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NUCLEOLUS ORGANISER VARIATION IN WHEAT AND RYE REVEALED BY *IN SITU* HYBRIDISATION

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SUMMARY

The technique of *in situ* hybridisation has been used to demonstrate that some wheat varieties have very different numbers of ribosomal RNA genes clustered in homologous nucleolus organisers. In these experiments the labelled probe was RNA synthesized *in vitro* using wheat ribosomal DNA cloned in a bacterial plasmid as template. Homologous nucleolus organisers were identified by the use of intervarietal chromosome substitution lines. A variety of rye has been shown to be heterozygous for the number of rRNA genes clustered in its major pair of nucleolus organisers. The *in situ* hybridisation technique has also been used to demonstrate an anomalous deletion of rDNA in an aneuploid derivative of Chinese Spring wheat.

1. INTRODUCTION

A number of years ago it was shown that some varieties of hexaploid wheat and rye possess very different numbers of ribosomal RNA (rRNA) genes (Flavell and Smith 1974*a*, *b*). This was demonstrated by hybridising ribosomal RNA, radiolabelled *in vivo*, to denatured DNA on nitrocellulose filters.

A similar analysis of wheat intervarietal chromosome substitution lines in which chromosomes carrying nucleolus organisers from different varieties were substituted into the same genetic background suggested that the number of rRNA genes at each of the four nucleolus organisers in hexaploid wheat can vary in different varieties. This conclusion was drawn because the intervarietal chromosome substitution lines with a common genetic background had significantly different numbers of rRNA genes. However, the technique could not unequivocally prove that the number of rRNA genes differed in homologous substituted nucleolus organisers because changes in rRNA gene number in the genetic background of the substitution lines might have occurred. This possibility can, however, be tested by *in situ* hybridisation of labelled rRNA to individual metaphase chromosomes (Pardue and Gall, 1975).

In situ hybridisation of rRNA has been made considerably easier recently by the molecular cloning in our laboratory of the ribosomal genes from wheat and barley in bacterial plasmids (Gerlach and Bedbrook, 1979). RNA copies of the plasmid containing plant rDNA are easily made *in vitro* using *E. coli* RNA polymerase and the labelled RNA can be applied directly to the denatured metaphase chromosomes. This technique has been used here to prove:

(1) that different wheat varieties have homologous nucleolus organisers carrying different numbers of rRNA genes

(2) that there is heterozygosity for rRNA gene number in a variety of rye
(3) that an anomalous aneuploid stock of wheat carries a deletion of rDNA.

2. Materials and methods

(i) *Plant genotypes*

The substitution lines in which the 1A chromosomes of the hexaploid wheat (*Triticum aestivum*) variety Chinese Spring ($2n = 6x = 42$) have been replaced by their homologues from *Triticum spelta* ($2n = 6x = 42$) or from the variety Cappelle–Desprez were developed in this department by Dr C. N. Law and his colleagues (Law, 1968; Law and Worland, 1972). The aneuploid derivative of Chinese Spring which carries an additional pair of 5D chromosomes (tetrasomic 5D) was originally obtained from Dr E. R. Sears, University of Missouri, Columbia, Missouri, U.S.A. (Sears, 1954) but has been multiplied at this Institute for many years. The King II rye (*Secale cereale*, $2n = 2x = 14$) has been maintained as an outbreeding population.

(ii) In situ *hybridisation*

Root tip mitotic metaphase nuclei were accumulated in seedlings using colchicine or 1-bromonaphthalene treatments. The details of the *in situ* hybridisation procedure and preparation of autoradiographs are described elsewhere (Appels, Driscoll and Peacock, 1978; Bedbrook *et al.*, 1980). The nucleic acid probe used was tritium labelled RNA (specific activity about 2×10^7 cpm/μg) transcribed by *E. coli* RNA polymerase from the recombinant plasmid pTA71, as described by Gerlach and Bedbrook (1979). This plasmid consists of a single wheat ribosomal RNA gene repeating unit (*i.e.*, 18S and 25S rRNA genes + associated spacer DNAs) in the vector plasmid pAC 184 (Gerlach and Bedbrook, 1979).

Silver grain counts were made on at least 10 and in most cases 20 cells of each genotype in which the quality of the mitotic preparations was suitable for such analysis. Root tips from genotypes to be compared were similarly prepared, hybridised with the same amount of radioactive RNA under the same conditions and subjected to autoradiography in an identical way.

3. Results and discussion

(i) *Nucleolus organiser variation in hexaploid wheat*

The hybridisation of cRNA copies of wheat rDNA to metaphase chromosomes of four hexaploid wheats was studied autoradiographically. It was possible to distinguish the pairs of different nucleolus organisers from one another by the number of grains clustered over each site. In the variety Chinese Spring the mean number of grains over the two major sites (fig. 1a) was approximately 18, while over the two other sites it was approximately 13 (table 1). No other sites could be reliably recognised without a much longer autoradiographic exposure time (see table 2). In *Triticum spelta*, however, four pairs of sites were recognised in most but not all cells (see fig. 1b). Cappelle–Desprez and Cheyenne showed only three pairs of sites (table 1). The most likely interpretation of these results is that hexaploid wheats have at least four pairs of nucleolus organisers but in some varieties the number of

TABLE 1

Variation in in situ *rDNA hybridisation to different hexaploid wheats*

Nucleolus organiser	Mean silver grains per nucleolus organiser in the four hexaploids			
	Chinese Spring	*Triticum spelta*	Cappelle–Desprez	Cheyenne
1	18·3±0·6	21·5±1·2	14·2±0·7	20·6±0·8
2	18·1±0·6	21·1±1·2	13·1±0·6	19·1±0·8
3	13·0±0·6	16·1±1·0	9·9±0·4	14·3±0·6
4	13·6±0·5	17·0±0·8	10·2±0·5	14·7±0·5
5	—	11·0±0·7	4·4±0·4	4·1±0·5
6	—	10·9±0·6	3·8±0·5	3·2±0·4
7	—	5·5±0·4	—	—
8	—	5·1±0·5	—	—

The slides were treated as described in Materials and Methods. The autoradiographic exposure time was 140 hrs. The nucleolus organisers are bracketed into pairs on the basis of the silver grain counts.

rRNA genes clustered at some of the organisers is so few that they are not recognised in these *in situ* hybridisation experiments.

Cytological studies of micronuclei formed during telophase of meiosis in the complete set of monosomic aneuploids of the variety Chinese Spring have indicated that chromosomes 1B, 6B, 1A and 5D carry nucleolus organisers (Crosby, 1957). Biochemical studies on this variety, however, have indicated that 90 per cent of the rRNA genes are found in the nucleolus organisers on chromosomes 1B and 6B (Flavell and O'Dell, 1976). Thus the *in situ* hybridisation results support the biochemical findings.

Chromosomes 1B and 6B carry major nucleolus organisers in *Triticum spelta* and Cappelle–Desprez (Flavell and Smith, 1974*a*). The other large nucleolus organisers in these hexaploid wheats should therefore be on chromosomes 1A or 5D in Cappelle–Desprez and on both 1A and 5D in *Triticum spelta*. The presence of major nucleolar organisers on 1A chromosomes was investigated by *in situ* hybridisation using plants in which 1A chromosomes from *Triticum spelta* and Cappelle–Desprez have been substituted into Chinese Spring. The results in fig. 1c and table 2 clearly show the presence of additional clusters of rRNA genes in these lines compared with Chinese Spring. This result agrees with the significant increase in the number of rRNA genes associated with these chromosome substitutions (Flavell and Smith, 1974*b*) and also with the frequency with which nucleoli large enough to be easily visible in the light microscope are associated with these substituted chromosomes (see table 2 and Flavell and O'Dell, 1979). In some Chinese Spring cells, after an autoradiographic exposure time of 570 h, evidence of a third site of rRNA hybridisation could be discerned (table 2). This site is probably that on chromosome 5D. It contained considerably fewer silver grains than was recognised over the minor site in the chromosome 1A substitution lines after an autoradiographic exposure time of only 258 h (table 2).

(ii) *Heterozygosity for rRNA gene number in King II rye*

Rye is an outbreeding species with one major pair of nucleolus organisers. Previous studies have shown that different varieties may differ

TABLE 2

In situ *hybridisation to chromosome 1A substitution lines of Chinese Spring*

Genotype	No. of rRNA[a] genes	Mean no. of[a] nucleoli/cell	Autoradiographic exposure time (hr)	Mean number of silver grains per nucleolus organiser					
				1	2	3	4	5	6
Chinese Spring	9,100	2·55	258	16·6±0·7	16·5±0·8	11·5±0·6	11·2±0·6	—	—
			570	sat	sat	19·8±1·9	19·6±2·0	1·1±0·2*	0·7±0·1*
CS (*T. spelta* 1A)	12,600	3·20	258	16·1±0·7	14·8±0·7	10·8±0·7	8·6±0·5	3·8±0·7*	2·7±0·6*
			570	sat	sat	18·0±1·8	16·8±1·9	7·8±1·0	6·4±0·8
CS (Cappelle–Desprez 1A)	12,100	2·87	258	18·3±1·1	17·9±1·0	13·3±0·9	13·8±1·0	1·3±0·4*	0·9±0·5*

[a] Data taken from Flavell and O'Dell (1979)

— denotes no sites with more than one grain consistently recognised

sat sites were saturated with silver grains and so could not be counted

* These values like all the others are the mean number of silver grains over these sites, in *all* the cells scored. In some cells of these genotypes however sites 5 and 6 could not be recognised because not more than 2 grains were located in the correct position on the short arm of the chromosomes. These mean values are therefore underestimates of the number of silver grains used to identify the site.

markedly in rRNA gene number (Flavell and Smith, 1974*a*) and considerable variations in rRNA gene number may occur between individual plants in an inbred population (Flavell and Rimpau, 1975). These observations predict that heterozygosity may occur frequently in the number of rRNA genes in rye. This is illustrated in fig. 2 for a plant of the variety King II. In an analysis of 20 sets of mitotic chromosomes, the mean number of silver grains over the larger nucleolus organiser was $17{\cdot}0 \pm 1{\cdot}0$ while over the smaller nucleolus organiser the mean number was $7{\cdot}6 \pm 0{\cdot}6$.

(iii) *Ribosomal DNA deletions in the wheat aneuploid stock tetrasomic for chromosome 5D*

In a study of rRNA genes in some aneuploid derivations of the variety Chinese Spring (Sears, 1954), several anomalies in rRNA gene number were discovered which could not be explained by the aneuploidy *per se*. For example, in the stock possessing two additional 5D chromosomes, approximately 40 per cent of the rRNA genes were deleted (Mohan and Flavell, 1974; Flavell and O'Dell, 1976; 1979). It was concluded this stock has deletions in one or both of the major clusters of rRNA genes on chromosomes 1B and 6B. Figure 1d shows that the tetrasomic 5D stock shows only one major pair of rRNA gene clusters, in contrast to the two pairs in Chinese Spring. This confirms that the tetrasomic 5D stock carries a major deletion in the nucleolus organiser of either chromosome 1B or 6B.

4. Concluding Remarks

This paper demonstrates the value of the *in situ* hybridisation technique for recognising and quantitatively evaluating genetic variation in clustered repeated sequences. The molecular mechanism responsible for creating the variation has not been studied, although unequal crossing over (Smith, 1976) is a likely explanation. The variation recognised emphasises that in inbreeding organisms with more than one nucleolus organiser or in outbreeding organisms, measurement of total rRNA gene number in the genome is not a complete description of the variation. Localisation of the variation to particular nucleolus organisers is also essential if the variation is to be described more completely.

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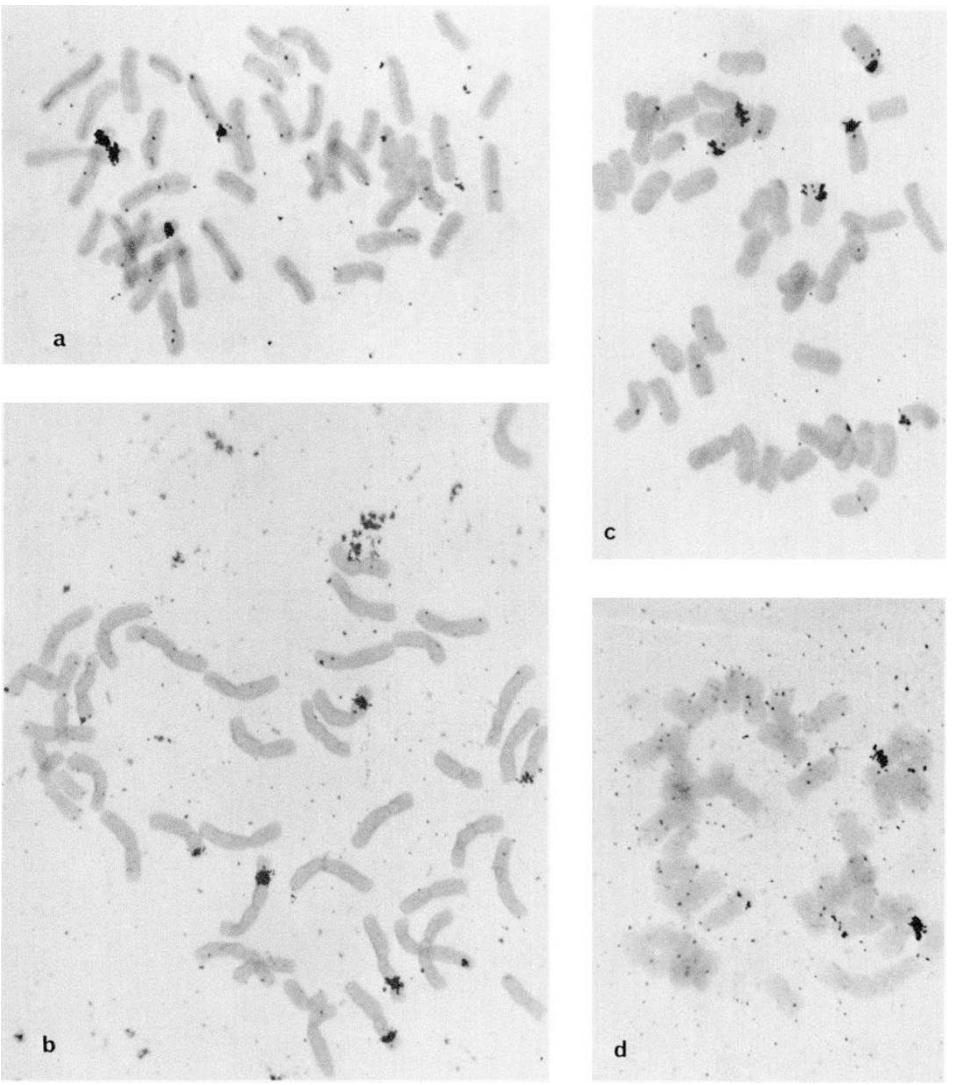

FIG. 1.—*In situ* hybridisation of rRNA to different wheat genotypes.

a Chinese Spring showing two pairs of nucleolus organisers.
b *Triticum spelta* showing three pairs of nucleolus organisers. The fourth pair is not obvious in this particular cell.
c Chinese Spring with 1A chromosomes from *Triticum spelta* substituted into it.
d A Chinese Spring aneuploid stock with an additional pair of 5D chromosomes.

Photographs a and b were taken from the experiment summarised in table 1. Photograph d was obtained from tetrasomic 5D cells also included in this experiment. Photograph c was taken from the experiment summarised in table 2 (autoradiographic exposure time 258 h).

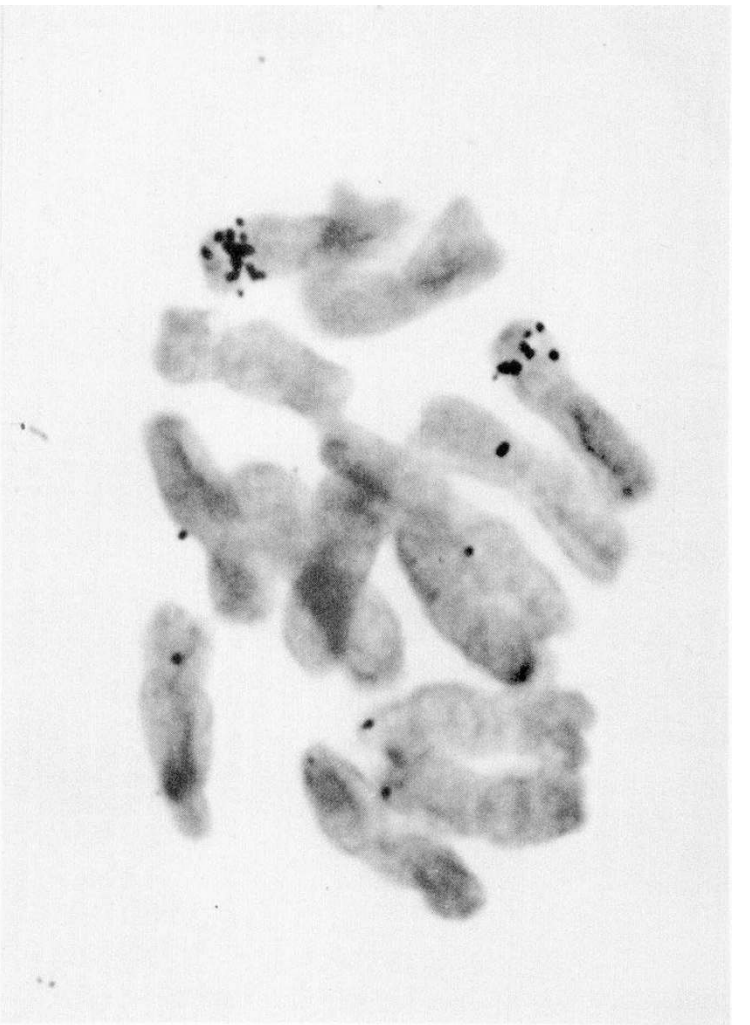

FIG. 2.—*In situ* hybridisation of rRNA to King II rye. The autoradiographic exposure time was 570 h.