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Allosyndetic recombination between a chromosome of *Aegilops umbellulata* and wheat chromosomes

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Allosyndetic recombination was induced between chromosome 1U of *Aegilops umbellulata* and wheat chromosomes by producing plants monosomic for this alien chromosome and homozygous for the mutant *ph1b* allele, which permits homoeologous chromosome pairing. Recombinants were selected among the progeny of such plants by observing the disruption of the *Glu-1-Gpi-1-Gli-1* linkage on both the alien chromosome as well as along wheat chromosomes 1B and 1D. A frequency of 8·0 per cent recombination between the *Gpi-U1* and the *Gli-U1* loci was estimated when chromosomes 1U and 1B were simultaneously monosomic, whereas the rate of recombination was only 4·6 per cent when 1B was present as a disome. Some double homoeologous recombinants between the three loci *Glu-1*, *Gpi-1* and *Gli-1* were also isolated. Control populations, where homoeologous pairing was suppressed by the presence of *Ph1*, did not produce any allosyndetic recombinants between *Gpi-1* and *Gli-1*.

INTRODUCTION

It is widely recognised that scientific plant breeding tends to narrow the germplasm available within crop species. In addition some alien species are endemic in environments where their cultivated relatives cannot at present be grown. Thus the introgression of genetic variation from wild relatives has become an important goal in plant breeding. The wild relatives of wheat include species with one or more genomes in common with hexaploid bread wheat, and introgression from these is reasonably straightforward, requiring only hybridisation and selection for homologous recombinants. However, when the donor alien species is less closely related, it is necessary to interfere with meiotic pairing in order to induce allosyndesis between homoeologous chromosomes. Since homoeologous chromosome pairing in wheat is suppressed largely by the action of the *Ph1* gene on chromosome 5B (Sears and Okamoto, 1958; Riley and Chapman, 1958), allosyndesis can be achieved by the deletion of 5B (Riley and Kimber, 1966), the mutation of the *Ph1* locus (Sears, 1977), or the suppression of the action of *Ph1* by an alien genome (Riley *et al.*, 1968).

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The frequency of allosyndetic pairing between wheat and alien chromosomes in a *Ph1* deficient background depends heavily on the evolutionary separation between the wheat and alien genomes. Thus it can reach quite high levels in some wheat-*Aegilops* spp. hybrids, while remaining rather low in the case of wheat-rye hybrids (Riley and Kimber, 1966). The *Aegilops* genus is known to contain an extensive reservoir of useful genetic variation, particularly with respect to genes for resistance to phytopathogens (Frauenstein *et al.*, 1985; Valkoun *et al.*, 1985), and some progress towards introgression from these species has already been made (Riley and Kimber, 1966; Riley *et al.*, 1968; Dvořák, 1977; Ceoloni, 1984).

In this paper, recombination between chromosome 1U of *Ae. umbellulata* and wheat is described. Law (1984) has briefly reported work along similar lines, but the procedures employed and the details of the results achieved have not to date been published. Chromosome 1U 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 1U(1D) substitution line is reported to be meiotically unstable at low temperatures (Chapman *et al.*, 1975), and has a tendency to form shrivelled grain (Shepherd,

unpubl.). The *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 present work describes the production and identification of a substantial number of *ph1b* induced intergeneric recombinants.

MATERIALS AND METHODS

Plant materials

(a) Disomic substitution lines 1U(1B) and 1U(1D), having chromosome 1U of *Ae. umbellulata* replacing one wheat homoeologue in cv. Chinese Spring (Shepherd, 1973).
(b) *ph1b* mutant (Sears, 1977); two stocks used: (1) derivatives of Ac. 7876 with an unknown number of generations of self-fertilisation since the original isolation, and (2) Ac. 9821, a reselection from a *Ph1bph1b* stock obtained from Prof. C. J. Driscoll. Accession numbers are those of Dr K. W. Shepherd (WARI, Adelaide, S. Australia).
(c) An accession of *Ae. variabilis* (Ac. 7069) obtained from Dr K. W. Shepherd.

Electrophoresis

SDS-PAGE was conducted on both unreduced and reduced extracts of mature endosperm to score for the *Gli-1* and *Glu-1* loci, respectively, following established methods (Singh and Shepherd, 1984, 1985). Reduced extracts were obtained by adding 10 μl of 25 per cent v/v 2-mercaptoethanol to the remaining unreduced sample and incubating overnight at 4°C and for 2 h at 60°C on the day of the run.

Isoelectric focussing (IEF) and cellulose acetate electrophoresis (CAE) on Cellogel 250 (Chemetron) were used to score for *Gpi-U1* and *Gpi-B1*, respectively. A small piece of mature endosperm was crushed and incubated overnight in 50 μl of distilled water at 4°C. Prior to electrophoresis, the samples were briefly centrifuged to obtain a clear supernatant. IEF gels were prepared by pouring a 0·5 mm thick gel (5 per cent T, 3 per cent C) containing 2 per cent Servalyt 3–10 on a silanised glass plate. The gels were run at 1 W/cm with the voltage limited to 2000 V; the run consisted of 500 Vh prefocussing, 1000 Vh with the samples left on the gel approx. 1cm from the cathode, and a further 1500 Vh (total run 3500 Vh). The samples were prepared by saturating a 0·4× 1·0 cm piece of Whatman 3 MM chromatography paper with approx. 15 μl of supernatant. Staining was achieved by laying over the gel a strip of Cellogel 250 soaked in a mixture containing 2 ml 0·3 M Tris-HCl pH 8, 0·4 ml 0·3 M $MgCl_2$, 0·4 ml 0·2 per cent w/v MTT, 0·4 ml 0·05 per cent w/v PMS, 6 mg D-fructose-6-phosphate, 4 mg β-NADP and 10 μl G-6-PDH (263 U/ml—Sigma). After staining the overlays were fixed in 7 per cent glacial acetic acid, bathed for 20 min in 20 per cent v/v glycerol and air-dried. For CAE, a strip of Cellogel 250 was equilibrated in electrode buffer (0·025 M Tris, 0·2 M glycine) for 15 min, and 2 μl of sample was loaded onto each track with a Super-Z Applicator (Helena Labs). Two rows of samples (each of 12 samples) were applied onto each 9× 10 cm strip. The gels were run at 350 V for 45 min at 4°C, and stained, fixed and dried as described above.

Cytology

Standard Feulgen squashes of anthers at meiotic metaphase I were employed to analyse chromosome configurations in the pollen mother cells (pmcs) of selected plants.

Production of populations containing wheat-Aegilops allosyndetic recombinants

A scheme similar to that described in Koebner and Shepherd (1985) was used to obtain plants containing both one dose of wheat chromosome 1B (or 1D) and of chromosome 1U in a homozygous *ph1b* background. The mutant was used as the male parent in the cross to each substitution line to minimise transmission of unbalanced gametes, resulting from translocations accumulated in the *ph1b* stock (Koebner and Shepherd, 1985). In addition, for the cross involving the 1B substitution, a selection of *ph1b* from a heterozygote showing 21 regular bivalents at meiotic metaphase I was utilised to reduce further the extent of translocations within the male parent. The resulting F_1 plants were allowed to self-fertilise and both unreduced and reduced endosperm protein extracts of individual F_2 grains were analysed by SDS-PAGE to select individuals which presumably carried both 1U and the respective wheat chromosome on the basis of the presence of the appropriate protein bands. Approximately 20 such individuals from each F_2 population were grown and meiotically

analysed to select for *ph1b* homozygosity, using a reduction in chiasma frequency as the major indicator (Koebner and Shepherd, 1985). Furthermore, as chromosome 1U could be easily recognised in meiotic chromosome preparations (fig. 1), 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 to produce the populations to be screened for allosyndetic recombination. The wheat × *Ae. variabilis* hybrids were grown and analysed for the presence or absence of homoeologous pairing in pmcs at metaphase I. control populations, from plants of constitution *Ph1bph1b* where homoeologous pairing was suppressed, were obtained from the F_2 generation of the cross of each substitution line × *ph1bph1b*.

The progeny of plants having one dose of 1U and of constitution *ph1bph1b* will be hereafter referred to as test (T) populations, while those from *Ph1bph1b* parents will be referred to as control (C) populations.

Screening for wheat-Aegilops recombinants

Initially, the *Gli-1* and *Glu-1* phenotype of each T and C seed was established. The genes controlling these two protein types are located on the short and long arms, respectively, of the group 1 chromosomes (reviewed by Payne *et al*, 1982). 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), it was assumed that the same gene order existed on chromosome 1U as on 1B and 1D. Individuals with dissociated protein markers (*e.g.*, Glu-B1^{+}Gli-B1^{-}or Glu-U1^{-}Gli-U1^{+}) thus represent the products of either misdivision of the centromere or homoeologous

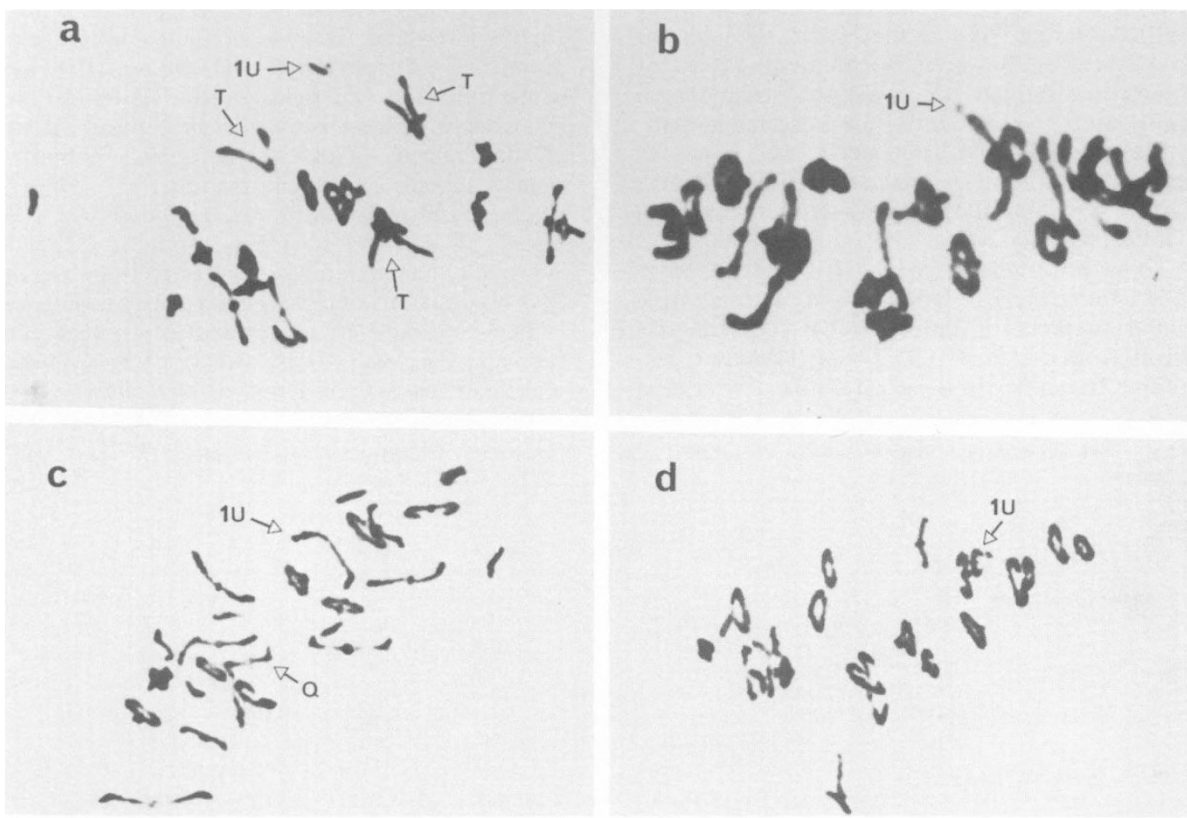

Figure 1 Chromosome configurations at metaphase I in pmcs of presumptive *ph1b* homozygotes carrying one dose of chromosome 1U, showing this chromosome (a) unpaired, and (b), (c), (d) paired. (a), (b) from cross involving substitution 1U(1B); (c), (d) from cross involving substitution 1U(1D). Pmc showing 1U unpaired: (a) Pmc showing $4' + 5^{[\prime\prime]} + 10^{(\prime\prime)} + 3'''$ (T = trivalent), Pmcs with 1U paired in a rod bivalent: (b) Pmc with probable configuration $1' + 8^{[\prime\prime]} + 13^{(\prime\prime)}$, (c) Pmc showing $4' + 8^{[\prime\prime]} + 9'' + 1^{ir}$ (Q = quadrivalent), Pmc with 1U paired in a ring bivalent: (d) Pmc showing $3' + 4^{[\prime\prime]} + 16^{(\prime\prime)}$.

recombination. *Gpi-D1* is located interstitially between *Gli-D1* and *Glu-D1* (Chojecki *et al.*, 1983) in wheat, and therefore, assuming gene synteny, analysis of the *Gpi-1* phenotype of the dissociated protein selections allows homoeologous recombinants in the interval *Gpi-1-Gli-1* to be distinguished from misdivision products and recombinants in the interval *Glu-1-Gpi-1*. Later the *Gpi-1* phenotype of a sample of the undissociated protein individuals was also analysed to search for double recombinants.

RESULTS

Selection of F_2 individuals homozygous ph1b and carrying endosperm protein markers for both chromosomes 1U and 1B (or 1D)

The F_1 hybrids from crosses between substitution line 1U(1B) × *ph1b* mutant and 1U(1D) × *ph1b* mutant were expected to be monosomic for both 1U and the wheat chromosome replaced in the substitution line. Pmcs in the hybrid involving the 1B substitution showed most commonly 20″ + 2′ as expected, 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.

Three presumptive *ph1bph1b* selections were made among the F_2 plants carrying both storage protein markers of chromosomes 1U and 1B derived from the cross 1U(1B) × *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-*), 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 1). Chromosome 1U usually remained unpaired at metaphase I (fig. 1(a), but a heteromorphic rod bivalent was observed in two pmcs of plant 46-1 and in one pmc in plant 52-1 (fig. 1(b), table 1). The homozygous *ph1b* status of these selections was confirmed by analysing the meiotic pairing in their hybrids with *Ae. variabilis* (Koebner and Shepherd, 1985). 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.

The overall level of chromosome pairing in the F_2 derived from 1U(1D) was lower than in the equivalent 1U(1B) families. This difference is thought to be related to the two different stocks of the mutant parent crossed to the two substitution lines (cf. plant materials). Two *ph1bph1b* selections (plants 114-1 and 115-1) were made among the F_2 plants derived from the 1U(1D) cross on the basis of their chromosome pairing (table 2). Plant 114-1 was shown to have been *ph1bph1b* when all five of its hybrids with *Ae. variabilis* exhibited homoeologous pairing at metaphase I. Only a single viable hybrid with *Ae. variabilis* was produced from plant 115-1 and it showed homoeologous metaphase I pairing. The observation of a rod bivalent involving chromosome 1U in one pmc, and a ring bivalent in another pmc (table 2; figs. 1(c)(d) provided strong evidence that this plant was of genotype *ph1bph1b*.

Table 1 Mean chromosome configurations at metaphase I in pmcs of selected F_2 plants from a cross between U(1B) × *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

* 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.

Table 2 Mean chromosome configurations at metaphase I in pmcs of selected F_2 plants from a cross between 1U(1D) × *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[a] (1-9)	14·2[a] (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 1 pmc with a bivalent involving 1U. univ. = univalents, biv. = bivalents, triv. = trivalents, quad. = quadrivalents, > quad = higher multivalents. χ = chiasmata.

Analysis of the 1U(1B) derived T and C populations

Isolation of individuals having dissociated *Gli-1* and *Glu-1* markers

All three presumptive *ph1b* homozygotes selected from the F_2 of the cross 1U(1B) × *ph1b* mutant were highly self-fertile. A total of 1322 seeds were analysed by SDS-PAGE to determine their Gli-1 and Glu-1 phenotypes. The Gli-1 phenotypes of the two parents were distinguished by the presence of clearly-separated bands on unreduced SDS-PAGE gels (fig. 2A(a)) and these bands could be reliably scored in the progeny seed (Fig. 2A(b)). The Glu-1 phenotypes were scored in reduced SDS-PAGE gels and the glutelin subunits known to be controlled by chromosomes 1B (Payne and

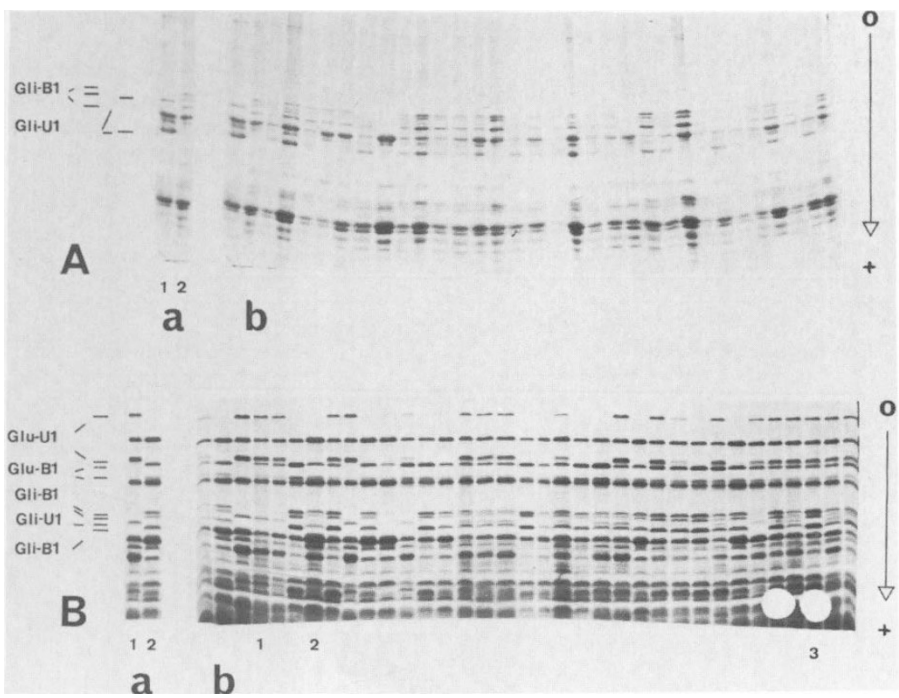

Figure 2 Endosperm protein phenotype of parents and segregating progeny derived from the cross substitution 1U(1B) *ph1b* mutant. Patterns obtained by (A) unreduced, and (B) reduced SDS-PAGE. A. (a) parental phenotypes: 1. 1U(1B)(Gli-B1$^-$Gli-U1$^+$), 2. Euploid Chinese Spring (CS)(Gli-B1$^+$Gli-U1$^-$); (b) progeny phenotypes. B. (a) parental phenotypes: 1. 1U(1B)(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$^-$.

Lawrence, 1983) and 1U (Lawrence and Shepherd, 1981) could also be easily distinguished in both parents and progeny (fig. 2B(a), (b)). Although the Gli-1 phenotypes could often be scored on the same gels, the relevant bands sometimes overlapped other wheat bands, and therefore protein extracts were routinely analysed in both unreduced and reduced gels.

From the segregation patterns of the Gli-1 and Glu-1 proteins in these progeny (table 3), it was concluded that plant 52-1 was disomic for 1B and monosomic for 1U, while both 45-2 and 46-1 were monosomic for both these chromosomes. 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 *Ph1bph1b* parent, the segregation of the markers controlled by these chromosomes can be considered separately, giving rise to four phenotypic classes for both the 1B and the 1U controlled proteins (table 3).

It was not possible to pool the data from all three T populations because of the different behaviour of plant 52-1. Furthermore, although the segregation patterns of the progeny of plants 45-2 and 46-1 were homogeneous for the chromosome 1U classification ($\chi^2 = 0{\cdot}30$, 3 df $0{\cdot}95 < p < 0{\cdot}98$), they were heterogeneous for the chromosome 1B classification ($\chi^2 = 11{\cdot}25$, 3 df $0{\cdot}01 < p < 0{\cdot}02$). 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 ($\chi^2 = 38{\cdot}2$, 3 df $p < 0{\cdot}01$) or for the chromosome 1U classifications ($\chi^2 = 16{\cdot}6$, 3 df $p < 0{\cdot}01$). For simplicity, the pooled 45-2 and 46-1 progeny will be referred to hereafter as the T1 population, and the progeny of plant 52-1 as the T2 population.

Progeny with dissociated *Gli-1* and *Glu-1* markers were detected in both the T and the C populations. In the control population such individuals are expected to arise only from chromosome misdivision and transmission of a telocentric arm, as any homoeologous pairing in the presence of *Ph1* is very rare; by contrast, in the T populations allosyndetic recombination also contributes to this class. Therefore it was surprising that the frequency of dissociated 1B markers was more frequent in the C population (13·6 per cent) than in the T1 population (11.1 per cent). This apparent anomaly may be a consequence of the absence of pairing of chromosome 1B in the meiocytes of the *Ph1bph1b* parent, while some pairing does presumably occur in the *ph1bph1b* parents, thus allowing a higher rate of misdivision in the former plant. The frequency of the Glu-B1$^+$Gli-B1$^-$ types was higher than Glu-B1$^-$Gli-B1$^+$, and this is probably due to differences in the transmission rate of the long and short arm telocentrics.

Fewer dissociated 1U marker individuals were obtained in the C population (8·0 per cent) than in the T1 (11·0 per cent) or T2 (11·1 per cent) populations, and also fewer than were recorded

Table 3 Endosperm storage protein phenotypes and their frequencies in the T and C populations derived from the cross 1U(1B) × *ph1b* mutant

	Endosperm storage protein phenotype									
	Chromosome 1B markers					Chromosome 1U markers				
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
%	28·9	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	

* Dissociated 1B protein individuals ^ dissociated 1U protein individuals.
† 5 individuals analysed for 1B phenotype not analysed for 1U phenotype, T: *ph1bph1b*-derived populations C: *Ph1bph1b*-derived population

with the 1B markers. The lower recovery rate of individuals having dissociated 1U markers may be due to a lower rate of misdivision of chromosome 1U compared to 1B, or a lower transmission of 1U misdivision products through the gametes. Unlike with the 1B markers, the 1U markers showed no marked difference in the frequency of the two classes of dissociated protein type. However the frequency of progeny carrying at least one of the 1U markers was much greater in the T1 population (68·6 per cent) than in the T2 population (50·9 per cent), probably reflecting the expected difference in transmission of an alien chromosome when present as a univalent in a 42-chromosome double monosomic rather than as the extra chromosome in a 43-chromosome monosomic addition line.

Gpi phenotype of dissociated *Gli-1*, *Glu-1* selections

From the C population, 73 progeny with dissociated chromosome 1B markers and 42 with dissociated 1U markers were detected. Among the T populations, there were 100 such 1B selections and 147 1U selections (table 3). The Gpi phenotypes of these individuals were analysed to distinguish allosyndetic recombinants in the interval *Gpi-1-Gli-1* from misdivision products and recombinants in the interval *Glu-1-Gpi-1*. The CAE phenotype of normal wheat gives four strong bands and a fifth, faster, weak band (fig. 3A). In the absence of chromosome 1B, or its short arm, the slowest two bands are lost (see Hart, 1979). *Gpi*-U1 controls the most anodal band on the IEF gels (Chojecki and Gale, 1982) and was used to score for the presence of this locus (fig. 3B). The gel shown in fig. 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. The frequencies of the Gpi-1 phenotypes among the selected progeny are given in table 4.

Due to a technical fault, 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 for *Gpi-U1-Gli-U1* (table 4), as expected if 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 T populations showed a disruption in the *Gpi-1-Gli-1* association, indicating the occurrence of allosyndetic recombination, presumably induced by the loss of normal

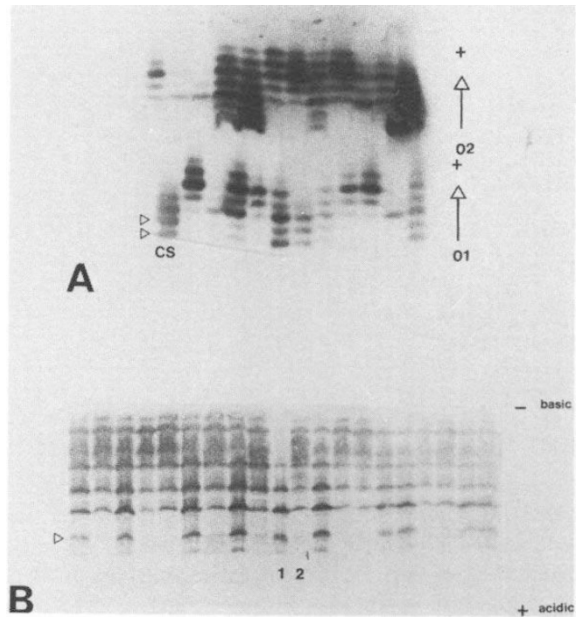

Figure 3 Glucose phosphate isomerase phenotypes of parents and segregating progeny derived from the cross substitution 1U(1B) × *ph1b* mutant. A. Cellulose acetate zymogram. CS-Euploid Chinese Spring. Other samples represent progeny segregating for *Gpi-B1* and *Gpi-U1*. Bands controlled by *Gpi-B1* 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 1U(1B), 2. Euploid Chinese Spring. Other samples represent progeny segregating for *Gpi-U1*. Band used for scoring Gpi-U1 phenotype shown by ▷.

Ph1 activity. Of the 15 recombinants in the segment *Gpi*-B1-*Gli*-B1, 13 were Glu-B1^{+}Gpi-B1^{-}Gli-B1^{+}, while 2 were of the reciprocal type (table 4). Since the former type retained much more 1B chromatin than the latter, it is likely that they will be more easily transmitted through the pollen than the other type. The proportion of *Gpi-U1-Gli-U1* recombinants (42 out of 147 = 29 per cent) was, surprisingly, much more frequent than that observed for the equivalent segment of chromosome 1B (15 out of 100 = 15 per cent), but in the former case there was less of a difference in the frequency of the reciprocal recombinant types (25 versus 17, table 4).

Despite the identification of these definite allosyndetic recombinants, the great majority of the T population selections had parental combinations of these short arm markers—85 per cent for chromosome 1B and 71·4 per cent for chromosome 1U (table 4). This group of progeny will include all those possessing misdivision products, and

Table 4 Gpi phenotypes of individuals selected as having dissociated endosperm protein markers in the T and C populations

	Endosperm protein and Gpi 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
(ii) C population										
Family 178-2	37	0	11	0	48*	21	0	21	0	42

^ Recombinants in the interval *Gpi-1-Gli-1*.
* 25 individuals in these endosperm protein classes not scored for Gpi-B1 T: *ph1bph1b*-derived populations C: *Ph1bph1b*-derived population

others having allosyndetic recombination in the interval *Glu-1-Gpi-1*. These two types are indistinguishable in the absence of other markers in this chromosome segment without extensive cytological analyses.

The above analysis of recombination was based on the assumption of a maximum of one homoeologous single cross-over per chromosome. In order to test this assumption, a sample of 211 from the T1 and 116 from the T2 populations having undissociated storage protein markers (*i.e.*, Glu-1$^+$Gli-1$^+$and Glu-1$^-$Gli-1$^-$), was analysed for Gpi phenotype. Both Gpi-U1 and Gpi-B1 phenotypes were scored on IEF gels for this purpose, and the results are given in table 5. 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. Although the majority of seeds sampled showed a Gpi phenotype consistent with there having been no double cross-over between the three loci under consideration, a few double cross-overs were detected along chromosome 1U (5·7 per cent in the T1, and 4·3 per cent in the T2 populations) and along 1B (1·9 per cent in the T1 population). 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 earlier.

Analysis of the 1U(1D) derived populations

A sample of 268 progeny from each of the two plants identified as *ph1bph1b* from the cross 1U(1D) × *ph1b* mutant was analysed electrophoretically, to compare with the results obtained with the 1U(1B) cross. The observed frequencies of the Gli-1 and Glu-1 storage protein phenotypes determined by SDS-PAGE are listed in table 6, and typical patterns are illustrated in fig. 4A, B. Progeny of plant 115-1 (with somatic chromosome number of 43, table 2) did not segregate for either *Gli-D1* or *Glu-D1* and this plant was therefore thought to be disomic for chromosome 1D. However progeny from both of the selected plants segregated for *Gli-U1* and *Glu-U1*.

Table 5 Frequency and phenotype of double homoeologous cross-over individuals involving (a) chromosome 1B, and (b) chromosome 1U, sampled from the T populations

	Endosperm phenotype		% occurrence
(a) Chromosome 1B	(Glu-B1$^+$Gpi-B1$^-$Gli-B1$^+$)	(Glu-B1$^-$Gpi-B1$^+$Gli-B1$^-$)	
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 *ph1pbh1b* plant 52-1.

Table 6 Storage protein phenotypes and their frequencies in progeny of two *ph1bph1b* plants selected in the F_2 generation from the cross 1U(1D) × *ph1b* mutant

	Endosperm protein phenotype										
	Chromosome 1D markers					Chromosome 1U markers				
	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

* Dissociated 1D protein individuals. ^ Dissociated 1U protein individuals. + = protein present, − = protein absent.

The equivalent F_2 control population was not analysed as before, as the previous experiment had already shown that no allosyndetic recombinations occurs in the presence of one dose of *Ph*1.

The progenies of 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 1U(1B) cross. Thus the plant which was monosomic for chromosome 1D gave a higher proportion of progeny containing all or part of chromosome 1U (59 per cent) than the disomic 1D parent where this proportion was only 39 per cent (table 6). Among the individuals having dissociated 1U storage protein phenotypes, the Glu-U1$^-$Gli-U1$^+$ phenotype was more frequent than its reciprocal phenotype (13·1 per cent versus 6·7 per cent). A preponderance of this phenotype, although less marked, was also noted in the progeny from the 1U(1B) 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 (table 7). A total of 23 further recombinants involving the segment of chromosomes 1U between *Gpi-U1* and *Gli-U1* were thus identified.

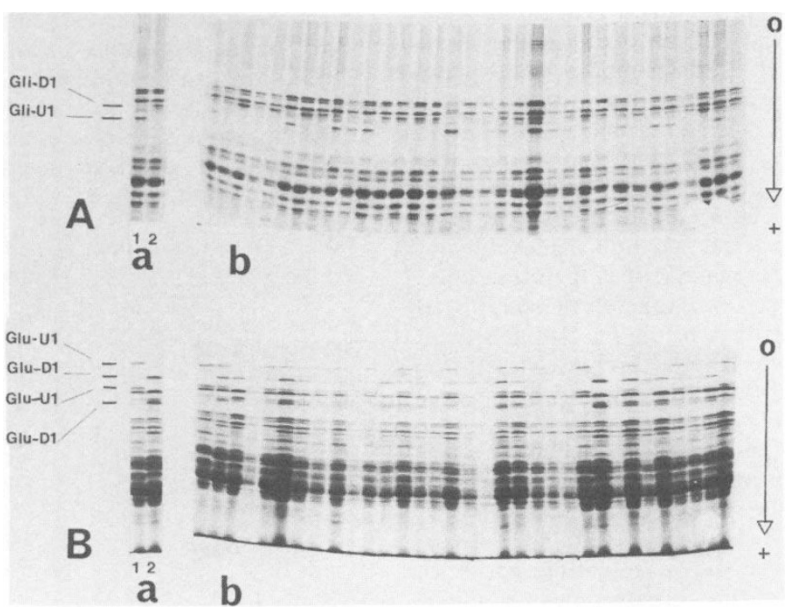

Figure 4 Endosperm protein phenotype of parents and segregating progeny derived from the cross substitution 1U(1D) × *ph1b* mutant. Patterns obtained by (A) unreduced, and (B) reduced SDS-PAGE. A. (a) parental phenotypes: 1. 1U(1D)(Gli-D1$^-$Gli-U1$^+$), 2. Euploid Chinese Spring (CS)(Gli-D1$^+$Gli-U1$^-$); (b) progeny phenotypes. B. (a) parental phenotypes: 1. 1U(1D)(Glu-D1$^-$Glu-U1$^+$), 2. Eucploid CS (Glu-D1$^+$Glu-U1$^-$); (b) progeny phenotypes.

Table 7 Gpi phenotypes of progeny selected as having dissociated 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

* Recombinants in the interval *Gpi-U1-Gli-U1*.

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. However, to obtain this, 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 T1 and the T2 populations are shown separately in table 8, 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 8 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 "– – –" ($m_0 f_0$) 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 (*i.e.*, $1 - m_0$), 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 (tables 1, 2) and the

Table 8 Relative frequencies of chromosome 1U controlled phenotypes in the T1 and T2 populations (1U(1B) cross)

Progeny phenotype Glu-U1	Gpi-U1	Gli-U1	Relative frequency 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.

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 9) 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.

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 in the interval *Gpi-1-Gli-1* was greater for chromosome 1U than for 1B (table 4). Moreover, the relative frequency of double cross-overs was substantially higher for chromosome 1U as compared to chromosome 1B (table 5). 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* × *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* (N. K. Singh, personal communication) provide circumstantial 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 two independent chromosome 1D mapping experiments (Chojecki *et al.*, 1983; Koebner, unpubl.), allelic variation between two wheat cultivars in *Gpi-D1* and *Gli-D1* on chromosome 1DS gave similar recombination values (34·5 per cent and 25·6 per cent, respectively) or three to four times greater than the rate estimated for homoeologous recombination along chromosome 1U (8·0 per cent table 9). 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.

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

Table 9 Estimated relative frequencies of allosyndetic recombination in the interval *Gpi*-U1-*Gli*-U1 on chromosome 1U in the T1 and T2 populations (1U(1B) cross)

	T1	T2
r_2	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 chromsome *ph1bph1b* plant 52-1.
r_1 = refers to phenotype *Gpi*-U1$^+$*Gli*-U1$^-$.
r_2 = refers to phenotype *Gpi*-U1$^-$*Gli*-U1$^+$.

is still in the form of seed (less a small portion of endosperm). The embryos representing the non-dissociated storage protein markers are also still intact, and residual entire 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 (c.f. Koebner *et al.*, 1986), 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 loci *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.*, MacKey, 1954; 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.

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