of the

homoeologous group 1 chromosomes (reviewed by Payne *et al.*, 1982a). Glutenin proteins are thought to impart elasticity to a bread dough, while the more viscous gliadins promote extensibility (Payne *et al.*, 1984a).

Zeller *et al.* (1982) have suggested that the dough quality defect of the 1BL-1RS translocation lines, which is expressed as 'sticky' dough, derives from deleterious gene(s) present on the rye arm. The more pronounced deterioration in dough quality observed with 1DL-1RS translocation lines would then imply that genes on 1BS are less able to counteract the negative effects of the rye genes than can genes on 1DS. An alternative explanation for these observations is that the loss in quality is due more to the loss of wheat genes than to the gain of rye genes, with 1DS genes being more important for dough quality than 1BS genes. This hypothesis is supported by the earlier findings of Welsh and Hehn (1964) who demonstrated that 1D was by far the most important chromosome in determining dough quality when measured by the wheatmeal fermentation test (Pelshenke, 1933). The value of this test for prediction of breadmaking quality has, however, since been questioned (e.g. Monsivais *et al.*, 1983). Irrespective of the cause of the quality problem associated with 1RS translocations, induction of recombination between 1RS and, ideally, 1DS would be expected to overcome it. Recombinant lines, in which the deleterious genes had been lost, or in which the critical wheat genes had been restored and while simultaneously retaining the gene for resistance to stem rust from 1RS, would offer the breeder the use of an alien gene for disease resistance without any attendant quality defect. Although chromosome arm 1RS is homoeologous with both 1DS and 1BS, the presence of the *Ph1* gene on wheat chromosome 5B prevents any recombination occurring between these chromosome arms in normal wheat backgrounds. In order to induce such recombination, it is necessary to introduce the translocation into either a mutated or a deleted *Ph1* background. In the present work this was achieved using both the *ph1b* mutant of Sears (1977) and nullisomy for chromosome 5B.

4.2. Plant materials and methods

4.2.1. Plant materials

All of the wheat genotypes used possessed a Chinese Spring background unless otherwise noted.

a. Translocation lines: 1DL-1RS (Shepherd, 1973);

1BL-1RS and double translocation 1BL-1RS / 1DL-1RS in cv. Gabo (Shepherd, unpubl.).

b. *ph1b* mutant (Sears, 1977). Stock used derived from Accession 7876 of Dr. K.W. Shepherd.

c. Chromosome 5B stocks: monosomic 5B (mono 5B) (Sears, 1954); nullisomic 5B-tetrasomic 5A (N5BT5A), nullisomic 5B-tetrasomic 5D (N5BT5D) (Sears, 1966).

d. Ditelosomic 1DL (Dit 1DL) (Sears and Sears, 1978).

e. Substitution line (1D) 1R (Shepherd, 1973).

f. An accession of *Ae. variabilis* (Ac. 7069) obtained from K.W. Shepherd.

4.2.2. Marker loci used in the selection and characterisation of recombinants

Chromosome 1RS is marked by a gene for a prolamin-like storage protein *Sec-1* (Shepherd and Jennings, 1971), the gene for resistance to stem rust (*SrR*), and a structural gene for the isozyme glucose phosphate isomerase (Gpi) (Chojecki and Gale, 1982; Koebner, unpubl.).

Chromosome arm 1DS carries genes for the ω-gliadin storage protein Gli-D1 (Shepherd, 1968), the LMW glutelin subunit Glu-D3 and the triplet proteins Tri-1 and Tri-2 (Singh and Shepherd, 1985); and structural genes for the isozymes Gpi (Chojecki and Gale,

1982) and leaf peroxidase (Per) (Ainsworth *et al.*, 1984).

Chromosome arm 1BS carries the ω-gliadin gene *Gli-B1* (Shepherd, 1968).

4.2.3. Phenotypes of parental lines

4.2.3.1. Unreduced SDS-PAGE patterns

The unreduced SDS-PAGE and 2D SDS-PAGE phenotypes of euploid Chinese Spring and the homoeologous group 1 aneuploids have been described by Singh and Shepherd (1985). Chromosome 1DS controls three endosperm storage protein loci, *Tri-D1*, *Gli-D1* and *Glu-D3*, the first two of which can be simultaneously visualised on single dimensional SDS-PAGE, the third requiring 2D SDS-PAGE in order to first remove the overlapping gliadin bands. The absence of *Tri-D1* causes the loss of Tri-1 and Tri-2, which are sharply stained bands against a region of heavy streaking in the cathodal half of the gel. Tri-1 is wholly controlled by *Tri-D1*, while Tri-2 is a hybrid molecule formed from the gene products of *Tri-D1* and *Tri-A1*, a homoeolocus of *Tri-D1*, located on chromosome arm 1AS. The product of *Gli-D1* (a prolamin protein) appears as a single band of greater mobility than the Tri proteins. The gene *Sec-1*, located on the short arm of rye chromosome arm 1RS, codes for a group of at least four proteins (prolamins) with higher mobility than Gli-D1. These patterns are illustrated in Figure 4.1. *Gli-B1*, the gliadin locus on chromosome arm 1BS codes for a group of three proteins which run on the cathodal side of Gli-D1 in cv. Chinese Spring (Figures 4.4, 4.9). In some gels the slowest two bands are not well separated.

4.2.3.2. Leaf peroxidase

The genetic control of certain peroxidase isozymes has been elucidated by Ainsworth *et al.* (1984), who give a numbering system to the bands produced when these isozymes are separated by isoelectric focussing. In the absence of chromosome arm 1DS, bands 6 and 7 are lost. However a number of the figures shown by Ainsworth *et al.* (1984) support the experience in this work that band 6 is not a reliable marker for *Per-D1* ; rather band 7 has been taken to be diagnostic of the presence of this locus (Figure 4.7).

4.2.3.3. Glucose phosphate isomerase

In cv. Chinese Spring, three bands in the central portion of the gel are lost when chromosome 1DS is removed (bands W1, W2, W3). When this chromosome arm is replaced by 1RS, as in the translocation line 1DL-1RS, at least one new band (R1) is produced (Figure 4.8).

4.2.4. Selection of a 1D/1DL-1RS translocation heterozygote, homozygous for *ph1b*

Since there is no recombination between wheat and rye chromosomes in the presence of the *Ph1* allele, and since prolamin protein phenotypes exhibit codominance, it can be inferred that seeds carrying both the wheat and rye prolamins are translocation heterozygotes. An F_2 population derived from the cross *ph1b* mutant x translocation homozygote 1DL-1RS segregated for the Gli-D1 band from the female parent and for the Sec-1 bands from the male, and translocation heterozygotes were selected from this population by unreduced SDS-PAGE (Chapter 3.1.1). Twenty such F_2 individuals were grown in pots in the glasshouse. At meiosis anthers with pmcs at metaphase I were taken and their chromosomal configurations, stained by the Feulgen technique (Chapter 3.2.3),

were analysed in an attempt to isolate homozygous *ph1b* plants. One putative *ph1b* homozygote was pollinated by Dit 1DL to produce a test-cross population to be analysed for wheat-rye recombination. Dit 1DL, which lacks all 1DS marker genes, was chosen as the pollen parent so that any recombination involving chromosome arm 1DS in the female gamete would not be masked in the test-cross progeny.

4.2.5. Selection of translocation heterozygotes 1D/1DL-1RS and 1B/1BL-1RS nullisomic for 5B

Mono 5B was crossed as female with both translocation lines and progeny with 2n=41 were identified by root tip mitotic chromosome counts (Chapter 3.2.1). The chromosome constitution of these selections was verified at meiosis ($20'' + 1'_{5B}$). They were then pollinated with N5BT5A or N5BT5D and progeny were screened by unreduced SDS-PAGE to select for translocation heterozygotes, followed by acid PAGE (Chapter 3.1.3) to allow those nullisomic for 5B to be identified. The resulting plants were allowed to self-fertilise to give populations suitable for detection of wheat-rye recombination.

4.2.6. Screening for wheat-rye recombination

Individual seeds were subjected to unreduced SDS-PAGE and scored for the protein bands Tri-1, Gli-D1 and Sec-1 (1DL-1RS material) and Gli-B1 and Sec-1 (1BL-1RS material). This scoring presented no difficulty and examples of the gels for the 1DL-1RS and the 1BL-1RS materials are shown in Figures 4.1 and 4.9, respectively. The corresponding embryo halves were then sown into boxes in ordered positions and the seedlings tested for reaction to stem rust (Chapter 3.3). The susceptible and resistant reactions are illustrated in Figure 4.2. The stem rust resistance from rye present in the translocation line is

characterised by small pustules which are surrounded by chlorosis of leaf tissue, in contrast to the susceptible reaction where the pustules enlarge and later merge without any leaf chlorosis; infected leaves of susceptible plants usually wither, while leaves of resistant plants tend to remain green.

Any individual shown to possess a phenotype differing from the parental combination of characters was progeny tested to confirm the initial classification, and tested for the spectrum of the isozymes Gpi (using IEF with a pH gradient flattened around pH7) and Per (Chapter 3.1.4) and in some cases for the LMW glutelin subunit Glu-D3 (Chapter 3.1.2).

4.3. Results

4.3.1. Identification of heterozygous 1D/1DL-1RS, *ph1bph1b* plants and analysis of test-cross progeny

Plants homozygous for *ph1b* were expected to show multivalent formation at metaphase I of meiosis (Sears, 1977), but this criterion alone proved unsatisfactory, as in the majority of plants examined cytologically some of the pmcs had multivalents. The *ph1b* mutant stock used to make the initial cross had itself undergone an unknown number of generations of self-fertilisation since its initial isolation, and because the deletion of *Ph1* allows allosyndesis, it is likely that numerous homoeologous translocation events had occurred within the stock. Thus when a cross is made between the mutant and a normal wheat, any translocation differences between the maternal and the paternal gametes are expected to give rise to multivalents in the meiocytes of the progeny and some of these differences will be transmitted to the F_2 generation. The F_2 plants will often therefore show multivalents unrelated to their constitution at the *Ph1* locus. A similar result in hybrids of nullisomic 5B x euploid was noted by Riley and Chapman (1958a).

A more reliable indicator of *ph1b* homozygosity was a reduction in chiasma frequency as documented by Yacobi *et al.* (1982) and which is also characteristic of nullisomy for 5B (Driscoll *et al.*, 1979). Among the 16 plants which were cytologically investigated, only one was positively identified as being homozygous for *ph1b*. The mean pairing of this plant (82-58-2) at metaphase I was 1.37' (0-6) + 5.37'' (1-8) + 13.67'' (11-17) + 0.26''' (0-1) + 0.44 ^{iv} (0-1) in 27 pmcs (range in brackets). Up to 10 rod bivalents and frequent univalents were also seen in pmcs which could not be analysed fully. In none of the other plants were more than four univalents per pmc observed and the relative number of rings and rods among the bivalents was close to the mean values of approximately 19

and 2, respectively, observed in normal wheat (Driscoll *et al.*, 1979). A more detailed comparison of the effect of *ph1b* homozygosity on chromosome pairing was obtained in subsequent experiments (see Chapters 5 and 6).

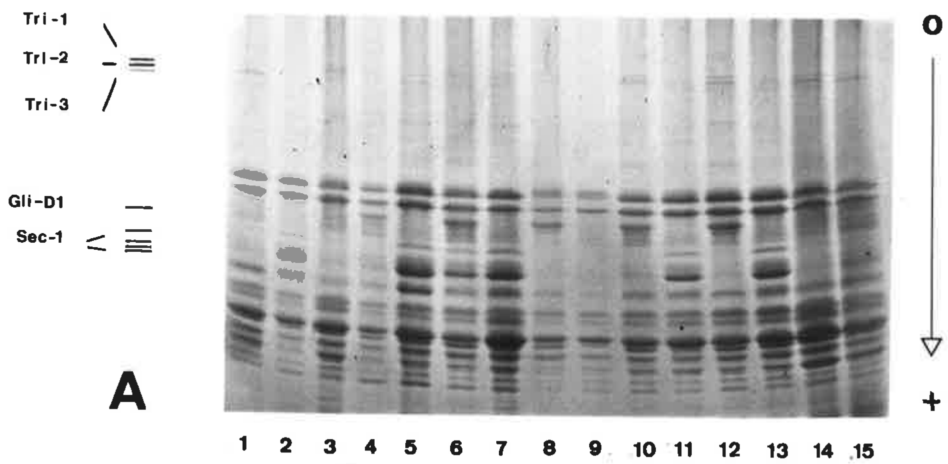
A total of 397 test-cross progeny was obtained by pollinating plant 82-58-2 with Dit 1DL. SDS-PAGE phenotypes were obtained for 394 of these; the remaining three seeds were shrivelled and produced abnormal banding patterns on the gel. The different phenotypic patterns obtained from the test-cross population are illustrated in Figure 4.1A and the frequency of their occurrence is given in Table 4.1. The two parental phenotypes

Table 4.1: Observed frequency of progeny from the test cross 1D/1DL-1RS heterozygote *ph1bph1b* x Dit 1DL having the endosperm protein phenotype indicated.

Endosperm protein phenotype			No. observed	Classification
Tri-1	Gli-D1	Sec-1		
+	+	-	159	parental
-	-	+	152	parental
-	-	-	40	hypoploid
+	+	+	39	hyperploid
+	-	-	3	recombinant
-	+	+	1	recombinant

+ : protein present - : protein absent

represent the transmission of either a normal 1D or a translocation chromosome 1DL-1RS from the heterozygote, and these two chromosomes were transmitted with an equal frequency through the female gamete. Transmission of neither chromosome (hypoploidy) and transmission of both chromosomes (hyperploidy) occurred with a similar frequency and aneuploid products represented 20% of the test-cross population. A high level of aneuploidy was expected following the observation of a high incidence of univalents induced by *ph1b* homozygosity in the female parent. The phenotypes of the four progeny classified as recombinant are shown in Figure 4.1A (tracks 3, 6, 14, 15). Three of these possessed *Tri-D1* but had lost *Gli-D1* and did not carry *Sec-1* (tracks 3, 14, 15), while


Tri-1
Tri-2
Tri-3
Gli-D1
Sec-1
A
O
+
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

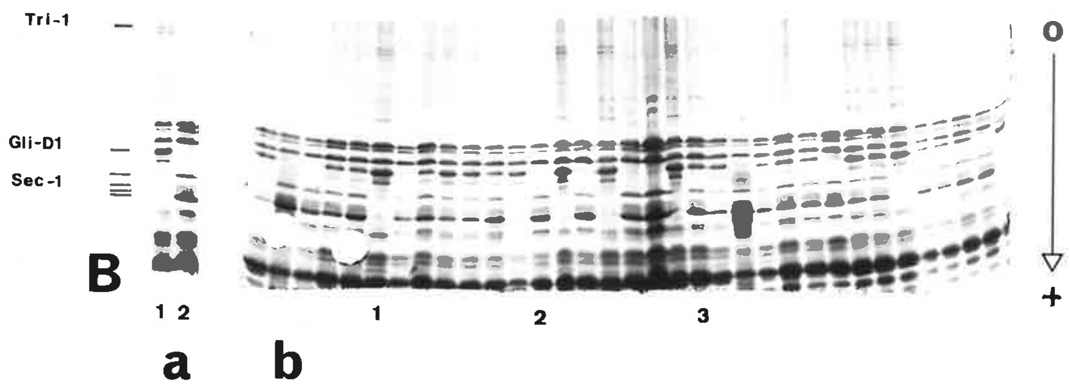

Tri-1
Gli-D1
Sec-1
B
O
+
1 2
1
2
3
a
b

Figure 4.1

Endosperm storage protein phenotypes of parents and progeny segregating for loci on chromosome arms 1DS and 1RS. Patterns obtained by unreduced SDS-PAGE.

A. Parents, and progeny derived from the test cross 1D/1DL-1RS heterozygote *ph1bph1b* x Dit 1DL.

Parents - Lane 7: Translocation 1DL-1RS (Tri-1^- Gli-$D1^-$ Sec-1^+), Lane 8: Chinese Spring (CS) euploid (Tri-1^+ Gli-$D1^+$ Sec-1^-), Lane 9: Dit 1DL (Tri-1^- Gli-$D1^-$ Sec-1^-).

Test-cross progeny - Lanes 1-6, 10-15. Note non-parental phenotype of samples in lanes 3 (plant 82-177), 6 (plant 82-180), 14 (plant 82-178) and 15 (plant 82-179).
Lanes 3, 14, 15: Tri-1^+ Gli-$D1^-$ Sec-1^-; Lane 6: Tri-1^- Gli-$D1^+$ Sec-1^+.

B. Progeny from the F2 of the cross (mono 5B x translocation 1DL-1RS) x N5BT5A. Parent plant selected to be a 1D/1DL-1RS heterozygote, nullisomic for chromosome 5B.

a - parental phenotypes: 1. Euploid CS 2. Translocation 1DL-1RS
b - progeny phenotypes: 1, 2, 3 are examples of the three most frequent phenotypes recovered.
1. Tri-1^+ Gli-$D1^+$ Sec-1^- (disomic 1D entire) 2. Tri-1^- Gli-$D1^-$ Sec-1^+ (translocation homozygote) 3. Tri-1^+ Gli-$D1^+$ Sec-1^+ (translocation heterozygote)

the fourth had lost *Tri-D1* but retained both *Gli-D1* and *Sec-1* (track 6). For ease of reference, the first three recombinants will be called type I, and the fourth, type II.

The embryos corresponding to the four selections were grown and the resulting plants were designated 82-177, -178 -179 and -180, respectively. The linkage between *Gli-D1* and the LMW-glutelin locus *Glu-D3* (Singh and Shepherd, 1985) was maintained in all four lines - that is, the three lacking *Gli-D1* also lacked *Glu-D3* while the seed which retained *Gli-D1* also possessed *Glu-D3* . Analysis of leaf peroxidase phenotypes indicated that 82-177, -178 and -179 all possessed the isozyme controlled by 1DS, while 82-180 was null (Figure 4.7, Table 4.2). No rye isozyme was detected on these gels,

Table 4.2. Phenotypes of four recombinant plants derived from the test cross, translocation heterozygote 1DL-1RS *ph1bph1b* x Dit 1DL with respect to marker loci on chromosome arms 1DS and 1RS.

	1DS markers				1RS markers			Recombinant
Plant no.	Tri-1	Gli-D1	Gpi-D1	Per-D1	Sec-1	Gpi-R1	SrR	type
82-177	+	-	+	+	-	-	-	Ia
82-179	+	-	+	+	-	-	-	Ia
82-178	+	-	-	+	-	-	-	Ib
82-180	-	+	-	-	+	+	+	II

+: protein present -: protein absent

although Ainsworth *et al.* (1984) reported that chromosome 1RS both from rye culti[illegible] King II and that present in 1BL-1RS translocations Veery "S" and Hahn "S" produc[illegible] peroxidase isozyme that is distinguishable from those of wheat. The glucose phosph[illegible] isomerase phenotypes of the progeny of these plants showed that while 82-177 and [illegible] both retained *Gpi-D1*, 82-178 was null. Thus the type I recombinant can be subdivi[illegible] into Ia and Ib (Table 4.2). 82-180 had *Gpi-R1* rather than *Gpi-D1* (Figure 4.8).

A total of 347 (including the four recombinants discussed above) of the 394 test[illegible] progeny analysed by SDS-PAGE produced seedlings which could be rust tested. Th[illegible] reaction showed complete linkage with the Sec-1 phenotype, that is, all lines lack[illegible] rye protein bands were stem rust susceptible, while those carrying these bands [illegible]

a　　　　　　　　b

Figure 4.2

Stem rust reaction of progeny seedlings from the test cross 1D/1DL-1RS heterozygote *ph1bph1b* x Dit 1DL.

a - susceptible reaction (equivalent to reaction of Chinese Spring euploid parent).

b - resistant reaction (equivalent to reaction of Chinese Spring 1DL-1RS translocation parent).

the translocation-type resistance.

The phenotypes of the four recombinant plants recovered in this population with respect to 1DS and 1RS markers are summarised in Table 4.2.

4.3.2. Further analysis of the four putative recombinant lines induced by *ph1bph1b*

The protein phenotype of each of the four putative recombinants was confirmed by testing the progeny obtained by self-fertilisation of the plants 82-177, -178, -179 and -180 by SDS-PAGE. In each case some of these progeny had the same phenotype as Dit 1DL. This was expected as each plant received one dose of telosome 1DL from the test-cross male parent. The frequency of recovery of the Dit 1DL phenotype was approximately one in six in each case, which is rather higher than the recovery of approximately one in sixteen of Dit 1DL among the progeny obtained from self-fertilisation of a plant monotelodisomic for this chromosome arm (N.K. Singh, unpubl.). The remainder of the progeny from each plant had the same protein phenotype as that of the respective parent, thereby confirming the original recombinant classification. Among the progeny of plant 82-180, the Gli-D1 and Sec-1 bands always remained associated, so that the two loci controlling these bands are present on the same chromosome in this line. However, no conclusion as to their proximity could be made as there was no homologous arm available for normal crossing-over to occur in the recombinant plant 82-180. Singh (1985) has estimated the map distance between the centromere and *Tri-D1* to be 15.4 cM, while the *Tri-D1* to *Gli-D1* distance was measured in an independent 1DS mapping experiment to be 45.5 cM (Koebner, unpubl.), so that the 82-180 recombinant appears to have had a segment of 1RS replaced by a segment of 1DS, distal to the *Tri-D1* locus, but including the *Gli-D1* locus.

In order to gain a clearer idea of how much of the rye arm is present in this recombinant (type II, Table 4.2), progeny of plant 82-180 was supplied to Dr. R. Appels (CSIRO, Division of Plant Industry, Canberra) in order to assay for the presence of

molecular markers associated with this rye chromosome arm. By analysis of the hybridisation of DNA from this line with molecular probes for the rye nucleolar organising region (Appels *et al.*, 1980) and the rye 5S RNA genes, it was found that both these regions of rye chromatin are present on this recombinant chromosome (Appels, pers. comm.). *In situ* hybridisation studies have shown that the 5S RNA gene complex is located within the satellite of 1RS (Appels, pers. comm.), so that it seems that the 82-180 chromosome consists mostly of rye chromatin on its short arm, and that the amount of wheat chromatin present in this recombinant is likely to be very limited. The physical location of *Gli-D1* on chromosome arm 1DS is not known, as no deletion lines involving this arm have to date been available, and *in situ* hybridisation with radioactive cDNA clones of the gene has not been reported, although such clones for the linked γ-gliadin sequences are now available (Harberd *et al.*, 1985). However, because *Gli-D1* has been retained in 82-180, it is likely that this locus lies near the telomere of 1DS, and this is consistent with the position of the homoeoloci *Gli-B1* and *Sec-1* within the satellites of their respective chromosomes (Payne *et al.*, 1984c; Lawrence and Appels, 1985). Since both *Gpi-B1* and *Gpi-R1* also lie within the satellite of their respective chromosomes, it seems likely that the type Ia recombinant chromosome (Table 4.2), which retains *Gpi-D1* , involves only a small loss of 1DS chromatin, while the type Ib chromosome, which has lost *Gpi-D1* , involves a breakpoint closer to the centromere than that in type Ia.

In an attempt to find whether the three recombinant lines 82-177, -178 and -179 contain any rye chromatin, they were each crossed to the substitution line (1D) 1R and the pmcs of F1 plants which carried the *Tri-D1*- coded protein bands were analysed by C-banding (Chapter 3.2.3.) to search for any evidence of pairing between the 1R chromosome which is marked by a heterochromatic telomere on the long arm (Gill and Kimber, 1974a) and any chromosome from the recombinant lines. In each case, chromosome 1R was not paired in at least 20 pmcs, and it was therefore concluded that the three plants 82-177, 82-178 and 82-179 contained either no, or at most very little, rye chromatin.

To further elucidate the nature of the three recombinant plants apparently not possessing rye chromatin, they were each crossed to plant 82-180. Using SDS-PAGE patterns, only those F1 progeny with the phenotype Tri-1$^+$ Gli-D1$^+$ Sec-1$^+$ (that is, those possessing both recombinant chromosomes) were selected, and these were test crossed to Dit 1DL. The test-cross progeny were analysed by SDS-PAGE and seven different phenotypes were recovered as shown in Table 4.3.

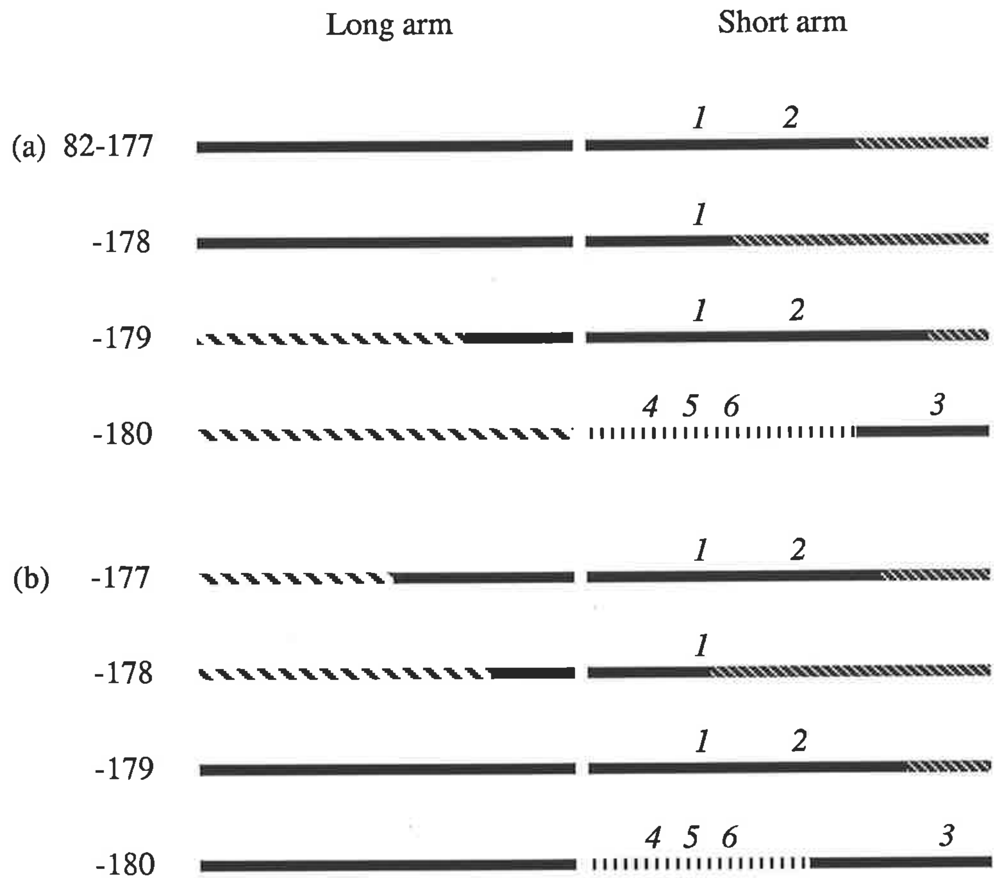
Table 4.3. Endosperm protein phenotypes and their frequency among the progeny from the three test crosses (82-177, 82-178 and 82-179 x 82-180) x Dit 1DL.

		Endosperm protein phenotype							
	Tri-1	+	-	+	-	-	+	+	
	Gli-D1	-	+	+	-	-	-	+	
Test-cross combination	Sec-1	-	+	+	-	+	+	-	Total
(178x180) x Dit 1DL		27	31	26	25	0	0	0	109
(177x180) x Dit 1DL		33	28	18	15	3	1	0	98
(179x180) x Dit 1DL		47	61	2	0	9	0	5	124

+ : presence of protein - : absence of protein

In the cross 178 x 180 there was no recombination between *Gli-D1* and *Sec-1* but these two loci recombined freely with *Tri-D1* leading to a 1:1:1:1 distribution among the four phenotypic classes obtained. This evidence is taken to indicate that *Tri-D1* in 82-178 and *Gli-D1, Sec-1* in 82-180 are present on different chromosomes. Thus either *Tri-D1* has been separated from *Gli-D1* by allosyndetic recombination between chromosome 1DS and a wheat homoeologue to give the 178 recombinant chromosome, while the 180 chromosome consists of a segment of wheat chromatin other than 1DL on the long arm, with a short arm composed of parts of 1RS and 1DS; this could have arisen following the formation of a homoeologous trivalent made up of chromosomes 1B or 1A joined with 1DL-1RS and 1D (Figure 4.3a); or the180 chromosome has retained 1DL, and has a short arm composed of parts of 1RS and 1DS resulting from a single homoeologous cross-over on the short arm between 1DL-1RS and 1D, while the 178 chromosome involves the

Figure 4.3. Two alternative structures for the recombinant chromosomes present in plants 82-177, -178, -179 and -180.



Note common segment of 1DS chromatin in -179 and -180. Chromosomes with segments from two homoeologues have derived from a homoeologous bivalent; those with segments from three homoeologues from a homoeologous trivalent.

transfer of an interstitial segment containing *Tri-D1* to a wheat homoeologue (Figure 4.3b).

The phenotypic distribution of the test-cross progeny involving 82-177 resembles that from 82-178, except for four individuals with two additional phenotypes. The "+-+" individual (Table 4.3) was grown and allowed to self-fertilise and the segregation of its progeny indicated that the gametic constitution of this plant was "+--" and "--+". Since the latter gamete is identical to that produced by 1DL-1RS, it was concluded that the "+-+" individual had been produced by accidental use of pollen from a 1DL-1RS rather than a Dit1DL plant; this conclusion was supported by the observation that, of the three "--+" individuals, two were produced on the same spike as the "+-+" individual. Thus the recombinant chromosome present in plants 82-177 and 82-178 appear to be similar, except that the breakpoint on chromosome 1DS in 82-178 is closer to the centromere as this line lacks the structural gene *Gpi-D1a* which is located between *Tri-D1* and *Gli-D1* with map distances (Singh, 1985; Koebner, unpubl.) in centimorgans as shown in the sketch below.

The test-cross progeny involving plant 82-179 showed a markedly different segregation pattern from the crosses involving the other two plants, where instead of independent segregation, most of the progeny fell into one or other of the parental phenotypic classes (Table 4.3). The few "--+" and "++-" individuals were considered to be cross-over products following chiasma formation in a short common length of chromatin along 1DS (see Figure 4.3). The two "+++" individuals were progeny tested and were found to be hyperploid - that is possessing both the 82-180 and the 82-179 recombinant chromosomes. Therefore it is concluded that the 82-179 chromosome arose following homoeologous synapsis between 1DS and another group 1 wheat chromosome.

In an attempt to identify the changed chromosomal location of *Tri-D1* in 82-177 and 82-178, both these lines were crossed and backcrossed to the double translocation line 1DL-1RS/1BL-1RS. The recurrent parent lacks both the short arms of 1B and 1D and thus, in the first cross, both *Tri-D1* and *Gli-B1* are present in only one dose, while in the backcross they will be expected to segregate jointly only if they are linked on the same chromosome arm. Thus if *Tri-D1* had been translocated onto 1BS, then in any BC_1 seed, the Tri-1 and Gli-B1 bands would be either both present or both absent. However, these bands segregated independently in both cases, and the tracks corresponding to BC_1 progeny where Tri-1 is present without Gli-B1 are marked with an asterisk in Figure 4.4. From the negative evidence obtained with 1BS, and since *ph1b* homozygosity is expected to induce homoeologous translocations, it was thought likely that plants 82-177 and 82-178 contained 1AS-1DS exchanges. This conclusion was supported by the observation that certain derivatives of these two lines lacked Tri-3, coded for by *Tri-A1*, and when seeds without this protein were subjected to 2D SDS-PAGE they were found to still possess the LMW glutelin subunits controlled by *Glu-A3*. This suggests that *Tri-A1* had been replaced by *Tri-D1* in these two lines. Since the map distance between *Tri-1* and *Gli-1* is similar on chromosomes 1A and 1D (Singh, 1985), a substantial length of chromatin is available on chromosome arm 1AS for such homoeologous exchanges to occur.

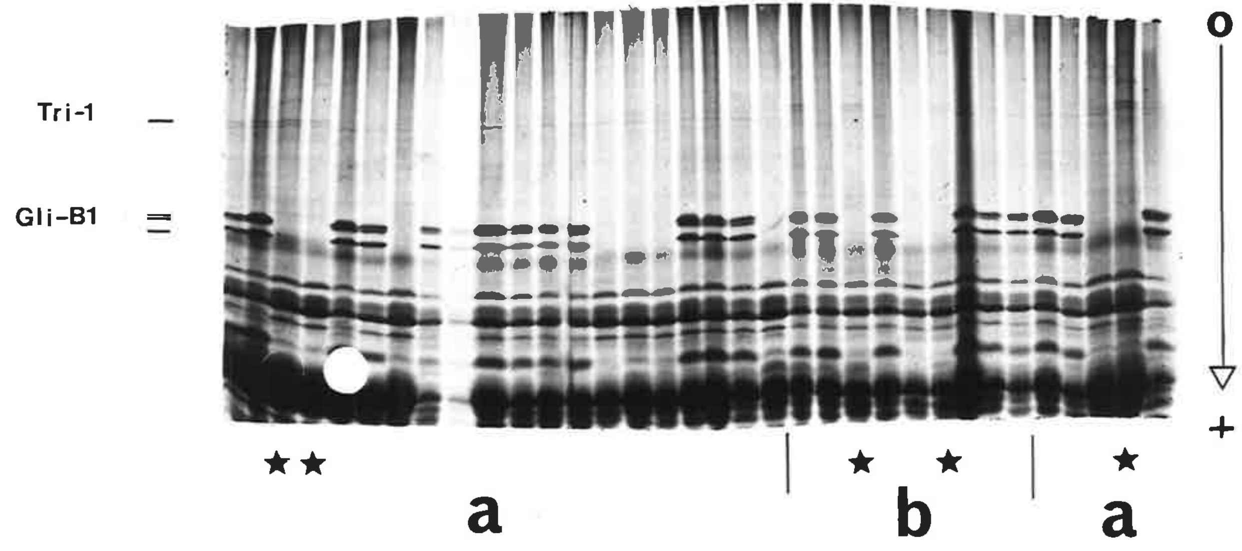

Tri-1
Gli-B1
o
+
a
b
a

Figure 4.4

Endosperm storage protein phenotypes of BC1 progeny from the two crosses: recombinant 82-177 and 82-178 x (double translocation 1BL-1RS/1DL-1RS)[2]. Patterns obtained by unreduced SDS-PAGE.

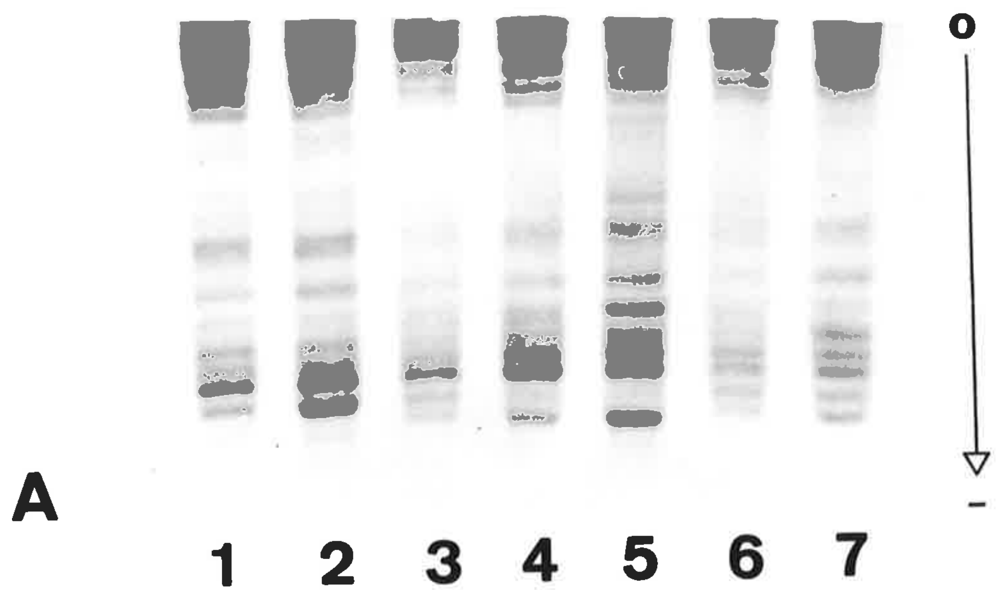
Asterisks mark critical BC1 progeny from (a) 82-178 and (b) 82-177 with phenotype Tri-1^{+} Gli-B1^{-} (see text).

4.3.3. Use of acid PAGE to detect nullisomy for chromosome 5B

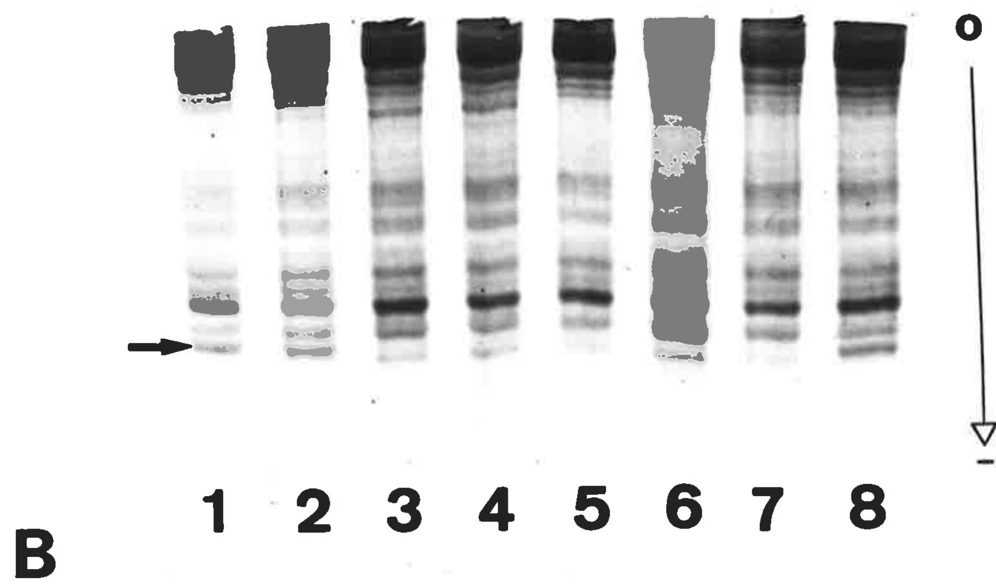
A urea soluble protein which migrates rapidly in the starch gels described by Shepherd (1968) can be shown by standard aneuploid analysis to be controlled by a gene on the long arm of chromosome 5B, while another protein of slightly lower mobility is controlled by a gene on chromosome 5DL (Shepherd, unpubl.). Similar patterns are obtained on polyacrylamide gels (for methods, see Chapter 3.1.3). The banding patterns of cv. Chinese Spring aneuploids of homoeologous group 5 obtained in acid PAGE are shown in Figure 4.5A. The absence of a particular band in genotypes nullisomic for 5B (tracks 1, 2) and of a different band in 5D nullisomics (tracks 4, 5), and the reinforcement of these bands in tetrasomic 5B (track 5) and tetrasomic 5D (track 2) respectively, support the conclusion that structural genes controlling these proteins are located on these two chromosomes. The pattern shown by the two respective long arm ditelosomic lines (tracks 3, 6) resembles that of euploid Chinese Spring (track 7) and hence these genes must be present on the long arms of chromosomes 5B and 5D. The pattern obtained from seed from the *ph1b* mutant (not shown) is indistinguishable from that of euploid wheat. The absence of chromosome 5A has no observable effect on the banding pattern in these gels. Two dimensional electrophoretic separation of salt-soluble proteins from wheat endosperm has revealed a protein species controlled by a gene on chromosome 5B (Fra-Mon *et al.*, 1984), but as no 5D effect was reported, it is uncertain whether this protein is identical to the one identified on acid PAGE described above.

4.3.4. Identification of 1D/1DL-1RS heterozygotes nullisomic for 5B and screening for allosyndetic recombination in their progeny

Twenty-seven F_1 seeds were produced from the cross (mono 5B x 1DL-1RS) x N5BT5A or x N5BT5D. These crosses were difficult to make as the nullisomic 5B male parent



o
A
-
1 2 3 4 5 6 7



o
-
1 2 3 4 5 6 7 8
B

Figure 4.5

Urea soluble endosperm protein phenotypes of euploid Chinese Spring (CS) and critical homoeologous group 5 aneuploids. Patterns obtained by acid PAGE.

A. Homoeologous group 5 aneuploids and euploid CS.

1. N5BT5A 2. N5BT5D 3. Dit 5BL 4. N5DT5A 5. N5DT5B 6. Dit 5DL

7. Euploid CS

B. Parents, control, and progeny from the F_2 of the cross (mono 5B x translocation 1DL-1RS) x N5BT5A.

Parents - 1. Euploid CS 5. N5BT5A

Control - 6. N5BT5D

Progeny - 2, 3, 4, 7, 8

Samples 3-7 do not carry the chromosome 5B-encoded marker (arrowed).

produced little pollen. Analysis of these hybrids by SDS-PAGE revealed that 11 carried both Gli-D1 and Sec-1 and these were subjected to acid PAGE to select individuals which had not received chromosome 5B from the female parent. Six putative nullisomic 5B seeds were identified by this means (Figure 4.5B), and the corresponding embryo halves were grown out in pots in the glasshouse and the plants allowed to self-pollinate. One of these plants (83-39) set much more seed than its five sibs and a spike on a late tiller was crossed to *Ae. variabilis* in order to confirm whether the plant was truly nullisomic for chromosome 5B. High levels of allosyndetic pairing occur in nulli-5B hybrids of wheat x *Ae. variabilis* whereas very little pairing results when *Ph1* is present (Driscoll, 1968). While the pmcs from two of the resultant interspecific hybrids showed allosyndetic pairing at meiotic metaphase I, one plant had pmcs which showed mainly univalents with an occasional rod bivalent and this parent plant was inferred to have been monosomic rather than nullisomic for chromosome 5B; its progeny was therefore used as a control population for assessing the effectiveness of nullisomy for 5B in inducing allosyndetic recombination. A few hybrid seeds from the cross to *Ae. variabilis* were obtained from the other five putative nullisomic 5B selections, and these all showed allosyndetic pairing at meiotic metaphase I; however since the expected transmission of a 5B monosome through the female gamete is approximately only 30% (Tsunewaki, 1963), many such hybrids need to be tested to differentiate between a nulli- and monosomic 5B plant, and it was not possible to produce sufficient of these hybrids without severely reducing the number of progeny needed for screening for allosyndetic recombination. The five sib plants of plant 83-39 were of moderate to very poor self-fertility. While *de novo* nullisomic 5B plants have been reported to have zero self-fertility (Riley and Chapman, 1958a), extra dosage of homoeologues is known to partially restore self-fertility (Sears, 1966); thus the observed variation in self-fertility of the five nullisomic 5B plants (Table 4.4) might be due to differences in dosage of 5B homoeologues present in these plants.

The five presumed nullisomic 5B plants produced a total of 544 progeny, while the control plant produced in excess of 300 seeds and 227 of these were analysed. The

SDS-PAGE phenotypes (Figure 4.1B) of all the control progeny tested and all but 13 of the nullisomic 5B progeny were scorable; these 13 seeds were shrivelled and gave abnormal banding patterns. Six different phenotypes were observed among the 531 seeds derived from presumed nullisomic 5B parents, while only three of these phenotypes were recovered in the control population, as shown in Table 4.4.

Table 4.4. Endosperm protein phenotypes and their frequency in progeny from 1D/1DL-1RS heterozygotes presumed nullisomic for 5B (pooled data of five plants) and presumed monosomic for 5B (one plant).

Dosage of 5B in parent	Endosperm protein phenotype						
	Tri-1 + Gli-D1 + Sec-1 -	+ + +	- - +	+ - +	- + +	- - -	Total
nullisomic	162	249	105	10	4	1	531
monosomic	67	113	47	0	0	0	227

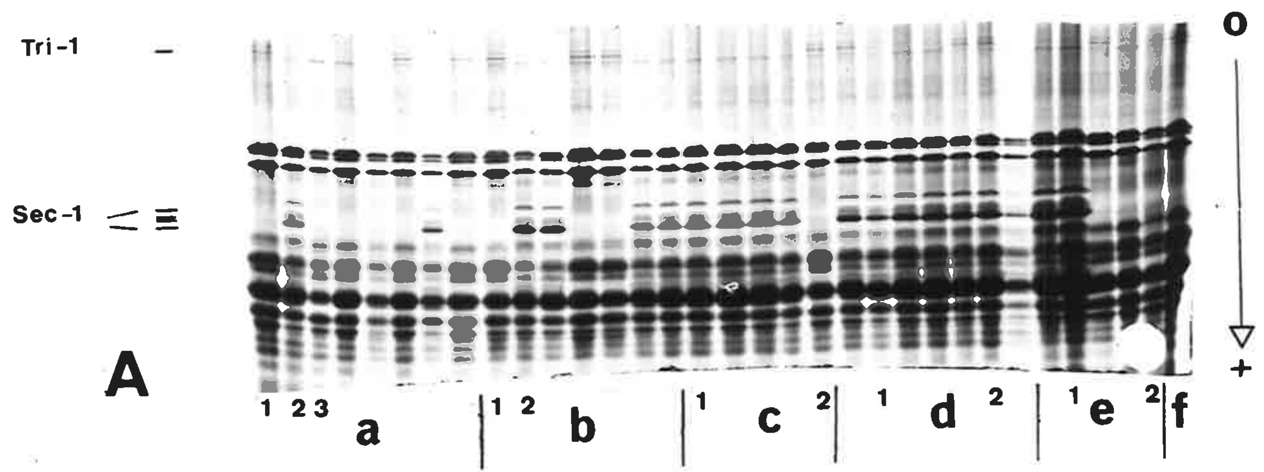
+: protein present -: protein absent

In order to assess whether the phenotypic segregations of the five presumed nullisomic 5B derived progeny were statistically homogeneous, the three rare classes present only in these families ('+-+", "-++", and "---"), were pooled. There was no evidence of heterogeneity between the five families (χ^2= 16.03, 12 d.f. 0.1<p<0.2). Inferring the chromosome constitution of the progeny within the control population from their SDS-PAGE phenotype, a segregation pattern of 1.42: 2.40: 1 for normal 1D homozygote : 1D / 1DL-1RS heterozygote : 1DL-1RS homozygote was observed. Since the female transmission of both normal 1D and translocation 1DL-1RS is approximately 0.5 (Table 4.1, section 4.3.1), then the departure from a 1:2:1 segregation ratio in the F_2 must be due to differential male transmission of the two chromosomes. Given equal transmission through the female gamete, the relative male transmission frequencies can be calculated to be 0.61 and 0.39 for 1D and 1DL-1RS, respectively. The higher male transmission of a

gamete bearing 1D is probably due to the greater vigour of pollen carrying a normal 1D over that bearing the translocation chromosome.

The three storage protein phenotypes recovered in the control population correspond to the two parental homozygotes and the heterozygote between them, and thus no recombinant for the 1DS chromosome was detected in the progeny of a plant possessing chromosome 5B. Fifteen non-parental progeny arose from the nullisomic 5B parents. One of these lacked markers for either 1DS or 1RS ("---") and it was considered to be hypoploid rather than recombinant. Although aneuploid female gametes were frequently detected in the test-cross experiment (Table 4.1), functional aneuploid male gametes are expected to be infrequent, so that recovery of nullisomic progeny is rare.

Of the remaining 14 non-parental progeny, the embryos of two (one "+-+", one "-++") failed to germinate and so their identity could not be verified by progeny testing; however the others were grown and gave progeny either by self-fertilisation, or by pollination with Dit 1DL. The progeny were analysed by SDS-PAGE to confirm the identification of the recombinants and to elucidate their gametic constitutions. The protein phenotypes of progeny from the crosses of 11 of the presumed recombinants x Dit1DL and the progeny by self-fertilisation of two of these are shown in Figure 4.6, and the phenotypic classes obtained in these progeny tests from all 12 recombinants are given in Table 4.5. The nature of these recombinants with respect to 1DS and 1RS markers could be deduced from the progeny tests. A number of the recombinants gave rise to "+--" progeny similar to the three plants 82-177, -178 and -179 recovered in the *ph1bph1b* experiment, and these were therefore assigned a recombinant type I classification. Two of the three "-++" selections (I-45 and I-66) carry a chromosome in which *Sec-1* and *Gli-D1* have become associated, as in plant 82-180, and these are thus type II recombinants. There were two recombinant types which did not arise in the *ph1bph1b* experiment: in plant VI-59 *Gli-D1* is no longer associated with *Tri-D1* (type III), but unlike the type II recombinant, *Sec-1* is not present, and the remaining selection (I-93) carries a chromosome in which *Sec-1* and *Tri-D1* are associated, but *Gli-D1* is absent (type IV).


Tri-1
Sec-1
A
o
+
1 2 3 a
1 2 b
1 c 2
1 d 2
1 e 2 f

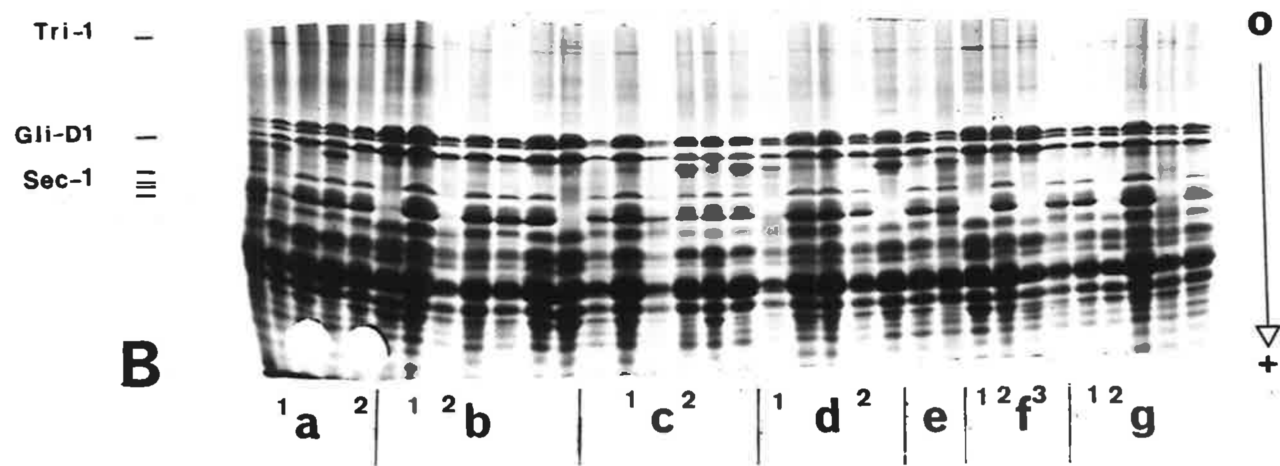

Tri-1
Gli-D1
Sec-1
B
o
+
1 a 2
1 2 b
1 c 2
1 d 2
e
1 2 f 3
1 2 g

Figure 4.6

Gametic analysis of presumptive recombinants selected among the progeny of 1D/1DL-1RS heterozygotes, nullisomic for 5B. Samples shown represent progeny from the test cross: presumptive recombinant x Dit 1DL, or from self-fertilisation of the presumptive recombinant. Patterns obtained by unreduced SDS-PAGE.

A

	plant no.	
a	VII-36 *	1. Tri-1^{+} Gli-D1^{-} Sec-1^{-} (R) 2. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 3. Tri-1^{-} Gli-D1^{-} Sec-1^{-} (H)
b	VII-53 *	1. Tri-1^{+} Gli-D1^{-} Sec-1^{-} (R) 2. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T)
c	VII-98 *	1. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 2. Tri-1^{+} Gli-D1^{-} Sec-1^{-} (R)
d	I-93 *	1. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 2. Tri-1^{+} Gli-D1^{-} Sec-1^{+} (R)
e	IV-14 *	1. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 2. Tri-1^{+} Gli-D1^{-} Sec-1^{-} (R)
f	II-83 *	

B

a	I-66 †	1. Tri-1^{-} Gli-D1^{+} Sec-1^{+} (R) 2. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T)
b	III-95 *	1. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 2. Tri-1^{+} Gli-D1^{-} Sec-1^{-} (R)
c	I-45 *	1. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 2. Tri-1^{-} Gli-D1^{+} Sec-1^{+} (R)
d	VI-59 *	1. Tri-1^{-} Gli-D1^{+} Sec-1^{-} (R) 2. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T)
e	I-66 *	
f	III-94 *	1. Tri-1^{-} Gli-D1^{-} Sec-1^{-} (H) 2. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 3. Tri-1^{+} Gli-D1^{-} Sec-1^{-} (R)
g	II-83 *	1. Tri-1^{-} Gli-D1^{-} Sec-1^{+} (T) 2. Tri-1^{+} Gli-D1^{-} Sec-1^{-} (R)

* test-cross progeny † progeny from self-fertilisation

R = recombinant T = translocation 1DL-1RS H = hypoploid

Table 4.5. Endosperm protein phenotypes and their frequency obtained in the progeny of (a) the test cross: presumed recombinant x Dit 1DL, and (b) from self-fertilisation of two presumed recombinants.

	Endosperm protein phenotype						
	Tri-1	-	-	-	-	+	+	
Recombinant	Gli-D1	+	+	-	-	-	-	
plant no.	Sec-1	+	-	-	+	-	+	Recombinant type *
(a) Test-cross progeny:								
II-83					3	3		I
III-94				1	2	1		I
III-95					3	3	1	I
IV-14				1	2	2		I
VII-36				3	2	3		I
VII-53					4	3		I
VII-98					4	1		I
I-45		2			4			II
I-66		1			1			II
VI-59			2		3			III
I-93				1	7		7	IV
(b) 'Selfed' progeny:								
III-1						2	4	I
I-66		3			2			II

* see Table 4.2

+: protein present -: protein absent

A total of 528 of the progeny derived from nullisomic 5B parents and 221 of the control progeny could be germinated and these were inoculated with stem rust. The reaction to stem rust followed the same pattern as had been obtained in the test-cross experiment - that is, resistance to the fungus was displayed only by those individuals whose endosperm carried Sec-1. Thus in no case did 5B nullisomy induce a break in the linkage between *Sec-1* and *SrR*.

The Per and Gpi phenotypes of the 12 recombinant lines listed in Table 4.5 were ascertained (Figures 4.7, 4.8) and these, along with the storage protein phenotypes and stem rust reaction were used to infer genotype at the relevant loci (Table 4.6). Three further recombinant types were identified in addition to those described in Table 4.2. All

type I recombinants possessed *Gpi-D1* and were thus classified as type Ia. Plant I-66 differed from the type II recombinants 82-180 and I-45 in possessing *Gpi-D1* and hence is designated as type IIb, while 82-180 and I-45 are designated as type IIa. As the rye Gpi band R1 migrates to a similar position on the gel as wheat band W2 (Figure 4.8), it is not possible to ascertain whether plant I-66 carries *Gpi-R1* as well as *Gpi-D1*. Of the 16 recombinants identified in the two experiments, only four (plants I-45, I-66, I-93 and 82-180) definitely involved homoeologous recombination of rye with wheat chromatin, while the remainder probably resulted from wheat-wheat homoeologous pairing.

Table 4.6. Genotype at marker loci on 1DS and 1RS of recombinant lines induced by nullisomy for 5B.

Plant	Marker loci on recombinant chromosome							Recombinant type
	Tri-D1	*Gli-D1*	*Gpi-D1a*	*Per-D1*	*Sec-1*	*Gpi-R1*	*SrR*	
I-45	-	+	-	-	+	+	+	IIa
I-66	-	+	+	-	+	+?	+	IIb
I-93	+	-	-	+	+	+	+	IV
II-83	+	-	+	+	-	-	-	Ia
III-1	+	-	+	+	-	-	-	Ia
III-94	+	-	+	+	-	-	-	Ia
III-95	+	-	+	+	-	-	-	Ia
IV-14	+	-	+	+	-	-	-	Ia
VI-59	-	+	+	-	-	-	-	III
VII-36	+	-	+	+	-	-	-	Ia
VII-53	+	-	+	+	-	-	-	Ia
VII-98	+	-	+	+	-	-	-	Ia

+: marker present -: marker absent

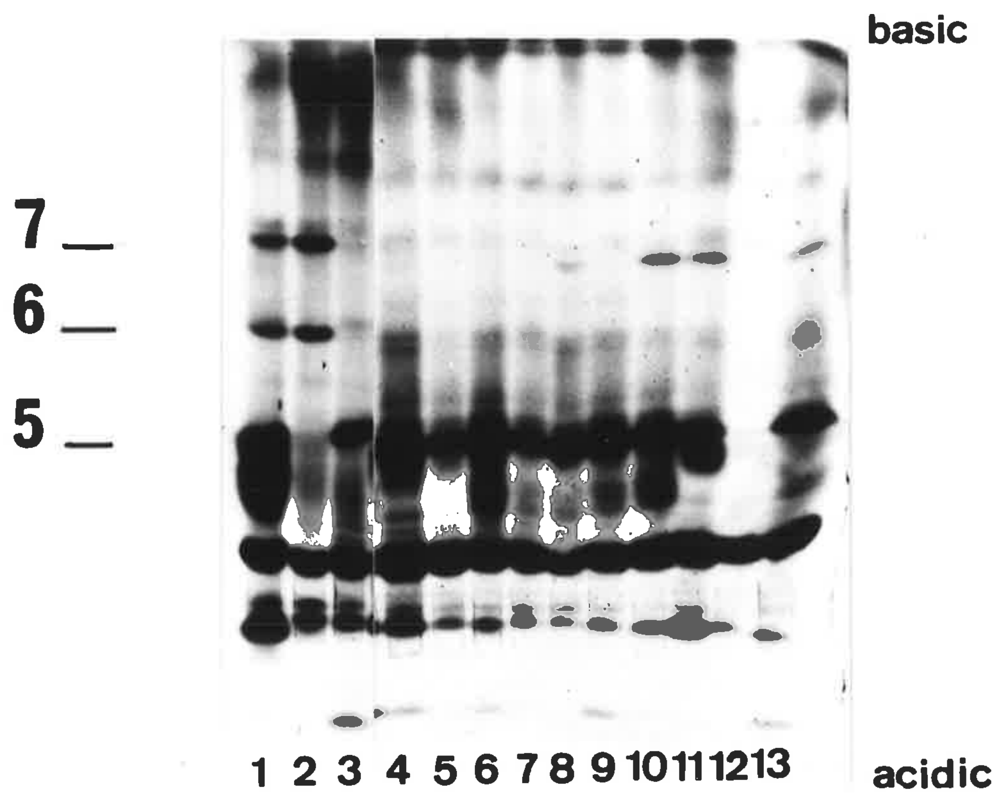

basic
7
6
5
1 2 3 4 5 6 7 8 9 10 11 12 13
acidic

Figure 4.7

Leaf peroxidase zymogram of euploid wheat, selected group 1 aneuploids and recombinants. Band numbers indicated follow Ainsworth *et al.* (1984).

Parents and Chinese Spring (CS) aneuploids:

1., 13. Euploid CS (bands 5, 6, 7) 2. Dit 1BL (bands 6,7) 3. Dit 1DL (band 5)

4. Translocation 1DL-1RS (bands 5, 6?) 12. Double translocation 1BL-1RS/1DL-1RS (-)

Recombinants:

5. 82-180 (bands 5, 6?) 6. I-45 (bands 5, 6?) 7. I-66 (bands 5, 6?)

8. I-93 (bands 5, 6?,7) 9. VI-59 (bands 5, 6?) 10. 82-177 (bands 5, 6?, 7)

11. 82-179 (bands 5, 6?, 7)

Other recombinants (see text Tables 4.2, 4.6) had similar phenotype to lanes 10, 11.

Band 7 used to score for presence of *Per-D1* .

Samples focussed on LKB Pag plates, pH 3.5-9.5

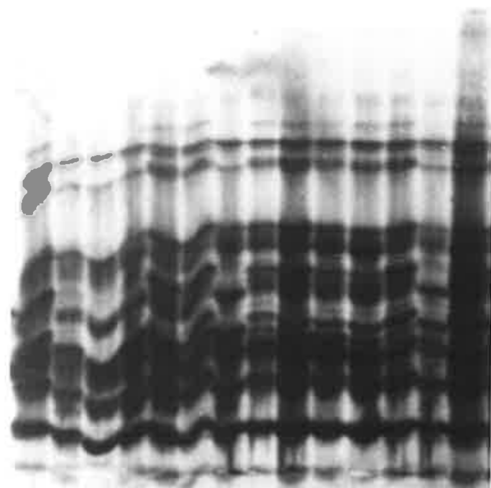

acidic
basic
1 2 3 4 5 6 7 8 9 10 11 12 13 14
A
W1
W2
W3
a
R1
b
B

Figure 4.8

Glucose phosphate isomerase zymogram of parents and selected recombinants.

A. IEF gel

Parents - 1., 6., 12. Euploid Chinese Spring; 2., 13. Translocation 1DL-1RS

Recombinants - 3. 82-180 4. I-45 5. I-66 7. I-93 8. VI-59 9. VII-36 10. VII-53
11. VII-98 14. IV-14.

B. Interpretation of the banding pattern in the central portion of the gel in A of (a) Euploid Chinese Spring, and (b) translocation 1DL-1RS. Major wheat bands denoted by W1, W2, W3; major rye band by R1. Bands W1, W2, W3 controlled by *Gpi-D1*. Band R1 controlled by *Gpi-R1*.

All other recombinants except 82-178 had the same pattern as lanes 8 - 12 in A. Phenotype of 82-178 resembles that of Dit 1DL (missing bands W1, W2, W3 and R1).

Patterns obtained by IEF on gels with pH gradient flattened around pH7.

4.3.5. Identification of 1B/1BL-1RS heterozygotes nullisomic for 5B and screening their progeny for recombinants

Four plants heterozygous 1B/1BL-1RS and nullisomic for 5B were selected from the cross (mono 5B x 1BL-1RS) x N5BT5A in the same manner as for 1DL-1RS (section 4.3.4), except that *Gli-B1* was used as the marker for 1BS. The embryo halves of these plants were planted in pots in the glasshouse, grown and allowed to self-fertilise. The progeny seeds were analysed by SDS-PAGE for the presence of Gli-B1 and Sec-1 (Figure 4.9). There is no equivalent protein controlled by a gene on chromosome arm 1BS to that coded by *Tri-D1* and *Tri-A1* on chromosome arms 1DS and 1AS, respectively (Singh and Shepherd, 1985). A total of 647 progeny were obtained and their SDS-PAGE phenotypes are listed in Table 4.7. The families were found to exhibit significant heterogeneity for the

Table 4.7. Endosperm protein phenotypes and their frequency in progeny from 1B/1BL-1RS heterozygotes, presumed nullisomic for 5B.

	Endosperm protein phenotype				
Family no.	Gli-B1 + Sec-1 -	+ +	- +	- -	Total
84-128-1	54	128	52	0	234
84-129-1	8	9	5	0	22
84-129-2	69	82	19	1	171
84-130-1	58	116	46	0	220
Total	189	335	122	1	647

+: protein absent -: protein absent

frequency of their endosperm protein phenotypes (χ^2= 19.34, 6 d.f. p<0.01). Although pooling of the data across the four families is therefore not justified, it appears that once again there is some selection against gametes carrying the translocation chromosome. From the evidence of Singh (1985) that female transmission of the 1BL-1RS translocation

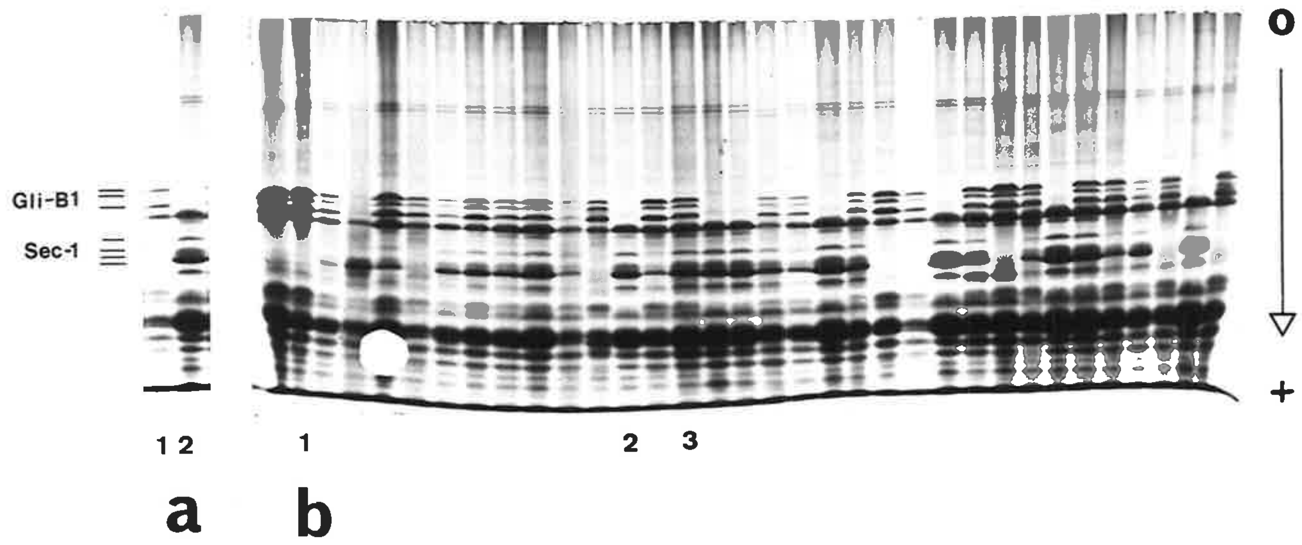

Gli-B1
Sec-1
o
+
1 2
1
2
3
a
b

Figure 4.9

Endosperm storage protein phenotypes of parents and progeny segregating for loci on chromosome arms 1BS and 1RS. Progeny from the F_2 of the cross (mono 5B x translocation 1BL-1RS) x N5BT5A. Parent plant selected to be a 1B/1BL-1RS heterozygote, nullisomic for chromosome 5B. Patterns obtained by unreduced SDS-PAGE.

a - parental phenotypes: 1. Euploid CS (Gli-B1$^+$ Sec-1$^-$)

2. Translocation 1BL-1RS (Gli-B1$^-$ Sec-1$^+$)

b - progeny phenotypes: 1, 2, 3 are examples of the three major phenotypes recovered

1. Gli-B1$^+$ Sec-1$^-$ (disomic 1B entire) 2. Gli-B1$^-$ Sec-1$^+$ (translocation homozygote)
3. Gli-B1$^+$ Sec-1$^+$ (translocation heterozygote).

chromosome is normal, pollen bearing this translocation must suffer a competitive disadvantage. The three major phenotypic classes of the progeny (Table 4.7) were assumed to be composed predominantly of normal 1B homozygotes ("+-"), 1B / 1BL-1RS heterozygotes ("++") and 1BL-1RS homozygotes ("-+"), while the single "--" individual from 83-129-2 was thought to have been a rare nullisomic progeny.

Forty of the 647 progeny seeds tested by SDS-PAGE failed either to germinate or to produce a vigorous seedling and the 607 resultant plants were inoculated with stem rust. All but four of these showed complete linkage between the presence of the Sec-1 bands and resistance to stem rust. The endosperm of two progeny seeds (C-89, D-94) lacked the Sec-1 proteins, but the resultant seedlings were resistant to stem rust, while plants F-39 and C-62 possessed Sec-1 in their endosperm, but were susceptible to stem rust.

All four putative recombinants were progeny tested to confirm their phenotypic classification. Six progeny from each plant were tested by SDS-PAGE for the presence of Sec-1. Where self-fertility allowed the use of seed derived from self-fertilisation, this procedure tested 12 gametes (F-39, C-62 and D-94); plant C-89 produced only one such progeny and five seeds produced by pollination with a rust susceptible euploid cultivar were tested in addition (total of 7 gametes). None of the progeny of C-89 or D-94 carried Sec-1. If either parent carried a single dose of *Sec-1* , then, assuming equal transmission of the chromosome carrying this gene and its homologue, the probabilities of obtaining these results by chance were $(0.5)^7$ (= 0.008) and $(0.5)^{12}$ (= 0.0002), respectively. Thus, in these two plants the linkage between *Sec-1* and *SrR* appeared to have been broken. Out of the six progeny tested from the stem rust susceptible plant C-62, three possessed Sec-1, and therefore this plant also contains a wheat-rye recombinant chromosome. All progeny tested from the other susceptible plant (F-39) lacked Sec-1; in this case either the parent had been wrongly classified for protein phenotype or the chromatin bearing the rye protein gene had not been transmitted to the progeny. Steinitz-Sears (1966) has observed that a telosome known to be present in root tip meristematic cells was not seen in the meiocytes and had therefore been lost in somatic division.

4.4. Discussion

It has long been known that the use of F2 progeny is more efficient than test-cross progeny in the estimation of linkage between two or more tightly linked loci, although the efficiency of F2 mapping declines as the loci become more loosely linked (Mather, 1938). *Tri-D1* and *Gli-D1* on chromosome arm 1DS are genetically separated by a map distance of 45.5 cM (Koebner, unpubl.) and the extent of the linkage between *Sec-1* and *SrR* on 1RS was unknown at the outset of these experiments, but it was realized that, in the absence of homologous partners, crossing-over would be rare and hence all loci on a single chromosome would behave as if tightly linked, irrespective of their relative map location. Thus the use of self-fertilisation to generate the material to be used for screening of wheat-rye recombination was theoretically preferable, as well as having the practical advantage of avoiding the necessity of emasculation and hand-pollination. Self-pollination, given adequate male fertility, also gives more seeds per spike than hand-pollination, as the latter technique necessitates removal of the lowest and the highest few spikelets on each spike, together with the tertiary and higher florets in the remaining spikelets.

Populations derived through self-fertilisation are most efficient in the detection of recombination when the alleles or characters to be scored are codominant, as each individual progeny carries two independent gametes which can each be characterised (see Chapter 5); in contrast the test-cross procedure only allows one (female) gamete from the critical parent to be analysed. However, when the characters are fully dominant, some recombinant progeny will not be detected. For example, in the nullisomic 5B experiment involving the 1DL-1RS translocation, recombinants of chromosome 1D were selected on the basis of a break in the linkage between *Tri-D1* and *Gli-D1*, but recombinant gametes could not be identified when a complete chromosome 1D was transmitted through the other gamete. Similarly, an individual composed of a normal 1BL-1RS gamete and a

recombined gamete having *Sec-1* but not *SrR* (or vice versa) will be wrongly classified as a parental translocation homozygote. However all of those recombinant individuals derived from the union of a Sec-1^+ SrR^- (or vice versa) gamete with one carrying a normal chromosome 1B will be identified in these populations. Individuals with one dose of chromosome 1B represent 50% of the progeny if the normal and translocated chromosomes are transmitted equally; but as the translocation chromosome has been shown to suffer a reduction in gametic transmission (see section 4.3.5), more than half the population is of the 'correct' type in this case.

Analysis of the interaction between *Ph1* and the pairing promoter gene(s) on the opposite arm of 5B led Riley and Chapman (1967) to conclude that maximal homoeologous pairing would be achieved when *Ph1* was removed or suppressed while the 5BS pairing promotion activity was left unimpaired. It would therefore be predicted that the frequency of recovery of recombinants would be higher when the *ph1b* mutant is used to induce allosyndesis rather than nullisomy for 5B. In the present study, allosyndetic recombination in the interval *Tri-D1* to *Gli-D1* among the test-cross progeny from the *ph1b* homozygote was measured to be four gametes out of 394, or a gametic recombination frequency of 1.0%. To obtain an estimate of this recombination rate from the nullisomic 5B experiment, it is assumed that the female transmission of the 1DL-1RS chromosome is 0.5, so that the male transmission of this chromosome is 0.39 (see section 4.3.4). Since a recombination in the interval *Tri-D1* - *Gli-D1* cannot be detected when the recombinant gamete unites with a gamete carrying an entire chromosome 1D, fusion with a translocation gamete is necessary to recover a recombinant. If the recombinant chromosome suffers no selective disadvantage in either mega- or microsporogenesis, and recombination occurs with a probability p in either gamete, then the expected relative frequency of recombinant progeny is given by $(0.5 + 0.39)p$. This can be equated to the observed relative frequency of $14/531 = 0.026$, giving a value for p of 2.9%, or almost three times that achieved in the *ph1bph1b* test-cross experiment. It appears that the use of nullisomy for 5B significantly increases the rate of allosyndetic recombination over that

achieved by *ph1b* , but it is not possible to directly compare the recombination rates derived from the two experiments in order to assess the relative efficacy of *ph1b* and nullisomic 5B in inducing allosyndetic recombination, as the experimental material was grown at different times, and different methods of obtaining the progeny were employed. The use of 5B nullisomy has the further advantage that it is relatively easy to identify those critical individuals which lack chromosome 5B, while the identification of *ph1b* homozygotes by meiotic chromosome configuration is prone to error, and test crosses to *Ae. variabilis* need to be made for verification (Chapter 5). The major disadvantage of the nullisomic 5B method lies in the poor fertility of some of these plants; however the problem of low seed yield can be overcome by simply selecting a greater number of nullisomic 5B parents.

The efficiency with which recombinants are detected relies on the type, number and spread of the genetic markers available for the chromosome segment to be recombined. At present there are few suitable markers in wheat and rye. A leaf peroxidase gene is reported to be present on 1RS (Ainsworth *et al.*, 1984; Schmidt *et al.*, 1984; Vahl and Müller, 1984) but it could not be identified in the present material, possibly because the genotype of rye used to produce the translocation had a different allele from that present in the rye genotypes used by these workers; Imperial rye may possess an allele which codes for an isozyme of similar isoelectric point to one of those in wheat. No non-wheat band is visible in the peroxidase phenotype of the double translocation line 1BL-1RS/1DL-1RS, despite the presence of four doses of the rye arm and the loss of all the enzyme activity associated with 1BS and 1DS (Figure 4.7, track 12). A series of hexokinase (E.C. 2.7.7.1) genes has been reported by Ainsworth (1983) to be located on homoeologous group 1 chromosomes, but no rye isozyme was identified in his study. Recently, a structural gene coding for a grain protease inhibitor was located on chromosome 1R, but arm location was not assigned (Hejgaard *et al.*, 1984); whether this gene is present in the wheat-rye recombinant lines has not yet been determined. No other genes controlling biochemical characters have been located on group 1 chromosomes, and other physiological and

morphological genes listed by McIntosh (1983) have no known homoeoloci in rye.

The short arm of 'Imperial' rye chromosome 1R is reported to be cytologically marked by a satellite (Sybenga, 1983), but the satellite is fused with the rest of the arm in hexaploid triticale (Merker, 1973) and is also not visible in Feulgen stained somatic chromosome spreads of the 'Veery' 1BL-1RS translocation (Merker, 1982). Two heterochromatic bands, one telomeric, the other slightly proximal to the telomere in the nucleolar organising region can be seen in Giemsa C-banded karyotypes of the 'Imperial' rye 1R addition line to wheat cv. Chinese Spring (Darvey and Gustafson, 1975) and in 1BL-1RS translocation lines (Bennett and Smith, 1975; Merker, 1982). However both Feulgen staining and C-banding of the 1DL-1RS translocation line failed to reveal the 1RS telomere although a repeated DNA sequence diagnostic for this telomere is still present in this line (Appels, pers. comm.). The lack of telomeric heterochromatin may represent another example of the modification of rye chromosomes in wheat backgrounds, already noted in addition lines (Singh and Röbbelen, 1976) and in triticale (Merker, 1975). The telomere of chromosome arm 1RL was deleted following tissue culture (Lapitan *et al.*, 1984) and also was spontaneously lost in a normal plant as reported elsewhere in this thesis (see Chapter 5), but loss of the short arm telomere has not previously been reported. Recently molecular probes have been developed which hybridise to the rRNA gene complex in the nucleolar organising region on 1RS (Appels *et al.*, 1978; 1980), to the rye 5S RNA genes (Lawrence and Appels, 1985) and to a repeated sequence located in the heterochromatic regions of rye (Appels and Moran, 1984) and the four recombinant lines known to involve rye chromatin from the 1DL-1RS experiments (82-180, I-45, I-66 and I-93) are currently being studied to determine whether they still possess these segments of rye chromatin.

Recombination between *SrR* and *Sec-1* on chromosome arm 1RS was not obtained in either of the experiments involving the 1DL-1RS translocation, although as yet unconfirmed evidence suggests that this recombination had occurred in three out of 647 individuals in the case of 1BL-1RS. Linkage mapping of the 1DL-1RS chromosome has

indicated that the likely gene order along the rye arm is: centromere - *Sec-1* - *SrR* - telomere, and that *Sec-1* and *SrR* are tightly linked (Singh, 1985). Thus the probability of inducing an interstitial homoeologous crossover between these two rye loci is low.

Rye chromosome arm 1RS was recombined with a wheat chromosome four times in the 1DL-1RS experiments (82-180 : Table 4.2; I-45, I-66, I-93: Table 4.6). The gametic wheat-rye recombination frequency obtained in the *ph1bph1b* experiment can be calculated directly, as each test-cross gamete was analysed (1/394 = 0.3%). To obtain this frequency in the nullisomic 5B experiment, a similar calculation to the one described earlier for the *Tri-D1* - *Gli-D1* interval must be performed; thus, a recombinant could only be detected when fusion with a gamete bearing a normal chromosome 1D occurred, so that the expected frequency of recombinant progeny is given by (0.5 + 0.61)p which is equated to the observed relative frequency of 3/531 = 0.0056, giving a value for p of 0.5%.

In three of these wheat-rye recombinant lines, *Sec-1* has been transferred to the same chromosome as *Gli-D1* while *Tri-D1* has been lost, and in the fourth line *Sec-1* has become associated with *Tri-D1* with the loss of *Gli-D1*. Previously it was assumed that *Gli-D1* and *Sec-1* were homoeoloci on the basis that the proteins that they code for have similar physical properties and, more strikingly, as their N-terminal aminoacid sequences show substantial homology (Shewry *et al.*, 1984a). However, the present results are strong evidence that these prolamin genes are genetically located along their respective chromosomes at dissimilar positions, since, if these genes occur in strictly homoeologous regions, it should not be possible to recombine them in one chromosome. Data of Singh (1985), obtained from mapping of 1R in a wheat background, shows that *Sec-1* maps much closer to the centromere than does *Gli-D1*. Thus, although these loci are almost certainly homoeologous, map distances between two sets of homoeoloci may not be comparable. A similar lack of consistency in map distance between rye and wheat homoeoloci has been found for the *Glu-1* genes, in which the rye locus is more tightly linked to the centromere than the wheat loci (Payne *et al.*, 1982b; Singh and Shepherd, 1984). The structural gene *Gpi-R1* is located in the satellite of 1RS, interstitially between

Nor-R1 and *Sec-1* , with the map distance of *Sec-1 - Gpi-R1* estimated at 21.8 cM (Lawrence and Appels, 1985); from its storage protein phenotype, the type IIb recombinant (Table 4.6) appears to possess the proximal segment of 1RS, at least as far as the *Sec-1* locus, and would therefore also be expected to possess *Gpi-R1*. It is not possible, however, to visualise the product of this gene when *Gpi-D1* is present, using the electrophoretic techniques described. If this recombinant does possess both *Gpi-R1* and *Gpi-D1* on a single chromosome, this provides yet another example of different genetic locations of homoeoloci on related chromosomes.

The recombinant 1DS chromosomes produced in this study may provide further information on the genetic control of the dough quality problem associated with the 1DL-1RS translocation. Lines possessing the Tri-1^{+} Gli-D1^{-} Glu-D3^{-} recombinant chromosome in a suitable wheat background will demonstrate the effect on flour quality of the absence of this particular gliadin and low molecular weight glutelin subunit, and the single line with the Tri-D1^{-} Gli-D1^{+} Glu-D3^{+} recombinant chromosome will similarly provide information on the effect of the 'triplet' proteins Tri-1 and Tri-2 on quality. The wheat-rye recombinants can be used to test the suggestion of Zeller *et al.* (1982) that the gene product of *Sec-1* may be the cause of the sticky dough problem in 1BL-1RS lines. Since the Tri-1^{+} Gli-D1^{-} Sec-1^{+} SrR^{+} and Tri-1^{-} Gli-D1^{+} Sec-1^{+} SrR^{+} chromosomes share some rye chromatin, the rye segment can now be further shortened by homologous recombination according to the scheme suggested by Sears (1981). A genotype possessing all five loci should be obtainable; this chromosome will possess all the known 1DS seed storage protein genes, and may therefore be free of the dough quality problem associated with the 1DL-1RS translocation if the cause of the defect is loss of wheat genes rather than the acquisition of deleterious effects due to rye genes. If there are deleterious genes affecting dough quality on the full rye arm, it is possible that one or more of the four recombinant lines will have lost these genes and will not produce sticky dough.

The influence of the 1DL-1RS translocation on flour quality cannot be reliably measured in a Chinese Spring background, as this cultivar itself produces a dough which

is very weak (Shepherd, pers. comm.). The first wheat-rye recombinant isolated (82-180) was therefore crossed and backcrossed twice to cv. Gabo, chosen as the recurrent parent because it has been the breadmaking quality standard for Australian wheats. Lines homozygous for the recombinant chromosome, and sister lines homozygous for normal 1D and for the translocation 1DL-1RS were isolated and subjected to the SDS sedimentation test (Axford *et al.*, 1978) as an indicator of the effect of the recombination on dough quality. The results of this test were inconclusive, as there was little discrimination between the lines homozygous for 1D and those homozygous for 1DL-1RS, although these chromotypes are known to give very different flour qualities. Several factors were thought to have been responsible for this. The seed was multiplied out of season and variation in maturity times among the lines produced uneven ripening of seed. Also the high nitrogen status of the soil in which the plants were grown led to a high seed protein percentage (around 19%), which is beyond the range for using this test as a prediction of dough quality (Preston *et al.*, 1982). In addition, some of the lines were affected by sprouting which is known to substantially affect baking quality (Meredith and Pomeranz, 1985). The backcrossing programme is being continued with the first recombinant line and a similar procedure has been initiated with the three other wheat-rye recombinants.

The recombinant lines will also be useful in deducing gene order along 1DS, and the order *Tri-D1* - *Per-D1* - *Gpi-D1* - *Gli-D1* has already been obtained in this way. The order of the latter three loci agrees with that found on chromosome 1BS by Ainsworth *et al.* (1984), providing a further example of the conservation of gene synteny resulting from the assumed common ancestry of the individual genomes of wheat. To date the phenomenon of gene synteny conservation has relied purely on chromosome arm location, but this result extends the relationship further to gene order.

The two presumptive recombinants arising from 1BL-1RS, in which *SrR* has been separated from *Sec-1*, will require more extensive progeny testing to confirm that *SrR* has been transmitted to the progeny generation. They will be then be backcrossed to cv. Gabo

to assess whether the novel translocations have improved the quality characteristic of the parent translocation stock. If these lines produce flour of an adequate strength, then the rye gene for stem rust will be available for immediate inclusion into conventional wheat breeding programmes. An intercross between the two recombinant types (Sec-1^{-} Sr^{+} and Sec-1^{+} Sr^{-}) will enable the rye segment to be further shortened if the recombinant chromosomes carry any common rye chromatin, but genotypes deriving from homologous crossing-over within the common rye segment will not carry *SrR* , and thus will not be of direct use in wheat breeding at present.

Chapter 5: Wheat-rye recombination

II. The long arm of rye chromosome 1R

5.1. Introduction

The frequency with which rye chromatin can be recombined with that of wheat is low, as demonstrated in Chapter 4 of this thesis, but not zero as had been suggested by a number of previous workers (see Chapter 2.5). The ability to detect these rare recombinants depends on the availability of markers, and is optimised when these markers are as well-spread along the alien chromosome as possible. The earlier experiments aimed at recombining genes on the short of arm of 1R, described in Chapter 4, involved rye markers, which were later revealed to be tightly linked; furthermore, as discussed above, the use of dominant markers precluded complete analysis of the gametic constitution of every individual progeny, and it is therefore likely that some recombination events remained undetected.

To obtain further genetic evidence to confirm that the *ph1b* mutant can induce wheat-rye homoeologous recombination, and to permit a more meaningful estimate of the frequency of this induction, a segment of rye chromatin having well-spread, codominant markers was required. Rye chromosome 1RL fulfilled these criteria, as it carries an endosperm storage protein gene (*Glu-R1*) closely linked to the centromere (Singh and Shepherd, 1984) and is cytologically marked by a prominent heterochromatic telomere (Gill and Kimber, 1974a). It would have been possible to attempt to induce allosyndesis in a genotype containing the complete chromosome 1R by initially crossing a whole chromosome substitution line to the *ph1b* mutant. However, a translocation line involving 1RL and wheat chromosome 1DS was preferred, based on the notion that, in a plant heterozygous for this translocation, the normal pairing of the wheat chromatin common to the unchanged wheat short arm of the translocation chromosome and a complete chromosome 1D would bring the homoeologous arms 1RL and 1DL physically

together at zygotene/pachytene and thereby encourage synapsis of these two arms. The frequency of coalignment of the homoeologous chromosome arms at meiotic prophase I, brought about by using such a translocation heterozygote, was expected to be greater than that induced by the secondary association of homoeologous bivalents noted by Kempanna and Riley (1964) and recently confirmed by Yacobi *et al.* (1985).

There is some lack of consistency in gene nomenclature for the rye glutelin locus. Shewry *et al.* (1984b) refer to the gene as *Sec-3* , while Singh and Shepherd (1984), working with rye segments in a wheat background, prefer the terminology *Glu-R1* to retain consistency with the nomenclature of the wheat homoeoloci. This latter system has been chosen in this work for the same reason.

5.2. Plant materials and methods

5.2.1. Plant materials

a. Translocation line 1DS-1RL (Lawrence and Shepherd, 1981) in cv. Chinese Spring.

b. *ph1b* mutant (Sears, 1977) in cv. Chinese Spring; stock used derived from Accession 7876 of Dr. K.W. Shepherd.

c. An accession of *Ae. variabilis* (Ac. 7069) obtained from Dr. K.W. Shepherd.

5.2.2. Production of populations for screening of wheat-rye recombination

The long arms of chromosomes 1D and 1R carry the homoeoloci *Glu-D1* and *Glu-R1* which code for high molecular weight glutelin subunits (Lawrence and Shepherd, 1980; 1981). These genes are expressed codominantly and thus endosperm protein phenotypes obtained by reduced SDS-PAGE (Chapter 3.1.1) were used to infer the presence of normal 1D and 1DS-1RL translocation chromosomes in individual grains in the segregating F_2 progeny derived from the cross *ph1b* mutant x translocation 1DS-1RL homozygote. Individuals possessing both Glu-D1 and Glu-R1 were assumed on this basis to be translocation heterozygotes and 20 selected embryo halves with phenotype Glu-D1$^+$ Glu-R1$^+$ were grown in pots in the glasshouse. Their meiotic configurations, stained by the Feulgen technique, were analysed in pmcs at metaphase I in order to select those homozygous for *ph1b*. One spike of each plant suspected of being *ph1bph1b* was pollinated by *Ae. variabilis* , a species used by Sears (1977) to identify *ph1b* ; the remaining spikes on these plants were allowed to self-fertilise. Four progeny from the intergeneric cross wheat x *Ae. variabilis* from each putative *ph1bph1b* selection were grown and pmcs from the resulting hybrid plants were analysed for the presence of homoeologous pairing at metaphase I in order to confirm the constitution of the selections

at the *Ph1* locus. The two populations used as control progeny were derived by self-fertilisation of plants known to be of genetic constitution *Ph1bph1b*. One of these was the same F1 plant, from the cross *ph1b* mutant x translocation 1DS-1RL, which produced the progeny from which homozygous *ph1b* translocation heterozygotes were selected; while the other was a sib F1 plant.

5.2.3. Reduced SDS-PAGE phenotypes of parental lines

The wheat gene *Glu-D1* codes for two high molecular weight glutenin protein subunits which can be visualised on SDS-PAGE when the protein extracts are reduced by the addition of 1% 2-mercaptoethanol (Lawrence and Shepherd, 1980). Some polymorphism has been noted for this locus and in cv. Chinese Spring, the slower and the faster moving bands representing the two subunits obtained upon reduction of the gene product(s) of *Glu-D1* are numbered 2 and 12 respectively (Payne *et al.*, 1980; Payne and Lawrence, 1983). The long arm of rye chromosome 1R carries the gene *Glu-R1*, which codes for the high molecular weight glutelin subunits of rye (Lawrence and Shepherd, 1981). Upon reduction and separation by SDS-PAGE, the products of this gene are visualised as two major bands, one on either side of band 2 of wheat. These patterns are illustrated in Figure 5.4A.

5.2.4. Marker characters used in screening for wheat-rye recombination

Chromosome arm 1RL was marked proximally by the endosperm protein gene *Glu-R1* (Singh and Shepherd, 1984) and terminally by a prominent heterochromatic telomere (Gill and Kimber, 1974a). Chromosome arm 1DL was marked by the endosperm protein gene *Glu-D1*.

5.2.5. Recovery and verification of wheat-rye recombinants

Progeny derived by self-fertilisation of homozygous *ph1b* plants, selected in the F_2 generation of the cross *ph1b* mutant x translocation 1DS-1RL, represent individual F_3 populations, while the control populations were progeny of a *Ph1bph1b* parent, derived by self-fertilisation of the same, or sib, F_1 plants, and therefore represented F_2 populations. Although these two types of population are derived from different generations, as they are both the product of self-fertilisation of a translocation heterozygote, they are genetically equivalent. Thus, for simplicity, the *ph1bph1b*-derived population will hereafter be referred to as the T population, while the *Ph1bph1b*-derived population will be called the C population.

Individuals from the T and C populations were analysed for their glutelin subunit constitution by reduced SDS-PAGE, scoring for the presence of Glu-D1 and Glu-R1. Assignment of glutelin phenotype by reduced SDS-PAGE presented no difficulty (Figure 5.4A). The embryo halves were immediately germinated (Chapter 3.2.1) and Giemsa-stained preparations were obtained from a single root tip (Chapter 3.2.2) in order to ascertain the number of rye telomeres present in the seedling. The large heterochromatic dots in the Giemsa-stained interphase cells, which have been shown in *Allium cepa* to correspond to the C-bands in contracted mitotic metaphase chromosomes (Fussell, 1977), were each considered to represent a single telomere of 1RL. Wheat chromosomes have only weak C-bands (Gill and Kimber, 1974b) which therefore give rise to only small dots in interphase nuclei, many of which are visible in these interphase preparations. Hence the rye telomere content of individual progeny could be determined by counting the number of large Giemsa-stained dots in interphase cells obtained from the root-tip meristem. Many such cells were present in each preparation, and, except in a single case described below in section 5.3.3.3, each cell from a given root-tip displayed the same number of dots. At least five cells per root-tip were analysed for the assignment of rye telomere number.

The T and C populations segregated for both glutelin subunit constitution and rye telomere number. Seedlings were classified as parental if they retained the combination of glutelin and telomere number present in either a normal 1D homozygote (Glu-D1$^+$ Glu-R1$^-$, 0 rye telomere), a translocation heterozygote (Glu-D1$^+$ Glu-R1$^+$, 1 rye telomere) or a translocation homozygote (Glu-D1$^-$ Glu-R1$^+$, 2 rye telomeres); other phenotypic combinations were classified as non-parental. This classification ignores the two possibilities that the phenotypes classified as parental could be due either to a recombinant gamete occurring simultaneously in both male and female gametes to produce a double recombinant zygote, or to one gamete possessing a double homoeologous cross-over chromosome. However, both these possibilities were considered to be remote, given the low expected rate of homoeologous recombination between rye and wheat chromatin, and therefore the probable error in this classification was not likely to have been great.

Considering only the reference chromosome pair (normal 1D and translocation 1DS-1RL), four different euhaploid gametes are expected to be produced in the T population. Two of these are the parental types 1D entire and translocation 1DS-1RL, and the other two are recombined chromosomes involving replacement of either a distal or a proximal segment of 1RL by a segment of 1DL or some other wheat chromosome. Due to the high incidence of univalents in the meiocytes of these *ph1bph1b* plants, aneuhaploid gametes may also be produced. The phenotypes of the zygotic products of the likely combinations of these gametes are shown in Figure 5.1. Some fusion products are not included in this figure as the probability of obtaining a recombinant chromosome in both gametes simultaneously is expected to be negligible, and it is assumed that aneuhaploid gametes only function in the female gamete, as pollen certation tends to favour male gametes with the normal chromosome complement.

All seedlings having a non-parental phenotype were transplanted into pots in the glasshouse and were allowed to self-fertilise in order to give progeny for confirmatory testing. These progeny, where available, were analysed for glutelin subunit constitution and rye telomere number as above, and this test made it possible to distinguish which

♂ gamete

♀ gamete

1D (parental)	+ − 0 p	+ + 1 p	+ − 1 np	+ + 0 np
1DS-1RL (parental)	+ + 1 p	− + 2 p	+ + 2 np	− + 1 np
(recombinant)	+ − 1 np	+ + 2 np	*	
(recombinant)	+ + 0 np	− + 1 np		
	+ − 0 p	+ + 1 p	*	
	+ + 1 p	+ + 2 np		
	+ + 2 np	− + 3 np		
	+ − 0 p	− + 1 np		
iso 1RL	+ + 2 np	− + 3 np		

parental

recombinant

euploid* progeny

aneuploid* progeny

Figure 5.1. Gametic output of 1D/1DS-1RL heterozygote, *ph1bph1b* , and phenotypes of resulting progeny by self-fertilisation.

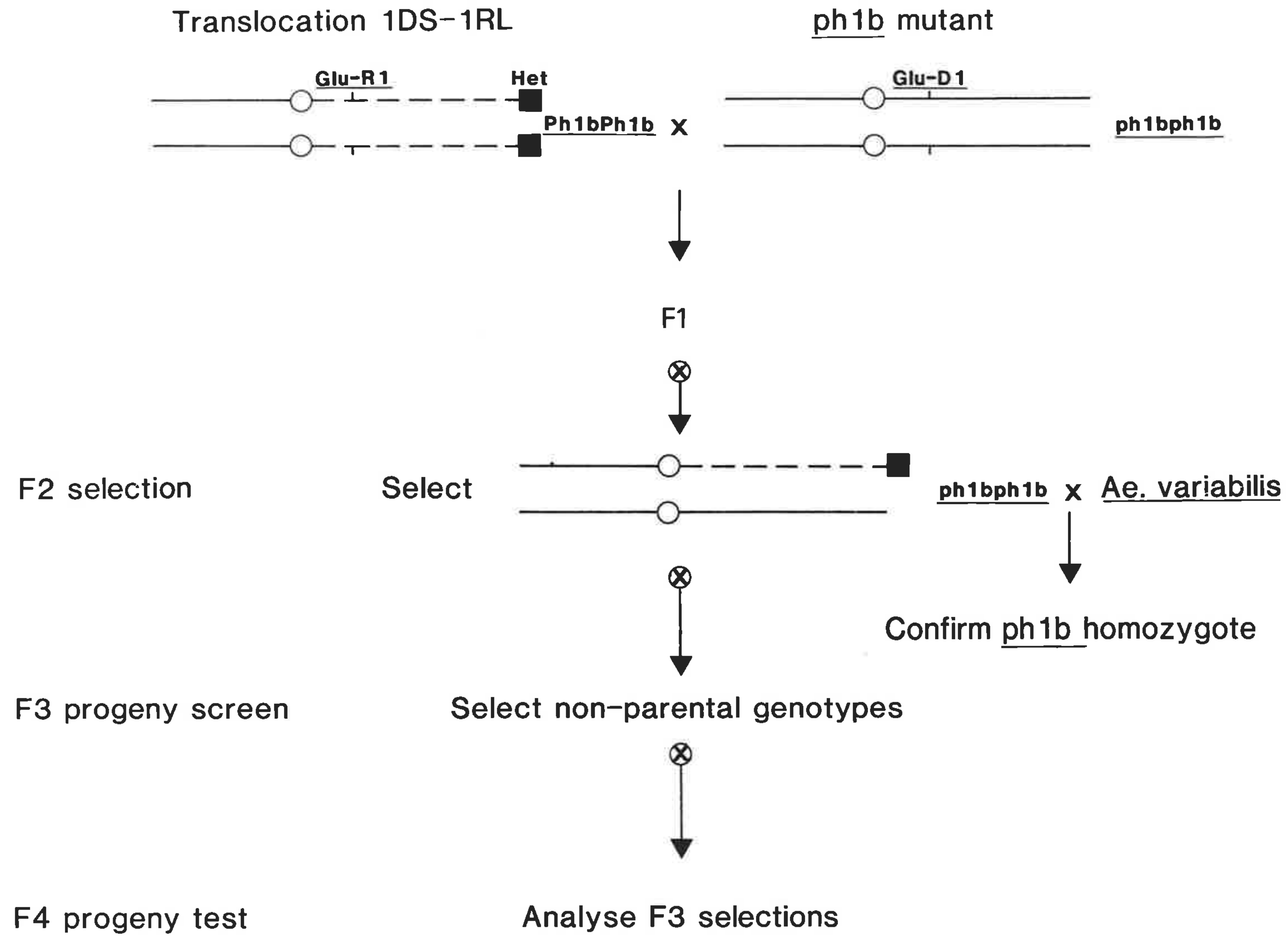
* with respect to the reference chromosome pair (1D and 1DS-1RL).

* excluded from the figure due to low probability of occurrence.

Aneuploid male gametes assumed not to function through certation.

event (aneuploidy, chromosome misdivision or wheat-rye recombination) had been responsible for the non-parental phenotype.

A summary of the steps involved in the recovery and verification of wheat-rye recombinants is shown in Figure 5.2.



Translocation 1DS-1RL
ph1b mutant
Glu-R1
Het
Ph1bPh1b
x
Glu-D1
ph1bph1b
F1
F2 selection
Select
ph1bph1b
x
Ae. variabilis
Confirm ph1b homozygote
F3 progeny screen
Select non-parental genotypes
F4 progeny test
Analyse F3 selections

Figure 5.2. Scheme for production, selection and verification of allosyndetic wheat-rye recombinants involving chromosome arm 1RL.

5.3. Results

5.3.1. Selection of F2 plants homozygous *ph1b* and heterozygous 1D/1DS-1RL

As discussed in Chapter 4, the presence of multivalents at meiotic metaphase I is not a satisfactory sole criterion for selection of *ph1b* homozygosity, since translocations induced within the *ph1b* stock will be expressed as multivalents when crosses are made to a wheat line with an undisturbed genome. Instead, a depression in the chiasma frequency manifested by unusually large numbers of univalents and a high proportion of rods to rings among the bivalents was taken to be diagnostic for *ph1b* homozygosity, based on the data of Driscoll (1979), who noted a decrease in chiasma frequency in pmcs of plants nullisomic for chromosome 5B. A similar effect has been noted to be the result of *ph1b* homozygosity (Yacobi *et al.*, 1982; Giorgi, 1983). Large multivalents were occasionally seen in the pmcs of presumptive *ph1bph1b* selections (Figure 5.3a) and some pmcs contained more than one multivalent (Figure 5.3b), but no pmcs with more than two multivalents were observed. Three plants were classified as presumptive *ph1b* homozygotes by analysis of their mean meiotic pairing, which is shown for two of the selections in Table 5.1, together with the average pairing in eight sib plants presumed to be *Ph1bph1b* or *Ph1bPh1b* (i.e. *Ph1b* -). The meiotic preparations from the third plant did not allow sufficient pmcs to be fully analysed, but up to 10 rod bivalents were observed in some pmcs and univalent frequency was abnormally high. One spike from each of the three putative *ph1bph1b* selections was pollinated by *Ae. variabilis* and the meiotic pairing behaviour of four progeny from each intergeneric cross was investigated using Feulgen squashes of pmcs at metaphase I. In each case, all four hybrids showed a high level of allosyndetic pairing (Figure 5.3d), in contrast to the lack of pairing shown when *Ph1* is present (Figure 5.3c). The transmission of *ph1b* through the male gamete from a *Ph1bph1b* heterozygote has been estimated by Sears (1977) to be 0.386; the

P
a

T
Q
b

c

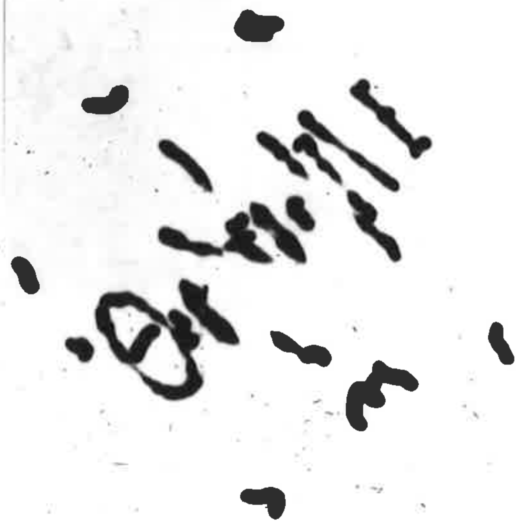

d

Figure 5.3. Chromosome configurations at metaphase I in pmcs of (a), (b) presumptive *ph1b* homozygotes, and (c), (d) of intergeneric wheat x *Ae. variabilis* hybrids.

(a) Pmc showing 3' + 3ʃ'' + 14⊙ + 1^{v} (P = pentavalent)

(b) Pmc showing 1' + 3ʃ'' + 14⊙ + 1''' + 1^{iv} (T = trivalent, Q = quadrivalent)

Effect of *Ph1* in intergeneric hybrid

(c) *Ph1* present. Pmc shows 35' (no allosyndetic pairing)

(d) *Ph1* absent. Pmc shows 1^{iv} + 2⊙, at least 5ʃ'' and univalents (allosyndetic pairing)

transmission through the female gamete was not measured, but it is likely to lie between this value and 0.5, as there is generally less selection against abnormal gametes among female than among male gametes. Thus the probability of transmission of *ph1b* rather than *Ph1b* by chance in four consecutive intergeneric hybrids from each of the three presumptive *ph1bph1b* selections, if the selections had instead been *Ph1bph1b*, lies between the limits of 0.022 (= 0.386^4) and 0.062 (= 0.5^4). These probabilities were considered sufficiently low to discount the possibility of wrong classification.

Table 5.1. Mean chromosome pairing in pmcs at metaphase I of two presumptive *ph1b* homozygotes and eight sib plants carrying at least one dose of *Ph1*, obtained in the F2 from the cross *ph1b* mutant x translocation 1DS-1RL.

plant no.	univ.	biv.		triv.	quad.	>quad.	χ per pmc	no. of pmcs
		rod	ring					
(a) *ph1bph1b* :								
60-2	1.2 (0-6)*	5.5 (2-13)	14.5 (8-19)	0.10 (0-1)	0.16 (0-1)	-	35.2	50
70-2	1.5 (0-6)	4.8 (2-10)	15.1 (11-18)	0.07 (0-1)	0.07 (0-1)	0.04 (0-1)	35.1	28
(b) *Ph1b* -: (mean of 8 plants)	0.4 (0-4)	2.5 (0-7)	18.0 (13-20)	0.03 (0-1)	0.16 (0-1)	-	39.0	234

* Range in brackets
univ.= univalents, biv.= bivalents, triv. = trivalents, quad.=quadrivalents, >quad.= higher multivalents, χ = chiasmata

5.3.2. Glutelin phenotype and root tip telomere number of the T and C populations

The three confirmed homozygous *ph1b* F2 selections produced 272, 303 and 156 (total 731) F3 seed respectively, while 292 and 249 (total 541) of the control progeny obtained from the two *Ph1bph1b* parents were analysed. Four different glutelin phenotypes were obtained from the T population and three from the C population, and the segregation of

these is summarised in Table 5.2(a). A typical SDS-PAGE gel showing segregation for these patterns is shown in Figure 5.4A. The data for the three T populations and for the two C populations were internally homogeneous for glutelin subunit phenotype in each group ($\chi^2 = 9.05$; 4 d.f.; $0.05<p<0.1$, and 4.09; 2 d.f.; $0.1<p<0.2$ respectively) and thus only pooled values for the two types of population are given in Table 5.2(a).

Table 5.2. Individual segregation of glutelin phenotype and rye telomere number in the T and C populations

(a) Glutelin phenotype

Population	Glu-D1 Glu-R1	+ -	+ +	- +	- -
T		264	364	102	1
C		170	290	81	0

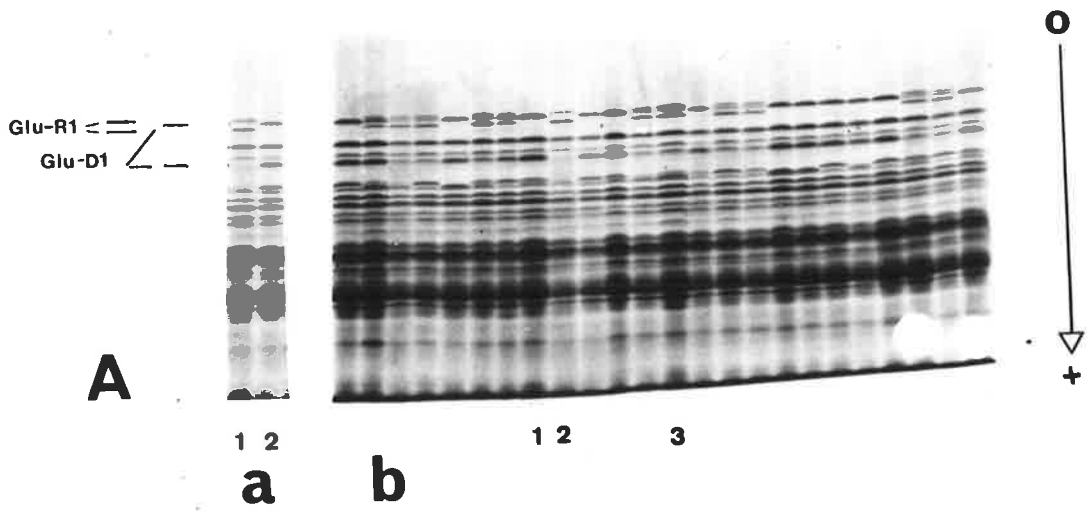
+: protein present -: protein absent

(b) Rye telomere number

Population	0	1	2	3
T	264	356	109	2
C	.171	283	87	0

T: *ph1bph1b*- derived populations C: *Ph1bph1b*- derived populations

The chromosome constitution of the individuals from the T and C populations was inferred from their seed storage protein phenotype (Chapter 4). The segregation ratio for homozygous 1D: translocation heterozygote: translocation homozygote would be 1:2:1 if there was no competition between gametes in their formation and transmission, but the observed ratios were 2.6:3.6:1 for the progeny of *ph1bph1b* parents and 2.1:3.6:1 for the progeny of *Ph1bph1b* parents. Although the female transmission rate of gametes bearing the translocation chromosome is unknown, it is likely that this observed distortion in the segregation ratio is due to the lower vigour of male gametes bearing the 1DS-1RL


Glu-R1
Glu-D1
o
+
A
1 2
a
b
1 2
3

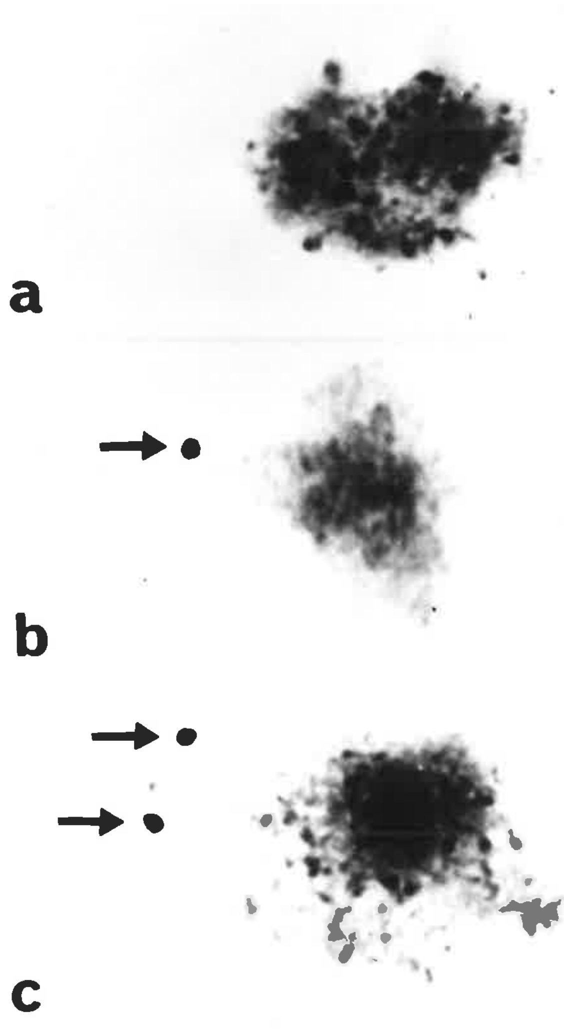

a
b
c
B

Figure 5.4. Markers used to score progeny derived from the cross *ph1b* mutant x translocation 1DS-1RL.

A. Endosperm storage protein phenotype derived by reduced SDS-PAGE.

a - parental phenotypes:
1. Translocation 1DS-1RL (Glu-D1$^-$ Glu-R1$^+$)
2. Euploid Chinese Spring (Glu-D1$^+$ Glu-R1$^-$)

b - progeny phenotypes:
1. (Glu-D1$^+$ Glu-R1$^-$) 2. (Glu-D1$^-$ Glu-R1$^+$)
3. (Glu-D1$^+$ Glu-R1$^+$)

B. Rye telomere number visualised by Giemsa C-banding of root tip interphase cells.

a - 0 rye telomere b - 1 rye telomere c - 2 rye telomeres

Rye telomeres arrowed.

translocation compared to those bearing a normal 1D, as was found with the translocations involving 1RS (Chapter 4).

The number of rye telomeres in the root tip interphase nuclei normally varied from 0 to 2 (Figure 5.4B a,b,c) and rarely 3 (not shown). The pattern of segregation for this marker is shown in Table 5.2(b). As for glutelin phenotype, there was no evidence of heterogeneity for rye telomere number either in the three T populations (χ^2= 9.96; 4 d.f.; $0.02<p<0.05$) or in the two C populations (χ^2= 4.03; 2 d.f.; $0.1<p<0.2$) and hence pooled data only are given in Table 5.2. The joint segregation of the two characters is shown in Table 5.3. The major difference between the T and the C populations lies in the greater diversity of non-parental classes recovered and the larger number of individuals within each such class in the former families; these numbers remain relatively larger even after allowing for the greater population size analysed for the T population.

Table 5.3. Joint segregation of glutelin phenotype and rye telomere number in the T and C populations.

	Classification of phenotype									
	Parental			Non-parental						
Glu-D1	+	+	-	+	+	+	-	+	-	-
Glu-R1	-	+	+	-	+	+	+	+	+	-
Rye telomere no.	0	1	2	1	0	2	1	3	3	0
Population										
T	258	337	88	6	5	21	13	1*	1^	1
C	170	282	80	0	1	7	1	0	0	0

*iso-1RL + 1DS-1RL ^plant died before maturity
+: protein present -: protein absent
T: *ph1bph1b*- derived populations C: *Ph1bph1b*- derived populations

All non-parental plants were grown and allowed to self-fertilise in order to permit a progeny test. This was undertaken both to confirm the original classification and because with the two non-parental phenotypes Glu-D1$^+$ Glu-R1$^+$, 2 rye telomeres and Glu-D1$^-$ Glu-R1$^+$, 1 rye telomere, it was necessary to distinguish whether they had arisen from aneuploidy, chromosome misdivision or wheat-rye recombination (see Figure 5.1).

5.3.3. Confirmatory progeny tests of non-parental plants

It was not possible to progeny test all the non-parental plants as some produced too few seed and others were completely self sterile. This low fertility seemed to be genetic in origin since there was variation in its extent between sib plants grown in the same pot. It is likely that the genomic structure of these sterile plants had been sufficiently disturbed following two generations of self-fertilisation in the homozygous *ph1b* condition to prevent or reduce the production of viable gametes. The original mutation itself was characterised by reduced fertility (Sears, 1977).

5.3.3.1. Glu-D1^{+} Glu-R1^{-}, 1 rye telomere phenotype

Individuals lacking the *Glu-R1* locus should also lack the rye telomere unless the rye arm has been recombined with wheat. Six plants of phenotype Glu-D1^{+} Glu-R1^{-}, 1 rye telomere were isolated from among the T population, but this phenotype did not occur among the C population (Table 5.3). Five of the six non-parental plants of this type gave sufficient seed to allow a progeny test. In each case all the progeny lacked Glu-R1 and in only one case was there segregation for Glu-D1; however the rye telomere number was seen to vary from 0 to 2 in individual seedlings (Table 5.4). In these five plants the linkage between the two rye markers has been broken, and they therefore carry a chromosome involving allosyndetic recombination between wheat and rye. The occurrence of progeny from plant 269-23 lacking both *Glu-D1* and *Glu-R1* suggests that some group 1 homoeologue other than 1DL is involved in this recombinant, whereas 1DL may be recombined with 1RL in the other four plants. The new chromosomal location of the 1RL telomere, resulting from homoeologous recombination, can be simply investigated by crossing the recombinant lines bearing it to the group 1 ditelocentric stocks and observing, by means of Giemsa C-banding, which telosome pairs with the

heterochromatin-bearing chromosome at meiotic metaphase I.

Table 5.4. Confirmatory progeny tests of non-parental selections from the T population, having the phenotype Glu-D1^{+} Glu-R1^{-}, 1 rye telomere.

Selected plant no.	No. progeny tested	Endosperm protein phenotype Glu-D1 + / Glu-R1 -	Glu-D1 + / Glu-R1 +	Glu-D1 - / Glu-R1 +	Glu-D1 - / Glu-R1 -	No. germinated	Rye telomere no. 0	1	2
269-23	8	5	0	0	3	2	1	1	0
283-9	23	23	0	0	0	23	11	11	1
290-20	20	20	0	0	0	19	5	12	2
290-23	6	6	0	0	0	6	1	3	2
294-16	15	15	0	0	0	14	8	5	1

+: protein present -: protein absent

5.3.3.2. Glu-D1^{+} Glu-R1^{+}, 0 rye telomere phenotype.

In the presence of the *Glu-R1* locus, at least one rye telomere should be present unless recombination or some other event has occurred to separate these markers. Six plants of the Glu-D1^{+} Glu-R1^{+}, 0 rye telomere phenotype were recovered among the T and C populations, five from the former and one from the latter (Table 5.3). All gave sufficient seed for progeny testing. This genotype could have arisen from three different events: recombination between the rye arm and a wheat chromosome, so that the rye glutelin locus was no longer linked to the rye telomere; spontaneous loss of heterochromatin, as has been observed in both triticale (Merker, 1975) and in wheat-rye addition lines (Singh and Röbbelen, 1976); or chromosome mosaicism between the endosperm tissue and the root tip cells, whereby the rye chromatin is present in the endosperm but not in the zygote, or is lost during the development of the root tip, as discussed in Chapter 4. The progeny segregated for Glu-R1 (Table 5.5), thereby excluding chromosome mosaicism as the origin of the non-parental individuals. Only those possessing the rye protein were germinated in order to determine their rye telomere content, as plants without the rye glutelin were not expected to have any rye telomeres, and none of these seedlings were

found to possess any rye telomeres. In the absence of other genetic markers for the rye arm, it is not possible to distinguish between a recombination event and spontaneous loss of heterochromatin. Since allosyndesis is expected to be suppressed in the plants which generated the control populations, the single individual of this phenotype selected among the control progeny was likely to have originated from spontaneous loss of the rye telomere.

Table 5.5. Confirmatory progeny tests of non-parental selections from both the T and the C populations, having the phenotype Glu-D1^{+} Glu-R1^{+}, 0 rye telomere.

Selected plant no.	No. progeny tested	Endosperm protein phenotype Glu-D1 + Glu-R1 -	Glu-D1 + Glu-R1 +	Glu-D1 - Glu-R1 +	No. germinated*	Rye telomere no. 0	1	2
(a) T population:								
262-31	7	5	2	0	2	2	0	0
270-2	7	1	6	0	3	3	0	0
272-19	20	8	9	3	3	3	0	0
284-12	10	2	6	2	7	7	0	0
289-8	15	11	2	2	3	3	0	0
(b) C population:								
308-17	15	6	8	1	9	9	0	0

* Glu-R1^{-} individuals not selected for germination
+: protein present -: protein absent
T: *ph1bph1b*- derived populations C: *Ph1bph1b*- derived populations

5.3.3.3. Glu-D1^{+} Glu-R1^{+}, 2 rye telomere phenotype

These plants differ from normal translocation heterozygotes in having an extra rye telomere. Twenty-one such plants were isolated in the T population (ten progeny tested) and seven in the C population (five progeny tested) (Tables 5.3, 5.6). Possible origins of this non-parental phenotype include wheat-rye recombination, hyperploidy (i.e. having two doses of 1DS-1RL and one of 1D) or the formation of an isochromosome involving 1RL. Recombinant plants can be distinguished from hyperploids by checking the rye

Table 5.6. Confirmatory progeny tests of non-parental selections from both the T and the C populations, having the phenotype Glu-D1^{+} Glu-R1^{+}, 2 rye telomeres.

Selected plant no.	No. progeny tested	Endosperm protein phenotype Glu-D1 + / Glu-R1 -	Glu-D1 + / Glu-R1 +	Glu-D1 - / Glu-R1 +	Rye telomere no.: No. germ#	0	1	2	Classification of selection*
(a) from T population :									
268-16	45	0	26	19					H
269-29	33	3	23	7	3	0	3	0	R
272-10	32	0	21	11					H
274-11	32	12	13	7	12	1	3	8	R
275-3	19	0	16	3					H
284-14	17	0	11	6					H
284-30	20	4	16	0	4	4	0	0	H
292-11	17	0	16	1					H
292-12	53	16	35	2	9	9	0	0	H
289-16	16	11	5	0	11	0	0	11	R
(b) from C population:									
295-1	13	2	10	1					I
295-17	15	0	14	1					H
300-15	15	0	13	2					H
329-25	12	2	8	2	2	2	0	0	H
330-9	11	0	11	0					H

\# Only Glu-R1^{-} seed selected for germination
* H: hyperploid, R: recombinant, I: isochromosome
+: protein present -: protein absent
T: *ph1bph1b*- derived populations C: *ph1bph1b*- derived populations

telomere content of progeny which lack Glu-R1: with recombinants, these progeny will have one or two rye telomeres (plants 269-29, 274-11 and 289-16), whereas with hyperploids Glu-R1^{-} progeny will have no rye telomeres (plants 284-30, 292-12 and 329-25 : Table 5.6). Furthermore, with a hyperploid, the frequency of Glu-R1^{-} progeny will be very low, compared to their frequency among progeny derived from recombinants where there is an expectation that 25% of the progeny will lack *Glu-R1*. In some selections (plants 275-3, 284-14 , 292-11 from the T population, and 295-17, 300-15 and 330-9 from the C population), progeny lacking Glu-R1 were not obtained in the limited progeny available; the phenotypic distributions of their progeny were similar to one

another and not inconsistent with the segregation expected from a hyperploid parent, and these plants were therefore classified as hyperploids.

One selection from the C population (plant 295-1) gave progeny which carried Glu-R1 but lacked both Glu-D1 and Gli-D1, the product of the ω-gliadin locus *Gli-D1*, located on the short arm of 1D (see Chapter 4); this locus is readily scorable on SDS-PAGE separations of reduced proteins (Singh and Shepherd, 1984). Furthermore, in some progeny of this plant, different interphase cells from the same root tip showed either one large dot, or two normal-sized ones. These observations led to the conclusion that an isochromosome for 1RL was present, accounting for the extra telomere in this selection, and the occurrence of occasional large heterochromatic dots was caused by fusion of the telomeres in the isochromosome. A similar isochromosome was observed at mitosis in the root tip of another selection, but this plant died before maturity (see Table 5.3).

It is concluded from these analyses that three recombinants were obtained from the T population but none from the C population (Table 5.7).

Table 5.7. Classification of Glu-D1$^+$ Glu-R1$^+$, 2 rye telomere selections.

Population	No. of selections	No. selections progeny tested	Classification of non-parental plant		
			Recombinant	Hyperploid	Isochromosome
T	21	10	3	7	0
C	7	5	0	4	1

T: *ph1bph1b*- derived populations   C: *ph1bph1b*- derived populations

5.3.3.4. Glu-D1⁻ Glu-R1⁺, 1 rye telomere phenotype.

Plants having *Glu-R1* but lacking *Glu-D1* will normally be translocation homozygotes, and therefore those with this glutelin subunit constitution are expected to have two rye telomeres. However, thirteen progeny from the T population (ten progeny tested) and one from the C population (not progeny tested) had this glutelin constitution but possessed only one rye telomere. Such plants could have arisen from hypoploidy (one dose of 1DS-1RL, zero of 1D), wheat-rye recombination or spontaneous loss of heterochromatin. The progeny test was able to differentiate between hypoploidy and the other two possible origins, but as mentioned previously, recombination and heterochromatic loss could not be distinguished.

All progeny lacked Glu-D1, and the progeny from the three plants classified as hypoploid (plants 266-10, 266-16 and 267-3 : Table 5.8) segregated for Glu-R1 in a

Table 5.8. Confirmatory progeny tests of non-parental selections from the T population, having the phenotype Glu-D1⁻ Glu-R1⁺, 1 rye telomere.

Selected plant no.	No. progeny tested	Endosperm protein phenotype (all Glu-D1⁻) Glu-R1⁺ Rye tel. no. 0	1	2	Glu-R1⁻ 0	Classification of non-parental plant*
263-17	21	0	11	3	7	W/W
266-10	21	0	13	8	0	H
266-16	22	0	10	10	2	H
267-3	22	0	13	8	1	H
270-12	18	1	6	4	6	W/R
271-18	18	0	9	3	6	W/W
271-19	17	0	6	4	6	W/W
272-12	10	2	5	2	1	W/R
280-22	20	6	6	7	0	W/R
290-21	20	0	9	0	10	W/W

* W/W: wheat-wheat recombinant, W/R: wheat-rye recombinant, H: hypoploid

pattern typical of monosomics, almost all possessing at least one dose of *Glu-R1*, while the majority of the progeny possessed one rye telomere and were, therefore, themselves monosomic. A few progeny from these plants possessed neither rye glutelin nor any rye

telomere, and were thus nullisomic for the rye arm. Three of the other non-parental selections in this category (plants 270-12, 272-12 and 280-22) were classified as wheat-rye recombinants (or spontaneous loss of heterochromatin) because some of their progeny had Glu-R1 but no rye telomere. As many as one third of the progeny of the remaining three non-parental plants (plants 263-17, 271-18 and 271-19) besides lacking Glu-D1, also lacked Glu-R1. Unlike the rare progeny of the same glutelin phenotype derived from plants classified as hypoploid, these progeny still possessed the short arm of 1D, as shown by the presence of *Gli-D1* . The possibility that the parents of these progeny carried chromosomes 1DS and 1RL as independent telosomes can be eliminated as there was no segregation for 1DS. These three plants were therefore considered to have arisen through wheat-wheat homoeologous exchange, causing disruption of the usual association of *Glu-D1* and *Gli-D1* . The single individual with a non-parental phenotype from the control population could not be progeny tested, but it is thought likely to have been hypoploid as were three out of the ten analysed plants from the T population.

The conclusions derived from the progeny tests are shown in Table 5.9.

Table 5.9. Classification of Glu-D1$^-$ Glu-R1$^+$, 1 rye telomere selections.

Population	No. of selections	No. selections progeny tested	Classification of non-parental plant		
			Wheat-wheat recombinant	Wheat-rye recombinant	Hypoploid
T	13	10	4	3	3
C	1	0			

T: *ph1bph1b*- derived populations C: *ph1bph1b*- derived populations

5.4. Discussion

Having established that the *ph1b* mutant can effect recombination between rye chromosome 1RL and wheat chromosome/s, it is of interest to estimate the frequency of this allosyndetic recombination.

Plants having one rye telomere in the absence of *Glu-R1* (section 5.3.3.1) can only have been derived from crossing over along the rye chromosome arm; five plants of this type were confirmed and one remained unverified through lack of progeny. Spontaneous translocation of rye telomeres to wheat chromosomes has been reported in triticale (Sapra and Stewart, 1980) and in derivatives of triticale x wheat hybrids (Lukaszewski and Gustafson, 1983); however these conclusions are based solely on C-banded karyotypes and are not supported by any genetic evidence that these novel chromotypes are not further examples of whole arm exchanges, commonly seen in genomic mixtures where univalent frequency is high. No Glu-D1^{+} Glu-R1^{-}, 1 rye telomere individual was obtained in the control population, so that this non-parental phenotype was most probably induced by the *ph1bph1b* condition.

Those plants with *Glu-R1* but lacking the rye telomere (section 5.3.3.2) could have arisen from wheat-rye recombination or from the spontaneous loss of heterochromatin. Five individuals of this phenotype from the T population and one from the C population were isolated. Spontaneous loss of telomeric heterochromatin has been reported a number of times (e.g. Merker, 1975; Singh and Röbbelen, 1976), although it has been questioned whether the apparent loss of the rye telomere is not rather an example of wheat-rye translocation (May and Appels, 1980). In either case, a spontaneous change within the rye arm could explain the isolation of one Glu-D1^{+} Glu-R1^{+} 0 rye telomeres phenotype in the control population, but the frequency of its occurrence (1 in 541, 0.18%) was too low to account for the appearance of five such plants in 731 *ph1bph1b*-derived progeny (0.68%) unless *ph1b* homozygosity promotes this change other than by induction of allosyndetic pairing. To derive a maximal estimate of the number of

wheat-rye recombinants in this phenotypic class, it was therefore assumed that all five non-parental plants had originated from an allosyndetic recombination event.

Among the individuals with the Glu-D1$^+$ Glu-R1$^+$, 2 rye telomere phenotype individuals (section 5.3.3.3), it was possible to identify three recombinants and seven hyperploids but eleven isolations remained unclassified through lack of progeny (Table 5.7). Extrapolating the proportion of known recombinants to aneuploids to these eleven unclassified individuals gives an estimate of a further three recombinants in this phenotypic class.

A similar extrapolation can be applied to the unclassified individuals in the Glu-D1$^-$ Glu-R1$^+$, 1 rye telomere phenotypic class (section 5.3.3.4). Three wheat-rye recombinants were identified among ten classified individuals while three plants remained unanalysed (Table 5.9), giving an estimate of one further recombinant among these plants.

The overall maximal estimate for the frequency of wheat-rye recombinants is therefore six, five, six and four from each of the non-parental phenotypic classes, respectively, giving a total of 21 in 731 progeny analysed, or 2.9%. Since all gametes could be fully classified in this experiment, the gametic recombination frequency is 1.4%. This compares with the figures of 0.3% from *ph1bph1b* induced, and 0.5% from nullisomic 5B induced allosyndetic recombination along what may be a relatively short segment of rye chromosome arm 1RS (Chapter 4).

The two markers used in the present study were *Glu-R1*, a locus only 4.6±1.0 cM from the centromere (Singh and Shepherd, 1984) and the telomere, and therefore cross-overs occurring along practically the entire genetic length of the chromosome arm would be identifiable. Of all the rye chromosomes, Naranjo (1982) has observed that 1R, and in particular its long arm 1RL, has the greatest pairing affinity with its wheat genome homoeologues. Thus the estimate of 1.4% obtained in this study is expected to represent an upper limit for the frequency of allosyndetic recombination between wheat and rye chromatin along a chromosome arm.

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In order to more fully characterise the wheat-rye recombinants, and, in particular, to establish how much rye chromatin has been lost among those that no longer possess the heterochromatic telomere, more markers for the rye arm are required. Structural genes for lipopurothionins (Fernandez de Caleya *et al.*, 1976), grain lectins (Stinissen *et al.*, 1983) and the isozyme malate dehydrogenase (E.C. 1.1.1.37) (Benito and Salinas, 1983) have been reported to be located on the group 1 chromosomes in wheat, but, of these, only a gene for lipopurothionins has as yet been located on 1R (Sanchez-Monge *et al.*, 1979); the assay for this marker requires 1g of seed and so can only be used to characterise recombinants which first have been selected using other markers. Allelic differences within rye have allowed a gene coding for the isozyme 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) to be located on chromosome arm 1RL (Lawrence and Appels, 1985) but the electrophoretic mobility of the product of the rye allele present in the 1DS-1RL translocation is indistinguishable from that of the wheat isozyme/s and therefore cannot be used as a marker for this chromosome arm in a wheat background.

Riley and Kimber (1966) observed homoeologous pairing in a nullisomic 5B amphiploid of wheat x *Ae. longissima* , but concluded that full homologues paired preferentially, so that the level of allosyndetic crossing-over was low. As wheat-wheat homoeologous recombination was observed both in the present study (Table 5.9), and between 1DS and other wheat chromosomes (Chapter 4), it may be that allosyndesis, at least between wheat homoeologues, is more common in *Ph1* deficient backgrounds than has been supposed. Few of the wheat chromosomes possess distinct morphological characters when stained by the conventional Feulgen method, and so only those bivalents composed of chromosomes differing substantially in length or arm ratio can normally be distinguished as homoeologous, rather than homologous associations. Multivalent formation occurs in *ph1bph1b* genotypes, but with a low frequency and, as discussed earlier, these multivalents may not be formed as a direct result of *Ph1* deficiency. Thus cytological observation of metaphase I pairing cannot effectively allow the full extent of non-homologous chromosome associations to be visualised, unless individual

chromosomes can be unambiguously identified at meiosis using differential staining techniques such as C- and N-banding, or by *in situ* hybridisation with radioactive DNA probes. Furthermore, the chromosome pairing observed at metaphase I is only a fraction of the synapsis which takes place at prophase I (Fu and Sears, 1973). If desynapsis of homoeologous bivalents occurs, then these paired chromosomes will appear as univalents at metaphase, so that at least some of the univalents observed at this time were synapsed at pachytene/zygotene. In this study, the high proportion of rods to rings among the bivalents, and the high frequency of univalents observed in *ph1b* homozygotes (Table 5.1) are taken to indicate the occurrence of homoeologous pairing, where some of the rod bivalents represent homoeologous associations, leaving the homologous partners unpaired as univalents. The loss in fertility of the *ph1b* stock noted by Sears (1977) could then be due to a combination of the production of unbalanced gametes and the detrimental effect of accumulated chromosomal translocations mentioned earlier (Chapter 4).

No agronomically useful genes are known to be located on rye chromosome arm 1RL, and thus the agricultural value of the wheat-rye recombinants produced in this study is limited. Nevertheless, they may prove useful in the elucidation of gene order along this chromosome arm, as they are genetically equivalent to deletion lines with respect to an intact chromosome arm. While gene mapping is conventionally carried out by exploiting allelic variation at multiple loci, deletion analysis is useful when such allelic variation is rare.

Naranjo (1982) has suggested that chromosome 1A is more closely related than 1B to chromosome 1R, and the new chromosomal locations of the rye chromatin in the recombinant lines will provide some test for this idea, as it would be predicted that the majority of the recombinants will involve 1A/1R exchanges. The diagnostic crosses necessary to determine where the recombined rye chromatin is now located have been made, and some of the material is presently being analysed.

Chapter 6: Wheat-*Aegilops* recombination: Chromosome 1U of *Aegilops umbellulata*

6.1. Introduction

The frequency of allosyndetic pairing between wheat and rye chromosomes is low (Chapter 2.5), and hence the expected recovery of genetic recombinants involving chromatin from these two species is rare, although it has now been shown in Chapters 4 and 5 not to be zero. The frequency of allosyndetic pairing in *Ph1* deficient hybrids of wheat x *Aegilops* spp. varies considerably, but reaches high levels in some species (Riley and Kimber, 1966). The ease with which this homoeologous pairing can be induced has been exploited in the production of Compair from *Ae. comosa* (Riley *et al.*, 1968a, b), and introgression has also been achieved from *Ae. bicornis* (Riley and Kimber, 1966) and *Ae. umbellulata* (Kimber, 1967b; Law and Payne, 1983). Early work on the introgression of chromatin segments from *Aegilops* spp. most often was aimed at transferring genes which conferred resistance to various foliar leaf diseases into wheat, and the transfers were identified by the presence of the alien resistance in a wheat in which the alien chromosome could not be observed as a univalent at metaphase I of meiosis. Other phenotypic characters, such as grain colour and quantitative changes in height, maturity and grain size, and some karyotypic changes were noted in derivatives of crosses of wheat with *Ae. bicornis*, and these alterations in plant phenotype were taken as evidence of the incorporation of alien chromatin (Riley and Kimber, 1966). More recently, attempts have been made to transfer certain endosperm storage proteins, controlled by a chromosome of *Ae. umbellulata*, into wheat (Law and Payne, 1983).

Chromosome 1U of *Ae. umbellulata* has been disomically substituted for each of its wheat homoeologues (Shepherd, 1973; Chapman *et al.*, 1975) and all three lines are fertile and show near normal phenotypes, although the (1D)1U substitution line is

reported to be meiotically unstable at low temperatures (Chapman *et al.*, 1975). This *Aegilops* chromosome carries both a *Glu-1* and a *Gli-1* locus (Shepherd, 1973; Lawrence and Shepherd, 1981) and a *Gpi-1* locus (Chojecki and Gale, 1982), all of which code for gene products electrophoretically distinct from those of their wheat homoeoloci in cv. Chinese Spring. This alien chromosome is thus well suited to a study of induced allosyndetic recombination. The recombination frequency was expected to be higher than that induced between wheat and rye chromatin, given that wheat and *Aegilops* spp. are more closely related than are wheat and rye. Recombination of chromosome 1U with wheat chromosomes has been reported briefly by Law (1984), but the procedures employed and the details of the results achieved have not to date been published. The present work describes the production and identification of a substantial number of *ph1b* induced interspecific recombinants, expanding the methodology developed in the previous chapters of this thesis. In contrast to the previous work with rye chromosome segments, where allosyndetic recombination was restricted to a single chromosome arm, the use of the whole chromosome of *Ae. umbellulata* allowed the possibility of pairing along an entire alien chromosome, with markers for the *Aegilops* chromosome available on both chromosome arms.

6.2. Plant materials and methods

6.2.1. Plant materials

a. Substitution lines (1B) 1U and (1D) 1U in cv. Chinese Spring (Shepherd, 1973).
b. *ph1b* mutant (Sears, 1977); two stocks used: (i) derivatives of Ac. 7876 with an unknown number of generations of self-fertilisation since the original isolation, and (ii) Ac. 9821, a reselection from a *Ph1bph1b* stock obtained from Prof. C.J. Driscoll. Accession numbers are those of Dr. K.W. Shepherd.
c. An accession of *Ae. variabilis* (Ac. 7069) obtained from Dr. K.W. Shepherd.

6.2.2. Production of populations for screening of wheat-*Aegilops* allosyndetic recombination

A scheme similar to that described in Chapters 4 and 5 was used to obtain plants containing both one dose of wheat chromosome 1B (or 1D) and of chromosome 1U in a homozygous *ph1b* background. In order to minimise the extent of translocations which may have accumulated in the *ph1b* mutant parent (Chapters 4 and 5), the mutant was used as the male, rather than the female, parent in the cross to each substitution line, based on the premise that competition between male gametes in fertilisation would tend to exclude those with severely disturbed genomes. In addition, for the cross involving the 1B substitution, a reselection of the mutant (see section 6.2.1.b) was utilised to reduce further the extent of translocations within the mutant parent. The resulting F_1 plants were allowed to self-fertilise. The endosperms of individual F_2 grains were analysed by both unreduced and reduced SDS-PAGE, and the presence of the appropriate protein bands (see below, section 6.2.3) was used to select individuals which presumably carried both 1U and the respective wheat chromosome. Approximately 20 such individuals from each

F_2 population were grown and meiotically analysed to select for *ph1b* homozygosity.

Chromosome 1U carries a prominent satellite on its short arm (Kimber, 1967b) and could be easily recognised in meiotic chromosome preparations (Figure 6.1). Thus any allosyndetic pairing which involved 1U could be identified and the occurrence of such allosyndetic pairing was a further criterion used to aid in the selection of *ph1bph1b* individuals. One spike from each presumptive homozygous *ph1b* plant was pollinated by *Ae. variabilis* and the remaining spikes were left to self-fertilise, and these progeny formed the populations to be screened for allosyndetic recombination. Where possible, at least four of the intergeneric wheat x *Ae.variabilis* hybrid seeds from each selection were grown and the pmcs of the resulting plants were analysed for the presence or absence of homoeologous pairing at metaphase I.

Control populations, where homoeologous pairing was suppressed, were obtained from the F_2 generation of the cross of each substitution line x *ph1bph1b* , which represent progeny from plants known to be *Ph1bph1b* . The same terminology will be applied to these populations as was developed in Chapter 5. Thus the progeny of plants of constitution *ph1bph1b* will be referred to as T populations, while the control progeny will be referred to as C populations.

6.2.3. Phenotypes of parental lines for biochemical markers

6.2.3.1. Unreduced and reduced SDS-PAGE phenotypes

The SDS-PAGE phenotypes of the gene products of the wheat ω-gliadin loci *Gli-B1* and *Gli-D1* and the wheat glutenin locus *Glu-D1* have been described in Chapters 4.2.3 and 5.2.3. The wheat glutenin locus *Glu-B1* is polymorphic and the various alleles known are described in Payne and Lawrence (1983). In cv. Chinese Spring, chromosome arm 1BL codes for two high molecular weight protein subunits which appear as bands 7 and 8

on SDS-PAGE gels of reduced protein extracts (Figure 6.2B). Chromosome 'B' of *Aegilops umbellulata* (Kimber, 1967b) has been allocated to homoeologous group 1 on the basis of its ability to substitute successfully for wheat chromosomes of this group, and as it carries genes coding for glutelin protein subunits (*Glu-U1*) (Lawrence and Shepherd, 1981), a prolamin protein (*Gli-U1*) (Shepherd, 1973) and for the isozyme glucose phosphate isomerase (Chojecki and Gale, 1982). The Gli-U1 protein appears as two bands on unreduced SDS-PAGE gels (Figures 6.2A, 6.4A). The slower Gli-U1 band can often be scored on reduced SDS-PAGE gels (Figure 6.2B), but in some runs it overlaps with Gli-B1 and in others it is not reliably expressed (Figure 6.4B); therefore the faster band was used to score for the presence of this locus, and so both unreduced and reduced extracts needed to be analysed. Two *Ae. umbellulata* glutelin subunits appear on reduced SDS-PAGE gels (Lawrence and Shepherd, 1981) as shown in Figures 6.2B and 6.4B. Chromosome 1U also codes for a "triplet" protein; however the mobility of this protein is identical to that coded for by *Tri-D1* and therefore it could not be used in this study.

6.2.3.2. Gpi phenotypes

Gpi phenotypes were visualised on nominal pH 3-10 IEF gels (for the *Gpi-U1* product) (Chapter 3.1.4) and on cellulose acetate gels (*Gpi-B1* product) (Chapter 3.1.5). The wheat isozymes are dimeric and form both homo- and heterodimers (Hart, 1979b; Chojecki and Gale, 1982) but the monomers controlled by *Gpi-U1* do not appear to form heterodimers with the wheat monomers (Chojecki and Gale, 1982). Thus the most anodal band on the gel is thought to be controlled entirely by *Gpi-U1* and it was therefore used to score for the presence of this locus (Figure 6.3B). The Gpi-B1 phenotype can also be scored using IEF, but electrophoresis in cellulose acetate gels provides a cheaper and simpler method for determination of this phenotype. In this system, normal wheat gives four strong bands and a fifth, faster, weak band (Figure 6.3A). In the absence of

chromosome 1B, or its short arm, the slowest two bands are lost, resembling the results of Hart (1979b) who used extracts of coleoptile and young leaf tissue and separated the isozymes on starch gels; starch gel electrophoresis however only allows the three slower bands to be visualised when extracts are made from scutellar tissue. The gel shown in Figure 6.3A also reflects segregation for *Gpi-U1*, and thus more than four strong bands are visible in some samples; however this system did not prove sufficiently reliable for the analysis of segregation for this locus.

6.2.4. Marker loci used in the detection of wheat-*Ae. umbellulata* recombinants

The genes controlling the endosperm storage proteins Gli-1 and Glu-1 are present on the short and long arms, respectively, of chromosomes 1B and 1D in wheat, and a similar arm location on 1U is likely. Structural genes for the isozymes of glucose phosphate isomerase (Gpi) are located on chromosomes 1BS and 1U (Hart, 1979b; Chojecki and Gale, 1982).

6.2.5. Screening for wheat-*Aegilops* recombination

As large numbers of progeny needed to be analysed, the sample preparation method was streamlined to allow 134 samples to be run easily per day. A small piece of the brush end of the endosperm was removed from each progeny seed derived from both *Ph1bph1b* and *ph1bph1b* parents in order to establish their storage protein phenotype by first unreduced and then reduced SDS-PAGE. This procedure allowed a number of storage protein phenotypes to be scored, as detailed in Table 6.1.

Gpi-D1 is located interstitially between *Gli-D1* and *Glu-D1* (Chojecki *et al.*, 1983) in wheat, and it was therefore assumed, on the basis of the widespread evidence for the maintenance of gene synteny groups within wheat and its relatives (e.g. Hart *et al.*, 1980), that the same gene order existed on both chromosomes 1B and 1U; if this is

Table 6.1. Endosperm protein marker loci scored by SDS-PAGE in the F2 progeny of plants derived from the two crosses, substitution (1B)1U x *ph1b* mutant and (1D)1U x *ph1b* mutant.

	(1B)1U cross	(1D)1U cross
Unreduced SDS-PAGE	Gli-B1, Gli-U1	Gli-D1, Gli-U1
Reduced SDS-PAGE	Glu-B1, Glu-U1	Glu-D1, Glu-U1

correct, individuals which possessed *Gli-1* but lacked *Glu-1* (or vice versa) would represent the products of either misdivision of the centromere or homoeologous recombination, and these seeds were scored for Gpi phenotype. To do this, another small portion of endosperm was removed from the selected grains and a crude enzyme extract was obtained as described earlier (Chapter 3.1.4), except that only 30 µl of distilled water was added to the crushed endosperm fragment. The Gpi phenotype of individuals with the two storage protein phenotypes Glu-U1^{+} Gli-U1^{-} and Glu-U1^{-} Gli-U1^{+} was analysed by IEF, while those with phenotypes Glu-B1^{+} Gli-B1^{-} and Glu-B1^{-} Gli-B1^{+} were scored for Gpi phenotype using cellulose acetate electrophoresis, as explained above. In the populations segregating for chromosome 1B, some individuals showed non-parental combinations of Gli-1 and Glu-1 involving simultaneously both chromosomes 1B and 1U; in these cases the one extract for Gpi analysis was run on both IEF and cellulose acetate. All samples analysed for Gpi were re-extracted with SDS and run on unreduced SDS-PAGE gels in order to confirm their Gli-1 phenotype, as the Gli-U1 bands were not totally clear in some gels, and also as a guard against accidental misnumbering of samples. Very few Gli-U1 phenotypes needed to be changed from their original classification after this step.

6.3. Results

6.3.1. Selection of F2 individuals homozygous *ph1b* and carrying endosperm protein markers for both chromosomes 1U and 1B (or 1D)

The F1 hybrids from crosses between substitution line (1B)1U x *ph1b* mutant and (1D)1U x *ph1b* mutant were expected to be monosomic for both 1U and the wheat chromosome replaced in the substitution line. The meiotic pairing of the hybrid involving the 1B substitution was as expected, showing most commonly 20" + 2', although an occasional trivalent or quadrivalent was seen and a few pmcs had as many as six univalents. Chromosome 1U remained unpaired in all pmcs analysed. The equivalent hybrid involving the 1D substitution was not meiotically analysed, as it was expected to have been less regular, since the *ph1b* mutant parent in this cross had been maintained as a homozygote over more generations than was the parent in the (1B)1U cross.

The somatic chromosome number of the selections from the F2 of the substitution line x *ph1b* mutant positive for both storage protein markers (*Gli-1* and *Glu-1* respectively) of chromosome 1U (assumed to mark the long and short arms of this chromosome) and for those of the relevant wheat chromosome, varied from 40 + iso to 44, as shown in Table 6.2. This variation is thought to have arisen primarily from irregular segregation of monosomes in progeny derived from parents of chromosome constitution 20" + $1'_{wheat}$ + $1'_{alien}$, as shown by several workers (Smith, 1963; Knott, 1964; Johnson, 1966; Gupta, 1969); male and female transmission rates of the univalents differ and the two most common zygotes possessing both chromosomes have the constitutions 20" + $1'_W$ + $1'_A$ and, less frequently, 21" + $1'_A$. Irregular meoitic configurations resulting from *Ph1* deficiency or accumulated translocations may also have contributed to this variation in somatic chromosome number, as illustrated by the more pronounced variation observed in the derivatives of the (1D)1U cross, which involved a *ph1b* mutant parent expected to be more affected by genome disturbance than that used in the (1B)1U cross.

Table 6.2. Somatic chromosome number of F2 progeny, bearing Gli-1 and Glu-1 proteins controlled by chromosomes 1U and either 1B or 1D respectively, selected from the crosses substitution line (1B)1U x *ph1b* mutant and (1D)1U x *ph1b* mutant.

	Somatic chromosome number				
Cross	40 +iso	41	42	43	44
(1B)1U x *ph1bph1b*	-	-	9	4	2
(1D)1U x *ph1bph1b*	1	2	7	5	-

As discussed in Chapter 5, the major criterion used for selection of *ph1b* homozygosity was a depression in chiasma frequency in the pmcs at metaphase I. Three presumptive *ph1bph1b* selections were made among the F2 plants carrying both storage protein markers of chromosomes 1U and 1B derived from the cross (1B)1U x *ph1b* mutant. The chiasma frequency in these plants was lower than the mean observed in eight sib plants presumed to be of constitution *Ph1bph1b* or *Ph1bPh1b* (i.e. *Ph1b* -). The lower chiasma frequency was manifested mainly by a higher average incidence of univalents and a marked increase in the proportion of rods to rings among the bivalents compared to the control (Table 6.3a). Although chromosome 1U usually remained unpaired at metaphase I (Figure 6.1a), a rod bivalent involving the cytologically distinct chromosome 1U was observed in two pmcs of plant 46-1 and in one pmc in plant 52-1 (Figure 6.1b), but not in plant 45-2, where only a low number of pmcs could be analysed (Table 6.3a). The homozygous *ph1b* status of these selections was confirmed by analysing the meiotic pairing in hybrids with *Ae. variabilis* (Chapter 5). High levels of homoeologous pairing were observed at metaphase I in pmcs of five, four and five such intergeneric hybrid plants obtained from plants 45-2, 46-1 and 52-1, respectively.

Among the F2 plants derived from the cross (1D) 1U x *ph1b* mutant, the depression in chiasma frequency of the presumptive *ph1bph1b* selections, compared to that of the *Ph1b* - controls, was less marked (Table 6.3b). It is possible that the lower average chiasma frequency in the control progeny is attributable to the more disturbed genomic

Table 6.3a. Mean chromosome configurations at metaphase I in pmcs of selected F2 plants from a cross between (1B)1U x *ph1b* mutant

Plant no.	Somatic chr. no.	univ.	biv. rod	biv. ring	triv.	quad.	> quad.	χ per pmc	No. pmcs
(i) *ph1bph1b* selections									
45-2	43	3.4 (2-5)*	4.1 (1-7)	14.0 (11-17)	0.47 (0-1)	0.47 (0-1)	-	34.5	17
46-1	42	3.3 (1-7)	4.5[a] (2-8)	14.4 (12-18)	0.13 (0-3)	0.11 (0-1)	0.02 (0-1)	34.5	53
52-1	43	2.5 (1-7)	5.6[b] (2-10)	14.2 (10-18)	-	0.17 (0-2)	0.02 (0-1)	34.6	41
(ii) *Ph1b* - : (mean of eight plants)									
	42	2.3 (1-6)	2.4 (0-7)	17.2 (13-20)	0.07 (0-1)	0.06 (0-1)	-	37.1	231

Table 6.3b. Mean chromosome configurations at metaphase I in pmcs of selected F2 plants from a cross between (1D)1U x *ph1b* mutant.

Plant no.	Somatic chr. no.	univ.	biv. rod	biv. ring	triv.	quad.	> quad.	χ per pmc	No. pmcs
(i) *ph1bph1b* selections:									
114-1	42	2.8 (1-8)	4.6 (1-9)	14.5 (9-18)	0.19 (0-1)	0.12 (0-1)	-	34.3	65
115-1	43	2.4 (1-9)	5.4[b] (1-9)	14.2[b] (9-20)	0.04 (0-1)	0.26 (0-1)	0.04 (0-1)	34.9	46
(ii) *Ph1b* - : (mean of four plants)									
	42	2.7 (2-6)	2.9 (0-9)	16.2 (10-20)	0.13 (0-1)	0.21 (0-1)	-	36.2	108

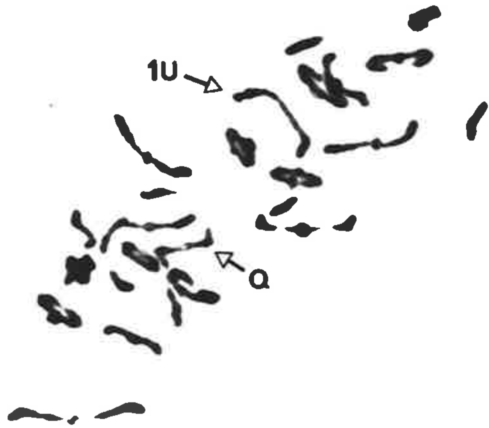
* range. [a] includes 2 pmcs with a bivalent involving 1U, [b] includes 1 pmc with a bivalent involving 1U.
univ. = univalents, biv. = bivalents, triv. = trivalents, quad. = quadrivalents, > quad = higher multivalents. χ = chiasmata

structure of the *ph1b* mutant parent used for this cross, compared to that used in the (1B)1U cross. Initially, four presumptive *ph1b* homozygotes were selected from this F2 population, but later, one of them (2n=43, mean chiasmata/pmc = 35.4) was shown to

a

b

c



d

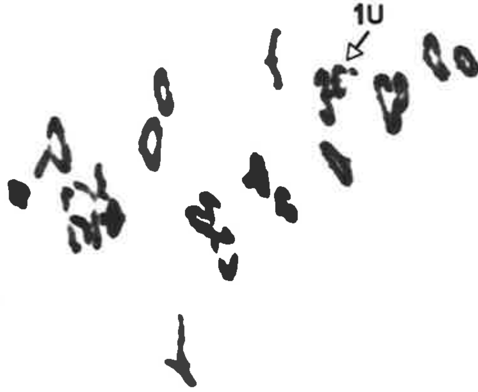


Figure 6.1. Chromosome configurations at metaphase I in pmcs of presumptive *ph1b* homozygotes carrying one dose of chromosome 1U, showing this chromosome (a) unpaired, and (b), (c), (d) paired.

(a), (b) from cross involving substitution (1B)1U;

(c), (d) from cross involving substitution (1D) 1U.

Pmc showing 1U unpaired:

(a) Pmc showing 4'+ 5('' + 10⊙''+ 3''' (T = trivalent)

Pmcs with 1U paired in a rod bivalent:

(b) Pmc with probable configuration 1' + 8('' + 13⊙''

(c) Pmc showing 4' + 8('' + 9⊙''+ 1^{iv} (Q = quadrivalent)

Pmc with 1U paired in a ring bivalent:

(d) Pmc showing 3' + 4('' + 16⊙''

have been *Ph1bph1b* as two of its hybrids with *Ae. variabilis* lacked homoeologous pairing in pmcs at metaphase I; while a second (2n = 40 + iso, mean chiasmata/pmc = 32.2) was only marginally self-fertile and gave too few progeny to be further analysed. Plant 114-1 was shown to have been *ph1bph1b* when all five hybrids with *Ae. variabilis* exhibited homoeologous pairing at metaphase I. Only a single viable hybrid with *Ae. variabilis* was produced from plant 115-1. Although this plant showed homoeologous metaphase I pairing, this was insufficient evidence to conclude that plant 115-1 was of genotype *ph1bph1b*. However the observation of a rod bivalent involving chromosome 1U in one pmc, and a ring bivalent in another pmc (Table 6.3b; Figures 6.1c, d) provided strong evidence that this plant was in fact *ph1bph1b*.

6.3.2. Endosperm storage protein and glucose phosphate isomerase phenotype of the T and C populations

6.3.2.1. Endosperm storage protein phenotype of progeny from the (1B)1U substitution line

All three presumptive *ph1b* homozygotes selected from the F_2 of the cross (1B)1U x *ph1b* mutant were highly self-fertile. A total of 1322 seeds (499, 400 and 423 from each of the three F_3 families 45-2, 46-1 and 52-1 respectively) were analysed by SDS-PAGE to determine their Gli-1 and Glu-1 phenotypes (Figure 6.2A, B). Residual seed not analysed remained in each family. The protein markers associated with chromosome 1U segregated in each family, but those on chromosome 1B did not segregate in the progeny of plant 52-1, and this 43 chromosome plant (Table 6.3a) was thought therefore to be disomic for 1B. As the two non-homologous chromosomes 1B and 1U are expected to only pair infrequently in *ph1b* homozygotes, and not at all in the control *Ph1b ph1b* parent, the segregation of the markers controlled by these chromosomes can be considered separately in the two plants where both chromosomes were segregating. This

segregation of endosperm protein markers gave rise to four phenotypic classes for both the 1B and the 1U controlled proteins (Table 6.4).

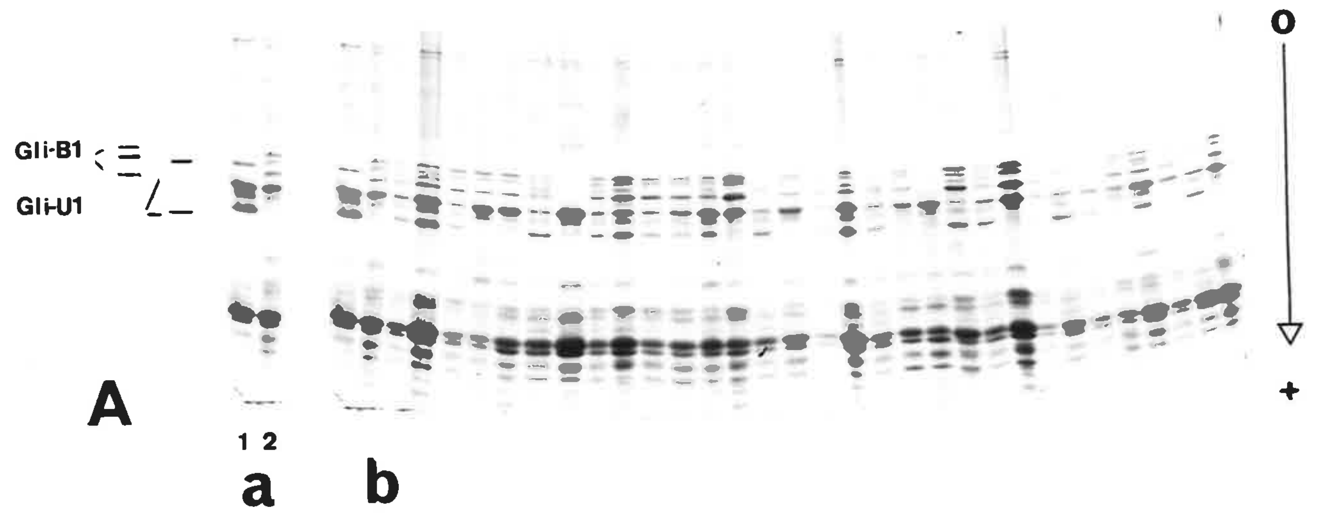
It was not possible to pool the data from all three T populations as the segregation pattern in the progeny of plant 52-1 is clearly different from that found in the other two families. However, the segregation patterns of the progeny of plants 45-2 and 46-1 were homogeneous for the chromosome 1U classification (χ^2=0.30, 3 d.f. 0.95<p<0.98), but since they were marginally heterogeneous for the chromosome 1B classification (χ^2=11.25, 3 d.f. 0.01<p<0.02), the data for these two families has not been pooled in Table 6.4. The pooled data obtained from the two T populations 45-2 and 46-1 were clearly not homogeneous with those from the C population (progeny of plant 178-2) for either the chromosome 1B (χ^2=38.2, 3 d.f. p<0.01) or for the chromosome 1U classifications (χ^2=16.6, 3 d.f. p<0.01).

Table 6.4. Endosperm storage protein phenotypes and their frequencies in the T and C populations derived from the cross (1B)1U x *ph1b* mutant.

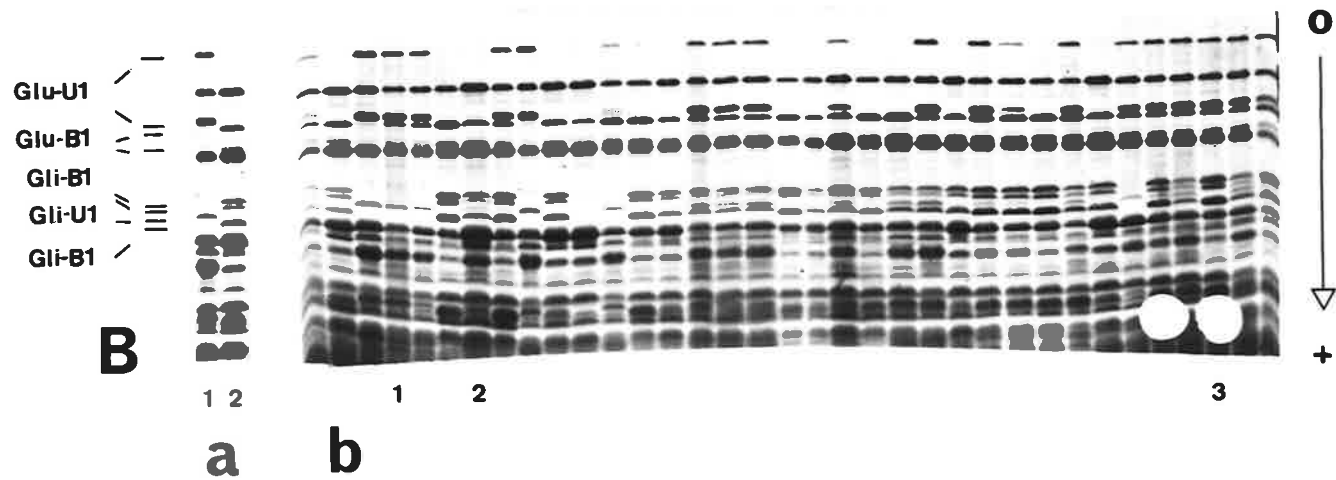
Endosperm storage protein phenotype

	Glu-B1	+	-	+	-	Glu-U1	+	-	+	-	
	Gli-B1	+	-	-	+	Gli-U1	+	-	-	+	Total
(i) T populations :											
Family 45-2		430	22	40	7		287	159	24	29	499
Family 46-1		315	32	39	14		231	123	22	24	400
Total		745	54	79	21		518	282	46	53	899
%		82.9	6.0	8.8	2.3		57.6	31.4	5.1	5.9	
Family 52-1		No segregation					163	212	22	26	423
%							37.7	49.1	5.1	6.0	
(ii) C population :											
Family 178-2*		398	64	60	13		267	221	21	21	530
%		74.4	12.0	11.2	2.4		50.4	41.7	4.0	4.0	

* 5 individuals analysed for 1B phenotype not analysed for 1U phenotype
T: *ph1bph1b*- derived populations C: *Ph1bph1b*- derived population



Gli-B1
Gli-U1
o
+
A
1 2
a
b



Glu-U1
Glu-B1
Gli-B1
Gli-U1
Gli-B1
o
+
B
1 2
1
2
3
a
b

Figure 6.2. Endosperm protein phenotype of parents and segregating progeny derived from the cross substitution (1B)1U x *ph1b* mutant. Patterns obtained by (A) unreduced, and (B) reduced SDS-PAGE.

A

a - parental phenotypes: 1. (1B)1U (Gli-B1$^-$ Gli-U1$^+$)

2. Euploid Chinese Spring (CS) (Gli-B1$^+$ Gli-U1$^-$)

b - progeny phenotypes

B

a - parental phenotypes: 1. (1B)1U (Glu-B1$^-$ Glu-U1$^+$)

2. Euploid CS (Glu-B1$^+$ Glu-U1$^-$)

b - progeny phenotypes 1. Glu-B1$^+$ Glu-U1$^+$ Gli-B1$^-$ Gli-U1$^+$

2. Glu-B1$^+$ Glu-U1$^-$ Gli-B1$^+$ Gli-U1$^+$

3. Glu-B1$^+$ Glu-U1$^+$ Gli-B1$^+$ Gli-U1$^-$

6.3.2.2. Segregation of marker loci

Before analysing the pattern and frequency of recombination in these populations, two points of interest arising from these segregation data will be considered. The relative frequency of the Glu-B1$^+$ Gli-B1$^+$ phenotype is somewhat higher in the T populations than in the control population (82.9% against 74.4%, Table 6.4). It is suggested that this difference is due to the formation of occasional homoeologous bivalents involving the 1B monosome in the plants where *Ph1* is absent; thus, whereas chromosome 1B always remains unpaired in the meiocytes of a *Ph1bph1b* plant, so that the univalent is distributed randomly to the gametes and is frequently lost, in *ph1bph1b* individuals the chromosome has a chance of becoming involved in a bivalent (or multivalent), and normal disjunction of these homoeologous chromosome associations will result in a higher probability of the chromosome reaching the pole at anaphase I. A similar argument can be applied to explain the higher frequency of the Glu-U1$^+$ Gli-U1$^+$ phenotype observed in the T as opposed to the C populations (57.6% compared to 50.4%, Table 6.4). While direct cytological observation of homoeologous bivalents involving chromosome 1B was not possible, chromosome 1U was seen paired with a wheat chromosome in 3 out of 111 pmcs analysed in the three *ph1bph1b* parents (Table 6.3a).

In both the T and the C populations, the proportion of individuals having both endosperm storage protein markers of chromosome 1U is much less than that having both the chromosome 1B markers. In the C population, the Glu-1$^+$ Gli-1$^+$ phenotype controlled by a particular chromosome arises from transmission of at least one dose of this chromosome to the progeny seed. It can therefore be concluded that chromosome 1B is transmitted more frequently than chromosome 1U. Although chromosome 1U compensates well for the absence of chromosome 1B as a disomic substitution (Shepherd, 1973), it is apparent that substitution gametes do not compete successfully against normal gametes, where both gametic species arise in the same plant. Sears (1956) found little differential selection between 21 and 22 chromosome female gametes derived

from a monosomic addition line of a different *Ae. umbellulata* chromosome, and it is thus assumed that the gametic selection probably occurs in the male gamete through competition between pollen grains at fertilisation.

In both the 45-2 and the 46-1 families the relative frequency of the phenotype Glu-U1$^+$ Gli-U1$^+$ was almost twice that of Glu-U1$^-$ Gli-U1$^-$, but in the progeny of plant 52-1, which did not segregate for chromosome 1B, the recovery of the Glu-U1$^+$ Gli-U1$^+$ phenotype was less than that of Glu-U1$^-$ Gli-U1$^-$. Two factors are thought to be responsible for this difference. In the first place, plant 52-1 was a monosomic alien addition individual, and thus its gametes (excluding the effects of homoeologous pairing) would have a chromosome number of either 21 or 22. Certation of pollen would be expected to ensure that the 21 chromosome male gamete competed more successfully than the addition gamete, thus reducing male transmission of the alien chromosome. Furthermore, as the plant was disomic for chromosome 1B, there would be fewer homoeologous univalents available for allosyndetic pairing with the 1U monosome. Thus chromosome 1U would remain unpaired at meiosis more frequently in this plant than in the double monosomic plants 45-2 and 46-1, and therefore the female transmission of chromosome 1U would also be reduced in comparison with that from plants 45-2 and 46-1.

6.3.2.3. Analysis of progeny with dissociated endosperm protein markers

A small number of progeny in both the T and the C populations had protein phenotypes where the Gli-1 and Glu-1 markers had become dissociated. In the control population, 13.6% had dissociated 1B markers, compared to only 11.1% of the progeny of plants 45-2 and 46-1 (Table 6.4). In the absence of allosyndetic recombination, dissociation of *Glu-B1* from *Gli-B1* can only occur through chromosome misdivision, and it was surprising that this dissociation was more frequent in the C than in the T populations, where both recombination and misdivision can occur. It is thought that this anomaly is a

consequence of the lack of pairing of chromosome 1B in the meiocytes of the *Ph1bph1b* parent, while some pairing does presumably occur in the *ph1bph1b* parents; thus 1B can be expected to misdivide more frequently in the former plant, giving rise to more progeny with dissociated protein markers. Most of these selected progeny are of phenotype Glu-B1^{+} Gli-B1^{-} rather than Glu-B1^{-} Gli-B1^{+}, and this is probably due to differences in the transmission rate of the two opposite arm telocentrics.

Misdivision products of chromosome 1U were also obtained in the C population, but these occurred less frequently than with chromosome 1B (8.0% compared to 13.6%, Table 6.4). The lower recovery rate of individuals having dissociated 1U markers may be due to infrequent misdivision of chromosome 1U (Shepherd, pers. comm.) but it is not possible to use these data to compare the true misdivision rates of the two chromosomes, as the lower overall transmission of chromosome 1U is likely to result in an underestimate of its rate. The preponderance of the supposed long arm 1B telocentric (phenotype Glu-B1^{+} Gli-B1^{-}) among the misdivision products is not repeated for 1U, as there was an equal frequency of each of the two reciprocal dissociated protein phenotypes. Since there appears to be little selection against transmission of complete chromosome 1U in female gametes (and hence probably of its misdivision products also), and male transmission is low, there is no reason to expect that long arm telocentrics would be favoured over short arm telocentrics. Although the relative frequency of progeny having dissociated 1B markers was greater in the control population than in the T populations, the opposite is the case with the 1U markers, and this is taken to reflect the low frequency of misdivision (or low transmission of misdivision products) of this chromosome. The relative frequency of dissociation of 1U markers was similar in the two types of T population (45-2/46-1 and 52-1, hereafter referred to as T1 and T2, respectively), and the occurrence of the Glu-U1^{+} Gli-U1^{-} phenotype was slightly, although consistently, less frequent than that of the reciprocal type.

The frequency of a single homoeologous cross-over in the segment *Glu-1* to *Gli-1* was expected to be low, and therefore the chance of a double cross-over event was initially

discounted. The result of a single cross-over in this segment would be the dissociation of *Glu-1* and *Gli-1* and therefore only progeny of phenotype Glu-1$^+$ Gli-1$^-$ or Glu-1$^-$ Gli-1$^+$ were initially analysed for their Gpi phenotype (Figure 6.4). Extrapolating from the linkage data available for chromosome arm 1DS (Chojecki *et al.*, 1983; Koebner, unpubl.), the structural genes *Gpi-B1* and *Gpi-U1* are expected to lie on the short arm of their respective chromosome, between the centromere and *Gli-1* .

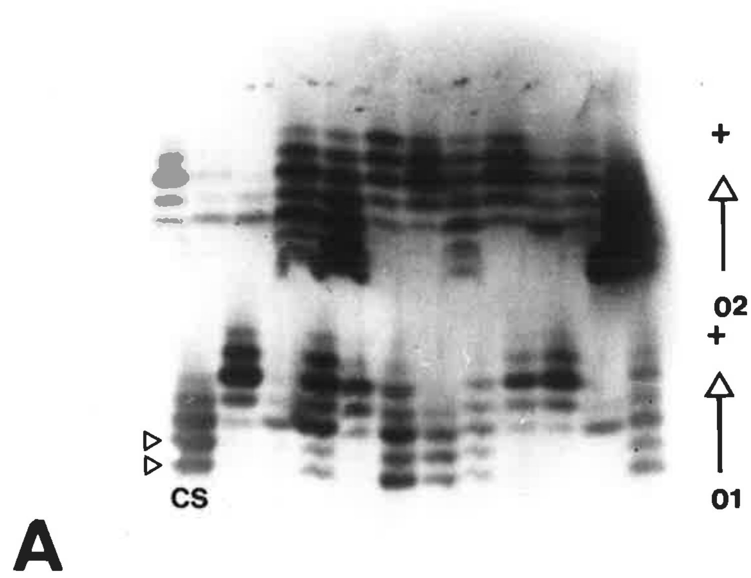
From the C population, 73 progeny with dissociated chromosome 1B markers and 42 with dissociated 1U markerswere detected. Among the T populations, there were 100 such 1B selections and 147 1U selections (Table 6.4). The Gpi phenotypes of these individuals are given in Table 6.5.

Table 6.5. Gpi phenotypes of individuals selected as having non-parental combinations of endosperm protein markers in the T and C populations.

	Non-parental phenotype									
	(Glu-B1$^+$ Gli-B1$^-$)		(Glu-B1$^-$ Gli-B1$^+$)			(Glu-U1$^+$ Gli-U1$^-$)		(Glu-U1$^-$ Gli-U1$^+$)		
	Gpi-B1$^-$	Gpi-B1$^+$	Gpi-B1$^+$	Gpi-B1$^-$		Gpi-U1$^-$	Gpi-U1$^+$	Gpi-U1$^+$	Gpi-U1$^-$	
(i) T populations:					Total					Total
Family 45-2	36	4	6	1		13	11	21	8	
Family 46-1	30	9	13	1		12	10	20	4	
Family 52-1						18	4	21	5	
Total	66	13	19	2	100	43	25	62	17	147
(b) C population:										
Family 178-2	37	0	11	0	48*	21	0	21	0	42

* 25 individuals in these endosperm protein classes not scored for Gpi-B1
T: *ph1bph1b* -derived populations C:*Ph1bph1b*- derived population

Due to a technical fault in the electrophoretic procedure, a number of the 1B selections from the C population could not be scored for the presence of Gpi-B1, but all of the 48 analysed retained the linkage *Gpi-B1* - *Gli-B1* as did all 42 of the 1U selections


CS
+
02
+
01
A

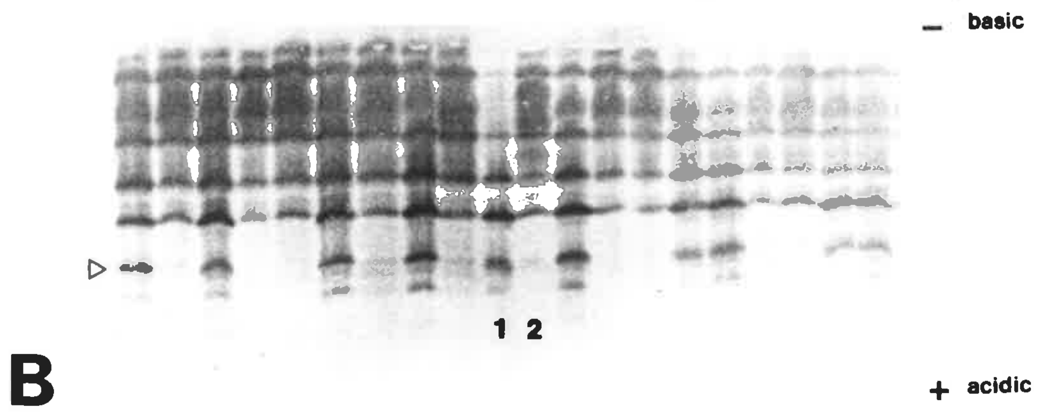

— basic
1 2
B
+ acidic

Figure 6.3. Glucose phosphate isomerase phenotypes of parents and segregating progeny derived from the cross substitution (1B)1U x *ph1b* mutant.

A. Cellulose acetate zymogram.

CS - Euploid Chinese Spring.

Other samples represent progeny segregating for *Gpi-B1* and *Gpi-U1*.

Bands controlled by *Gpi-B1* shown by

01: loading start for bottom row of samples; 02: loading start for top row of samples.

B. Isoelectric focussing zymogram (nominal pH gradient 3-10).

Parental phenotypes: 1. Substitution (1B)1U 2. Euploid Chinese Spring

Other samples represent progeny segregating for *Gpi-U1*.

Band used for scoring Gpi-U1 phenotype shown by ▷

for *Gpi-U1* - *Gli-U1* (Table 6.5). These observations are consistent with the expectation that all of the progeny of plant 178-2 with dissociated endosperm protein markers resulted from chromosome misdivision, and not allosyndetic recombination. In contrast, many of the selections obtained from the *ph1bph1b* families showed a disruption in the *Gpi-1* - *Gli-1* association, and these individuals must therefore have originated from allosyndetic recombination, presumably as a result of the loss of normal *Ph1* activity. The majority of the selections derived from the *ph1bph1b* parents had the parental combinations of these short arm markers - 85% for chromosome 1B and 71.4% for chromosome 1U (Table 6.5). This group of progeny will include all of the misdivision chromosomes, and any products of allosyndetic recombination in the interval *Glu-1* - *Gpi-1* , which are indistinguishable from misdivision products in the absence of other markers in this chromosome segment. Out of the 100 selected progeny involving chromosome 1B, only 15 were recombinants in the segment *Gpi-B1* - *Gli-B1* , with 13 retaining *Glu-B1* and *Gpi-B1* but lacking *Gli-B1* , while 2 were of the reciprocal type (Table 6.5). Assuming that a single cross-over gave rise to these recombinants, the Glu-B1$^+$ Gpi-1$^+$ Gli-B1$^-$ individuals retain all of the long arm and a segment of the short arm of 1B, while the reciprocal type retains much less 1B chromatin. A possible explanation for the unequal recovery of these reciprocal recombinant phenotypes is that the one retaining more native 1B chromatin is more easily transmitted than the other type through the male gametes.

The rate of recombination between the *Gpi-U1* and the *Gli-U1* loci on chromosome arm 1US was, surprisingly, much more frequent than that observed for the equivalent segment of chromosome 1B. Among 147 selected individuals, 42 (29%) were recombinants in this interval (Table 6.5) and there is little difference between the frequencies of the reciprocal phenotypes (25 versus 17), in contrast to the observations with the recombined chromosome 1B, mentioned above.

A total of 24 individuals (6 from the control population, 11 derived from plant 45-2 and seven from plant 46-1) possessed dissociated endosperm protein markers controlled

by both chromosome 1B and 1U. It is possible that, following simultaneous misdivision of chromosomes 1B and 1U, several of these individuals carry centric fusion products, which arise frequently when two monosomes misdivide in the same meiocyte (Sears, 1972a). These chromosomes may be favoured in gametic transmission over telocentrics, as gametes carrying centric fusion products will often be more genetically balanced than those bearing a telosome. There is, however, no cytological evidence yet available to substantiate this possibility. Among the T populations, one selection carries a recombined chromosome 1B and a recombined chromosome 1U, having the phenotype Glu-B1^{+} Gpi-B1^{+} Gli-U1^{+}. This phenotype is consistent with a plant containing a chromosome consisting of the long arm and a segment of the short arm of 1B linked to the distal segment of 1U. Many of the other recombinants may involve chromosome 1B/1U exchanges, but the recombinant phenotype of such chromosomes will only be expressed when neither an intact chromosome 1B nor 1U are present in the other gamete, and the transmission rate of such nullisomic gametes may be low.

As indicated earlier, the analysis of recombination was based on the assumption that the frequency of a homoeologous single cross-over would be so low that the chance of a double cross-over would be negligible. In order to test this assumption, a sample of the both the T1 and the T2 populations having parental combinations of storage protein markers (i.e. Glu-1^{+} Gli-1^{+} and Glu-1^{-} Gli-1^{-}), was analysed for Gpi phenotype. A larger sample was taken from the T1 population rather than from the T2 population as the single homoeologous cross-over frequency was greater in the former populations. Both Gpi-U1 and Gpi-B1 were scored on IEF gels for this purpose. If no double cross-overs had occurred, all progeny which possessed both Glu-B1 and Gli-B1 would also possess Gpi-B1, while those individuals lacking these storage proteins would also lack Gpi-B1, and similarly for the loci controlled by chromosome 1U. A sample of 211 of these progeny derived from the T1 population, and of 116 from theT2 population, were scored for Gpi phenotype, and while the majority of these showed no evidence of the occurrence of double cross-overs, there were 12 individual progeny which must have derived from a

double cross-over along chromosome 1U, and 4 from a double cross-over along 1B from the first family, and 5 double cross-overs along 1U in the second family. The phenotypes of these 21 individuals are shown in Table 6.6. The higher frequency of recovery of homoeologous double cross-overs involving chromosome 1U rather than 1B is consistent with the greater frequency of single cross-overs in the interval *Gpi-U1 - Gli-U1* compared to those in the interval *Gpi-B1 - Gli-B1* , as noted above.

Table 6.6. Frequency and phenotype of individuals resulting from a homoeologous double cross-over involving (a) chromosome 1B, and (b) chromosome 1U, sampled from the T1 and T2 populations.

	Endosperm phenotype		%
(a) Chromosome 1B	(Glu-B1^{+} Gpi-B1^{-} Gli-B1^{+})	(Glu-B1^{-} Gpi-B1^{+} Gli-B1^{-})	occurrence
T1 population	1	3	1.9
(b) Chromosome 1U	(Glu-U1^{+} Gpi-U1^{-} Gli-U1^{+})	(Glu-U1^{-} Gpi-U1^{+} Gli-U1^{-})	
T1 population	5	7	5.7
T2 population	2	3	4.3

T1: progeny of 42 chromosome *ph1bph1b* plants 45-2 and 46-1
T2: progeny of 43 chromosome *ph1bph1b* plant 52-1

6.3.2.4. Recombination in progeny from the (1D)1U substitution line

Although a large quantity of seed was produced on the two F2 plants selected as *ph1bph1b* from the cross (1D)1U x *ph1b* mutant, only a sample of 268 progeny from each selection was analysed, to check if they gave similar results to those obtained with the (1B)1U cross described above. The equivalent F2 control population was also available, but no attempt was made to analyse this population, as the previous experiment

had already demonstrated that no allosyndetic recombination occurs in the presence of one dose of *Ph1*. The observed frequencies of the different storage protein phenotypes determined by SDS-PAGE are listed in Table 6.7, and examples of the gels which were used to score these patterns are illustrated in Figure 6.3. Progeny of plant 115-1 (shown to have a somatic chromosome number of 43, Table 6.3) did not segregate for either of the storage proteins controlled by chromosome 1D and this plant was therefore thought to be disomic for chromosome 1D. However progeny from both of the selected plants segregated for the 1U controlled storage proteins.

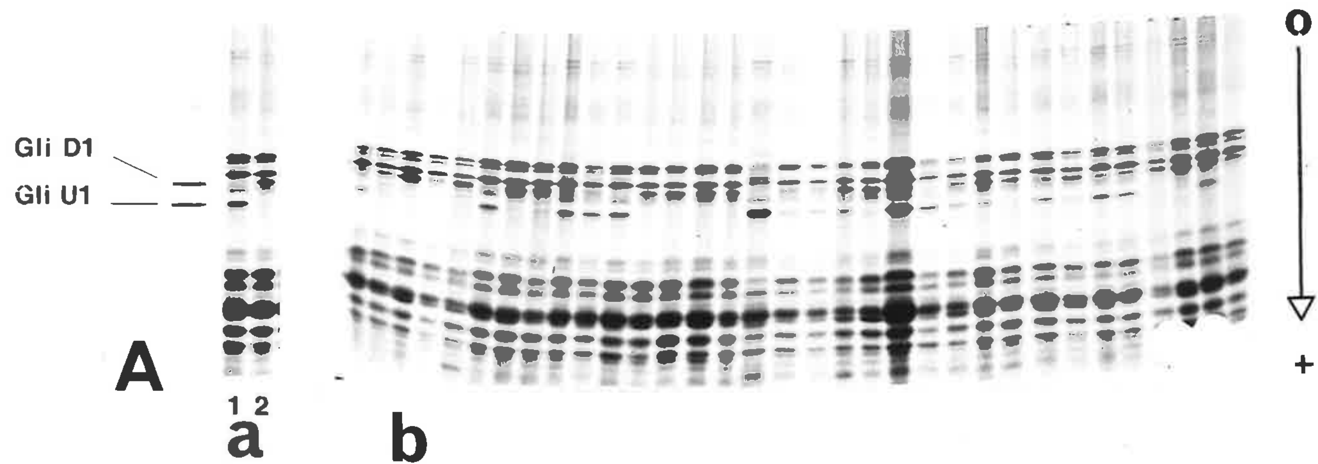
Table 6.7. Storage protein phenotypes and their frequencies in progeny of two *ph1bph1b* plants selected in the F_2 generation from the cross (1D)1U x *ph1b* mutant.

	Endosperm protein phenotype									
Glu-D1	+	-	+	-		Glu-U1 +	-	+	-	
Gli-D1	+	-	-	+		Gli-U1 +	-	-	+	
Family					Total					Total
114-1	225	11	23*	9	268	118	110	12	28	268
115-1	No segregation					92	163	6	7	268

+: protein present -: protein absent

* includes 2 individuals Tri-1$^+$ Gli-U1$^-$ Glu-U1$^-$

The relative distribution of the 1D controlled phenotypic classes in the progeny of plant 114-1 was similar to that observed for the 1B controlled phenotypes in the T1 populations (compare Tables 6.4 and 6.7). Most of the progeny (83.9%) carried both 1D markers, and only few (4.1%) lacked both markers. Among the selections having dissociated 1D markers, most were Glu-D1$^+$ Gli-D1$^-$, rather than Glu-D1$^-$ Gli-D1$^+$ (8.6% versus 3.4%). Two individuals lacking Gli-D1, Gli-U1 and Glu-U1 but possessing Glu-D1 were found to also have the protein Tri-1, controlled by either the structural gene *Tri-D1* in wheat (Singh and Shepherd, 1985) or by the homoeolocus *Tri-U1* in *Ae.*


Gli D1
Gli U1
A
1 2
a
b
o
+

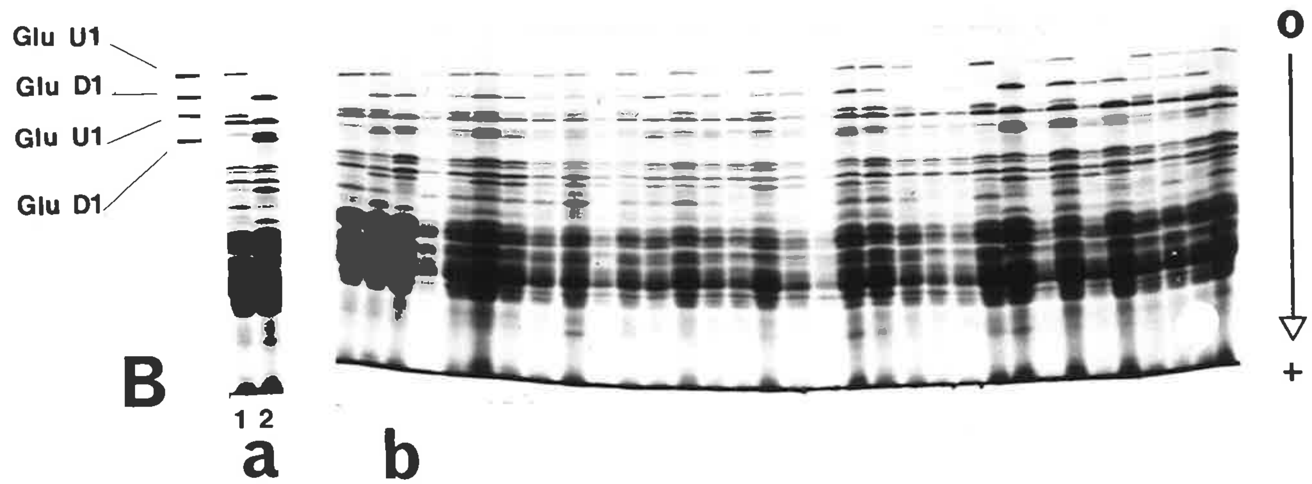

Glu U1
Glu D1
Glu U1
Glu D1
B
1 2
a
b
o
+

Figure 6.4. Endosperm protein phenotype of parents and segregating progeny derived from the cross substitution (1D)1U x *ph1b* mutant. Patterns obtained by (A) unreduced, and (B) reduced SDS-PAGE.

A

a - parental phenotypes:

1. (1D)1U (Gli-D1$^-$ Gli-U1$^+$)
2. Euploid Chinese Spring (CS) (Gli-D1$^+$ Gli-U1$^-$)

b - progeny phenotypes

B

a - parental phenotypes:

1. (1D)1U (Glu-D1$^-$ Glu-U1$^+$)
2. Euploid CS (Glu-D1$^+$ Glu-U1$^-$)

b - progeny phenotypes

umbellulata. It is not therefore possible to identify which *Tri-1* locus these two recombinant individuals possess, but the absence of Glu-U1 suggests that they are recombinants involving 1DS in the interval *Tri-D1 - Gli-D1*, similar to the type I recombinants recovered in the rye short arm experiments (Chapter 4).
The progenies from the 42 and 43 chromosome plants 114-1 and 115-1, respectively, gave different segregation patterns for the 1U controlled proteins, similar to the differences observed earlier with the T1 and T2 populations arising from the (1B)1U cross. Thus the plant which was monosomic for chromosome 1D gave a higher proportion of progeny containing all or part of chromosome 1U (59%) than the disomic 1D parent where this proportion was only 39% (Table 6.7). Among the individuals having dissociated 1U storage protein phenotypes, the Glu-U1$^-$ Gli-U1$^+$ phenotype was more frequent than its reciprocal phenotype (13.1% versus 6.7%). A preponderance of this phenotype, although less marked, was also noted in the progeny from the (1B)1U cross.

The 1D chromosomes showing a dissociated storage protein phenotype were not analysed further. However, the 53 individuals with dissociated 1U markers (18 Glu-U1$^+$ Gli-U1$^-$ and 35 Glu-U1$^-$ Gli-U1$^+$) were scored for their Gpi-U1 phenotypes, and the results are given in Table 6.8. As discussed earlier, individuals with phenotypes Glu-U1$^+$ Gpi-U1$^-$ Cli-U1$^-$ and Glu-U1$^-$ Gpi-U1$^+$ Gli-U1$^+$ include products both of chromosomal misdivision and allosyndetic recombination, and as expected, these classes were more frequent than the corresponding classes containing chromosomes recombined in the interval *Gpi-U1 - Gli-U1* (10 + 20 versus 8 +15, Table 6.8). This follows a similar pattern to that observed in the (1B)1U derived material (compare Tables 6.8 and 6.5). Thus, a total of 23 further recombinants involving the segment of chromosome 1U between *Gpi-U1* and *Gli-U1* were identified in this experiment.

Table 6.8. Gpi phenotypes of progeny selected as having non-parental combinations of endosperm protein markers in two *ph1bph1b*- derived families.

	Non-parental phenotype				
	(Glu-U1^{+} Gli-U1^{-})		(Glu-U1^{-} Gli-U1^{+})		
	Gpi-U1^{-}	Gpi-U1^{+}	Gpi-U1^{+}	Gpi-U1^{-}	
114-1	6	6	14	14	
115-1	4	2	6	1	
Total	10	8	20	15	53

6.4. Discussion

It would be of interest to derive an estimate of the recombination frequency induced by *ph1bph1b* between the various marker loci on chromosome 1U, to compare with the results achieved for rye chromosomes 1RS (Chapter 4) and 1RL (Chapter 5). However, to obtain such an estimate, the transmission rate of a complete, unaltered chromosome 1U through both the male and female gamete in a plant monosomic for this chromosome needs to be known, for the presence of such a complete chromosome 1U in a progeny seed will mask the presence of a recombinant chromosome in the same individual. The analysis of the segregation of a wheat and an alien chromosome, present as two monosomes, is complex when the plant is allowed to self-fertilise. However, studies of such systems indicate that if the alien chromosome can successfully substitute for the wheat monosome (i.e. the wheat and alien chromosomes are homoeologous), then the transmission of the alien chromosome through the female gamete is approximately 1/4, while the male transmission rate is 4/7 (Knott, 1964). The analysis of the present material is complicated by the occurrence of homoeologous pairing which reduces the frequency with which the two monosomes remain as univalents at metaphase I. It is therefore only possible to derive an approximate estimate of the frequency of allosyndetic recombination achieved between chromosome 1U and chromosomes of the wheat genome, and this estimate must be treated with some caution.

The observed relative frequencies of the various chromosome 1U controlled phenotypes recovered from the pooled progeny of the 42 chromosome plants 45-2 and 46-1 (T1 population) and from the progeny of the 43 chromosome plant 52-1 (T2 population) are shown separately in Table 6.9, as the distributions of these phenotypes have been shown to be different. The relative frequency of the chromosomes classified as parental ("+++" and "---") was adjusted in Table 6.9 by subtracting that of the double cross-overs which have the same storage protein phenotype. Taking m_0 and f_0 to be the relative frequencies with which male and female gametes lacking chromosome 1U function, equating the expected relative frequency of the phenotypic class "---" (m_0f_0)

Table 6.9. Relative frequencies of chromosome 1U controlled phenotypes in the T1 and T2 populations.

Progeny phenotype			Relative frequency	
Glu-U1	Gpi-U1	Gli-U1	T1	T2
-	-	-	0.282	0.475
+	+	+	0.552	0.368
+	-	-	0.028	0.043
-	+	+	0.046	0.050
+	+	-	0.023	0.009
-	-	+	0.013	0.012
+	-	+	0.024	0.017
-	+	-	0.032	0.026

+: presence of protein -: absence of protein
T1: pooled progeny of 42 chromosome *ph1bph1b* plants 45-2 and 46-1
T2 : progeny of 43 chromosome *ph1bph1b* plant 52-1

with its observed frequency and taking f_0 to be 0.75 (Sears, 1954; Tsunewaki, 1963), this gives an estimate for m_0 of 0.376 and 0.633 for each type of population, respectively. The male transmission rate of the alien monosome from the double monosomic 42 chromosome plants is thus estimated to be 0.624 (1 - m_0), and this accords well with the theoretical value of 4/7 (0.571).

Metaphase I pairing data is known to be a poor indicator of genetic recombination, since desynaptic univalents which were paired at prophase I are indistinguishable from asynaptic univalents which had never been paired (Fu and Sears, 1973). Homologous segments of alien chromosomes have been observed often to exhibit pairing failure when in a wheat background (Singh and Shepherd, 1984), and it is therefore likely that homoeologous bivalents are prone to such desynapsis, which is taken to explain the discrepancy between the low frequency of observed metaphase I bivalents involving chromosome 1U (Table 6.3) and the higher than expected numbers of recombinants. While it is recognised that desynapsis will result in differences in transmission of recombinant chromosomes through the male and female gametes, for simplicity it has been assumed in this analysis that the transmission of the two resulting recombinant chromosome types (phenotypes Gpi-U1$^+$ Gli-U1$^-$ and Gpi-U1$^-$ Gli-U1$^+$) is independent

of whether the gamete containing the recombinant chromosome is male or female. A recombinant gamete will be detected only if the other gamete is null for chromosome 1U, so that the expected relative frequency of such recombinants is given by $(m_0 + f_0)r$, where r is the probability of such a recombinant being formed and transmitted. Since chromosome 1U substitutes well for 1B (Shepherd, 1973; Chapman *et al.*, 1975), it is reasonable to assume that there is little selection against recombinant gametes, and thus r should represent a good estimate of the actual rate of allosyndetic recombination. The expression $(m_0 + f_0)r$ has been used to estimate the values of r for each of the two recombinant types in both the T1 and the T2 populations (Table 6.10) by equating it to the observed relative frequencies of cross-overs in the interval *Gpi-U1 - Gli-U1*. The frequency of occurrence of recombined progeny from the 42 chromosome (double monosomic) is nearly double that for the 43 chromosome (monosomic addition) plants. This result stresses the value of providing an alien chromosome with an unpaired homoeologue, and suggests that many of the recombinants in the T1 population involve exchange between chromosomes 1U and 1B.

Table 6.10. Estimated relative frequencies of allosyndetic recombination in the interval *Gpi-U1 - Gli-U1* on chromosome 1U in the T1 and T2 populations.

	T1	T2
r_1	0.048	0.025
r_2	0.032	0.021
total	0.080	0.046

T1: pooled progeny of 42 chromosome *ph1bph1b* plants 45-2 and 46-1
T2 : progeny of 43 chromosome *ph1bph1b* plant 52-1
r_1: refers to phenotype Gpi-U1$^+$ Gli-U1$^-$ r_2: refers to phenotype Gpi-U1$^-$ Gli-U1$^+$

The much higher allosyndetic recombination rate achievable with *Ae. umbellulata* chromosome 1U compared with rye chromosome 1RL is consistent with the extensive cytological evidence that rye chromosomes pair infrequently in *Ph1* deficient genotypes, while *Aegilops* spp. chromosomes can easily be induced to pair with wheat

homoeologues (reviewed in Chapter 2.5). The gametic recombination frequency over a long segment of the rye arm was estimated to be only 1.4%, whereas a much higher rate was estimated to occur over a relatively short segment of the *Aegilops* chromosome. Furthermore double cross-overs were detected in the interval *Glu-U1* - *Gli-U1*. This difference presumably reflects the closer relationship of the U genome, compared to that of the R genome, with the genomes of hexaploid wheat. Kimber (1967b) observed no evidence of allosyndetic pairing in the production of the wheat-*Ae. umbellulata* addition lines, but such pairing would have been suppressed by *Ph1* . However, some allosyndetic pairing was observed by Sears (1956) in the F_1 plants from the cross *T. aestivum* x (*T. dicoccoides* x *Ae.umbellulata*) and this was assumed to have involved pairing between the chromosomes of the D and U genomes.

It is of interest that, after correction of the chromosome 1U totals by subtracting the number of recombinants recovered in the progeny of plant 52-1 which did not segregate for 1B, the relative frequency of recombinants recovered in the interval *Gpi-1* - *Gli-1* was greater for chromosome 1U than for 1B (Table 6.5). Moreover, the relative frequency of double cross-overs was substantially higher for chromosome 1U as compared to chromosome 1B (Table 6.6). These observations are consistent with the hypothesis that chromosome 1U is more closely related to its wheat homoeologues than is chromosome 1B. Cytological evidence for the close relationship between the chromosomes of the U and D genomes has been provided by Kihara (1949), who found up to five bivalents per pmc in the hybrid *Ae. umbellulata* x *Ae. squarrosa* , the species now accepted to be the progenitor of the D genome in hexaploid wheat (Morris and Sears, 1967). The identical electrophoretic patterns of the gene products of the homoeoloci *Tri-D1* and *Tri-U1* provides some biochemical support to the hypothesis of a close relationship between chromosomes 1U and 1D. The finding that there are differences in pairing affinity between rye chromosome 1R and wheat chromosomes 1A and 1B (Naranjo, 1982) suggests that these two wheat chromosomes are not 'equidistant' in an evolutionary sense from chromosome 1R, and a similar difference may apply within the group of chromosomes 1A, 1B, 1D and 1U. Cytological studies with chromosome 1U,

similar to those of Naranjo (1982), are required to test this hypothesis.

In a chromosome 1D mapping experiment (Koebner, unpubl.), allelic variation between two wheat cultivars in *Gpi-D1* and *Gli-D1* on chromosome 1DS gave a recombination value of 25.6%, or three times greater than the rate estimated for homoeologous recombination along chromosome 1U (8.0%, Table 6.10). This reduction in the rate of recombination between two well-spaced markers underlines the difficulty of separating loci which are closely linked on an alien chromosome, without recourse to large progeny populations. Screening of large numbers of progeny requires highly efficient, rapid and reliable methods such as those described in this work, where approximately 2400 individual seeds were analysed. Many of the markers, biochemical, molecular and especially cytological, so far described in wheat, require techniques for their identification which are too time-consuming to be of general use in alien introgression. The procedures for these markers will need to be greatly streamlined before they can become applicable in this field.

A major feature of the populations produced in this study is that the two storage protein markers used initially to isolate single cross-over events are distantly separated genetically, so that any new marker obtained, which is interstitial to these two loci, can be applied to the same population, which is still in the form of dormant seeds (less a small portion of endosperm). The embryos representing the non-dissociated storage protein markers are also still intact, and residual entire progeny seed from each of the *ph1bph1b* selections is still available for further analysis. The overall number of wheat -*Aegilops* recombinants involving segments of the 1U chromosome other than in the interval *Gpi-U1* - *Gli-U1* cannot be estimated without further markers for chromosome 1U. A gene controlling a grain lectin characteristic for *Ae. umbellulata* has been located on this chromosome, but the gene product cannot yet be assayed on a single grain basis (Stinissen *et al.*, 1983). A series of molecular markers has been established for chromosome 1RS (cited in Chapter 4), and it is likely that the probes developed for regions on 1RS will be useful in characterising the *Gpi-U1* - *Gli-U1* recombinants from this study, as the rye homoeoloci *Gpi-R1* and *Sec-1* are both located in the region of

1RS most characterised by these molecular markers.

The potential agronomic value of the recombinant lines produced in this study is limited. The introgression of the gene(s) controlling the HMW glutelin subunits coded for by *Glu-U1* was thought worthwhile as a means of possibly improving the breadmaking quality of flour (Law and Payne, 1983), but the whole chromosome substitution lines involving 1U are known to suffer a yield disadvantage (Shepherd, 1973; Law and Payne, 1983). However glutenin subunits controlled by *Glu-D1* with electrophoretically very similar mobility on SDS-PAGE to the *Glu-U1* products have since been detected in five Japanese cultivars, although these proteins migrate to different gel positions under two dimensional electrophoretic separation (Payne *et al.*, 1983). No genes conferring disease resistance are known to be located on chromosome 1U. The value of the recombinant lines is likely to lie more in the opportunity they afford to map the group 1 wheat chromosomes. Genetic mapping requires allelic variation, and this variation is not common among biochemical characters in cultivated wheat. Deletion mapping presents an alternative procedure, and this has already been utilised to a limited extent in wheat (e.g. Ainsworth *et al.,* 1984). The wheat-alien recombination lines produced in this work make available a large number of genotypes which represent potentially many different breakpoints along the wheat chromosomes of homoeologous group 1; they are genetically equivalent to deletion lines providing the introgressed chromatin differs in its markers from those of wheat. They will therefore allow the establishment of gene order along these wheat chromosomes as various new markers located by aneuploid analysis to these chromosomes become available.

Chapter 7: General discussion

The work described in the preceding chapters of this thesis demonstrates the value of manipulating the *Ph1* system in wheat to effect allosyndetic recombination between the chromosomes of wheat and its alien relatives; and the greatly increased frequency with which this recombination occurs with a chromosome of *Aegilops* spp., rather than one from *Secale* spp., supports the conclusions from many cytological studies of chromosome pairing behaviour, which have shown that the genus *Aegilops* is more closely related to hexaploid wheat than is cereal rye. As discussed in Chapter 2, there has been some experience with induction of allosyndetic recombination, both from *Aegilops* and *Agropyron* spp., but the frequency of this recombination has not been previously reported except in the work of Sears (1972a, b; 1973; 1978; 1981) with *A. elongatum*.. When chromosome 7Ag was induced to pair with its wheat homoeologue, 12 transfer chromosomes were recovered, but the total population assayed was not given, so that no estimate of the allosyndetic recombination rate in this population can be made by the present author.

More complete data are available for the chromosome 3Ag derived material, where 20 transfers were identified out of 299 test-cross progeny, for a recombination rate of 6.7%. When only a telosome was used in place of the complete alien chromosome, a rate of 2.4% was recorded (Sears, 1978; 1981). The criterion used to distinguish a recombined chromosome from an unaltered alien chromosome was the retention of the gene *Lr24* , together with the lack of any univalent in at least some pmcs of hybrids between the presumptive recombinant and normal wheat. The frequency with which chromosome 3Ag appeared as a univalent in the pmcs of these plants was taken to be a measure of the amount of alien chromatin present in the transfer chromosome. A potential weakness in this criterion is that, in the presence of desynapsis, many distal cross-overs would not have been detected, as the resulting transfer chromosomes would usually be present as univalents at metaphase I. Desynapsis is likely to be frequent in this situation, given the

lack of agreement between metaphase I pairing data and genetic recombination frequency (Fu and Sears, 1973). The figure of 6.7% may therefore represent a substantial underestimate of the true recombination frequency induced. Neither the physical nor the genetic location of *Lr24* on the relevant arm of chromosome 3Ag is known, but one or more cross-overs anywhere along the chromosome would have resulted in the likely detection of a transfer chromosome in the experiment involving the full alien chromosome, so that the measured rate of recombination refers to the complete *Agropyron* chromosome. This should be considered when comparing the figure of 6.7% for chromosome 3Ag with 0.3-0.5% for rye chromosome arm 1RS (Chapter 4), 1.4% for a substantial genetic, but perhaps not physical, segment of the arm 1RL (Chapter 5) and 8.0% for the *Ae. umbellulata* chromosome segment bounded by the loci *Gpi-U1* and *Gli-U1* (Chapter 6). By extrapolation with the physical (Payne *et al.*, 1984c; Lawrence and Appels, 1985) and genetic (Chojecki *et al.*, 1983; Koebner, unpubl) locations of the wheat and rye homoeoloci for *Gpi-1* and *Gli-1*, this chromosomal segment in *Ae. umbellulata* is likely to be very short, and located within the satellite region of chromosome 1U. These comparisons suggest that the order of ease of alien introgression from the three genomes of *A. elongatum*, *Ae. umbellulata* and *S. cereale* is *Ae. umbellulata* > *A. elongatum* > *S. cereale*, in agreement with the expectations from cytological experience.

Evidence is accumulating which suggests that crossing-over may be restricted in the proximal segments of chromosome arms (Linde-Laursen, 1979; Dvorak and Chen, 1984; Snape *et al.*, 1985). The overwhelming majority of chiasmata at late prophase I in barley were located in the distal portion of the bivalents, although a much higher than average proportion of median or near centric chiasmata were observed with chromosome 7, a nucleolar chromosome (Rick, 1971). Although 1RS also contains the nucleolar organiser, all four of the wheat-rye recombinants involving 1RS appear to have been recombined in, or close to, the satellite region of the rye chromosome (Chapter 4). A distal localisation of crossing-over would be expected to produce a genetic map quite

different from the physical map of a given chromosome, and such a distortion has been noted in a number of plant species (Phillips, 1969; Rick, 1971; Dvorak and Chen, 1984; Lawrence and Appels, 1985; Snape *et al.*, 1985). For example, the segment of wheat chromosome 6BS between the centromere and the nucleolar organizing region accounts for 2/3 of the length of the mitotic metaphase chromosome, but these two regions are genetically very tightly linked, with an estimated map distance of 0.3-2.2 cM (Dvorak and Appels, 1985), while the distance from the centromere to the locus *Gli-B2* , located within the 6BS satellite, is as high as 20 cM (Dvorak and Chen, 1984). A similar situation prevails on the long arm of this wheat chromosome, where the awn inhibitor gene *B2* is very tightly linked to the centromere (Fu and Sears, 1973), but is absent in a deletion line in which as much as 1/3 of the metaphase length of the arm is still present (Giorgi, 1979).

The partial chiasmatype theory for crossing-over, whereby each chiasma at diplotene arises from an antecedent cross-over involving two non-sister chromatids, is now generally accepted as valid (Rhoades, 1961), so that, taken with the genetic evidence above, the majority of chiasmata must normally occur in the region of the telomere, agreeing with Fox's (1973) observation in the locust species *Schistocerca gregaria* , where chiasma frequency was highest in this region of the chromosome. An intriguing and important question which arises from this conclusion is what the relationship may be between the physical length of a particular chromosome segment between that at prophase I and that at metaphase I of meiosis. If the relative lengths of these segments remain conserved throughout meiosis, then substantial blocks of chromatin in the proximal region of the chromosome will be seldom affected by meiotic crossing-over, and will therefore themselves remain conserved; in this case, if alien chromatin is found to carry deleterious genes linked to gene/s, which are proposed to be introgressed into wheat, in such proximal segments, it will prove extremely difficult to induce recombination between them to separate them. On the other hand, if such regions are so highly conserved, it is likely that substantial gene homology within these regions still exists between species

with a common progenitor, and thus the probability that they include deleterious genes should be low. In contrast, it may be that the distal regions of the metaphase chromosome are relatively more condensed than the proximal regions, so that at prophase, where the chromosomes are not so coiled, the distal segment would appear much longer relative to the proximal segment. In this case, physical maps of chromosomes based on prophase karyotypes would allow a more meaningful comparison of genetic and physical location of given loci, while conclusions based on metaphase karyotypes would tend to be misleading. Hexaploid wheat prophase chromosomes are difficult cytological material, but diploid species such as maize, tomato and rye should lend themselves to the elucidation of this problem.

A further question that arises from the comparison of cytological and genetic recombination data in wheat concerns the number of chiasmata that are actually present on a given chromosome arm. Genetic data show that double cross-overs between homologues are not uncommon in wheat (e.g. Singh, 1985), although the average number of chiasmata per arm seen on metaphase bivalents is little greater than one (Kimber, 1962). However, if multiple chiasmata are concentrated in the distal region of the arm, only a small degree of terminalisation or pseudoterminalisation (Jones, 1978) will cause fusion of chiasmata, making them individually indistinguishable. Meiotic metaphase configurations only rarely include induced homoeologous associations, and yet genetic data presented in this thesis (Chapter 6) indicate that at least two homoeologous cross-overs can occur per bivalent. It is tempting to speculate that too few chiasmata on a chromosome arm are insufficient to prevent desynapsis. The mechanism for desynapsis remains uncertain. Precocious terminalisation of chiasmata has been suggested by Fu and Sears (1973), but there is disagreement as to whether chiasmata do in fact change their position in the course of meiosis (e.g. Fox, 1973; Hultén, 1974; Jones, 1978). Chromosomes which are genetically marked by several loci close to the telomere are required to test the hypothesis above, but due to the paucity of such markers in wheat, this species is unlikely to be suitable for such studies.

While the manipulation of the *Ph1* system offers the best prospect for alien introgression into wheat in the immediate future, other techniques may in future become available. Maintaining calli in culture for extended periods has been reported to induce karyotypic changes to wheat (Lapitan *et al.*, 1984), and less gross changes in chromosomal structure, such as deletions or inversions probably also occur in culture. It may be possible to induce small wheat-alien translocations in this way (Larkin and Scowcroft, 1983), but this possibility has not yet been tested. A further alternative to *Ph1* manipulation may be afforded by the 'genome restructuring' gene found in *Ae. longissima* by Feldman and Strauss (1983), but no information on its mode of action or capacity to induce translocations is at present available.

The recent advances in molecular biology have opened up new prospects for gene transfer between unrelated species. A number of the techniques developed in bacteria have been suggested as being applicable to introgression in higher plants; as these have been the subject of two recent reviews (Cocking *et al.*, 1981; Schilperoort, 1984) they will only be alluded to briefly here. The technology of genetic engineering, if it is indeed able to be applied to plant improvement, bypasses the requirement for a sexual hybrid between the recipient and the donor species before gene introgression can be initiated. Thus alien genomes may be combined by protoplast fusion (Evans and Flick, 1983); while small segments of DNA can be parcelled into an appropriate vector, such as the Ti plasmid of *Agrobacterium tumefaciens* (Depicker *et al.*, 1983) or the tobacco mosaic virus (Fraley and Horsch, 1983), and then transferred to the plant genome by infection with the engineered vector; it may even be possible to directly transform pollen grains (Schilperoort, 1984), and experiments demonstrating this possibility have had some as yet unconfirmed success in maize (E.S. Dennis, pers. comm.).

A problem common to all schemes aimed at introgression of alien genetic variation is the choice of gene/s to be transferred. Genes conferring disease resistance are important to plant breeders, and if present in near relatives to wheat, these should be readily accessible by manipulation of *Ph1* ; a priority in this area is to characterise many more

chromosome markers in wheat in order to facilitate selection of those transfer lines which involve the smallest possible disturbance to the wheat genome. There is potential for many new markers if restriction fragment polymorphisms can be found and exploited in wheat, as is beginning to be done in humans (Botstein *et al.*, 1980). The large number of biochemical systems utilised in human genetics (Harris and Hopkinson, 1976) suggests that many of these systems may be useful in wheat, and the uncovering of protein variation in wheat should therefore continue to be actively pursued.

The exploitation of genes controlling disease resistance and most other physiological or morphological characters from more exotic sources via genetic engineering techniques is hindered by the difficulty in recognising the appropriate gene product. An association between specific mRNAs and a disease resistance in pea has recently been reported (Riggleman *et al.*, 1985), but it is not clear whether these messages are a direct result, rather than merely a secondary effect of fungal infection. Other characters present in alien species which might be profitably transferred to wheat may involve complex inheritance, and thus may not be susceptible to transfer without causing substantial changes in the phenotype of wheat. Furthermore, there is no certainty that genetic material will inevitably be expressed at all in a wheat background, as mentioned in Chapter 2.

The challenge to plant breeders involved in broadening the genetic base of crops through alien introgression remains to choose among the wide range of alien variation that which is most likely to benefit wheat breeders in their search for ever greater yield capacity and stability.

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