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Intraspecific chromosome manipulation

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Whole chromosome manipulation within a crop species is restricted in the main to the hexaploid bread wheat, *Triticum aestivum* ($2n = 6x = 42$). Directed manipulation within this group is possible because aneuploidy is tolerated, and at least 60 monosomic sets, representative of many of the successful wheats of the world, are available. These permit, after recurrent backcrossing and cytological selection, the substitution of whole chromosomes from one variety into another.

Substitution lines have been used to identify chromosomes and genes responsible for varietal differences affecting a range of important agronomic characters, and provide the most efficient method of genetical analysis available in wheat or indeed in any crop species. The length of time taken to develop substitution lines is, however, a weakness of the approach. A method in which monosomic sets are crossed reciprocally overcomes this weakness and permits the identification of significant chromosomal effects in one to two generations. Modifications to the method enable chromosomal effects to be studied in any varietal combination even though monosomic sets are not available in all the varieties being studied.

The results obtained by intraspecific chromosome manipulation have been exploited in the development of a high-yielding spring wheat after recombination of a single chromosome substituted into a winter wheat. This example suggests that the methods may have direct applications to plant breeding where large chromosomal effects have been identified. This is particularly relevant where the variation concerned is difficult to assess.

These methods also have a major part to play in increasing the understanding of the genetic architecture of wheat. It is also likely that they will have value in transferring useful genes from distantly related varieties.

INTRODUCTION

In the main, whole chromosome manipulation within a crop species is confined to the polyploids, where the duplication of genes permits the loss of chromosomes without disastrous consequences on a plant's phenotype. Such aneuploids have been assembled in a number of polyploid crop species, e.g. cotton, oats and wheat (Brown 1966; Rajhathy & Thomas 1974). However, it is in the latter species that the use of aneuploids has been the most extensive, and an elaborate methodology has evolved for their use in genetical analysis and chromosome manipulation.

The bread wheat of agriculture, *Triticum aestivum* ($2n = 6x = 42$), is a hexaploid species, and a range of aneuploids (monosomics, tetrasomics, ditelocentrics, nullisomics) representing each of the 21 chromosomes of wheat have been assembled over the years. This collection was first made in the wheat variety Chinese Spring by Sears (1954) in the United States, but more recently complete sets of aneuploid lines have been systematically obtained in a number of varieties (Law & Worland 1973).

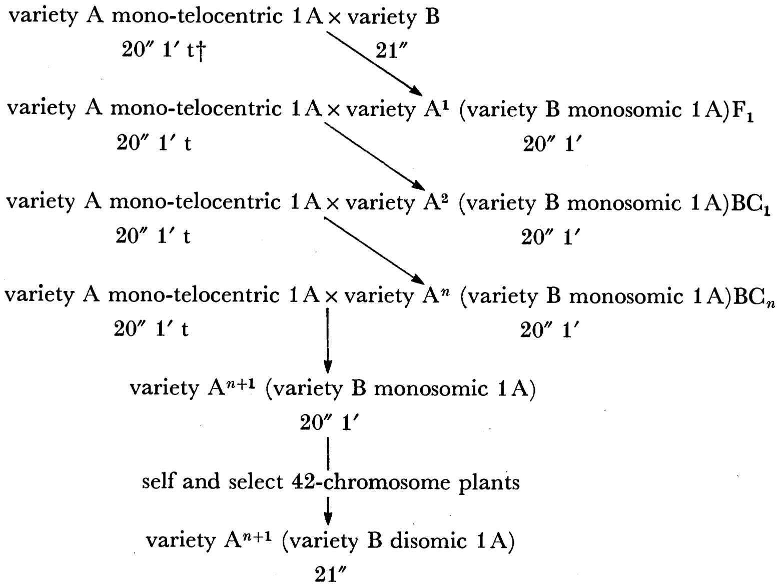
INTER-VARIETAL CHROMOSOME SUBSTITUTIONS

The ability to tolerate the loss of a chromosome and to produce cytologically recognizable marker chromosomes (telocentrics) has enabled the substitution of single chromosomes from a donor variety for their homologues in a recipient variety (Sears 1953; Unrau 1950). The method is illustrated in table 1.

By recurrent backcrossing to the monotelocentric line of the recipient variety and selecting in each generation for monosomic plants having only complete chromosomes, it is possible to ensure that the donor chromosome is maintained intact. After a number of backcrosses, the background will either be genetically identical or close to the recipient variety, so that by selfing, a disomic substitution line can be obtained that will be true-breeding and which can be treated as a normal variety in assessing the effect of the substituted chromosome. Since appropriate aneuploids exist for each of the 21 chromosomes of wheat, 21 inter-varietal chromosome substitutions are possible for each pair of recipient and donor varieties.

TABLE 1. DEVELOPMENT OF AN INTER-VARIETAL CHROMOSOME SUBSTITUTION LINE OF CHROMOSOME 1A BY USING A MONO-TELOCENTRIC LINE IN VARIETY A AS THE RECURRENT PARENT

(Selection is practised for monosomic plants after each hybridization. In this way the substituted chromosome is maintained intact and in the hemizygous condition until disomics are selected in the final selfing generation.)



† ″ refers to a bivalent and ′ refers to a univalent at first metaphase of meiosis.

Inter-varietal chromosome substitutions provide one of the best means of studying quantitative characters. The power of the method may be illustrated by referring to a recent study of the genetics of the semi-dwarfism that occurs in some Mediterranean wheats, for example the varieties Mara and Sava.

Semi-dwarfism has, of course, been a major objective of wheat breeders for many decades, and the introduction of the dwarfing genes *Rht*1 and *Rht*2 from the Japanese variety Norin 10 to produce the 'green revolution' wheats has been one of the major achievements of plant breeding (Borlaug 1968). However, the dwarfism of Norin 10 is genetically different from the dwarfism of Mediterranean wheats. There is therefore a need to understand the nature of this difference more clearly.

In crosses between the tall, W. European variety, Cappelle-Desprez and the Italian semi-dwarf variety, Mara, the F_2 segregation for height is continuous, suggesting that genes of large effect are not involved in this varietal difference. A series of chromosome substitution lines in which Mara chromosomes replace their homologues in Cappelle-Desprez have been produced, and the variation in height between these substitutions lines is shown in figure 1. This clearly indicates that chromosome 2D and a gene or genes on the short arm of 5B and/or 7B (5B^{s}–7B^{s}) are responsible for the semi-dwarfism of Mara.

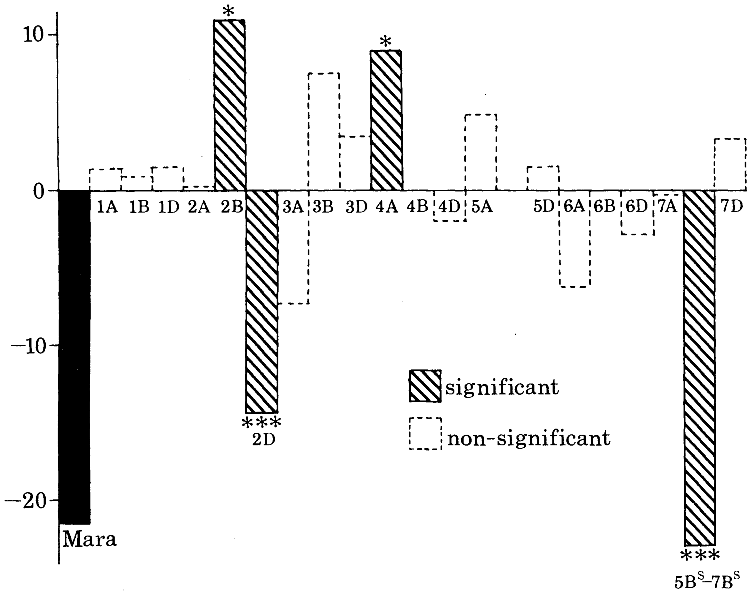


FIGURE 1. Deviations in final plant height from Cappelle-Desprez (103 cm) of chromosome substitution lines of Mara into Cappelle-Desprez. Because Cappelle-Desprez carries the reciprocal translocation, 5B^{L}–7B^{L} and 5B^{s}–7B^{s}, not present in Mara, it has not been possible to substitute the complete 5B or 7B chromosome into Cappelle-Desprez. The 5B^{s}–7B^{s} substitution line therefore refers to the transfer of only the short arms of 5B and 7B of Mara. The long arms of these two chromosomes could not be transferred because the 5B^{L}–7B^{L} monosomic of Cappelle-Desprez has yet to be obtained. *, $0.05 > p > 0.01$; ***, $p < 0.001$.

This analysis of course refers to whole chromosomes, so that it is possible that more than one gene is responsible for these major chromosomal effects and that these genes are loosely linked. The breakdown of these linkages might explain the absence of discontinuities in the F_2 generation. Such a possibility may be tested by crossing each of the critical substitution lines to Cappelle-Desprez and observing the F_2 products of this single chromosome segregation on a non-segregating background. This has not been done yet, although the method has been used successfully in other cases (Law 1966; Law *et al.* 1978). However, whatever the outcome, the results of this substitution exercise clearly demonstrates the power of inter-varietal chromosome substitutions in genetical analysis.

Height is, of course, an easy character to measure, but this is not so for many of the characters of interest to the wheat breeder. More often than not genetical estimates must be based upon large numbers of replicates and the use of elaborate techniques of estimation that are often time consuming and tedious. Under these circumstances, inter-varietal chromosome substitutions are again more efficient than conventional methods of genetical analysis.

One example of this advantage is the study of the genetical control of the eye-spot resistance of Cappelle-Desprez. This fungal disease is assessed by artificially infecting juvenile wheat plants with the fungus *Cercosporella herpotrichoides* and scoring the degree of penetrance of the fungus through the leaf sheaths. To detect resistance to the fungus, extensive replication is required, so that the method is labour-intensive and does not readily lend itself to easy selection or to detailed genetical studies by conventional procedures.

On the other hand, a study of the substitution lines in which Cappelle-Desprez chromosomes replace their homologues in the susceptible variety Chinese Spring indicated that the resistance of Cappelle-Desprez was controlled mainly by one chromosome, chromosome 7A (Law *et al.* 1976). Again, it is possible that this effect could be due to a number of genes concentrated on this chromosome. However, in terms of the future exploitation of this variation in breeding this need not matter, since once identified, the chromosome can be transferred intact by cytogenetical techniques into other varieties or breeding programmes without the need to resort to laborious screening methods. In this case, the 'useful' chromosome would be followed cytologically rather than by seeking to monitor the effects of the genes that it carries.

Reciprocal monosomics

These two examples illustrate the use of chromosome substitution techniques in genetical analysis and in exploiting the results of analysis in making directed contributions to breeding. The weakness of the approach is the length of time taken to develop substitution lines. To remove the possible confounding effects of background, at least four backcrosses, and in some cases as many as eight, have been found to be necessary.

This weakness can be overcome by crossing homologous monosomics from two varieties reciprocally. This method, first used by McKewan & Kaltsikes (1970), is shown in figure 2 and depicts the development of a pair of F_1 monosomics with identical backgrounds but different hemizygous chromosomes. Any phenotypic differences between these reciprocal hybrids, assuming the absence of cytoplasmic effects, must relate to the genetical differences between the two hemizygous chromosomes. Because large numbers of F_1 monosomic hybrids may not be easy to produce, assessment may be deferred to the F_2 generation, where the means of the two reciprocally derived F_2 populations may be compared. Disomics may also be selected in the F_2 generation so that those genes ineffective in the hemizygous state may be detected and their effects estimated.

The reciprocal monosomic method will permit the detection of chromosomes whose effects are large in relation to the segregation of genes on other chromosomes. The method will therefore identify those chromosomes that are likely candidates for the future development of substitution lines and for directed introduction into breeding programmes.

Two examples of this approach will suffice. The first concerns the different chromosomal control of ear-emergence time between the spring/winter variety Bersée and the winter wheat Cappelle-Desprez. The results of crossing the monosomics of these varieties reciprocally and scoring the F_2 monosomic populations in the field after a spring sowing are shown in figure 3.

Clearly a number of chromosomes are responsible for the differences between the two varieties, so that the character can be regarded as polygenic. However, a major proportion of the variation is associated with chromosomes of homoeologous group 5 and 7, the major effect being determined by chromosome 5A. It is known from other aneuploid studies that 5A carries *Vrn*1, a gene for vernalization requirement, which has a major effect on the control of winter and spring wheat differences (Halloran & Boydell 1967; Law *et al.* 1976; Snape *et al.* 1976).

The second example concerns the chromosomal location of *Rht*2, one of the dwarfing genes from Norin 10. Although the presence of this gene can now be detected relatively easily in plants by their lack of response to exogenous gibberellic acid (Gale & Marshall 1973), it nevertheless provides an excellent example of the effectiveness of the reciprocal monosomic method in detecting major effects on a quantitative character. Disomic F_2 plants were selected from each of the reciprocal crosses between monosomics 4D of Bersée and the semi-dwarf wheat Hobbit, and their final plant heights obtained. These indicated that those plants carrying 4D of Hobbit were on average 20% shorter than plants carrying the Bersée homologue, thus confirming the location of *Rht*2 on Hobbit 4D (Gale *et al.* 1975).

However, once a chromosomal effect of this magnitude has been detected among reciprocally derived F_2 disomics, it is then but a simple matter to look at F_3 lines to study the consequence of such a chromosomal effect on other characters. A number of F_3 lines from the Bersée 4D with

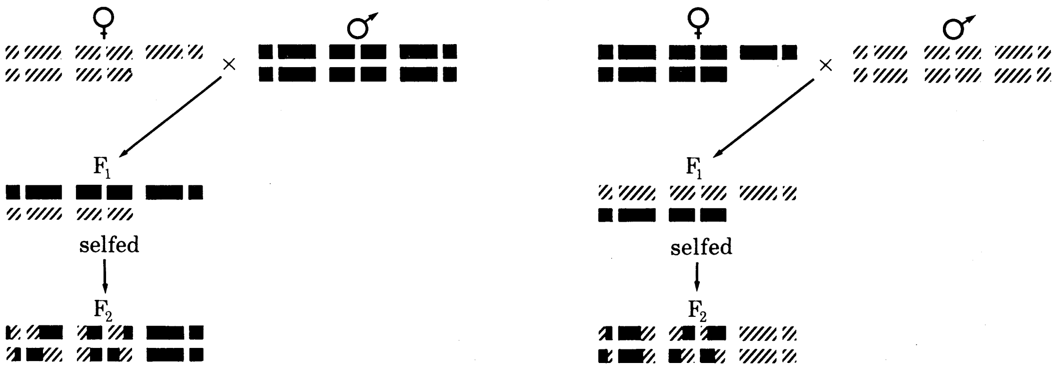


FIGURE 2. The development of F_1 monosomic hybrids with identical backgrounds but different hemizygous chromosomes, by crossing, reciprocally, homologous monosomics from two different varieties. For the sake of simplification, only three pairs of chromosomes are depicted instead of the normal 21. Different varietal chromosomes are shown as either hatched or in black.

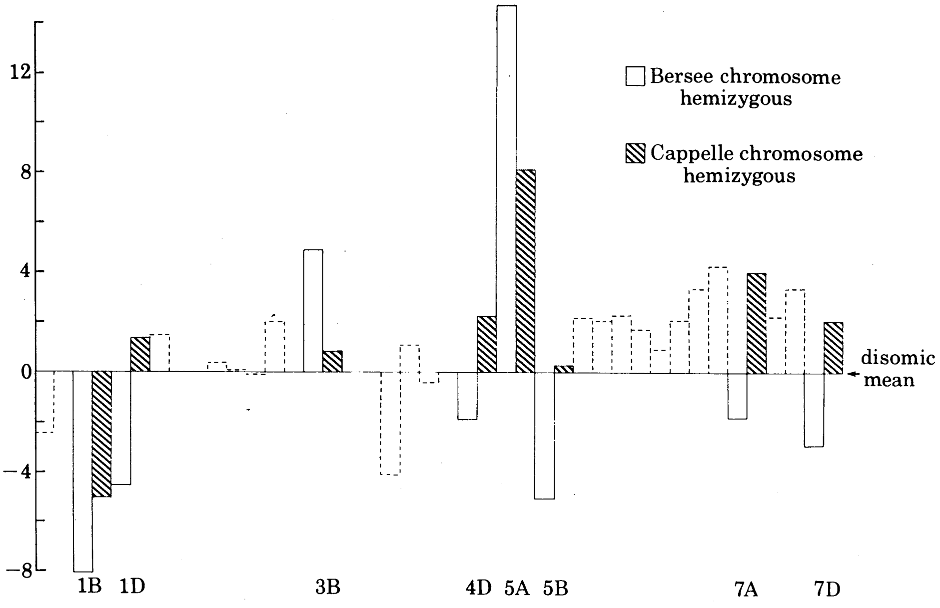


FIGURE 3. Mean ear-emergence times of F_2 reciprocal monosomic hybrids between Bersée and Cappelle-Desprez defined from the controlled disomic F_2 mean. Histograms of reciprocal monosomic pairs that are significantly different from each other are drawn with solid lines, and the chromosomes responsible for these differences are given at the bottom of the figure. (Law *et al.* (1979).)

Hobbit 4D cross were therefore grown; the different effects of the 4D chromosomes on a number of important agronomic characters are shown in table 2. Apart from the large effect on height, the effects on two of the yield components, grain number and grain size, were noteworthy. However, the effects were opposed so that their combined effect on overall yield was not significant.

Again, in these types of experiment it is not possible to state whether the effects are due to one or more genes, or that the same genes are affecting each of the characters. In the present case, after more detailed studies it was found that the effects are all due to the gene for dwarfism, *Rht2* (Gale 1978). Obviously for other situations, the numbers of genes and their relations between each other would need to be studied. However, in many cases the genetic associations will be such that the breakages of linked complexes will not be necessary, and the major benefits in plant breeding will be obtained by transferring the chromosome intact.

TABLE 2. MEANS OF F_3 LINES, HOMOZYGOUS FOR CHROMOSOME 4D OF EITHER BERSÉE OR HOBBIT, DERIVED FROM THE RECIPROCAL CROSS BETWEEN BERSÉE MONOSOMIC 4D AND HOBBIT MONOSOMIC 4D

	Bersée 4D	Hobbit 4D	difference
height/cm	106.80 ± 2.07	88.45 ± 2.47	+ 18.35***
tiller number	8.41 ± 0.21	8.77 ± 0.21	− 0.36
spike length/cm	9.92 ± 0.17	9.99 ± 0.20	− 0.07
percentage fertility 1 × 2 florets	79.97 ± 1.09	82.64 ± 1.19	− 2.67
grains per ear	60.89 ± 1.42	65.79 ± 2.20	− 4.90
1000 grain mass/kg	55.14 ± 1.00	48.87 ± 0.99	+ 6.27***
grain mass per plant/g	23.20 ± 0.60	21.47 ± 1.31	+ 1.73

***, $p < 0.001$.

TABLE 3. MONOSOMIC SERIES IN WHEAT

region	number of monosomic sets	noted varieties
W. Europe	11	Cappelle-Desprez, Hobbit, Caribo
E. Europe	7	Carola, Besostaya I, Mironovskaya 809
U.S.S.R.	14	Aurora, Besostaya II, Kavkaz, Saratovskaya 29
Mediterranean	6	Sava, Mara, Aragon 03
India	2	Kalyansona
Canada	10	Thatcher, Rescue, Redman
U.S.A.	6	Cheyenne, Wichita
Japan	3	Norin 10
Australia	3	Federation, Gabo, Spica
total	62	

Monosomic series

The method of reciprocal monosomics depends upon the existence of suitable monosomic series. These are not universally available. However, it is perhaps not widely known that a large number of monosomic series have been developed. As table 3 indicates, these include varieties produced from most of the major wheat-breeding programmes of the world, as well as many very successful varieties. Saratovskaya 29, for instance, is the major spring wheat of the Soviet Union. Besostaya I had for several years the biggest acreage of any wheat in the world. Caribo and Hobbit are grown widely in Europe. The opportunity therefore exists for screening chromosomal variation among some of the most important wheat germplasm of the world.

Backcross reciprocal monosomics

However, even in the absence of a particular monosomic series, a modified reciprocal monosomic method may be used. This is referred to as the backcross reciprocal monosomic method (Snape & Law 1980), and the details are shown in figure 4. This method requires a further generation of crossing compared with the previous method, and the selection of a number of backcross monosomics to allow for sampling of background variation. The method will, however, permit the detection of major chromosomal differences affecting a quantitative character.

An example of the application of this method is given in table 4. In this experiment, a comparison was made between the estimates of 5A chromosomal effects obtained either by the

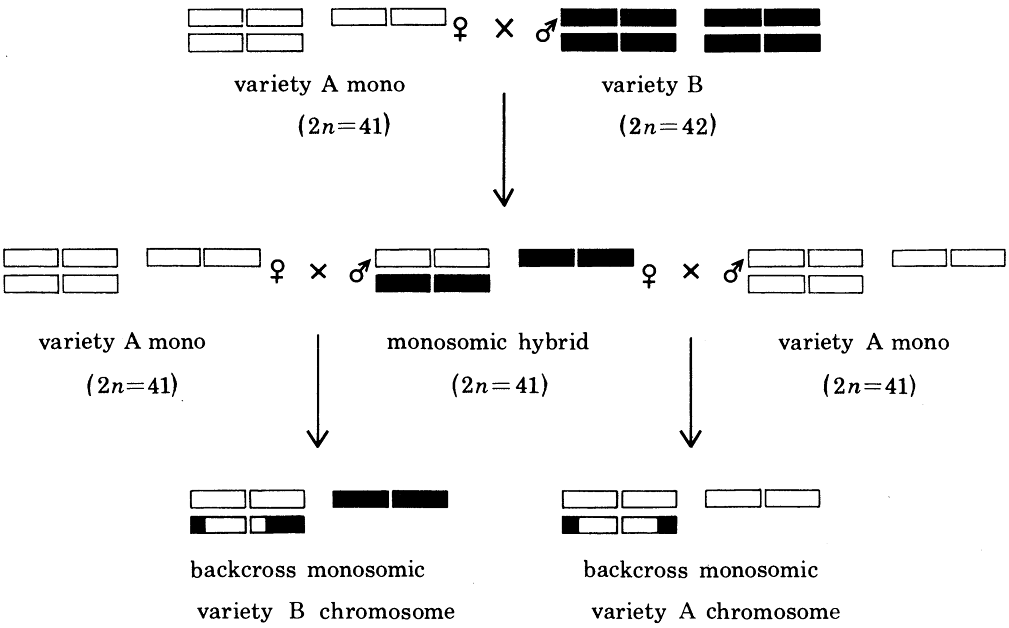


FIGURE 4. The development of backcross reciprocal monosomics by using variety A in which a monosomic series exists and variety B in which monosomics are not available, to give two populations of monosomics with comparable backgrounds but with different hemizygous chromosomes.

TABLE 4. COMPARISON BETWEEN ESTIMATES OF 5A CHROMOSOMAL EFFECTS FROM SUBSTITUTION LINES AND BACKCROSS RECIPROCAL MONOSOMIC (BRM) LINES

	chromosome	height/cm	yield/g
Chinese Spring (CS)	CS5A	107.2	7.01
Chinese Spring (Cap 5A)	Cap5A	99.1	9.89
difference		**+8.1***	**−2.88***
BRM lines	CS5A	122.7	14.73
BRM lines	Cap5A	114.9	15.15
difference		**+7.8***	**−0.42**
Cappelle-Desprez (Cap)	Cap5A	103.0	15.51
Cappelle-Desprez (Besostaya 5A)	Bes5A	111.2	16.95
difference		**−8.2*****	**−1.44***
BRM lines	Cap5A	109.1	15.16
BRM lines	Bes5A	115.2	21.15
difference		**−6.1***	**−5.99***

*, $0.05 > p > 0.01$; ***, $p < 0.001$.

backcross reciprocal monosomic method or by inter-varietal chromosome substitutions. The results show that the two sets of estimates are very close to each other. Thus, the effects of chromosome 5A from Chinese Spring compared with its homologue in Cappelle-Desprez and the comparative effects of the 5A chromosomes in Besostaya I and Cappelle-Desprez are very similar for height and, to a lesser extent, yield for both methods.

Direct use of these methods in plant breeding

The aneuploid methods described permit the detection and estimation of chromosomal effects with a precision and generality unattainable by other analytical methods at the moment. However, to what use can these methods be applied in plant breeding? An example of their direct exploitation comes from the work to genetically transform winter wheat into spring wheat. As already mentioned, chromosome 5A has a major effect on the control of winter–spring wheat differences. From the study of substitution lines, as well as the use of the monosomic techniques described already, a range of variation in ear-emergence time has been found for this chromosome (Law *et al.* 1976). At the 'lateness' end of the spectrum of variation is the 5A chromosome from winter wheats such as Cappelle-Desprez and Cheyenne, while at the other is 5A of *Triticum spelta* (a subspecies of *T. aestivum*). This chromosome conferred the smallest vernalization requirement of all the chromosomes studied, so that in converting a winter wheat into a spring wheat, *T. spelta* 5A is obviously the first choice. This chromosome was therefore substituted into the winter wheat Hobbit by using monosomic 5A of this variety as the recurrent parent. After five backcrosses, the Hobbit (*T. spelta* 5A) substitution line was obtained; it behaved exactly as expected and gave ear-emergence times comparable with other spring wheats after a spring sowing. Clearly the substitution of this chromosome had converted Hobbit into a spring wheat.

The substitution of *T. spelta* 5A into Hobbit introduced two major genes other than *Vrn1*. These are the genes for speltoid ears (*q*) and awning (*b1*). The speltoid character has adverse effects on threshability, so that it was important to remove the *q* gene by recombination. This was achieved by intercrossing the substitution line and Hobbit. By selecting among the products of this hybrid, or by backcrossing to Hobbit monosomic 5A, four true-breeding types of spring wheat were obtained that were either awned or awnless, or were either speltoid or square-headed. Assessments of the yield produced by these four classes showed variation within and between the four classes, suggesting that *T. spelta* 5A carries separate genes affecting yield not present on Hobbit 5A. The best yields were obtained for lines that were awned and square-headed.

In more extensive yield trials in 1979 and 1980, this spring Hobbit line has outyielded the present-day spring wheats, Highbury and Timmo, by an average of 12% (see table 5).

Table 5. Yield (grams per plot) of Spring Hobbit and two current spring wheat varieties, Timmo and Highbury

	1979	1980	mean	percentage of Highbury
Spring Hobbit	3636	3423	3530	112***
Timmo	3195	3123	3159	100
Highbury	3259	3038	3149	100

***, $p < 0.001$.

It should be stated that these favourable results do not necessarily mean that chromosome manipulation has in this case produced a new commercial variety, because, as might be expected, the Spring Hobbit line carries some of the weaknesses of Hobbit itself. Thus its level of mildew resistance is not sufficient and its bread-making quality is below the level expected for a spring wheat. The Spring Hobbit line must therefore be regarded as a breeders' variety rather than the finished article, and as such it is now being used by spring-wheat breeders as a parent in their breeding programmes.

In the development of Spring Hobbit, a lengthy backcross programme was necessary to substitute *T. spelta* 5A into a Hobbit background. Such a course of action is probably the exception rather than the rule. The more likely approach would be to substitute and fix a desirable chromosome during the early generations of a breeding programme, and then to allow conventional breeding procedures to take their course. In this way some of the genetical deficiencies of Spring Hobbit could have been removed by selection in the development of a new variety.

Conclusions

Intraspecific chromosome manipulation can be expected to contribute directly to breeding programmes under the following circumstances.

1. Where the chromosome has a large effect on a quantitative character and it is not easy to select for this effect by conventional procedures because the means of detection are tedious and labour or space intensive.

2. Where the chromosome has a large effect resulting from the combined effects of many genes that may be difficult to maintain intact by using normal breeding procedures. A special case of this will be the chromosome carrying genes affecting different characters.

The methods of chromosome manipulation may also have value as a screening procedure to detect useful genetic variation among unadapted varieties rarely used by breeders.

Over and above these possibilities of making direct contributions to breeding programmes, chromosome manipulation will continue to have considerable value in carrying out genetical analyses. Ultimately, such analyses should lead to a better understanding of the genetical architecture of wheat. It is from such understanding that opportunities will come for identifying new and useful gene combinations and predicting the outcome of further gene and chromosome manipulation to improving varietal performance.

References (Law *et al.*)

Borlaug, N. E. 1968 Wheat breeding and its impact on world food supply. In *Proc. 3rd Int. Wheat Genetics Symp.*, Canberra, Australia, pp. 1–39.

Brown, M. S. 1966 Attributes of intra- and inter-specific aneuploidy in *Gossypium*. In *Chromosome manipulations in plant genetics* (ed. R. Riley & K. R. Lewis), pp. 98–112.

Gale, M. D. 1978 The effects of Norin 10 dwarfing genes on yield. In *Proc. 5th Int. Wheat Genetics Symp.*, New Delhi, India, pp. 978–987.

Gale, M. D. & Marshall, G. A. 1973 Dwarf wheats and gibberellins. In *Proc. 4th Int. Wheat Genetics Symp.*, Columbia, Missouri, pp. 513–519.

Gale, M. D., Law, C. N. & Worland, A. J. 1975 The chromosomal location of a major dwarfing gene from Norin 10 in new British semi-dwarf wheats. *Heredity, Lond.* **35**, 417–421.

Halloran, G. M. & Boydell, C. W. 1967 Wheat chromosomes with genes for vernalisation response. *Can. J. Genet. Cytol.* **9**, 632–639.

Law, C. N. 1966 The location of genetic factors affecting a quantitative character in wheat. *Genetics, Princeton* **53**, 487–498.

Law, C. N. & Worland, A. J. 1973 Aneuploidy in wheat and its uses in genetic analysis. In *Plant Breeding Institute Annual Report, 1972*, pp. 25–65.

Law, C. N., Scott, P. R., Worland. A. J. & Hollins, T. W. 1976 The inheritance of resistance to eyespot (*Cercosporella herpotrichoides*) in wheat. *Genet. Res.* **25**, 73–79.

Law, C. N., Worland, A. J. & Giorgi, B. 1976 The genetic control of ear-emergence time by chromosome 5A and 5D of wheat. *Heredity, Lond.* **36**, 49–58.

Law, C. N., Young, C. F., Brown, J. W. S., Snape, J. W. & Worland, A. J. 1978 The study of grain protein control in wheat using whole chromosome substitution lines. In *Seed Protein improvement by nuclear techniques*, pp. 483–502. Vienna: I.A.E.A.

Law, C. N., Snape, J. W. & Worland, A. J. 1979 Reciprocal monosomic studies. In *Plant Breeding Institute Annual Report, 1978*, pp. 134–135.

McKewan, J. M. & Kaltsikes, P. J. 1970 Early generation testing as a means of predicting the value of specific chromosome substitutions into common wheat. *Can. J. Genet. Cytol.* **12**, 711–723.

Rajhathy, T. & Thomas, H. 1974 In *Cytogenetics of oats* (*Misc. Publs Genetics Soc. Canada* no. 2), p. 90.

Sears, E. R. 1953 Nullisomic analysis in common wheat. *Am. Nat.* **87**, 245–252.

Sears, E. R. 1954 The aneuploids of common wheat. *Mo. agric. exp. Stn Res. Bull.* no. **572**, p. 59.

Snape, J. W., Law, C. N. & Worland, A. J. 1976 Chromosome variation for loci controlling ear emergence time on chromosome 5A of wheat. *Heredity, Lond.* **37**, 335–340.

Snape, J. W. & Law, C. N. 1980 The detection of homologous chromosome variation in wheat using backcross reciprocal monosomic lines. *Heredity, Lond.* **45**, 187–200.

Unrau, J. 1950 The use of monosomes and nullisomics in cytogenetical studies of common wheat. *Sci. Agric.* **30**, 66–89.