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CYTOGENETICS AND PLANT BREEDING

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In dealing with the cytogenetic approach to plant breeding, I shall not attempt to discuss cytogenetics as an ancillary aid to the breeder, valuable though this may be in providing him with a rationale for, or a corrective to, procedures in which cytology is subsequently no longer necessary. Instead I will be concerned with those manipulative procedures that attempt to exploit gross changes of chromosome number or structure and that demand constant reference to the chromosome status of the breeding material. Further, I shall not attempt comprehensively to review work of the past, but will concentrate on current work and attempt to assess where effort might profitably be made in the immediate future.

Cytogenetic breeding methods depend upon a rather restricted range of changes including alterations in ploidy, aneuploidy, structural changes and the regulation of recombination. In the discussion that follows some procedures will be considered in which more than one type of change is used, but initially I shall be concerned with only one—changes of ploidy.

CHANGES OF PLOIDY

The products of changes in polyploidy occasionally afford improvements in the utility of a crop, or provide easier access to otherwise unattainable genetic variation. Both increases and reductions in ploidy have been exploited, although reductions have been less widely studied despite their apparent value in some crops. It is unnecessary to dwell on the extensive investigations of induced polyploids but mention may be made of the successful introduction to agriculture of such induced polyploids as autotetraploid rye,[6] seedless autotriploid water melon,[30] and autopolyploid sugar beet.[4] The problems already solved include the population dynamics involved in the self-cleaning of diploids from tetraploid stocks of rye, the production of triploid water melon seeds and the polyploid balance giving maximum root yield in sugar beet.

Some use has, therefore, already been made of induced autotetraploids and their more extended exploitation is limited primarily by low fertility. It has been suggested that the cytological causes of low fertility could be overcome by the development of lines with bivalent formation at meiosis. Such diploidization might be achieved either solely by the assembly, in the duplex state, of structural differences, or by the combination of such structural differentiation of chromosome sets with a genetic system restricting pairing specificity. The probability is slight of achieving diploidization in an existing autopolyploid. Nevertheless, heterozygosity for major structural inversions has been demonstrated to change the inheritance of particular loci in corn, from essentially tetrasomic to nearly

disomic.[29] Moreover, there are examples of diploidization following immediately the origin of tetraploidy, apparently due to the structural differentiation of the parents,[20] so that the development of structurally distinctive diploid parental lines might be a useful preliminary to the initiation of an autotetraploid breeding programme.

A relatively simple alteration of the genetic control of pairing specificity, like that responsible for the diploidization of wheat, could never achieve the same result in polyploids of non-hybrid parentage since it depends upon the genetical enhancement of an incipient differential affinity. However, structural differentiation sufficient to lead to only a moderate reduction in chiasma frequency may be great enough to preclude intergenomic synapsis in a genotype with restricted pairing specificity. This is illustrated by the chromosomes of distinct species of *Aegilops* that pair in hybrids at the diploid level but not when in the presence of the genotype of tetraploid wheat.[26, 10] It might, therefore, be worthwhile to search for mutants, with altered pairing specificity, in tetraploids derived from hybrids that have slightly lower chiasma frequencies than the parental diploids.

By contrast improved meiotic regularity might result from alterations in the frequency and distribution of chiasmata. In tetraploid rye, increases in fertility are associated with the occurrence of fewer univalents and either more bivalents or more quadrivalents at meiosis.[25] However, where selection increased bivalent frequencies, this would not result in diploidization since there would be polysomic inheritance, as in the bivalent-forming species *Lotus corniculatus* and *Phleum pratense*. This might not be important in terms of fertility but could reduce the speed and efficiency of subsequent selection at the polyploid level.

Synthetic allopolyploids have been of less success in agriculture than induced autopolyploids. This is because the latter represent mere variants of existing crops whereas the former are either complete novelties, or are reversions to the raw, superceded, forms of contemporary polyploids. Considerable effort has been devoted, for example, to the development of *Triticale* forms, combining wheat and rye, but nowhere have they been taken into cultivation. Nevertheless there have been successful synthetic allopolyploids like that of rape, *Brassica napus*,—resynthesized by Olsson[18]—which produces higher yields of oil in Sweden than current commercial varieties. Induced allopolyploids have also been used as intermediary bridging forms in the transfer of desirable attributes to cultivated polyploids from related diploids[28] and to some extent as horticultural novelties. However, unless synthetic allopolyploids provide an entirely novel product they are only likely to be of direct value in marginal areas, away from competition with the highly developed crops of industrialized agriculture. Thus *Triticale* might be useful in rye-growing areas.

Haploid crop plants, that is sporophytes with gametophytic chromosome constitutions, have already been employed in some breeding programmes. Chase employed genetic markers in the parents to recognize *Zea mays* haploids, which were treated with colchicine to produce homozygous diploids for use as parents in hybrid corn programmes.[2] Similar methods have been exploited for the production of *S* allele homozygotes for use in the production of hybrid kale, *Brassica oleracea*.[32]

To overcome the difficulties and slowness of breeding at the autotetraploid level in the potato, *Solanum tuberosum* ($2n=48$), Hougas and Peloquin suggested that the work could be carried out at the haploid ($2n=24$) chromosome level.[9] They were able to demonstrate that 24-chromosome haploids were interfertile, and fertile in crosses with natural diploid species.[19] Consequently potato breeding can apparently be carried out

at the lower level of ploidy with all the advantages of disomic inheritance. After appropriate genotypes have been synthesized, they can be transposed to the tetraploid state by the use of colchicine. However, ultimately this may be unnecessary since there have been suggestions of high tuber yields at the haploid level.:[31]

Certain clear benefits could result from the use of haploidy, particularly in inbreeding crops. For example, if it were possible to induce the F_1 plants of intervarietal crosses to set seeds with haploid embryos parthenogenetically, the first products of segregation and recombination could be isolated. After colchicining these could be made diploid and homozygous—so eliminating the heterozygous generations that normally intervene between the first hybridization and the release of a new variety. The application of such a system clearly demands techniques for the ready production of haploids, and the most likely way of increasing their frequency is probably by the use of selected pollinators, as demonstrated in corn by Coe.[3]

I am prompted, perhaps prematurely, to discuss this aspect of the use of haploids because of a situation recently discovered in a stock of *Triticum aestivum* variety Holdfast ($2n-1=41$) monosomic for chromosome I. In two successive generations monosomic individuals have produced a high frequency of haploids—28 in a total of 80 plants (Table 1). Naturally there is segregation for the presence of the monosomic chromosome in both haploid and non-haploid derivatives. The genetics of this situation are not yet understood but some aspects of the situation can be stated quite simply: (i) Only about 50 per cent of seeds germinate. (ii) Seeds which do not germinate usually have no embryo. (iii) Other isolates of monosomic I from Holdfast do not produce high frequencies of haploids. (iv) Haploid production is not necessarily associated with the monosomic condition. Speculating about this situation, it seems quite possible that pollen of this material permits the development of haploid embryos but normal endosperm. If this were so; and this stock were used to pollinate heterozygotes, haploidy could be used in wheat improvement and a new cytogenetic system of breeding developed.

TABLE 1. *Production of Haploids in the Selfed Progenies of a Stock of T. aestivum Holdfast monosomic*-I *($2n-1=41$)*

	Haploid			Non-haploid				
	20-chrom.	21-chrom.	total	40-chrom.	41-chrom.	42-chrom.	others	total
1961	11	2	13	3	25	5	1	34
1962	12	3	15	1	8	8	1	18
Total	23	5	28	4	33	13	2	52

INTRODUCTION OF ALIEN GENETIC VARIATION

A cytogenetic approach to the introduction of alien genetic variation is primarily necessary where there is no meiotic pairing and recombination between the chromosomes of the parents in hybrids between a crop species and a related species with useful genetic attributes. The situation has been most extensively studied in relation to the transfer of alien variation to wheat and tobacco.

TABLE 2. *Meiotic Regularity, Cytological Stability and Fertility of Disomic Substitutions of Chromosome II of S. cereale for the Chromosomes of Homoeologous Group 6 of T. aestivum*

		Pairing at M_1 of meiosis					Chrom. no. of progeny on selfing						Fertility (2 spikes per plant)	
		Plants scored	Cells scored	% with biv. only	% with 2 univ.	% with 4 univ.	Plants scored	41 %	42 %	43 %	44 %	45 %	Plants scored	% of 1st and 2nd florets fertile
	T. aestivum Holdfast	4	200	94	5	1	200	1	99	—	—	—	10	0.91 ± 0.02
6R	Holdfast with disomic addn. rye II	10	850	91	9	—	64	—	—	6	91	3	19	0.50 ± 0.11
(6A)6R	Substitution rye II/Holdfast VI	3	150	90	9	1	54	—	100	—	—	—	11	0.49 ± 0.03
(6B)6R	Substitution rye II/Holdfast X	3	150	95	5	—	33	12	85	3	—	—	14	0.12 ± 0.03
(6D)6R	Substitution rye II/Holdfast XIX	3	150	87	13	—	52	16	82	2	—	—	12	0.22 ± 0.05

TABLE 3. *Mean Pairing at First Metaphase of Meiosis in F_1's and Amphiploids of the Cross T. aestivum × A. longissima, with and without chromosome V (5B) of T. aestivum*

	Chrom. no.	Chrom. V (5B)	Mean pairing								Chiasmata per cell	Chiasmata per paired chrom.
			Univ.	Siv.	Triv.	Quad.	Quin.	Sex.	Sept.	Oct.		
F_1	28	present	24.20	1.78	0.08	—	—	—	—	—	1.94 ± 0.25	0.3926 ± 0.0321
	27	absent	6.36	6.18	1.30	1.04	—	—	—	—	14.18 ± 0.35	0.7058 ± 0.0135
Amphiploid	56	present	1.25	27.13	0.03	0.10	—	—	—	—	50.93 ± 0.89	0.9230 ± 0.0150
	54	absent	4.10	18.83	1.27	1.67	0.20	0.10	—	0.03	41.67 ± 0.72	0.8344 ± 0.0690

It was initially considered that the difficulty could be overcome by using backcross procedures to add single pairs of alien chromosomes to the full complement of a recipient crop species.[17] Although useful modifications of the phenotypes of recipient species occurred in the resulting addition lines other disadvantages, such as low fertility, cytological instability or disturbances of quality, prevented their direct use in agriculture.

It has been suggested that these disadvantages might be avoided by substituting the alien chromosome pair for a pair of the recipient crop species. Table 2 illustrates the fertility, stability and meiotic regularity of disomic substitutions of chromosome II of *S. cereale* for each pair, in turn, of homoeologous group 6 of *T. aestivum* Holdfast. These lines are vegetatively vigorous and the phenotypic modifications of the recipient variety caused by the addition of the rye chromosome, including disease resistance, persist in the substitution state, but fertility is sharply reduced. Similarly low fertilities usually occur in other substitutions and the limited exploration of the substitution condition, thus far made, indicates its severely restricted practical potential in crops grown for seed. In tobacco, where reduced seed fertility places little limit on crop production, substitutions of the chromosomes of *Nicotiana glutinosa* and of *N. plumbaginifolia* for those of *N. tabacum* have been used to incorporate resistance to mosaic disease and to black shank.[14]

It may be of interest briefly to consider the likely relationships between the participating chromosomes. Nullisomics are phenotypically abnormal and generally of poor vigour in *T. aestivum*[27] and are apparently not viable in *N. tabacum*.[1] Yet plants with disomic substitutions of alien chromosome pairs show little evidence of the abnormalities normally associated with nullisomy for the substituted chromosome. Consequently a genetic activity of the alien pair must compensate for the defects normally associated with the deficiency. It may be assumed that the genetic similarities thus indicated arise from evolutionary relationships between chromosomes, and that the substitutions do not take place at random. It seems reasonable to conjecture, therefore, in the absence of translocation differences, that every chromosome of a diploid donor species will substitute, with adequate compensation, for only one chromosome in every genome of a recipient polyploid species. It is significant that, in our work at Cambridge, we have only been able to substitute the nucleolar organizing chromosome II of rye for chromosomes of homoeologous group 6 of wheat. This presumably indicates genetic equivalences of evolutionary origin.

It was perhaps somewhat naive to expect that when a complete chromosome was transferred to another species the new genotype would function in a fully integrated manner. Sears demonstrated, however, that this genetic imbalance could be avoided by the use of induced translocations to introduce into wheat a small chromosome segment of *A. umbellulata* carrying a gene giving leaf rust resistance.[28] The need was thus removed for the transfer of whole chromosomes. Genes of *Agropyron* giving stem rust resistance, and of rye giving leaf rust resistance, have subsequently been translocated to wheat chromosomes.[5, 11]

By a logical sequence of steps, therefore, procedures have been evolved which enable alien genes to be incorporated in crop species despite the absence of appropriate meiotic recombination.

At the same time means have been proposed of creating situations in which recombination is more likely. Thus it has been shown that rare interspecific recombination will occur in tobacco in situations in which there are three sets of chromosomes of *N. tabacum* and one set of *N. plumbaginifolia*, since one set of each species is exposed to allosyndetic meiotic

recombination.[16] Also in tobacco, monosomic substitution often leads to recombination between the monosomic donor and recipient chromosomes, and so to segmental substitution.[15]

In wheat, recombination can be induced to occur in chromosomes that are normally meiotically isolated.[21, 22] This depends upon the knowledge that the activity of chromosome V is responsible for the absence of homoeologous recombination[24] and that the same activity also results in the absence of allosyndetic recombination in many hybrids.[23] Amphiploids deficient for chromosome V are multivalent-forming and have allosyndetic pairing and recombination, although this is absent from otherwise equivalent plants carrying V (Table 3). Such multivalent-forming amphiploids can be backcrossed to wheat, so restoring chromosome V and introducing allosyndetic recombinant chromosomes.

Alternatively, chromosome V-deficient interspecific hybrids, which are of low fertility, can be backcrossed to wheat again to introduce allosyndetic recombinant chromosomes. The products of this process among the derivatives of a cross between *T. aestivum* and *Aegilops bicornis* look most promising.

Thus the procedures developed to overcome the obstacles, created by the absence of meiotic pairing failures, may well ultimately be discarded. Moreover, the potential value of manipulations of the genetic regulation of meiotic pairing specificity, in wheat, should make us watch carefully for evidence of similar systems in other crops.

DUPLICATIONS

Obvious benefit could arise from the duplication of beneficial alleles with dose effects. Moreover, duplications could provide surplus genetic material so allowing scope for mutational changes without the loss of existing functions. From their work with corn, Gopinath and Burnham showed that it was possible to obtain segregants with duplicated regions from hybrids produced by crossing together plants homozygous for translocations of independent origins, and with different exchange points, but between the same two chromosome pairs.[7] The result of this process is to produce duplicated segments on otherwise dissimilar chromosomes. This system is being used to duplicate the locus responsible for the control of α amylase production in barley.[8] It has been suggested that the equivalent duplication of the two-row six-row locus, so that both alleles are in the duplex state, might allow the fixation of the heterotic affect on yield displayed by the locus.

The homoeologous recombination that occurs in wheat deficient for chromosome V may also permit the accumulation of duplications[20, 24] either directly or through the assembly of lines with different translocations between the same pairs. In this work, since *T. aestivum* nullisomic-V plants are sterile, it is convenient to use plants that are nullisomic-V and tetrasomic-XVIII, as the extra dosage of XVIII removes sterility without preventing homoeologous pairing.[24] After the accumulation of homoeologous recombinants, a convenient method of returning to the euploid condition is by the cross to the reciprocal genotype; that is nullisomic-V tetrasomic-XVIII × tetrasomic-V nullisomic-XVIII.

The immediate value of duplications attained by any method, however, must depend upon the usefulness of dose effects and on the production of duplications small enough not to interfere with normal bivalent formation.

INTERVARIETAL CHROMOSOME SUBSTITUTION

The possibility of transferring single intact chromosome pairs from one variety of wheat to another was made possible by the production of the full monosomic series by Sears,[27] and by the derivation, by backcrossing, of equivalent series in a number of varieties. Intervarietal substitution permits the transfer, by backcrossing, of beneficial genes which do not give clear cut phenotypic effects, using the monosomic chromosome as a marker for the gene.[33]

Considerable interest was stirred by the demonstration of pronounced changes in the expression of quantitative characters, including yield, following intervarietal substitutions of chromosomes.[12] However, the generalized application of this means of improving quantitative characters depends first upon the extent to which the substituted chromosome produces its effect purely additively. Alternatively, if the effect is interactive, substitutions would be easier to use if a pattern of interaction could be assigned to chromosomes related homoeologously or genomically.

Certain interactive effects between substituted chromosomes and the recipient background have been indicated, by my colleague Mr. C. N. Law,[13] to be due primarily to particular chromosomes of the D genome. However, it is clear that a considerable amount of basic information is necessary before the practical value of intervarietal substitution can be properly assessed. The study of substitution lines, necessary to assemble this information, offers unique opportunities for the investigation of quantitative inheritance. One possibility, proposed by Unrau, is the use of material heterozygous in a single chromosome pair to fix and make homozygous the first products of recombination.[34] Such contributions by cytogenetics to the understanding of quantitative inheritance must be of value in plant breeding research.

THE APPLICATION OF CYTOGENETICS

The participation of purely cytogenetic techniques in some breeding programmes is thus already considerable but certain areas in which valuable contributions might be made have been neglected, for example in the use of the effects of environmental differences on chiasma frequency. Nevertheless, it is clear that the increasing sophistication of cytogenetic method has improved its practical potentialities, for in the control which it gives in the synthesis of the improbable genotypes required by the plant breeder, the "engineering" approach of cytogenetics is unexcelled.

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