Theor. Appl. Genet. 63, 349–360 (1982)

Chromosome and Nucleotide Sequence Differentiation in Genomes of Polyploid *Triticum* Species

J. Dvořák
Department of Agronomy and Range Science, University of California, Davis, California (USA)

R. Appels
Division of Plant Industry, CSIRO, Canberra City, ACT (Australia)

Summary. The nature of genome change during polyploid evolution was studied by analysing selected species within the tribe *Triticeae*. The levels of genome changes examined included structural alterations (translocations, inversions), heterochromatinization, and nucleotide sequence change in the rDNA regions. These analyses provided data for evaluating models of genome evolution in polyploids in the genus *Triticum*, postulated on the basis of chromosome pairing at metaphase I in interspecies hybrids.

The significance of structural chromosome alterations with respect to reduced MI chromosome pairing in interspecific hybrids was assayed by determining the incidence of heterozygosity for translocations and paracentric inversions in the A and B genomes of *T. timopheevii* ssp. *araraticum* (referred to as *T. araraticum*) represented by two lines, 1760 and 2541, and *T. aestivum* cv. Chinese Spring. Line 1760 differed from Chinese Spring by translocations in chromosomes 1A, 3A, 4A, 6A, 7A, 3B, 4B, 7B and possibly 2B. Line 2541 differed from Chinese Spring by translocations in chromosomes 3A, 6A, 6B and possibly 2B. Line 1760 also differed from Chinese Spring by paracentric inversions in arms 1AL and 4AL whereas line 2541 differed by inversions in 1BL and 4AL (not all chromosomes arms were assayed). The incidence of structural changes in the A and B genomes did not coincide with the more extensive differentiation of the B genomes relative to the A genomes as reflected by chromosome pairing studies.

To assay changing degrees of heterochromatinization among species of the genus *Triticum*, all the diploid and polyploid species were C-banded. No general agreement was observed between the amount of heterochromatin and the ability of the respective chromosomes to pair with chromosomes of the ancestral species. Marked changes in the amount of heterochromatin were found to have occurred during the evolution of some of the polyploids.

The analysis of the rDNA region provided evidence for rapid "fixation" of new repeated sequences at two levels, namely, among the 130 bp repeated sequences of the spacer and at the level of the repeated arrays of the 9 kb rDNA units. These occurred both within a given rDNA region and between rDNA regions on non-homologous chromosomes. The levels of change in the rDNA regions provided good precedent for expecting extensive nucleotide sequence changes associated with differentiation of *Triticum* genomes and these processes are argued to be the principal cause of genome differentiation as revealed by chromosome pairing studies.

Key words: Chromosomes – Nucleotides – Evolution – Polyploids – *Triticum* – Heterochromatin – Wheat

Introduction

Chromosome pairing and the ensuing chiasma formation scored at metaphase I (MI) is the result of a complex set of events in meiosis. Despite this complexity, chromosome pairing at MI in plant interspecific hybrids has provided the only means, to date, for quantifying the degree of genome relatedness and for inferring the origin of polyploid plant species (see Kihara 1963). Detailed interpretation of this observation is difficult however because of the poor understanding of the biological events underlying chromosome pairing. In this paper we have examined chromosome changes within selected species of *Triticeae* at several levels viz. structural changes (translocations, inversions), heterochromatinization and nucleotide sequence change at the rDNA locus. These studies were aimed at analyzing the nature of genome change during polyploid evolution and thus assessing the validity of various models of genome differentiation developed on the basis of chromosome pairing studies in interspecific hybrids.

0040-5752/82/0063/0349/$ 02.40

Materials and Methods

Genotypes

Ditelosomics of *T. aestivum* cv. Chinese spring were obtained from E. R. Sears, University of Missouri, Columbia, Missouri, (USA). From these, double monotelodisomics of the homoeologous chromosomes of the A and B genomes were produced. Two lines of *Triticum timopheevii* ssp. *araraticum* used in hybridization were produced by self-pollination of a single plant selected from accessions G2541 and G1760 supplied by L. B. Johnson, the Department of Plant Science, University of California, Riverside (USA). Accessions of other *Triticum* species came either from the Department of Plant Science, Riverside (G. Wains), Agricultural Canada Station, Winnipeg (E. Kerber), Plant Breeding Institute, Cambridge, England (R. Johnson) or E. R. Sears. Populations of *Secale* species were obtained from V. Jaaska, Department of Botany, Tartu, University of Estonian SSR. Population of *Elytrigia elongata* (Host) Holub was supplied by L. Evans, University of Manitoba, Winnipeg, Canada whereas those of *E. scirpea* and *E. libanotica* were supplied by D. R. Dewey, USDA Research Station, Logan, Utah (USA). Populations of *T. speltoides* were collected by S. Jana, the Crop Science Department, University of Saskatchewan, Saskatoon, Canada in Syria and Turkey. Individual heads were randomly collected every 10 m.

Staining Techniques

Immature spikes were collected from plants grown in the greenhouse. They were fixed in Carnoy's ethanol – chloroform – acetic acid fixative (6 : 3 : 1, v/v) and stained with iron-acetocarmine (BDH).

For C-banded karyotypes root-tips were collected from three day old seedlings germinated in dark at 20 °C. Five to ten mm long root-tips were excised and placed in ice-cold distilled water in a staining dish and pretreated for 18 h at 2 °C in a refrigerated water-bath. The root-tips were then fixed in 45% acetic acid at room temperature from six to twelve hrs, then transferred directly into 0.2 N HCl, 60 °C, and hydrolyzed for 5 min. They were then washed in distilled water and treated with a mixture of 5% pectinase (Sigma, crude preparation), 5% cellulase (Sigma, Type II) and 5% hemicellulase (Sigma, Grade II). The digestion was monitored by making test slides and was terminated when a tap on the cover-glass resulted in dispersal of cells without them adhering to the slide (usually 30 to 45 min). The root-tips were then thoroughly washed in distilled water and either used immediately or stored in distilled water at 5 °C for 24 to 48 h. Squashes were made in 45% acetic acid. The cover-glass was removed by the dry-ice method and slides were dried at 45 °C. The slides were treated with saturated solution of $Ba(OH)_2$ for 6 min, transferred directly into 1 N HCl for 1 min to neutralize $Ba(OH)_2$, washed for 5 min in (several times changed) distilled water, and dried. The slides were then placed into 2×SSC, pH 7.0, at 60 °C. The progress of the development of bands was monitored by staining test slides. When the optimum length of the treatment was reached the slides were washed in distilled water and dried. They were then stained in freshly prepared 7% solution of azur B eosinate (Sigma) in 25 mM KH_2PO_4–Na_2HPO_4 buffer, pH 6.8. The staining solution was filtered prior to use. Stock solution of azur B eosinate was prepared by dissolving 1 g of dye in 50 ml of methanol at 50 °C, adding 50 ml of glycerol, and stirring at 50 °C for several hrs. The stained slides were rinsed in water for 10 sec, dried on the slide warmer, and mounted in permount.

Wheat rDNA Spacer Probes

The *T. aestivum* cv. Chinese Spring spacer that separates 26S and 18S rRNA genes in the clone pTA250 (Gerlach and Bedbrook 1979) was cloned in pBR322 as described in the preceding paper (Appels and Dvorak 1982). The plasmid pTA 250.4, containing the spacer region, was digested with BamH1 endonuclease to release the insert and fractionated electrophoretically on 1% agarose gel in the presence of ethidium bromide. The gel was viewed under UV light, the required band was cut out and electroeluted. Ethidium bromide was removed from the DNA sample by four extractions with water-saturated iso-amylalcohol. DNA was precipitated by adding 1/10 volume sodium acetate (pH 5.5), two volumes 95% ethanol and chilling to –70 °C, centrifuged at 10,000 rpm (30 min) in Sorval centrifuge, dried, redissolved in 0.01 M Tris-HCl, pH 8.0, 0.001 M EDTA (TE) and digested with HhaI endonuclease. The digested DNA was electrophoretically fractionated in 2% agarose gel. The "750" bp long fragment and the population of "130" bp repeated units (Appels and Dvořák 1982) were isolated as described above. These were ^{32}P-labelled by nick-translation as described in the accompanying paper.

ΔTm Determination of Hybrid DNA Molecules

DNAs were isolated from individual seedlings, populations of seedlings, or from seedlings grown from seeds harvested from open pollinated plants, utilizing a technique described in the preceding paper (Appels and Dvořák 1982). DNA hybridization with "130" bp and "750" bp probes and Tm determinations were carried out as described in the preceding paper. A single DNA preparation of Chinese Spring was used in all experiments as a standard of homologous reassociation.

Restriction Endonuclease Analysis of Genomic DNAs

DNA was digested with the required enzyme and subjected to electrophoresis in 1.0% or 2.0% agarose gels in the presence of ethidium bromide. The gels were photographed under UV light and denatured DNA was then transferred onto "gene screen" membranes (New England Nuclear) and hybridized with the required radioactive probes (see Appels and Dvořák 1982). The membranes were extensively washed in 2×SSC, 0.1% SDS, dried and autoradiographed.

Results

Chromosome Differentiation Between the T. timopheevii and T. turgidum Lineages

Polyploid wheat species can be allocated into two lineages, one is comprised of *T. turgidum* L. em. Morris et Sears (2n = 4x = 28, genome formula AAB^eB^e) and *T. aestivum* L. em. Thell (2n = 6x = 42, genome formula AAB^eB^eDD) whereas the other of *T. timopheevii* (Zhuk) Zhuk. (2n = 4x = 28, genome formula AAB^tB^t) and *T. zhukovskyi* Men. et Er. (2n = 6x = 42, genome formula $AAAAB^tB^t$). Most of the chromosomes of the A genome in both tetraploids pair well with the chromosomes of einkorn wheat, *T. monococcum* L. em. Bowden (Kihara 1924; Sachs 1953). However, in both

lineages the chromosomes of the B genome do not pair well with chromosomes of any diploid species, or with each other in the interlineage hybrids (Lilienfeld and Kihara 1934; Feldman 1966). Similar disparity in the extent of pairing with the chromosomes of the presumed ancestors and related genomes in other polyploid species, has been observed in most polyploid species in the genus *Triticum*. The two wheat lineages provide a suitable model system for the assessment of the significance of structural chromosome changes in the evolutionary differentiation of genomes.

Two lines of *T. timopheevii* ssp. *araraticum* (further referred to as *T. araraticum*), 1760 and 2541, used in the following experiments represent an extreme of translocation polymorphism observed in *T. araraticum* (Tanaka et al. 1979). A hexavalent (a chain) plus a quadrivalent (ring) were observed in their hybrid indicating that they differ by three translocations, two sharing a common chromosome and an independent one involving two other chromosomes.

The two lines were hybridized with Chinese Spring ditelosomics. The pairing behaviour of each telosome and total chromosome pairing in each hybrid were determined. A telosome derived from a Chinese Spring chromosome which did not differ by a translocation from the *T. araraticum* chromosome was expected to pair in a monotelo-bivalent ($t1^{II}$, see Fig. 1a–c). However, in exceptional cases a monotelo-trivalent ($t2^{III}$) or a monotelo-quadrivalent ($t3^{IV}$) could potentially occur as a consequence of homoeologous pairing involving chromosomes of the remaining genomes. A telosome derived from a Chinese Spring chromosome which differed by a translocation from the *T. araraticum* chromosome was expected to pair in a monotelo-trivalent or monotelo-quadrivalent in addition to pairing in a monotelo-bivalent. If the chromosome was involved in two or more translocations, the telosome was expected to pair in higher valency multivalents, such as $t4^{V}$ or $t5^{VI}$.

Data in Table 1 show that the chromosomes of Chinese Spring differed from the chromosomes of line 1760 by a number of translocations in both genomes. The present technique did not allow the determination of the specific arm of a chromosome involved in a

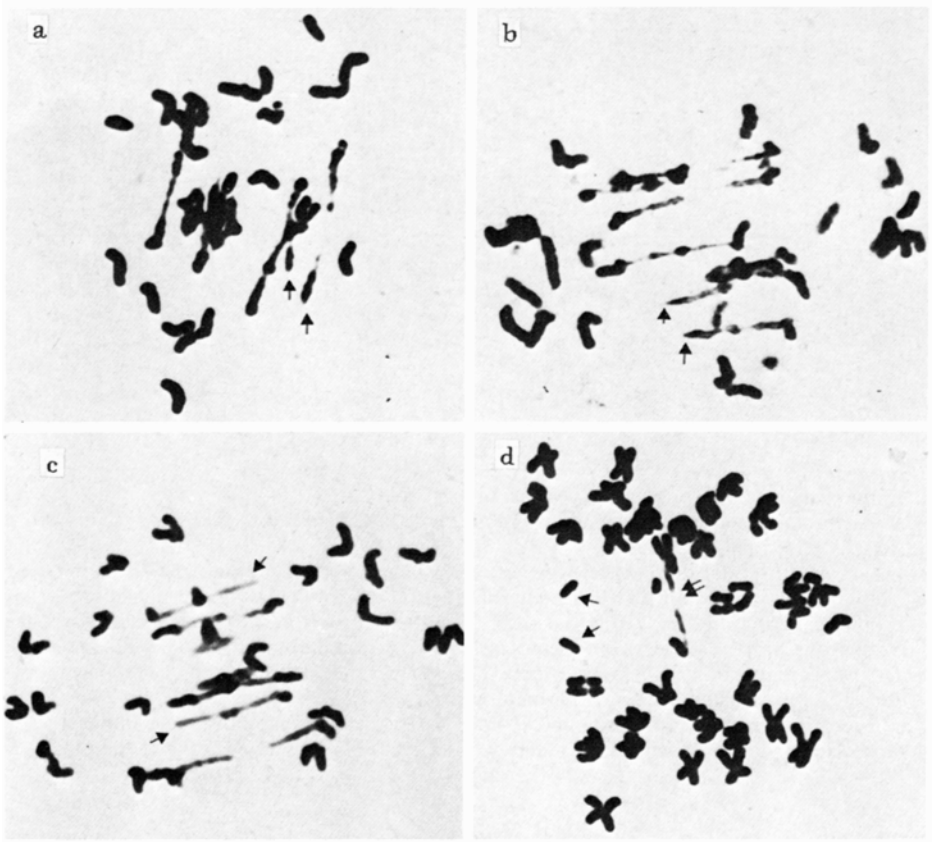


Fig. 1a–d. MI cells in hybrids, Chinese Spring double monotelosomics × *T. araraticum* 1760. **a** shows telosomes 1AL and 1BL pairing in different bivalents (arrows). Note that one of the telosomes is pairing with a satelited chromosome. **b, c** show telosomes 2AS and 2BL pairing in different bivalents, as well as 5AL and 5BL (respectively). **d** A1 cell in a hybrid (4AL×4Bα)×*T. araraticum* 1760 showing telosome 4AL involved in a bridge (double arrows). The univalent telosome 4Bα is dividing equationally (single arrows)

Table 1. Pairing of *Triticum aestivum* telosomes in hybrids between *T. aestivum* ditelosomics (DT) and *T. araraticum* lines 1760 and 2541

Hybrid	Plants	Cells	Percent of cells with telosome pairing in					Average number of configurations per cell						
			t^{I}	$t1^{II}$	$t2^{III}$	$t3^{IV}$	Other	I	II rod	II ring	III	IV	V	VI
DT 1AS × 1760	1	111	44.0	51.3	3.6	1.0		19.2	4.8	0.6	0.8	0.1	0.4	0.1
DT 1AL × 1760	4	203	18.1	78.3	3.6	0.0		15.9	5.3	0.8	1.1	0.5	0.2	0.1
DT 2AS × 1760	1	64	15.6	82.8	1.6	0.0		18.9	4.3	0.9	1.1	0.2	0.5	0.0
DT 3AL × 1760	3	128	31.6	16.2	4.2	46.6	0.6 ($t4^{V}$)[c] 0.8 ($t5^{VIII}$)	16.1	5.0	1.3	1.2	0.6	<0.1	0.0
DT 4AL × 1760[a]	2	90	78.9	15.6	5.6	0.0		17.9	4.1	1.3	1.2	0.2	0.4	<0.1
DT 5AL × 1760	3	139	9.1	90.4	0.5	0.0		17.0	5.4	0.8	1.0	0.3	0.3	<0.1
DT 6AL × 1760	3	113	12.6	75.1	8.5	3.5		13.5	5.2	1.2	1.1	0.4	0.5	0.1
DT 7AS × 1760	2	110	10.9	84.5	4.5	0.0		19.3	5.1	1.3	0.9	0.1	0.0	0.0
DT 7AL × 1760	2	78	21.9	29.6	20.5	28.1		20.0	4.4	1.3	0.5	0.4	<0.1	<0.1
DT 1BL × 1760	3	157	62.9	36.2	0.9	0.0		14.6	4.7	1.9	1.1	0.2	0.5	0.1
DT 2BL × 1760	3	143	48.9	49.0	2.0	0.0		18.0	4.0	1.6	1.2	0.2	0.3	<0.1
DT 3BL × 1760	1	71	84.5	9.8	5.6	0.0		14.7	5.1	1.3	1.0	0.5	0.5	0.0
DT 4Bα × 1760[a]	3	160	91.3	3.0	2.5	1.3	1.3($t4^{V}$)[c] 0.6($t5^{VI}$)	13.6	3.9	1.7	1.8	0.4	0.4	0.2
DT 5BL × 1760	2	116	67.2	32.7	0.0	0.0		19.7	4.1	1.2	0.9	0.2	0.2	0.0
DT 6BS × 1760	5	298	90.6	9.4	0.0	0.0		16.8	4.3	1.6	1.1	0.4	0.3	0.0
DT 7BS × 1760	1	55	90.4	7.3	0.0	1.8		15.4	4.9	1.5	0.9	0.4	0.5	0.0
DT 7BL × 1760	1	117	74.3	23.1	2.5	0.0		18.6	4.8	0.9	0.9	0.2	0.3	0.0
DT 1AL × 2541	3	149	17.5	82.5	0.0	0.0		21.0	4.4	1.7	0.6	<0.1	0.0	0.0
DT 2AS × 2541	1	53	32.1	66.0	1.8	0.0		19.8	5.1	1.6	0.6	0.0	0.0	0.0
DT 3AL × 2541	3	112	51.4	45.7	2.9	0.0		20.9	4.2	2.4	0.3	<0.1	0.0	0.0
DT 4AL × 2541[a]	4	234	90.6	9.4	0.0	0.0		21.3	4.3	1.8	0.5	<0.1	0.0	0.0
DT 5AL × 2541	3	113	18.3	80.9	0.8	0.0		20.7	5.0	1.5	0.4	<0.1	0.0	0.0
DT 6AL × 2541	3	149	30.0	53.6	15.4	1.0		20.4	4.6	2.3	0.4	<0.1	0.0	0.0
DT 7AS × 2541	3	166	39.0	61.0	0.0	0.0		22.3	4.3	1.4	0.4	<0.1	0.0	0.0
DT 1BL × 2541	2	83	91.6	8.4	0.0	0.0		22.1	4.1	1.9	0.3	0.0	0.0	0.0
DT 2BL × 2541	2	111	71.9	27.3	0.8[b]	0.0		22.1	4.5	1.5	0.3	0.0	0.0	0.0
DT 3BL × 2541	3	183	83.0	27.0	0.0	0.0		20.9	4.3	1.9	0.5	0.1	0.0	0.0
DT 4Bα × 2541[a]	1	177	96.6	3.4	0.0	0.0		19.5	4.9	1.9	0.6	<0.1	0.0	0.0
DT 5BL × 2541	3	291	88.8	11.2	0.0	0.0		24.3	3.7	1.1	0.3	0.0	0.0	0.0
DT 6BS × 2541	2	203	86.5	12.0	1.6[b]	0.0		23.0	3.6	1.7	0.4	0.1	0.0	0.0
DT 6BL × 2541	2	106	77.3	21.7	1.1[b]	0.0		22.0	4.4	1.5	0.4	0.0	0.0	0.0
DT 7BL × 2541	3	161	89.9	10.1	0.0	0.0		21.6	4.4	1.9	0.2	<0.1	0.0	0.0

[a] Assignment of chromosomes 4A and 4B into genomes was exchanged (Dvořák, in preparation)

[b] In view of the low pairing of the B genome chromosomes in hybrids involving line 2541, these figures are considered to reflect the presence of a translocation

[c] It could possibly be argued that finding of fewer translocation differences between Chinese Spring and line 2541 than between Chinese Spring and line 1760 was caused by poorer overall pairing which reduced the probability of crossing over in short translocated segments. Evidence answering this potential criticism comes from the observation that one of the two *T. araraticum* lines has additional translocations relative to the other one, importantly, two of them sharing a common chromosome. There seems to be little doubt that it is line 1760 which differs by more translocations from Chinese Spring. Quinquevalents and higher valency multivalents indicating the presence of two translocations sharing a common chromosome occurred only in hybrids involving line 1760. Data in the Table show that these two translocations involve chromosomes 3A, 4B, and 7A; the latter chromosome is implicated by the absence of the quinquevalent in hybrids lacking arm 7AL which presumably has the pairing segment leading to the quinquevalent and hexavalent formation

translocation and thus assignments were confined to the entire chromosomes. In the A genome translocations involved chromosomes 1A, 3A, 4A, 6A and 7A (as judged from the % of cells containing the $t2^{III}$, or higher, configuration). Chromosome 3A was implicated in two translocations. Cells with a telomultivalent observed in hybrids involving telosomes 2AS and 5AL were too infrequent (a single cell was observed in each case) to be attributed to a translocation heterozygosity. As discussed above they very likely originated from pairing

Table 2. Pairing of *Triticum aestivum* telosomes in hybrids between double monotelodisomics and *T. araraticum* lines 1760 and 2541

Hybrid	Cells	Percent of cells with telosomes paired in[b]:								
		t^{II}	$(t+t)1^{III}$	$t1^{II}+t1^{II}$	$t2^{III}+t1^{II}$	$t3^{IV}+t1^{II}$	$t1^{II}+t^{I}$	$t2^{III}+t^{I}$	$t3^{IV}+t^{I}$	$t^{I}+t^{I}$
(1BL×1AL)×1760	83	0.0	0.0	56.0	3.6	9.6	22.0	1.2	2.4	4.8
(2BL×2AS)×1760	83	0.0	0.0	33.0	0.0	0.0	48.0	1.2	0.0	18.0
(4AL×4Bα)×1760[a]	148	0.6	0.6	0.6	3.3	0.1	13.5	10.8	5.3	63.5
(5AL×5BL)×1760	138	0.0	0.0	14.5	0.0	0.0	68.8	>0.1	>0.1	14.5
(5AL×5BL)×2541	154	0.0	0.0	5.2	0.0	0.0	69.0	0.7	0.0	24.7

[a] Chromosomes 4A and 4B were reallocated in their genome assignment (Dvořák, in preparation)
[b] t stands for one telosome and the Arabic numerals specify the number of whole chromosomes in each pairing configuration. Superscripts specify the valency of the configurations (univalent, bivalent, trivalent, etc.)

with a homoeologous chromosome from the B or the D genome. In the B genome, the translocations involved chromosomes 3B, 4B, 7B, and possibly 2B. Of these, 4B appeared to be implicated in two translocations.

The total chromosome pairing was almost invariably lower in hybrids involving *T. araraticum* line 2541 than in those involving line 1760 (Table 1). The same trend was seen in the pairing frequencies of virtually all telosomes (Table 1). This indicated that the genotypes of the two *T. araraticum* lines differed in their influence on heterogenetic pairing; the interaction between the genotype of Chinese Spring and line 1760 apparently creates more favorable conditions for MI chromosome pairing than that between Chinese Spring and line 2541.

Hybrids involving line 2541 showed fewer translocation differences. The A genomes differed by break points in chromosomes 3A and 6A and the B genomes differ by a break point in chromosomes 6B and possibly in 2B. The two cells with a monotelo-trivalent that occurred in hybrids involving telosomes 2AS and 5AL (a single cell in each hybrid) probably did not reflect a translocation difference. This interpretation is based on the fact that these two telosomes paired with particularly high efficiencies (as judged from the frequency of $t1^{II}$).

Since the parental genotypes differed by several translocations, it was impossible to ascertain, by this technique, which two of the several possible chromosomes were involved in a specific translocation. It was however important, for the interpretation of the data, to determine whether or not the translocations occurred between homoeologous chromosomes since that could indicate that they originated from recombination due to heterogenetic pairing between A and B genome homoeologues. This was tested by crossing double monotelodisomics with *T. araraticum*. In these hybrids the A and B genome homoeologous chromosomes under investigation were marked by being telocentric. If a translocation involved homoeologous chromosomes, both telosomes were expected to occasionally pair in the same configuration, such as ditelo-trivalent, $(t+t)1^{III}$. Except for two cells in a hybrid having the telosomes of homoeologous group 4, telosomes did not pair in the same configuration, even though cells with both telosomes paired in different configurations were observed in all combinations (Table 2 and Fig. 1 a – c). In one of the two cells the telosomes paired in a ditelobivalent (t^{II}). This configuration could arise either due to residual homoeologous pairing or to the presence of a nonreciprocal translocation in *T. aestivum*, resulting in a duplication of originally homologous chromatin in homoeologous arms. In addition to pairing in the ditelobivalent the 4AL and 4Bα telosomes also paired in a telo-trivalent. Thus, of the chromosomes investigated 4A and 4B are the only candidates for the presence of a translocation between homoeologous chromosomes. Most of the other translocations that differentiate the A and B genomes of *T. aestivum* and *T. araraticum* (Table 1) occurred between nonhomoeologous chromosomes.

Since telosomes can be recognized from other chromosomes not only at MI but also at anaphase I (AI), they can be used as markers to identify chromosomes involved in the AI bridges which indicate heterozygosity for a paracentric inversion (Fig. 1 d). Both *T. araraticum* lines appeared to differ from Chinese Spring by an inversion in arm 4AL. A large fragment was always associated with the bridge (Fig. 1 d), thus, the inversion must be proximally located in the arm. In addition, line 1760 differed from Chinese Spring by another inversion in 1AL (Table 3). In the comparison involving line 2541, in addition to the inversion in 4AL, there was heterozygosity for an inversion in 1BL and possibly in 5BL (Table 3). Thus these data suggest that differences due to paracentric inversions are present in only a few of the chromosome arms. Consistent with this conclusion is the fact that a maximum of only 3 bridges per cell were observed even though 2447 cells were scored.

Table 3. Numbers of anaphase I cells with designated number of inversion bridges per cell in hybrids *Triticum aestivum* ditelosomics × *T. araraticum* lines 1760 and 2541

Telosome	1760				2541			
	No. of bridges in a cell				No. of bridges in a cell			
	0	1	2	3	0	1	2	3
1AS	43	3	0	0	–	–	–	–
1AL	124	17+2[b]	3	0	100	9	0	0
2AS	79	20	2	1	–	–	–	–
3AL	31	4	0	0	38	7	0	0
4AL[a]	91	9+4[b]	2	0	69	4+3[b]	1	0
5AL	68	15	1	1	54	5	0	0
6AL	5	3	0	0	71	8	0	0
7AS	138	27	1	0	99	7	0	0
1BL	84	15	0	0	95	9+1[b]	0	0
2BL	68	6	0	0	75	6	0	0
3BL	–	–	–	–	60	3	0	0
4B[a]	100	26	2	0	57	7	0	0
5BL	43	10	0	0	168	11	0	0
6BS	96	16	1	1	90	6	0	0
6BL	–	–	–	–	121	11	0	0
7BL	84	16	0	0	–	–	–	–

[a] Chromosomes 4A and 4B were realoated in their genome assignment (Dvořák, in preparation)
[b] The second number indicates the number of cells in which the telosome was involved in the anaphase I bridge

The Relationship Between Genome Differentiation and the Amount of Heterochromatin

In this section the question of whether changes occur in the amount of heterochromatin concomitant with the evolution of polyploids was examined. In particular it was of interest to find any consistent pattern between heterochromatinization of genomes and the loss of pairing between the respective chromosomes and the chromosomes of the ancestral species. To assay change in chromosomal heterochromatin all the diploid and polyploid *Triticum* species were C-banded.

While there is agreement, for wheat species, between the amount of heterochromatin in chromosomes and their ability to pair with the chromosomes of the ancestral species (Johnson and Dhaliwal 1978), no such relationship was evident in the remaining polyploids in the genus *Triticum* (all available data not shown). To illustrate this point, two examples are shown in Fig. 2. The chromosomes of the D genome in *T. ventricosum* Ces. (genome formula DDM^vM^v) pair well with the chromosomes of the ancestral *T. tauschii* (Coss.) Schmal (genome formula DD, Kihara 1940) whereas the chromosomes of the M genome pair poorly in all

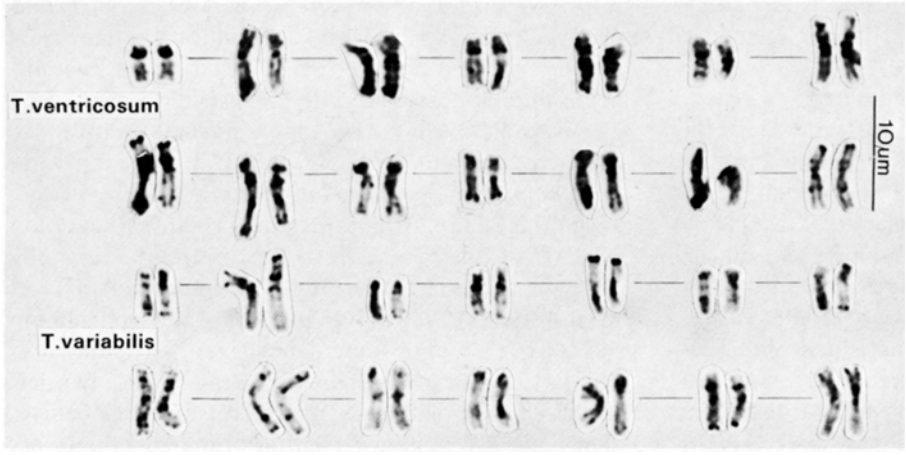


Fig. 2. C-banded chromosomes of *T. ventricosum* and *T. variabilis*

hybrid combinations investigated so far (Kihara 1963). All fourteen chromosome pairs in *T. ventricosum,* however, possess extensive (and approximately equal) amounts of heterochromatin (Fig. 2). A similar situation pertains in *T. variabilis* (Boiss.) comb. nor. (genome formula UUB^vB^v). The U genome chromosomes (originally designated by Kihara as C^u) pair well in all hybrids involving other species having this genome whereas the B^v genome chromosomes (originally designated by Kihara as S^v) consistently pair poorly (Kihara 1963), and yet all fourteen chromosome pairs of *T. variabilis* have similar amounts of heterochromatin.

The two species, *T. ventricosum* and *T. variabilis,* also provide good examples of major changes in the amount of chromosomal heterochromatin occurring in the evolution of *Triticum* polyploids. The D genome present in the diploid *T. tauschii* and polyploids such as *T. aestivum* (Gill and Kimber 1975; Gerlach 1977; Peacock et al. 1981), *T. crassum* (Boiss.) Aitch et Hensl, *T. vavilovii* (Zhuk.) McGuire et Dvořák and *T. juvenale* Thell. (results not shown) have little heterochromatin. In contrast *T. ventricosum* (Fig. 2) must contain a D genome with extensive regions of heterochromatin. The polyploid *T. variabilis,* like the polyploid wheats, has a B genome that evolved from a genome of a species of the section *Sitopsis* of the genus *Triticum* (Kihara 1963). The chromosomes of all the species in this section (*T. speltoides, T. longissimum, T. sharonense, T. searsii, T. bicorne*) are diploid and have prominent terminal, heterochromatic, bands (data not shown). Terminal C-bands are either small or absent in the polyploid wheat species (Gill and Kimber 1975; Dvořák unpublished) as well as in the polyploid *T. variabilis* (Fig. 2).

The Relationship Between the Nucleotide Sequence Change and Genome Differentiation

In the preceeding paper (Appels and Dvořák 1982) it was shown that the spacer nucleotide sequences that separate rRNA genes (rDNA) are polymorphic within the gene pools of *T. aestivum* and *T. turgidum* ssp. *dicoccoides* (further referred to as *T. dicoccoides*). Comparison of the wheat repeated "130" bp component and non-repeated "750" bp component of the Chinese Spring spacer with rye indicated that these nucleotide sequences must be evolving at a rate exceeding that of the genes they separate. The extent of differentiation of these sequences among closely related species was assessed by isolating DNA from all diploid *Triticum* species, except *T. tripsacoides* (Jaub. et Spach.) Bowden, and several representative species of the genera *Elytrigia* and *Secale*. The molecular hybrids between these DNA preparations and the "130" bp and "750" bp spacer sequences were examined (Figs. 3, 4). The investigation of several populations and a number of accessions of *T. dicoccoides* in the preceding paper (Appels and Dvořák 1982) indicated that polymorphism of both sequences (as measured by ΔTm's relative to a Chinese Spring standard) within the species did not exceed 2.2 °C. This ΔT_m translates to a maximum of 2.6% nucleotide difference, using a conversion factor 1 °C = 1.2% nucleotide mismatch (Appels and Dvořák 1982). The average ΔTm of the *T. dicoccoides* plants was 1 °C.

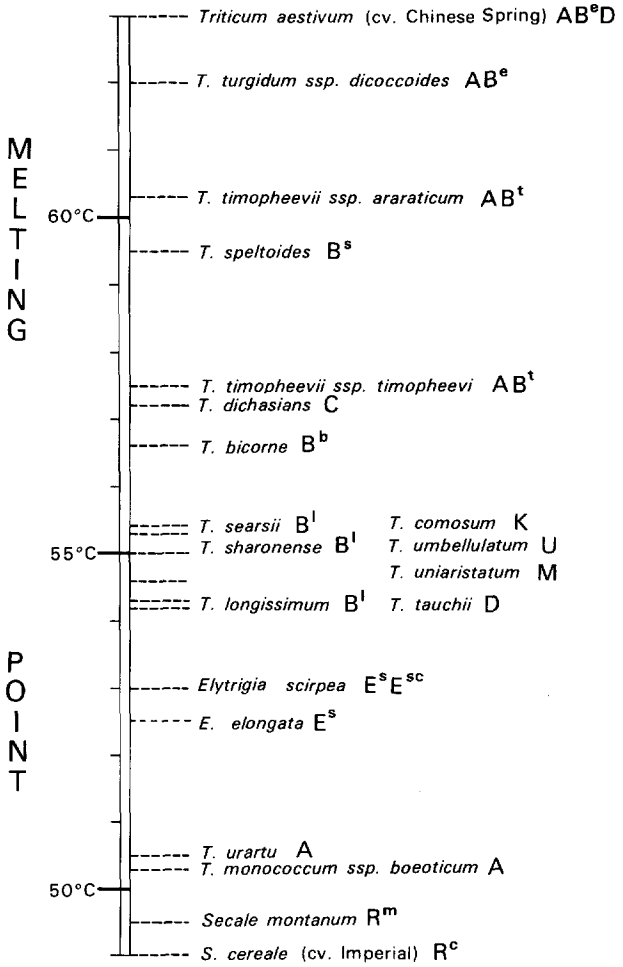


Fig. 3. Tm's of molecular hybrids between the '130' bp sequences of an rDNA spacer of *T. aestivum* cv Chinese Spring with the DNA's of designated species. The Tm of the hybrid with Chinese Spring (63 °C) is used as a standard. The values for *T. dicoccoides, T. timopheevii* ssp. *araraticum, T. speltoides* and *T. tauschii* are means based on the DNA preparations from 46, 6, 51 and 8 plants, respectively. The remaining values are based on single DNA preparations from a number of seedlings of a single accession. The letters following each species designate the respective genomic formulae

Molecules of "130" bp sequence reassociated with DNA of six individuals of *T. araraticum* (each selected randomly) had Tm's ranging from 60.2 °C to 61.0 °C, with a mean ΔTm of 2.5 °C, virtually all falling outside

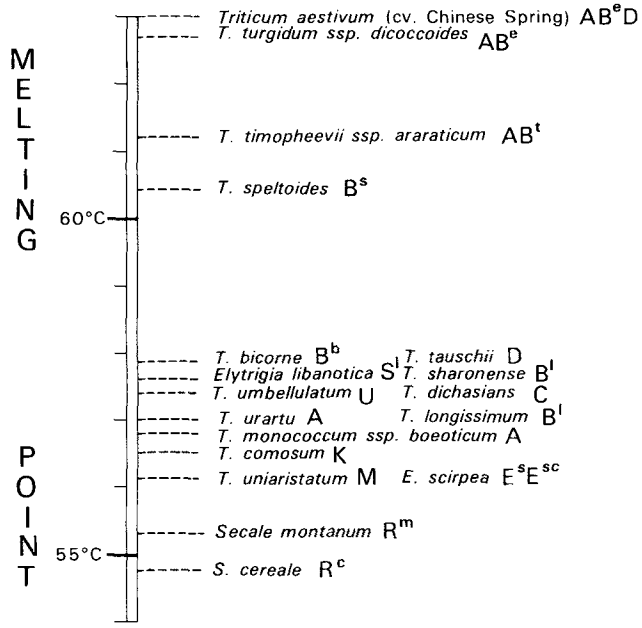


Fig. 4. Tm's of molecular hybrids of the '750' bp sequence with the designated DNA's. Chinese Spring (63 °C) is the standard. The value for *T. dicoccoides* is a mean based on the DNA preparations from 17 individuals while the remaining values are based on single DNA preparations from a number of plants of a single accession. The letters following each species designate the respective genomic formulae

of the range obtained for *T. dicoccoides*. Since in interspecific hybrids involving *T. araraticum* the Chinese Spring telosomes, 1BL (Fig. 1a) and 6BL (not shown) paired with satellited chromosomes, there is little doubt that the two major sites of rDNA in *T. araraticum* are in the B genome and correspond to those present in the 1BS and 6BS chromosome arms of Chinese Spring. Thus, the nucleotide sequences of the "130" bp repeated component (Fig. 3) and the "750" bp non-repeated component (Fig. 4) of rDNA spacer has diverged by an average of 3% and 2% of nucleotides, respectively, between the two wheat lineages. Interestingly, the "130" bp sequences of the single accession of the cultivar *T. timopheevii* ssp. *timopheevii* that was investigated showed a difference in Tm from those of Chinese Spring that was twice that of the "130" bp sequences of the wild ssp. *araraticum* (Fig. 3).

Among the diploid species the smallest ΔTm occurred in the hybrid DNA molecules involving *T. speltoides;* this was true for both "130" and "750" bp sequences. To assess polymorphism of these sequences in this species, DNAs were isolated from individuals randomly collected from 27 individuals of two populations of the awned form *ligustica* (populations Ha and Israel) and 23 individuals collected from three populations of the awnless form *aucheri* (populations San, G, and F) hybridised with wheat "130" bp sequences. The ΔTm's of these hybrid molecules varied among individual plants as in the *T. dicoccoides* populations. The *ligustica* populations showed larger ΔTm's from the Chinese Spring standard than the *aucheri* populations (Fig. 5). The populations Ha and San are large enough to permit a comparison. The mean ΔTm in the Ha population was 4.3 °C whereas it was 3.0 °C in the San population; the difference between the means is significant (t-test, $P = 0.05$).

The variation of ΔTm's within *T. speltoides* (Fig. 5) and *T. dicoccoides* can be used to estimate the polymorphism in *Triticum species,* assuming that these two species are not exceptional. The range of the variation was 1–3 °C. Taking this into account a clear implication from the ranking shown in Fig. 3 ("130" bp probe) and Fig. 4 ("750" bp probe) is that both the "130" bp and "750" bp nucleotide sequences of *T. speltoides* are more closely related to those in Chinese Spring than those of other species of the section *Sitopsis.* The *Sitopsis* species are, as a group, more related to the Chinese Spring sequences than the remaining diploid

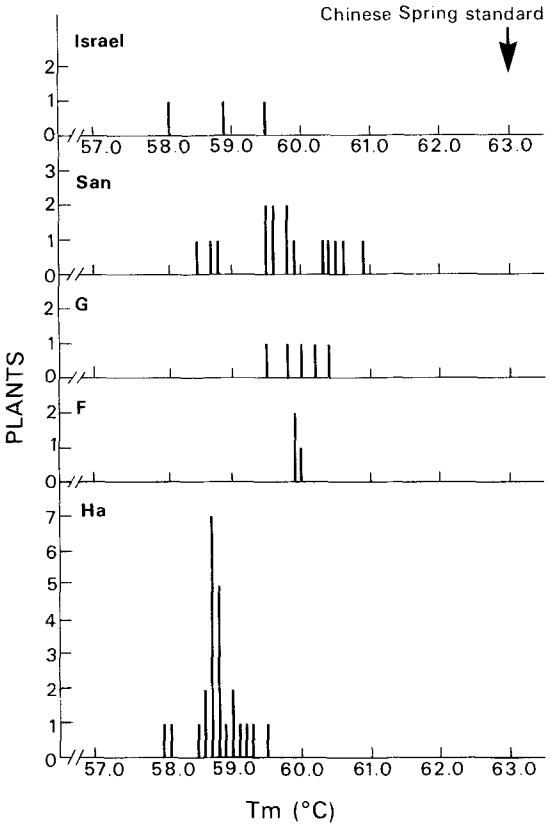


Fig. 5. Tm's of molecular hybrids of the '130' bp sequences with the DNA's of plants from 5 *T. speltoides* populations. The length of the lines specify the numbers of plants having a specific Tm

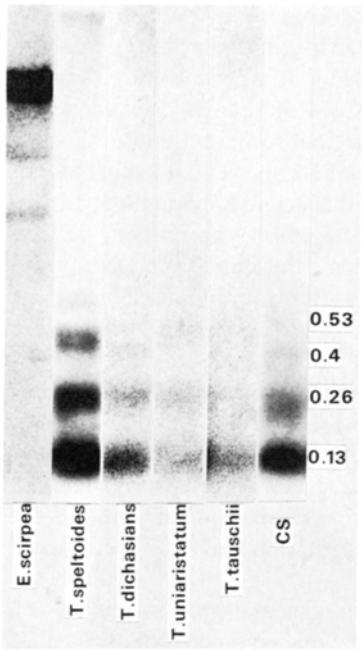
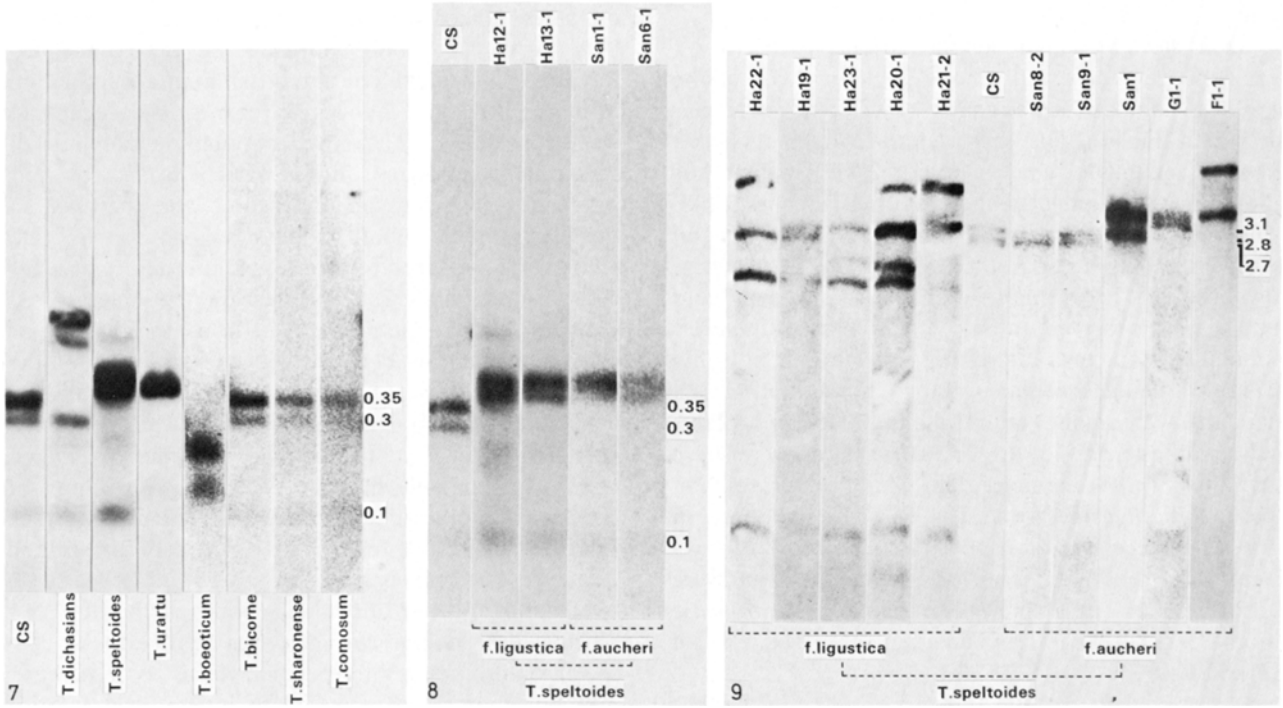


Fig. 6. Autoradiogram of a Southern analysis of DNA's from the species indicated, digested with Hae III and hybridized with the '130' probe

Triticum species. Particularly striking is the finding that the "130" bp sequences of *T. monococcum* and *T. urartu* are as differentiated from Chinese Spring as are those of the *Elytrigia* species.

Digestion of the genomic DNAs of diploid *Triticum* species with Hae III endonuclease, which cleaves wheat spacer into "130" bp repeats, and probing the digests with wheat "130" bp sequences showed that the investigated genotypes of all diploid goatgrasses have the "130" bp periodicity in their spacers (Fig. 6). In accession 3135 of *T. urartu* the Hae III site was entirely absent from the spacer and no indication of repeated organization was evident (results not shown). In tetraploid *Elytrigia scirpea* virtually all Hae III sites were absent from the "130" bp region of the spacer.

When the Hae III digests of the genomic DNAs were probed with the "750" bp wheat sequence it appeared that all species of *Sitopsis,* except for *T. speltoides,* had the same fragment length as Chinese Spring and the most common variant of *T. dicoccoides* (Fig. 7). Two of the three major fragments cleaved from the "750" bp sequences in *T. speltoides* were longer than in wheat (Fig. 8). All of the 50 plants investigated showed this pattern. The organization of the major variant in



Figs. 7–9. 7 Autoradiogram of a Southern analysis of DNA's from the species indicated after digestion with Hae III and hybridization with the '750' probe. **8** Autoradiogram of a Southern analysis of DNA's from individuals from a *T. speltoides* f. *ligustica* population (Ha) and f. *aucheri* population (San) after digestion with Hae III and hybridization with the '750' bp probe. All the individuals examined had the same pattern as the ones shown. **9** Autoradiograms of Southern analyses of DNA's isolated from individual plants from *T. speltoides* f. *ligustica* and f. *aucheri* populations, after digestion with Taq I and hybridization with the '750' bp probe

six accessions of *T. araraticum* was similar but not identical to that of Chinese Spring. Entirely different fragment patterns were observed in the remaining goatgrasses, and *T. monococcum* ssp. *boeoticum* (referred to as *T. boeoticum*) and *T. urartu* (Fig. 7). As in the ΔTm's the greatest similarity with wheat was again among the species of the section *Sitopsis*.

The genomic DNAs of seven accessions of *T. araraticum*, 42 individuals of *T. speltoides* (from five populations) and one from each remaining species of the section *Sitopsis*, seven from *T. tauschii* and one from *T. monococcum* were digested with Taq I endonuclease. This enzyme defines the major spacer fragment in *T. aestivum* and can be assayed by either the "130" or "750" bp sequences. Probing the digests with the "750" bp sequence showed that the minor 1.8 kb fragment present in Chinese Spring chromosome 5D (Appels and Dvořák 1982) was contributed by *T. tauschii* in which it is the most common length variant. The four accessions of *T. tauschii* that had this 1.8 kb fragment had also one or two minor fragments 2.3, 2.5, or 2.7 kb long. Three other major spacer variants were observed. In accessions RL 5495 and 5289, 1.8 and 1.9 kb fragments, respectively, were prominent and in RL 5257 only the 2.2 kb fragment was observed. Five of the seven DNAs of the *T. araraticum* accessions (all from Iran) had Taq I fragments 2.3 kb long, one 2.4 kb, and one 2.5 kb long.

In *T. speltoides* eight length variants of the Taq I fragment were observed among 22 individuals of the *ligustica* population designated Ha (some examples shown in Fig. 9). The most common variant was a 2.6 kb fragment to which a minor 2.4 kb variant was allelic. In the other locus the major variant was 3.1 kb long with allelic variants 3.5 and 4.4 kb long. Several other fragment lengths, 1.6 kb, 1.7 kb and 3.0 kb, were also observed. Five length variants, 2.7, 2.8, 3.1, 3.7 and 4.0 kb, were observed among 12 individuals of *aucheri* population San. It is interesting to note that not only ΔTm's of the *aucheri* plants, using Chinese Spring as a standard, were smaller than those of the *ligustica* plants but also the lengths of the Taq I fragments were more similar to those characterizing Chinese Spring than those of the *ligustica* form. The Taq I fragment length in a single accession of *T. searsii* was 2.7 kb. Two fragments occurring in an accession of *T. longissimum* were 2.6 and 2.7 kb. In a single accession of *T. sharonense* (D-61-33-1) two Taq I fragments were observed, 2.8 and 3.1 kb long.

Discussion

The understanding of chromosome evolution of the agronomically important *Triticum* species has relied almost entirely upon the analysis of pairing at metaphase I of meiosis in interspecific hybrids. The central observation that emerged from such analyses on various polyploid species of the genus *Triticum* was a markedly varying degree of differentiation between ancestral genomes of diploid species and corresponding genomes in polyploid species (Kihara 1963; Lilienfeld 1951). To explain this observation several mechanisms of chromosome differentiation were postulated. The differential introgression model (Zohary and Feldman 1962) assumed that hybridization between two polyploid species of, for example, genomic formulae AABB and AACC would allow the A genome to act as a buffer while the B and C genomes recombined. Although this hypothesis appears attractive there is no experimental evidence indicating that this process accounts for the uneven rate of genome differentiation in any of the polyploid *Triticum* species (Kihara 1963).

The differential heterochromatinization model (for a recent discussion see Dhaliwal and Johnson 1982) postulated that acquisition of extensive amounts of heterochromatin by a particular genome would result in a reduction of pairing with the original genome. The C-banding studies carried out here showed that most of the polyploid *Triticum* species were exceptions to the model, making it unlikely that heterochromatinization has a generalized effect on MI chromosome pairing.

A further possibility, that of uneven genome differentiation in polyploids by differential accumulation of major structural changes (inversions, translocations) was considered. The two parental genotypes of *T. araraticum* utilized, differed genetically in their influence on heterogenetic chromosome pairing. In the background of both genotypes good pairing with Chinese Spring occurred for A genome telosomes, except for 4AL, while all the B genome telosomes paired poorly. However the numbers of translocation and inversion differences between the chromosomes of the two species were not substantially greater in the B genome than in the A genome. Feldman (1966) obtained similar data for *T. timopheevii* ssp. *timopheevii*. Thus, it seems very unlikely that the different probabilities of pairing of the A and B genome chromosomes at MI in interspecific hybrids reflects the differential distribution of major structural changes.

Dvořák and McGuire (1981) observed that the poor pairing of B genome chromosomes relative to A genome chromosomes in interspecific hybrids observed here, and by others, also extended to intercultivar hybrids within *T. aestivum*. The authors postulated that the observations were most readily accounted for by either uneven rates of nucleotide sequence change in the two genomes or an uneven impact on chromosome pairing of similar levels of nucleotide sequence change. The C-

banding data (Fig. 2) indicates that polyploid genomes can be subjected to dramatic quantitative changes in the heterochromatin and thus, presumably, certain classes of repeated nucleotide sequences. Although in this instance the change did not correlate with the observed differential pairing of genomes it was of interest to assess whether other classes of sequences could also undergo such rapid changes and contribute to major nucleotide sequence differentiation between genomes.

The analysis of the rDNA region carried out here and in the preceding paper (Appels and Dvořák 1982) indicated: (i) high levels of polymorphism and, (ii) high rates of divergence among closely related species, in the nucleotide sequences of the spacer region. The comparison of sibling polyploid wheat species showed that they differed on average in 3% and 2% of nucleotides in the "130" bp and "750" bp regions of the spacer, respectively.

The rates at which new variation in the rDNA region, and very likely other classes of repeated nucleotide sequences, is generated appears to depend a great deal on processes other than spontaneous mutations. Data obtained in the preceding paper (Appels and Dvořák 1982) and here showed that the new variation in the rDNA region occurs by stepwise "fixation" of a mutational event within the hierarchy of arrays of repeated units.

(i) "Fixation" of a 130 bp Unit Within the Repeating Units of the Spacer Region

The evidence for this is most striking when the ΔTm's (relative to Chinese Spring) of the "130" bp sequence hybridized to *E. scirpea* and *T. uniaristatum* DNAs are compared to the Hae III digestions of DNAs of these species. The ΔTm's are 10 °C and 8.5 °C respectively (Fig. 3) and thus the species differ from *T. aestivum,* at the nucleotide sequence level, to approximately the same degree. The digestion of *T. uniaristatum* DNA with Hae III shows the same "130" bp ladder as is seen in *T. aestivum* (Fig. 6) while *E. scirpea* shows no "130" bp ladder. The all or none difference in the Hae III sites relative to a similar degree of sequence difference assayed by ΔTm suggests a "fixation" of certain sequence variants within the repeated array of "130" bp units. A similar argument can be put forward to account for the slightly larger than "130" bp periodicity of the ladder seen for *T. speltoides* (Fig. 6) – in this case a variant 5–10 bp longer appears to have been "fixed" in the spacer region.

(ii) "Fixation" of the 9 kb rDNA Repeating Unit

Concomitant with the preceding level of "fixation", a new variant can also spread throughout the 1,000–2,000, 9 kb, rDNA units found in a given rDNA region of cereal chromosomes. The evidence for this is most striking from the Taq I digests of the genomic DNA from individuals within populations of the species investigated. Extensive polymorphism for the length of the Taq I fragment (assayed by "750" bp probe) from the spacer was observed within every species investigated. In any one individual, the arrays of 9 kb units at an allelic rDNA region were usually monomorphic with respect to the length of the Taq I fragment. This very likely applies to the sequence per se of the respective spacer regions. The fact that differences in the Tm of the "130" bp probe hybridized to the rDNA spacers of different individuals of a species could be detected indicated that the arrays must be relatively homogeneous. Comparison of Hae III fragment patterns assayed using the "750" bp probe provided a further example of "fixation" at the level of the 9 kb rDNA unit. The two einkorn wheat species, *T. monococcum* ssp. *boeoticum* and *T. urartu,* are morphologically very similar and both show a ΔTm (relative to Chinese Spring) of 6 °C with the "750" bp probe and 12.5 °C with the "130" bp probe. The Hae III fragment patterns however show "fixation" of different size bands in the "750" bp region of the spacer in all units of the rDNA regions (it should be recalled that this probe assays the non-repeated part of a given spacer region).

(iii) "Communication" Between Non-allelic rDNA Regions

The organization of rDNA clusters on chromosomes 1B and 6B of *T. aestivum* is identical with respect to the location of Hae III sites in the "130" and "750" bp spacer components (Appels and Dvořák 1982). The 9 kb units on these chromosomes do show some differences in that the ones on 1B contain 2–3 "130" bp units more than the units on 6B, while at a sequence level the "130" bp unit regions of 1B have a ΔTm of 1 °C relative to the 6B regions. Evidence for "communication" between the non-allelic rDNA regions of chromosomes 1B and 6B derives from a comparison of *T. aestivum* with *T. araraticum.* Both of these species have major rDNA locations on chromosomes 1B and 6B (Appels and Dvořák 1982; Fig. 1a). Thus the two rDNA regions must have existed in the ancestor of the two wheat lineages represented by *T. aestivum* and *T. araraticum.* While the difference in Tm (using the "130" bp probe) between *T. aestivum* chromosomes 1B and 6B is 1 °C, the difference between *T. araraticum* and *T. aestivum* was 2.7 times as great. Although these differences are small they suggest the rDNA regions of chromosomes 1B and 6B are not as divergent as expected and we postulate that some form of "communication" has occurred to maintain a co-evolution of the sequences in these 1B and 6B rDNA regions.

The observations on the extent and nature of nucleotide sequence change in the rDNA region provide good precedent for expecting extensive nucleotide change within the cereal genomes in general; similar observations have been made recently in *Drosophila* (Coen et al. 1982). Nucleotide sequence change does not necessarily lead to a change in the chromosome pairing properties but at present it seems the most likely cause of genome differentiation assayed by chromosome pairing studies in interspecies crosses, as discussed by Dvořák and McGuire (1981).

Acknowledgements

The authors thank Lyndall Moran for her expert assistance in carrying out the work reported. One of us (J.D.) is grateful to Division of Plant Industry, CSIRO, for providing funds which facilitated a 9 month sabbatical in Canberra. We are grateful to Dr. W. L. Gerlach for stimulating comments on this manuscript.

Literature

Appels, R.; Dvořák, J. (1982): The wheat ribosomal DNA spacer region: its structure and variation in populations and among species. Theor. Appl. Genet. **63,** 337–348

Coen, ES.; Thoday, J.M.; Dover, G. (1982): Rate of turnover of structural variants in the rDNA gene family of *Drosophila melanogaster.* Nature **295,** 564–568

Dhaliwal, H.S.; Johnson, B.L. (1982): Diploidization and chromosome pairing affinities in the tetraploid wheats and their putative amphiploid progenitor. Theor. Appl. Genet. **61,** 117–123

Dvořák, J.; McGuire, P.E. (1981): Nonstructural chromosome differentiation among wheat cultivars, with special reference to differentiation of chromosomes in related species. Genetics **97,** 391–414

Feldman, M. (1966): Identification of unpaired chromosomes in F_1 hybrids involving *Triticum aestivum* and *T. timopheevii.* Can. J. Genet. Cytol. **8,** 144–151

Gerlach, W.L. (1977): N-banded karyotypes of wheat species. Chromosoma **62,** 49–56

Gerlach, W.L.; Bedbrook, J.R. (1979): Cloning and characterization of ribosomal DNA from wheat and barley. Nucleic Acids Res. **7,** 1896–1885

Gill, B.S.; Kimber, G. (1974): Giemsa C-banding and the evolution of wheat. Proc. Natl. Acad. Sci. USA **71,** 4086–4090

Johnson, B.L.; Dhaliwal, H.S. (1978): *Triticum urartu* and genome evolution in the tetraploid wheats. Am. J. Bot. **65,** 907–918

Kihara, H. (1940): Verwandtschaft der Aegilops-Arten im Lichte der Genomanalyse. Ein Überblick. Züchter **12,** 49–62

Kihara, H. (1963): Interspecific relationship in *Triticum* and *Aegilops.* Seiken Ziho **15,** 1–12

Lilienfeld, F.A. (1951): Kihara. Genome analysis in *Triticum* and *Aegilops.* X. Concluding remarks. Cytologia **16,** 101–123

McFadden, E.R.; Sears, E.R. (1946): The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J. Hered. **37,** 81–89

Peacock, W.J.; Gerlach, W.L.; Dennis, E.S. (1981): Molecular aspects of wheat evolution repeated DNA sequences. In: Wheat science-today and tomorrow (eds. Evans, L.T.; Peacock, W.J.). Cambridge: Cambridge University Press

Sachs, L. (1953): Chromosome behaviour of species hybrids with *Triticum timopheevi.* Heredity **7,** 49–58

Tanaka, M.; Kawahara, T.; Saro J. (1979): The evolution of wild tetraploid wheats, pp. 73–80. New Delhi: Proc. 5th Int. Wheat Genet. Symp.

Zohary, D.; Feldman, M. (1962): Hybridization between amphiploids and the evolution of polyploids in the wheat (*Aegilops – Triticum*) group. Evolution **16,** 44–61

Received June 26, 1982
Communicated by J. S. F. Barker and H. F. Linskens

Dr. J. Dvořák
Department of Agronomy and Range Science
University of California
Davis, CA 95616 (USA)

Dr. R. Appels
Division of Plant Industry CSIRO
Institute of Biological Resources
Black Mountain
P.O. Box 1600
Canberra City, ACT 2601 (Australia)