Proc. 6th International Wheat Genetics
Symposium, Kyoto, Japan, 1983: 5–12

THE TRANSFER TO WHEAT OF INTERSTITIAL SEGMENTS OF ALIEN CHROMOSOMES[1)]

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SUMMARY

The major problem in transferring desirable characters to wheat from its relatives is the failure of the alien chromosomes to pair with those of wheat. The most promising way to overcome this difficulty is the induction of homoeologous pairing through deficiency of the pairing suppressor *Phl* on chromosome 5B. However, unless the alien gene being transferred is located near the end of a chromosome arm, an induced homoeologous recombination that transfers the gene to a wheat chromosome will transfer also all the alien chromatin lying distal to the gene. A double crossover, which could transfer an interstitial alien segment, cannot be expected to occur between homoeologues. A simple solution is to produce a number of transfers, some resulting from recombination proximal to the alien gene concerned and others distal, and identify the one in each category resulting from recombination nearest the alien gene. When these two selected chromosomes are allowed to recombine with each other, they give rise to a wheat chromosome with an interstitial alien segment carrying the desired alien gene. Such a segment, carrying the *Agropyron elongatum* gene *Lr*24 for resistance to leaf rust, has been substituted for a homoeologous interstitial segment of chromosome arm 3DL.

Within the wheat genus, *Triticum*, and the genera closely related to it, there are many species with characters that could help protect cultivated wheat against pests, extend its range of adaptation, improve the quality of its grain, and increase its yield potential. Now that crossing techniques have been so greatly improved by the use of hormone treatment and embryo culture, wheat is being crossed with almost all of its close relatives and even with such distant species as cultivated barley. But the inability of the alien chromosomes to pair with those of wheat greatly complicates the task of making satisfactory transfers of characters to wheat from the relatives.

By means of simple backcrossing and selection, breeders can often obtain wheat-like plants that have a desired alien character, but almost always the gene concerned proves to be carried by a complete alien chromosome, either added to the wheat genome or substituted for a wheat homoeologue. Nearly always such an addition or substitution has side effects that are commercially unacceptable.

Since the fifties, radiation has been used to induce translocations between wheat and alien chromosomes, thereby making possible the recovery of wheat chromosomes with a segment replaced by an alien segment carrying the desired gene (Sears 1956; reviews by Knott 1971; Knott and Dvořák 1976). However, because breaks and reunions occur

[1)] Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 9417.

more or less at random, and because even a polyploid like wheat does not tolerate well the substitution of any except very short alien segments, the vast majority of the radiation-induced translocations involving a desired gene are unsatisfactory. Although radiation techniques have been employed successfully by several investigators, they are now seldom used.

The discovery by Okamoto (1957), Sears and Okamoto (1958), and Riley and Chapman (1958) that chromosome 5B carries a suppressor of chromosome pairing made it possible to induce recombination between alien chromosomes and their wheat homoeologues. Nullisomic 5B is male-sterile, but the fertile nulli-5B tetra-5A and nulli-5B tetra-5D were already available; and an induced mutation, *phlb*, an apparent deficiency for the *Phl* locus, was obtained in 1977 (Sears 1977). Since the chromosomes of the various species of the wheat group have apparently undergone little rearrangement during their evolution, recombination between homoeologues takes place in such a way that each alien segment replaces a homoeologous wheat segment. Since alien chromosomes tend to compensate for their wheat homoeologues, each alien segment compensates to some extent for the replaced wheat segment.

Sometimes an alien chromosome may carry two (or more) desirable genes located at a considerable distance from each other. In this case a transferred segment long enough to include both genes may be advantageous; but otherwise the shorter the alien segment, the better, since shortness reduces the likelihood of its carrying nearby deleterious genes. How difficult it may be to limit the length of the transferred alien segment depends on the location of the alien gene on its chromosome: If it is near the end of an arm, a simple distal crossover will result in an exchange of short, terminal segments, of which the alien one carries the desired gene. If, however, the alien gene has a median or proximal location, as is most likely, any simple exchange of a segment carrying the gene will necessarily consist of half or more of the arm concerned. A double crossover could of course transfer a short, interstitial segment, but unfortunately two crossovers near together occur only rarely between homologous chromosomes and cannot be expected at all between homoeologues.

One solution to the problem of introducing a short, interstitial alien segment is first to obtain a recombinant chromosome with the shortest possible terminal alien segment that includes the desired alien gene, then induce homoeologous recombination between the alien segment and the corresponding wheat segment, so as to replace a substantial terminal portion of the alien segment with the corresponding wheat chromatin. Another method, which is in some ways simpler, involves distinguishing between two types of recombinants, one the result of crossing-over proximal to the gene concerned and the other the result of distal crossing-over. In each class of recombinants the one with the least alien chromatin is then identified, and the two chromosomes concerned are allowed to pair and crossover. Pairing can only occur in the alien chromatin they have in common, and one product of their crossing-over is a wheat chromosome with an interstitial alien segment (Fig. 1). Its length will depend only upon how close the two original crossovers were to the alien gene.

My experiments on methods for using induced homoeologous pairing in the transfer of alien genes to wheat chromosomes began in 1967 (Sears 1972a) with two different *Agropyron elongatum* (Host) Beauv. substitution lines, each having the alien chromosome

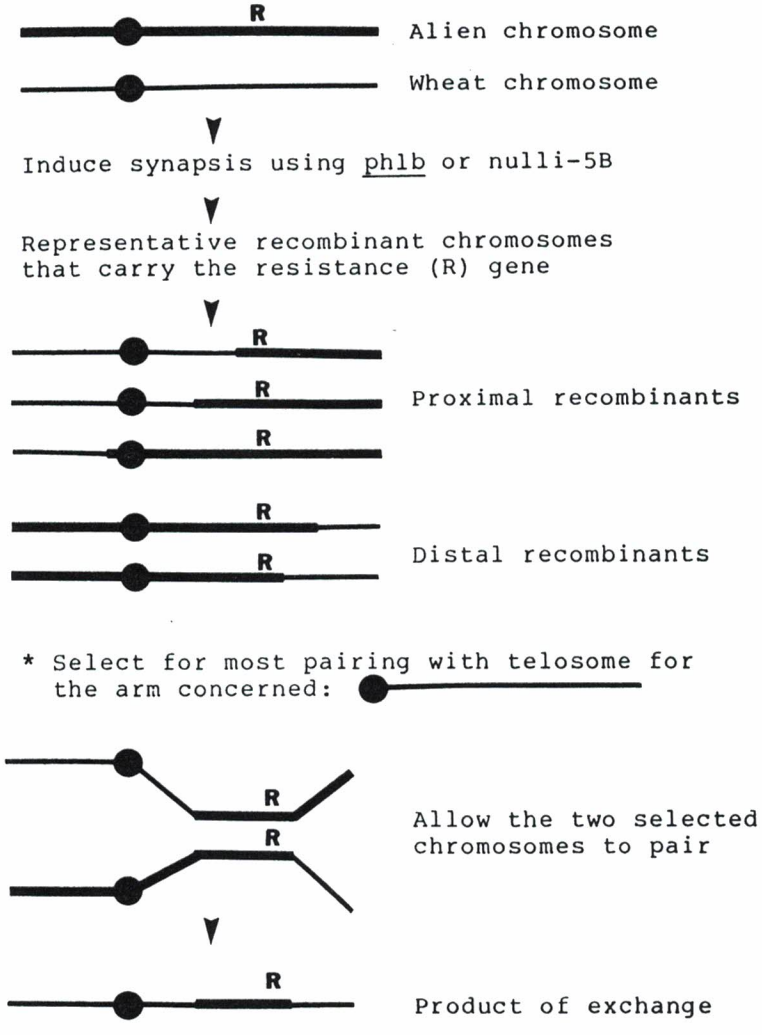

Fig. 1. Suggested procedure for transferring an interstitial segment of an alien chromosome to one of its wheat homoeologues.

marked by a gene for resistance to the leaf-rust fungus, *Puccinia recondita* Rob. ex Desm. The work with one of these lines, 'TAP 67', will be briefly reviewed here and brought up to date. This line, obtained from A. M. Schlehuber of Oklahoma State University, has a pair of *Agropyron* chromosomes substituted for wheat chromosome pair 3D.

The substitution line was first made monosomic for chromosome 5B, then pollinated by nulli-5B tetra-5D (Fig. 2). Most of the progeny from this cross were nulli-5B tri-5D and were also monosomic for both 3D and the *Agropyron* chromosome, designated 3Ag. The two homoeologous monosomes paired with each other in about 30% of the sporocytes. Pollination by euploid plants restored *Phl*, and offspring having the leaf-rust gene (*Lr*24) transferred to a wheat chromosome were tentatively identified by means of disease testing

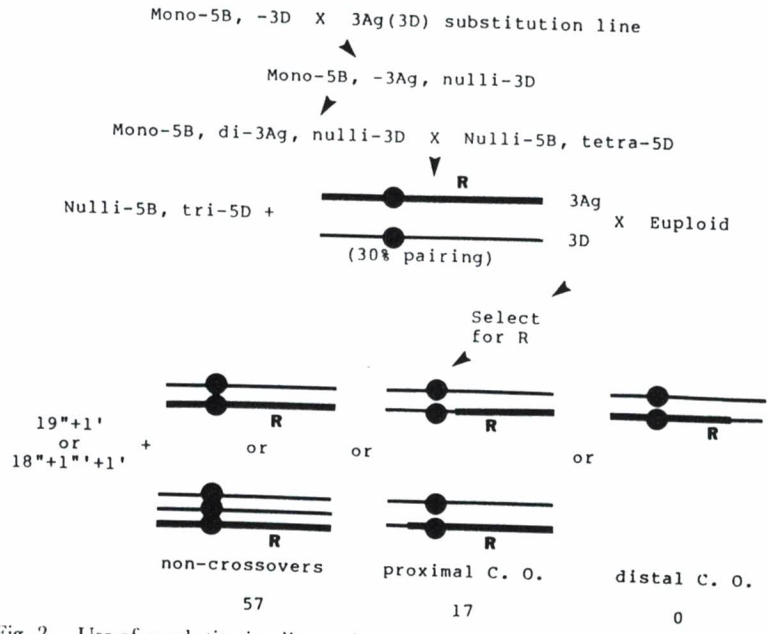

Fig. 2. Use of a substitution line and nulli-5B tetra-5D for the transfer of alien segments to wheat chromosomes by means of induced homoeologous pairing.

and cytological study of resistant segregates. Eventually it was shown that there were 17 different transfers from 3Ag to 3D, as well as 3 from 3Ag to 3B (Sears 1978, 1981). All of the 17 were tested for pairing with telosomes 3AgL, 3DL, 3DS and, where appropriate, 3AgS (Sears 1978, and unpublished). They were also scored (by Hart *et al.* 1976) for presence or absence of an allele on 3AgL for an isozyme of glutamate oxaloacetate transaminase (GOT) and (by Rodriguez-Loperena *et al.* 1976) for a 3AgS seed-protein allele.

It was established that all 17 3Ag–3D transfers had resulted from recombination proximal to *Lr*24. The relative lengths of the terminal *Agropyron* segments were best revealed by the frequency of pairing of the concerned chromosomes with telo-3DL. Such pairing occurred in from 0% to 70% of the microsporocytes. All the transfer chromosomes paired regularly with 3AgL, showing that even the one that showed 70% pairing with 3DL possessed a terminal 3AgL segment of considerable length. The presence of the 3D allele of the *R* (red-seed) gene in only the transfers having the most pairing with 3DL (R. A. McIntosh, pers. comm.) supported the conclusions drawn from chromosome pairing.

Because it was believed that recombination tends to be shifted distally in telocentric chromosomes (Sears 1972b), a second experiment was undertaken using telo-3AgL instead of the complete 3Ag. In this experiment (Fig. 3), the previously mentioned *phlb* mutation was used instead of nulli-5B tetra-5D. Use of telo-3AgL was advantageous in other ways than increasing recombination; namely, it enabled more accurate cytological observation of the amount of pairing with 3D, and it simplified the recognition of the recombinants involving 3AgL. Plants with a non-recombined 3AgL were identifiable as being resistant and having a non-pairing telosome; plants resulting from proximal recombination were

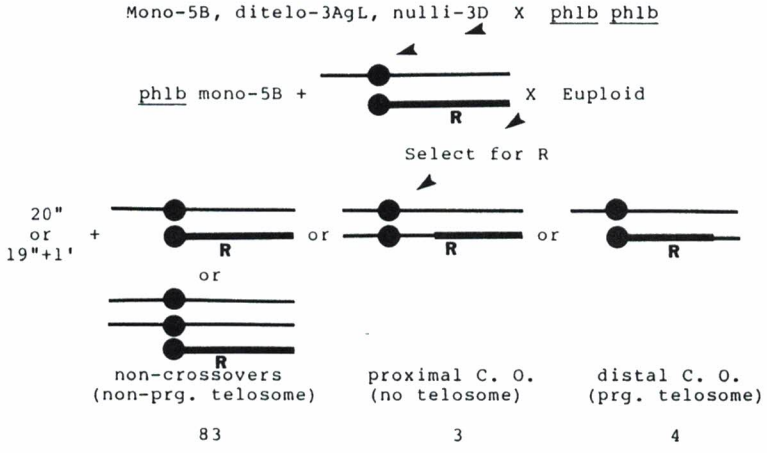

Fig. 3. Use of a ditelosomic substitution line and the *phlb* mutation for the transfer of alien segments to wheat chromosomes.

resistant but had no telosome; and distal recombinants were resistant and had a pairing telosome.

The experiment involving 3AgL was slightly larger than the one using a complete 3Ag, but it resulted in only 40% as many transfers (8 *vs.* 20); 7 of these involved chromosome 3D (*vs.* 17 from the complete 3Ag). The most striking difference between the two experiments was that with telo-3AgL, 4 transfers resulted from recombination distal to *Lr*24, whereas none of these were obtained when the complete 3Ag was used. One of the distally recombined chromosomes supported about 30% pairing with telo-3DL. None of the 3 proximal recombinants had as short a 3AgL segment as the two with the most telo-3DL pairing from the first experiment.

The next step (Fig. 4) was to combine a selected chromosome that was the result of a recombination proximal to *Lr*24 (the recombinant chromosome that had 70% pairing

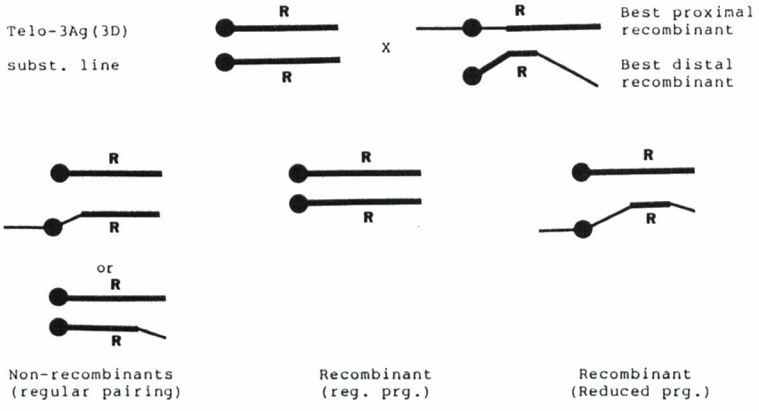

Fig. 4. Combining a proximal with a distal recombinant to obtain an interstitial alien segment through homologous pairing.

capability with telo-3DL) with the best distal-recombinant chromosome, in the presence of *Phl*. (These two chromosomes will hereafter be referred to as the original recombinants.) As mentioned before, the only possibility for pairing of these two chromosomes was in the *Agropyron* segment they possessed in common. The length of this segment, and the frequency of pairing of the two chromosomes with each other, depended upon how close to the *Lr*24 locus on opposite sides were the crossovers that had given rise to the two chromosomes. The segment's length proved to be substantial, sufficient to result in nearly 90% pairing. On the basis of the generally accepted assumption that every chiasma represents a crossover between two of the four chromatids, almost one-fourth of the chromosomes recovered were expected to be recombinants that were all 3D except for an interstitial 3Ag segment carrying *Lr*24. In crosses with the 3Ag substitution line, these would be identifiable as chromosomes pairing with 3Ag in fewer than 95% of microsporocytes. They were expected to pair as frequently with 3Ag as the two original complementary recombinants paired with each other (nearly 90%), since pairing in the two situations would be confined to the same segment. In fact, however, the five recovered interstitial-transfer chromosomes averaged only 79% pairing with 3Ag and were easily distinguished from non-recombinants, which showed more than 95% pairing with 3Ag. The five interstitials were recovered among 12 offspring of the 3Ag–3D substitution pollinated by plants carrying the two complementary recombinants. Only one telosome was transmitted.

Whether the apparent reduced pairing of the recovered interstitial-transfer chromosome with 3AgL, in comparison with that of the two parental chromosomes with each other, was genuine or an artifact remains to be seen. The observations were made at different times in different countries and thus are not strictly comparable. In any case, it is clear that the interstitial *Agropyron* segment is shorter than any previously obtained from the TAP 67 chromosome. (A spontaneous transfer identified by Schlehuber and designated 'Agent' was found by McIntosh (pers. comm.) to involve a terminal 3Ag segment long enough to include the *R* locus.) However, the *Agropyron* segment involved can scarcely be considered short.

Little difficulty is anticipated in shortening the alien segment. Advantage can be taken of the fact that any substantial reduction in the segment's length will lead to more pairing with telo-3DL. An interstitial-transfer line is being made mono-5B preparatory to pollinating it with *phlb phlb* (Fig. 5). The progeny of this cross will be heterozygous for the interstitial transfer, and most will be mono-5B, hemizygous for *phlb*. Following pairing in the interstitial segment, recombined chromosomes will be recovered in which either a proximal or a distal part of the *Agropyron* segment has been replaced by the corresponding wheat chromatin. If recombination occurs at random within the segment, a series of recombinant chromosomes should be recovered which have lost varying amounts of the alien chromatin, some losses proximal and some distal to *Lr*24, some long and some short, some including *Lr*24 and some not. The amount of pairing with telo-3DL and telo-3AgL will indicate how much of the segment remains, and tests for the presence of *Lr*24 will additionally characterize it. Whether *Sr*24 is present in the interstitial segment has not yet been checked; if it is present, it too can be used as a marker in differentiating among the derivatives.

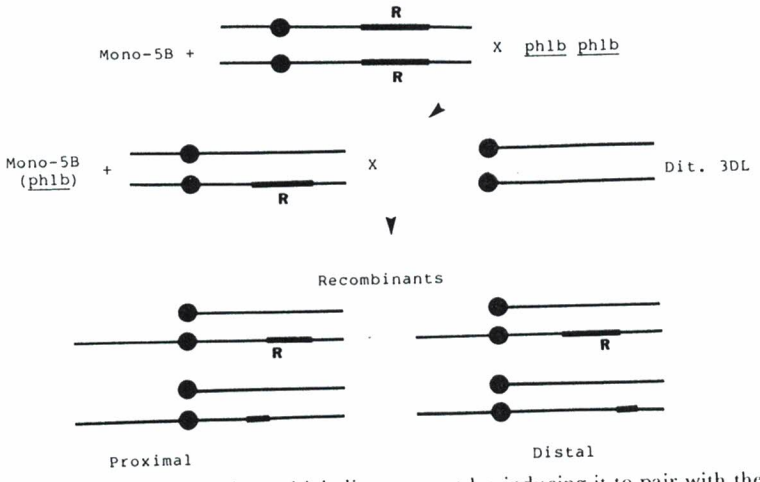

Fig. 5. Shortening an interstitial alien segment by inducing it to pair with the corresponding portion of its wheat homoeologue.

Currently there is no evidence as to the exact location of *Lr*24 in the alien segment. If it is located near one or the other end of the segment, a single crossover should suffice to reduce the segment to the desired shortness and still retain the gene. If, however, *Lr*24 lies near the middle of the segment, it will presumably be necessary to obtain two derivatives, one with the segment shortened on one end and the second shortened on the other end, and recover a recombinant between them, as was done with the two original recombinants.

In *phlb* plants carrying both 3D and the interstitial transfer chromosome, homoeologous recombination within the alien segment may differ from what it was when the segment was part of a complete *Agropyron* chromosome, for the occurrence of homologous pairing on both sides of the segment may affect homoeologous pairing and recombination within it. If there is no such effect, or if the effect is to reduce the amount of recombination, time and labor could presumably have been saved if larger initial experiments, particularly involving telo-3AgL, had been done. Proximal and distal recombinants with exchange points closer to *Lr*24 might then have been obtained that would have given rise to an interstitial segment requiring no shortening.

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