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CHROMOSOME MANIPULATION AND ITS EXPLOITATION IN THE GENETICS AND BREEDING OF WHEAT

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C. N. LAW, J. W. SNAPE and A. J. WORLAND

Plant Breeding Institute, Maris Lane
Trumpington, Cambridge CB2 2LQ, U.K.

SUMMARY

Chromosome manipulation in bread wheat uses aneuploid methods to transfer whole chromosomes or segments of chromosomes from one variety into another, or from related species into wheat. These methods have been applied extensively so that an incomparable range of aneuploid and whole chromosome substituion lines now exists in wheat. This has given rise to some of the most detailed genetic studies of economic characters carried out on a crop plant and include the analysis of the semi-dwarfism of Mediterranean wheat and the location of genes affecting grain protein amounts. The applications of this knowledge to wheat breeding is considered as well as the potential that these methods have for promoting the understanding of plant processes and of the molecular organisation of the wheat genome.

INTRODUCTION

Chromosome manipulation in hexaploid wheat, *Triticum aestivum*, uses aneuploid methods to transfer whole chromosomes or segments of chromosomes from one variety into another, or from related species into wheat.

The complete set of monosomics (2n-41), tetrasomics (2n=44) and almost all of the ditelosomics (2n=40+2t) and several nullisomics (2n=40) were first developed by SEARS (1954) in the variety Chinese Spring a number of years ago. This material has provided the foundation and the substance of much of wheat cytogenetics, and the use of this variety and its aneuploids has given a stimulus to the study of wheat cytogenetics throughout the world for over 40 years.

Wheat Cytogeneticists owe therefore a great deal to the pioneering efforts of ERNIE SEARS and the account that follows is, in a small way, a tribute to the vision and creativity that he has given to this area of biological science.

CHROMOSOME SUBSTITUTION AND CHROMOSOME ASSAYS

Apart from the development of single chromosome addition lines in which 'alien' chromosomes are transferred from a related species, chromosome manipulation in wheat depends upon the availability of monosomic lines. These provide the recurrent parents of a backcross programme in which a single chromosome from a donor wheat variety or related species can be substituted for its homologue or homoeologue (in the case of an 'alien' chromosome) in the recipient variety. The details of this method have been described frequently (SEARS, 1953; LAW & WORLAND, 1973). Modifications to the method involve the use of cytologically recognisable marker chromosomes, such as telocentric or isochromosomes, or marker genes so as to prevent the loss of the donor chromosome during backcrossing (SEARS, 1953; UNRAU, 1950).

Monosomic series have been developed in an impressive number of wheat varieties throughout the world (Table 1), mostly by backcrossing to the original monosomic series of Chinese Spring. Much of this investment of effort was stimulated by organisations such as the European Wheat Aneuploid Co-operative (EWAC) (LAW, 1968) and has resulted in the assemblage of monosomic series representative of much of the wheat germplasm in use by today's breeders.

Chromosome substitution lines provide the means of genetically assaying the effects of individual chromosomes on any character by comparing the phenotypes of the recipient variety with that of the substitution line. Because such lines are as true-breeding as a normal wheat variety, they can be treated in exactly the same way and character assessments made under conditions representative of normal (or abnormal) farming practice. They are thus ideal for studying the genetics of characters of agronomic importance, particularly those which are quantitative and are generally regarded as being of low heritability. The wide range of monosomic series also means that it is possible to assess the contributions of individual chromosomes to the phenotype in a range of different genetic backgrounds. Furthermore the growing of substitution lines in different environments affords the opportunity for studying interactions with the genotype with a detail rarely possible using conventional genetical techniques.

The methods used in carrying out chromosome assays are now well-established, as indeed are the methods used in identifying and estimating between-chromosome interactions by inter-crossing substitution lines amongst themselves and with their recipient and donor varieties (LAW & WORLAND, 1973: SNAPE et al. 1979).

An example of chromosome assay using substitution lines comes from the study of the semi-dwarfism of the Italian wheat Mara. This variety was developed indirectly from the early work of the famous Italian wheat breeder, STRAMPELLI, who preceded the semi-dwarf wheat revolution of the New World by some 30 years by introducing dwarfing genes from the Japanese variety Akagomughi into Italian wheats. These genes have had a

Table 1. A list of varieties of wheat in which monosomic series have been established or are in the process of development.

Country	Variety	Country	Variety
Australia	Federation	Netherlands	Starke
	Gabo	Poland	Grana
	Spica		Luna
	Kenya 744	Rumania	Besostaya Al
Bulgaria	Besostaya I		Favorit
Canada	Cadet	Spain	Aragon 03
	Kharkov		Ariana 8
	Red Bobs		Pane 247
	Rescue	Switzerland	Probus
	S615	UK	Holdfast
	Lemhi		Bersée
	Thatcher		Cappelle-Desprez
	Prelude		Hobbit 'sib'
	Redman		Koga II
	Winalta		Glennson
Egypt	Giza 144	USA	Chinese Spring
France	Courtot		Cheyenne
East Germany	Carola		Wichita
	Poros		Chris
	Alcedo	USSR	Diamant II
West Germany	Caribo		Saratovskaya 29
	Kranich		Opal
	Triticum spelta		Novastepniachka
	Saharense		Priboy
Hungary	Mironovskaya 808		Milturum 533
	Rannyaya 12		Kazakstanskaya 12
India	Kalyansona		Aurora
	C591		Besostaya 2
Italy	Mara		Kavkaz
Japan	Norin 10		Skorospelka 35
	Nanbu-komagi	Yugoslavia	Sava
	Shinchunaga		Novosadska Rana 1

significant impact on Italian wheat breeding and indeed on many Mediterranean breeding programmes where they are used extensively. The genes are different from those used widely in Mexico and in many parts of the world which were also derived originally from Japan via the variety Norin 10. The genetical basis of the Italian dwarfism is therefore of considerable interest to wheat breeders. In conventional studies, crosses between Mara and tall wheats have given rise to continuous

variation in the F_2 and later generations. Single chromosome substitution lines of Mara have therefore been developed in the tall wheat Cappelle-Desprez using the monosomic series of this variety as the recurrent parent (LAW *et al.*, 1981) and these lines (Table 2) showed clearly that two chromosomes, 2D and $5B^S$-$7B^S$ (a translocated chromosome involving the short-arms of 5B and 7B, present in Cappelle-Desprez but not in Mara), were responsible for the semi-dwarfism of Mara. Further work has established that the 2D effect is due to a single gene,

Table 2. Plant heights of lines in which chromosomes of Mara have been substituted for their homologues in Cappelle-Desprez (Cap). The development of these substitution lines was complicated by the presence of a reciprocal translocation in Cappelle-Desprez involving the two long-arms of chromosome 5B and 7B, $5B^L$-$7B^L$, and the two short-arms, $5B^S$-$7B^S$. Because of this translocation it has not been possible to establish both these substitution lines and only the transfer of the $5B^S$ and $7B^S$ arms has been achieved so far.

Substitution line	Height (cm)	Difference from Cappelle-Desprez
Cap(Mara 1A)	104.6	+ 1.4
Cap(Mara 1B)	104.1	+ 0.9
Cap(Mara 1D)	104.8	+ 1.6
Cap(Mara 2A)	103.5	+ 0.3
Cap(Mara 2B)	114.1	+10.9**
Cap(Mara 2D)	88.8	-14.3***
Cap(Mara 3A)	95.9	- 7.3
Cap(Mara 3B)	110.6	+ 7.4
Cap(Mara 3D)	106.7	+ 3.5
Cap(Mara 4A)	112.1	+ 8.9*
Cap(Mara 4B)	103.1	- 0.1
Cap(Mara 4D)	101.3	- 1.9
Cap(Mara 5A)	108.0	+ 4.8
Cap(Mara $5B^L$-$7B^L$)	-	-
Cap(Mara 5D)	104.7	+ 1.5
Cap(Mara 6A)	97.0	- 6.2
Cap(Mara 6B)	103.2	0.0
Cap(Mara 6D)	100.3	- 2.9
Cap(Mara 7A)	102.9	- 0.3
Cap(Mara $5B^S$-$7B^S$)	79.2	-24.0***
Cap(Mara 7D)	106.5	+ 3.3
Cappelle-Desprez	103.2	-
Mara	81.5	-21.7***

* P 0.05-0.01 ** P 0.01-0.001 *** P<=.001

Rht8, and that this gene is present in other Mediterranean wheats such as Sava (GALE et al., 1982). The genetical nature of the $5B^S$-$7B^S$ effect has still to be established.

The importance of this particular assay does not stop however with the identification and location of the genes involved

in the Italian semi-dwarfism. Once analysis using substitution lines has proceeded as far as this then the way is open to the study of the consequences of such genes on characters other than height. Do these genes have deleterious or advantageous properties on productivity characters such as yield? How do these genes compare with the *Rht1* and *Rht2* genes of Norin 10? Answers to these questions are possible once the genes are identified. Without the substitution method (and related aneuploid methods) it would be very difficult to unravel the genetics and assess pleiotropic effects using conventional methods of genetical analysis. Thus, chromosome manipulation has an important part in describing the detailed behavior of genes having major and quasimajor effects on economic characters.

Turning now to the use of substitution lines in identifying between-chromosome and chromosome-environment interactions. An example of the method comes from the study of two substitution lines, one of which carries an allele, *vrn3*, on chromosome 5D for increased vernalisation requirement and the other an allele, *ppd2* on chromosome 2B, for increased sensitivity to day-length, and their recipient variety, Chinese Spring. Altogether 21 different genetic families were produced from intercrossing the recipient and two substitution lines to produce F_1, F_2 and backcross generations. These allowed the estimation of the additive and non-additive genetical contributions of these two chromosomes to variation for each emergence time in different environments (SNAPE *et al.*, 1979).

In this particular case, two environments were chosen, Sydney, Australia and Cambridge, England, the former providing conditions of short day-lengths and reduced vernalisation requirement, whilst the latter environment was exactly the reverse. The results of this experiment and the estimates of a number of genetical parameters are given in Table 3. Clearly, the experiment showed that both chromosomes had major interactions with the environmental differences between Australia and England, variation for chromosome 2B being more apparent in Australia than in England, whereas chromosome 5D stands out in England but not in the same extent in Australia. This of course exactly reflects expectation, since Australia provides an environment where the response to day-length (determined by *ppd2* on 2B) is critical, whereas in England it is reduced vernalisation (determined by *Vrn3* on 5D) which is important. Interestingly, however, there was no evidence of between-chromosome interaction or that the various combinations of these two chromosomes was important in the reaction of the wheat plant to the two environments. This suggests that the two genetical systems on 2B and 5D act independently of each other on ear-emergence, possible because they affect different components of the flowering process.

RECOMBINANT LINES

In describing both these examples, the actual genes responsible for the different effects of the substitution lines had been identified previously. This of course is not usually

Table 3. Days to ear-emergence from an arbitrary date for Chinese Spring (CS), and two substitution lines, CS (Cappelle-Desprez 5D) carrying the gene *vrn3*, and CS (Lutescens 62 2B) carrying the gene *ppd2*, grown at Cambridge, England and Sydney, Australia. The estimates of the genetical parameters refer to additive chromosome effects (d), and within-chromosome interaction effects (h) and are based upon the means of 21 different families. The authors are grateful to Dr. R. A. McIntosh who recorded ear-emergence in Sydney.

Line	Cambridge, England	Sydney, Australia
Chinese Spring (CS)	31.80 ± 0.13	9.00 ± 1.41
CS(Cappelle-Desprez 5D)	54.10 ± 0.51	19.00 ± 0.95
CS(Lutescens 62 2B)	29.70 ± 0.41	22.84 ± 1.02
Estimates in genetical parameters		
m	41.62***	21.09***
d_{5D}	11.44***	6.44***
d_{2B}	-0.88	8.20***
h_{5D}	-8.19***	-2.46**
h_{2B}	-0.65	-1.35
$\chi^2[16]$	22.28	16.49
P	0.13	0.42

** P 0.01-0.001 *** P<0.001

the case in assaying the effects of a substitution line since it is the chromosome that is being assayed and as such this will reflect the summed activities of possibly a number of gene differences between the recipient and donor homologues. It is a relatively simple matter, however, to establish whether one or more genetical factors is responsible for the observed differences. This can be achieved by hybridising the substitution line with its recipient variety. Such a hybrid will consist of a single chromosome heterozygote (the substituted chromosome and its recipient homologue) in an otherwise homozygous recipient background. By backcrossing this hybrid to the recipient variety, monosomic for the chromosome being studied, monosomic derivatives can be selected which are hemizygous recombinants for this chromosome. By further selfing these selected lines, homozygous recombinant lines can be obtained which can be used in extensive experiments to study in more detail the variation between the substituted and recipient homologues (LAW, 1966, 1967). In many cases this method has resulted in the identification and mapping of single genetic factors controlling quantitative characters (LAW, 1966) and was indeed the method used in locating *Ppd2* on chromosome 2B (SCARTH & LAW, 1983) and *Vrn3* (Law *et al.*, 1976) in the substitution lines used in the experiment already described.

The genes *Ppd2* and *Vrn3* are however examples where the

variation is highly heritable. It should be emphasized that the use of homozygous recombinant lines can also provide important information about the genetical control of less heritable characters. A good example of this is the location of at least two genetical factors, *Pro1* and *Pro2*, on the chromosome 5D affecting the amount of protein in the grain (LAW, *et al.*, 1978). The recombinant lines used in this study were derived from two different types of crosses; firstly, from the cross of Chinese Spring with a substitution line in which chromosome 5D had been replaced by its homologue from the variety Hope, Chinese Spring (Hope 5D), and second, from the cross of Chinese Spring ditelocentric for the long-arm of 5D ($5D^L$) with CS (Hope 5D). This latter cross is of interest since in the hybrid, recombination would have been restricted to the long-arm of 5D. The derivatives could thus be potentially recombinant for the long-arm only, the short-arm in complete chromosome derivatives being non-recombinant and entirely Hope in origin. Comparisons between the two sets of derivatives will thus provide information about the location of genes with respect to chromosome arm. The analysis also benefited from the presence of two marker genes, *Vrn3*, the gene for vernalisation requirement located on the long-arm and *Ha*, the gene for grain hardness located on the short-arm.

The results of an experiment to study grain protein amounts based upon these recombinant lines grown in a replicated field trial is summarized in Fig. 1. It is evident that whilst the distribution of the lines is continuous the separation of the lines derived from the first cross on the basis of their marker genes indicates that the means of the two non-recombinant classes, *Vrn3Ha* and *vrn3ha* are slightly different from each other, but are closer together than the means of the recombinant classes, *Vrn3ha* and *vrn3Ha* which transgress them. The genes affecting protein amounts must therefore be located on 5D and at least two genes, *Pro1* and *Pro2*, must be involved with their alleles dispersed between the CS and Hope 5D chromosomes. The derivatives of the second cross segregated only two classes, *Vrn3ha* and *vrn3ha*, the means of which were lower than their counterparts from the first cross. One of the proposed genes, *Pro2*, must therefore be located on the short-arm and because of the lack of high protein lines in the second cross it would appear that *Pro2* is situated distally to *Ha*. Other evidence based upon the behavior of these lines grown under different vernalisation conditions indicated that *Vrn3* itself was probably the same as *Pro1*, since when the vernalisation requirements of the lines were completely satisfied, the effects of *Pro1* on protein amounts disappeared. The influence of *Pro2* could however still be detected.

ALTERNATIVE METHODS OF ASSAYING CHROMOSOMES

The application of aneuploid methods to the development and analysis of substitution lines is of course time-consuming since as many as seven generations of backcrossing may be necessary to achieve a satisfactory resolution of the genetical differences between two wheat varieties. Much effort has therefore been devoted to establishing methods which are less

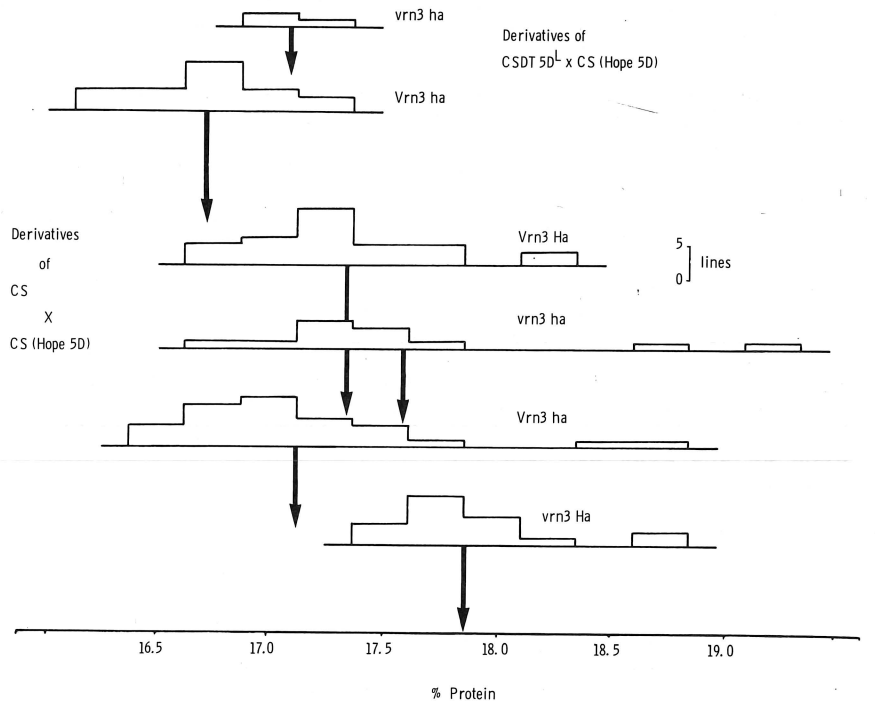

Figure 1. Mean percentage grain protein for homozygous recombinant lines from the cross of CS ditelocentric and CS (Hope 5D) and from the cross of Chinese Spring (CS) (CSDTSD^{L}) with CS (Hope 5D). The lines have been grouped into six classes, four for the first cross and two for the second, depending upon the markers, *Vrn3* and *Ha*. The mean of each of these groups is indicated by an arrow.

demanding on time and other resources. Three methods are currently used, each of which seeks to identify chromosome differences which have major effects on characters of economic importance.

a) Comparative studies between monosomic series

As Table 1 indicates, a large number of monosomic series are available for study in wheat. Major allelic differences between varieties may be detected by comparing the difference between a particular monosomic and the euploid of one variety with the difference between the same monosomic and its euploid in another variety. If the differences are not similar then either the monosomic chromosomes carry different alleles or there are different levels of interaction with the background.

Examples of this approach are given in Table 4 for the already established chromosomal locations of two dwarfing genes, *Rht2* on chromosome 4D, and the already mentioned *Rht8* on chromosome 2D. In the case of *Rht2*, the comparison is between the monosomic 4D and euploid of a tall wheat, Bersée, with that of a semi-dwarf wheat, Hobbit 'sib'. For the tall wheat, monosomic 4D is slightly shorter, whereas for the semi-dwarf wheat, the monosomic is much taller than the euploid. This confirms that the alleles for height on the two homologues are different, as well as indicating that the alleles on 4D of the semi-dwarf wheat are acting as inhibitors of height. In the second example, monosomic 2D and the euploid in the semi-dwarf variety Mara are similar in height, whereas this monosomic is much shorter than the euploid in Bersée. Again this confirms the presence of allelic differences between the two homologues, but in this case the action of the genes appears to be one of promoting height rather than inhibition. On this model, the allele on 2D of Mara is inactive giving little change in height in the monosomic, whereas the loss of the active allele as in Bersée monosomic 2D reduces height in the monosomic compared with the euploid.

Table 4. Comparisons between the heights of monosomics expressed as a percentage of the euploid value of two semi-dwarf wheats, Hobbit 'sib' and Mara, and the tall variety Bersée.

	Hobbit 'sib' (%) cm		Bersee (%) cm	
Monosomic 4D	95.80	(110.43)	127.83	(96.93)
Euploid	86.80		131.88	
	Mara (%)		**Bersée (%)**	
Monosomic 2D	56.39	(90.91)	97.75	(74.12)
Euploid	62.03		131.88	

This comparison between monosomics could be confounded with background interactions. However, in these particular examples, the monosomic comparisons predict exactly the presence of major allelic differences identified by the more penetrating substitution analyses. In an extensive analysis of several monosomic series, WORLAND & LAW (1984) have identified a number of chromosomes as likely sites of allelic differences affecting height. In several cases, it has been possible to confirm these locations from existing substitution line data.

b) Reciprocal monosomic crosses

The complications of interactions with the background are

removed if reciprocal crosses are made between the same monosomic from two varieties. The reciprocal F_1 monosomics that result have identical background chromosomes but the hemizygous homologous chromosomes are different. Comparisons between the two F_1 monosomic hybrids will thus reflect the difference between these two hemizygous chromosomes, each being derived from a different variety. By selfing such F_1 monosomics, disomic F_2 plants can be selected which on further selfing can give rise to two populations, comparison of which will also measure the allelic differences between the two chromosomes. This approach was first suggested by McKEWAN & KALTSIKES (1970) and has been used recently to analyse successfully the chromosomal control of a number of characters, including yield and its components, by LAW *et al.* (1981), LAW (1982) and LAW *et al.* (1983).

c) Backcross reciprocal monosomic crosses

Both methods a) and b) require comparisons between two or more monosomic series. An extension of the reciprocal monosomic method will allow comparisons to be made when only one of the varieties being studied is monosomic. By making the initial cross between a monosomic series in one variety and a number of other varieties, backcrossing of the resulting F_1 monosomic hybrids to the parental monosomic series carried out reciprocally, will produce backcross reciprocal monosomic plants or lines which on average have genetically identical backgrounds, but whose hemizygous chromosomes are different. Comparisons between the reciprocally derived lines will provide estimates of allelic differences between these homologous hemizygous chromosomes and this can be done for a range of varieties. An example of this method is shown in Table 5, for allelic variation distinguishing chromosome 5A of thirteen varieties for final plant height (SNAPE & LAW, 1980).

Comparisons between monosomic series, or reciprocally derived monosomic hybrids either at the F_1, F_2 or later generations and after backcrossing are thus useful approaches to identifying major components of chromosomal variation amongst varieties. Such prior analysis is also useful in deciding those chromosomes which are worth investigating further by developing substitution lines. In addition, they provide estimates of the extent of the variation that exists on any one chromosome amongst a sample of varieties.

EXPLOITATION OF CHROMOSOME MANIPULATION IN BREEDING

a) The identification and evaluation of agronomically important genes

The methods described give to the wheat geneticist the means of dissecting varietal differences in great detail. To this should also be added the analysis and description of chromosomes and segments introduced into wheat from its relatives. Genetic analysis itself is thus a major reason for a commitment to these methods. The identification and evaluation

Table 5. Mean heights of backcross reciprocal monosomic families in which the initial cross involved Cappelle-Desprez monosomic 5A (CD 5A) with twelve different varieties. In each cross the backcross reciprocal monosomic compares the effect of CD 5A against its homologue from the other variety.

Cross	Monosomic 5A Chromosome	Mean height (cm)	Difference	Probability of Difference
Chinese Spring x CD	Chinese Spring	122.72	7.78	0.001
	CD	114.94		
Besostaya I x CD	Besostaya I	115.15	6.10	0.01
	CD	109.05		
Mironovskaya x CD	Mironovskaya	123.42	5.06	0.02
	CD	118.36		
Sportsman x CD	Sportsman	111.50	4.09	0.07
	CD	107.41		
Atlas 66 x CD	Atlas 66	121.97	3.97	0.08
	CD	118.00		
Villein x CD	Villein	110.60	3.75	0.09
	CD	106.85		
Poros x CD	Poros	122.38	2.54	0.26
	CD	119.84		
Sicco x CD	Sicco	117.24	2.31	0.30
	CD	114.93		
Sava x CD	Sava	105.48	2.31	0.30
	CD	103.17		
Highbury x CD	Highbury	111.24	1.19	0.59
	CD	110.05		
Hobbit x CD	Hobbit	109.51	0.78	0.73
	CD	108.73		
Maris Ranger x CD	Maris Ranger	109.66	-0.95	0.67
	CD	110.61		

S.E.D. = 2.22

of genes is however central to much plant breeding effort. The chromosome manipulation methods offer a means of doing this more effectively than is possible by other available techniques in wheat. As already mentioned, the identification of dwarfing genes using these methods is a first step in evaluating these genes for their contributions to other characters.

A list of dwarfing genes ranked according to their effect on important agronomic characters would provide valuable information to breeders. The significance of the major adaptive genes for vernalisation and day-length response upon productivity is not known with any certainty at the moment. The identification and manipulation of these genes, as is possible using aneuploid methods, could lead directly to the development of isogenic and near-isogenic lines which would permit such evaluations to be made. A similar lack of information occurs for many of the genes affecting other agronomic characters.

b) Transfer of 'useful' genes by chromosome manipulation

Once chromosomes carrying 'useful' genes have been identified then these can be exploited using cytogenetic methods in the production of advanced breeders' lines or, even directly, in the development of varieties. This is particularly appropriate where the genetical effects identified are difficult to evaluate because the process of evaluation is costly in time, labour and equipment. A good example of this is to be found in the identification of the chromosomes responsible for resistance to eye-spot caused by the fungus *Pseudocercosporella herpotrichoides*. The assessment of resistance to this disease is extremely laborious and requires extensive replication of plant material under controlled environment conditions before reliable assessments can be made. It is therefore difficult to select for in any breeding programme. Using substitution lines of the resistant variety Cappelle-Desprez into the susceptible Chinese Spring, the resistance was shown to be due predominantly to chromosome 7A (LAW *et al.*, 1975). It is thus possible to transfer this chromosome into other varieties or advanced breeders' lines using the established methods of chromosome manipulation, but most importantly without the need for continual phenotypic assessment.

An interesting example of the transfer of 'useful' genes is the conversion of a winter wheat into a spring wheat using the methods of chromosome manipulation. This followed an in depth study of the variation determining reduced vernalisation requirement that existed amongst a sample of chromosome 5A's, the chromosome known to carry *Vrn1*, the gene mainly responsible for the differences between spring and winter wheats in Europe and N. America (LAW *et al.*, 1976). This identified a chromosome 5A from an accession of *Triticum spelta*, a rather unpromising source of useful variation from the breeding point of view, as determining the greatest reduction in vernalisation requirement. This chromosome was therefore substituted into the winter wheat, Hobbit 'sib', in which a monosomic series was already available. As expected, the derived substitution line, Hobbit sib (*T. spelta* 5A) was found to behave exactly as a spring wheat in the U.K. Moveover, and rather unexpectedly, once the unwanted speltoid ear character, determined by the gene *q* on *T. spelta* 5A, had been removed by recombination, the resulting Spring Hobbit line was found to outyield the best current spring wheats by 10 percent (Table 6).

This line is now being used as a breeders' line. The use-

Table 6. Yields in grams per plot of Spring Hobbit and Timmo and Highbury, two currently grown Spring wheat varieties.

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

*** P<0.001 and significantly different from Timmo and Highbury

fulness of this line as a parent illustrates the advances that can be made by 'backcross breeding' applied to whole chromosome transfers. It also illustrates the unexpected improvements in another character, yield, part of which has since been shown to be due to genes carried on *T. spelta* 5A (SNAPE *et al.* in prep.).

c) Synthesising new chromosomes

The analysis of homologous chromosomal variation leads to the recognition of recombinant chromosomes with advantageous combinations of genes. In many cases, it is unlikely that such combinations would so readily emerge as a consequence of selection practised in a normal breeding programme. The increased selection pressure that can be applied to a single chromosome using the methods described offer another potentially useful application of chromosome manipulation.

The synthesis of new and useful chromosomes is perhaps most likely to occur when there is a need to introduce a gene from a totally unrelated variety or alien species. Such wide-crossing is likely to lead to the transfer of disadvantageous combinations of genes along with those that are desirable. Unless the disadvantageous genes have large deleterious effects, then such combinations of genes are likely to be maintained for many generations despite selection. Chromosome manipulation techniques may thus find use in the 'upgrading' of particular chromosomes. At present there are no examples where such an approach has been carried out in wheat itself, although there are a number of possible candidates now beginning to appear.

d) 'Super-genes'

The selection of useful recombinant chromosomes is of course particularly relevant to the transfer of genes and segments of chromosomes from the wild relatives of wheat. For long it has been recognised that the transfer of such genes is restricted by the need to control pairing and recombination between chromosomes which are homoeologous and which under normal circumstances do not pair and recombine. The discovery of the

Ph gene suppressing homoeologous recombination has of course done much to overcome this problem (RILEY & CHAPMAN, 1958; SEARS & OKAMOTO, 1958) and homoeologous gene transfers have been achieved from a number of species into wheat by exploiting this system through chromosome manipulation and by other methods (RILEY *et al.*, 1968, SEARS, 1973).

It is usually considered that the objectives of these methods should be the transfer of a minimal amount of 'alien' chromosome and if possible just the new gene itself. Elaborate techniques have been devised to achieve this objective (SEARS, 1981). However, the converse, the transfer of larger segments of 'alien' chromosome, has perhaps not received the attention that it warrants. A particular virtue of any 'alien' chromosome segment is that once transferred it effectively remains intact and rarely ever recombined with a homoeologous wheat segment in the presence of a *Ph* gene. The segment therefore is transmitted as a unit and can thus be regarded as a 'super-gene'. It would seem reasonable to consider the possibility of constructing or selecting blocks of 'alien' chromosome which carry combinations of useful genes.

In work to transfer the gene *Yr8* from *Ae. comosa* into wheat there is evidence that such a 'super-gene' may already have been established. The gene *Yr8* was initially transferrred into the breeders' variety Compair by RILEY *et al.* (1968), the particular transfer involving a recombinant chromosome composed of most of the 2M chromosome of *Ae. comosa* and a part of the short-arm of chromosome 2D. This chromosome has been shown to be stable and is transmitted normally in crosses with wheat varieties. However, an additional virtue of this 2M-2D chromosome is that over and above its determination of resistance to yellow-rust, is an increased level of protein in the grain which is not attributable to any reduction in yield. Indeed, the 2M-2D chromosome has been shown to be comparable in its yielding ability to 2D homologues from a number of varieties (LAW *et al.* 1983). From the breeder's point of view such a chromosome has advantages, since it can be selected easily because of the effects of *Yr8* whilst at the same time the non-recombinable segment carrying *Yr8* will also confer increased protein to the grain.

The construction of blocks of genes or 'super-genes' may thus be one way of exploiting the discriminatory properties of the *Ph* gene in other ways than in making the initial transfer.

e) Wide-crossing

One of the problems of wide-crossing in wheat is the reduced likelihood that useful transgressive segregants will emerge. This is simply a consequence of the many gene differences between the two parents. Since most of the desirable alleles are normally in one of the parents, the standard practice for coping with this problem is to backcross, often repeatedly, to the better parent. This approach works reasonably well when few genes affecting qualitative characters are being transferred, although even here it is complicated if the trans-

ferred genes are recessive. For genes affecting quantitative characters however it is not a particularly rewarding or attractive approach. Undoubtedly, desirable genes affecting quantitative characters, not present in a breeders' population of acceptable varieties, exist in totally unrelated and unadapted material. Rarely will a breeder contemplate using such material except to transfer specific genes for disease resistance and other obvious and easily identifiable genes. On both counts, the transfer of qualitative and quantitative genes, the substitution of whole chromosomes using cytogenetic methods will provide a means of systematically transferring genes so that they can be identified and evaluated more effectively and often more rapidly than by simple backcrossing. The methods described should therefore have a role in screening and exploiting useful genes existing in distantly related varieties and land races.

EXPLOITATION OF CHROMOSOME MANIPULATION IN OTHER AREAS

The extensive range of aneuploids and substitution lines that already exists in wheat provides opportunities for advancing the understanding of other areas of scientific interest.

a) Plant development and physiology

Undoubtedly the use of genetics as a tool to understand plant processes can be an important aid to the plant physiologist. The use of aneuploid and substitution lines has for long been advocated as a means of studying wheat physiology (LAW, 1966; LAW & GALE, 1979). As mentioned previously, the detailed description of the effects of particular genes on characters other than that by which they were identified is important to the exploitation of genes in breeding, but it is also a valuable approach to understanding the mechanisms which underlie the changes in the characters observed. Physiologists have been slow to use this approach. This is regrettable since one consequence of the more penetrating character analysis that the physiologist will bring to such a study, is the improved understanding of a gene's phenotype from which more effective diagnostic tests should emerge for the use by geneticists and breeders. An excellent example of this is the exogenous gibberellic acid insensitivity of wheat plants carrying the dwarfing genes, *Rht1*, *Rht2* and *Rht3*. This was a feature first observed by RADLEY (1970) which was subsequently used by geneticists (GALE & MARSHALL, 1976) to solve the genetics of dwarfism and to develop rapid screening procedures for the identification of such genes at the seedling stage in breeding programmes (GALE & GREGORY, 1971).

b) Biochemical markers

Aneuploid and substitution lines have made an important contribution to the identification and chromosomal location of genes for isozyme variation in wheat (HART, 1979; CHOJECKI & GALE, 1982). This type of work will accelerate in the future as a consequence of the improving methods of isozyme detection

(FELDER, 1980). In conjunction with existing cytogenetic lines as well as by the use of the techniques described here, such developments will contribute to evolutionary studies but perhaps more important, will provide the opportunities to use isozyme variation as markers in genetic analysis. Ultimately such markers may find use in breeding if close linkages between an isozyme marker and a gene for improved agronomic performance can be detected. This would be particularly valuable if the agronomic character is normally difficult to evaluate. Isozyme markers must also be expected to improve the precision of chromosome engineering involving the introduction of 'alien' chromosome segments into wheat.

The understanding of the genetic of endosperm proteins has also relied very heavily on the availability of cytogenetic stocks of wheat. Indeed, the very rapid resolution of the genetics of these important proteins into nine loci, located on the group 1 and 6 chromosomes (PAYNE *et al.*, 1982) could hardly have taken place without the existence of such precisely defined chromosomal lines.

c) Molecular biology

The explosion of interest that is currently taking place in plant molecular biology has already exploited wheat cytogenetic stocks. The different numbers of ribosomal RNA genes (FLAVELL & SMITH, 1974) and their location by *in situ* hybridisation (HUTCHINSON *et al.* 1981) have been determined using aneuploid and substitution lines. Recently, the sites of the endosperm protein genes have been confirmed using cDNA probes hybridised to restriction enzyme digests from nullisomic-tetrasomics of the group 1 chromosomes (BARTELS et al. in preparation). As more and more DNA probes, particularly for single genes, emerge then there will be increasing opportunities for the identification of restriction enzyme polymorphisms within wheat. In this process of identification, aneuploid and substitution lines will be important in locating polymorphisms as well as exploiting them in genetic analysis.

It is possible that this may lead to the recognition of an almost limitless number of genetical markers in the future. In combination with increased numbers of biochemical markers, this may eventually lead to new methods of manipulating chromosomes without the need for the cytological selection used today.

CONCLUSION

This brief review of the methods of chromosome manipulation in wheat and their exploitation in breeding and science has one major underlying theme and that is the increased precision of genetic analysis that they provide. If directed genetic manipulation, either by the chromosome engineering described here or by the molecular genetic engineering being developed at the moment, is to be fully exploited then the genes that are worthy of manipulation need to be identified and their modes of operation understood. For the most part,

certainly for the major crop plants like wheat, this information is not available. There is therefore an urgent need for increasing effort in this area to overcome this major lack of knowledge, otherwise the techniques of manipulation will arrive well ahead of the opportunities for exploitation. This will certainly be the case in plant breeding. In this respect, chromosome manipulation in wheat, along with the other developing methods of analysis, has an important role to play in the future.

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