MOLECULAR ASPECTS OF WHEAT EVOLUTION: REPEATED DNA SEQUENCES

W. J. Peacock, W. L. Gerlach and E. S. Dennis

Polyploid wheats are of two basic types -- hexaploids with 21 pairs of chromosomes and tetraploids with 14 pairs of chromosomes. Cytogenetic analysis has established that the two genomes (A and B) of tetraploid wheat are different but homoeologous. Each has 7 pairs of chromosomes. These two genomes occur in the hexaploid wheats together with an additional genome (D), again of 7 pairs of chromosomes. The different genomes have become associated through hybridization events between different diploid species of grasses, the progenitors of the modern wheats.

Geneticists have been interested in identifying the diploid species which hybridized to form polyploid wheats not only because the evolution of cultivated wheats is closely associated with the social evolution of man, but also because knowledge of the progenitor species could be of importance in facilitating the introduction of genes into modern agricultural cultivars. Furthermore, the transition from diploid to tetraploid and hexaploid chromosome levels provides us with an opportunity to analyse and document changes that occur in plant genomes under the intense selection pressures associated with the development of agricultural crops.

The D genome of the hexaploid wheats is thought to have been derived from the diploid goat grass *Aegilops squarrosa*. A synthetic hexaploid formed by crossing *Ae. squarrosa* with the tetraploid wheat *Triticum dicoccoides* was morphologically indistinguishable from *T. aestivum* ssp. *spelta*, a naturally occurring hexaploid (McFadden and Sears, 1946). Meiotic behaviour in the hybrid between the synthetic and naturally occurring hexaploids was regular, indicating the equivalence of the *Ae. squarrosa* and D genomes. The origin of the other two genomes is by no means as certain. Morphological characters, isozyme variants and chromosome pairing characteristics have all been used in attempts to determine which, if any, of the diploid wheats or related grasses were the source of either the A or B genomes. The consensus is that the A genome was derived from a diploid *Triticum* species, the most favoured being the cultivated *T. monococcum* or the wild *T. boeoticum*. The identity of the B genome donor is very much an open question. Many different species have been proposed (Table 3.1) but none has all of the B genome properties, and different criteria have given conflicting indications.

Table 3.1
Species which have been proposed as donors of the B genome to tetraploid wheats and the basis for their proposition

Species	Basis of proposition	Reference
Aegilops speltoides	Spikelet morphology Chromosome pairing and karyological Esterase isozyme spectra Nuclear DNA content Nuclear-cytoplasmic interactions	Sarkar and Stebbins (1956) Riley *et al.* (1958) Jaaska (1980) Rees and Walters (1965) Suemoto (1978)
Ae. bicornis	Amphiploid with *T. monococcum* resembles tetraploid wheat	Sears (1956)
Ae. longissima	Amylase inhibitor spectra Nuclear DNA content	Vitozzi and Silano (1976) Nishikawa and Furuta (1978)
Ae. searsii	Chromosome pairing and karyological	Feldman (1978)
Triticum durartu	Seed protein electrophoretic profiles Anther morphology Spike and spikelet characters	Johnson (1975) Johnson and Dhaliwal (1978) Dhaliwal and Johnson (1976) Dhaliwal (1976)
Agropyron	Morphology	McFadden and Sears (1946)
Polyphyletic origin	Absence of a single demonstrable donor and the occurrence of introgression in polyploid plant series C-banding	Zohary and Feldman (1962) Natarajan and Sharma (1974)

It is possible that in seeking the identity of the wheat ancestors, both the methodologies and the questions have been inadequate. For example, meiotic chromosome pairing, which in many circumstances is a powerful indicator of chromosome homology, can be acutely affected by the action of modifying genetic elements (Riley and Chapman, 1958; Sears and Okamoto, 1958; Dover and Riley, 1972). Another potential problem is that the genomes as they now occur in the cultivated wheats may not reflect a single hybridization event. Additional hybridizations involving the initial amphiploids could lead to introgression and partial substitution of the original genomes (Zohary and Feldman, 1962).

We have approached the problem of the evolution of the modern wheats by looking at the molecular and chromosomal organisation of repeated DNA sequences isolated from the hexaploid genome. By examining segments of the chromosomal DNA molecules themselves, we avoid some of the uncertainties mentioned above and are able to trace particular chromosomal regions rather than genomes as a whole.

Repeated DNA sequences as markers of evolutionary events

DNA sequences which are repeated many times in the genome of hexaploid wheat can be detected in other polyploid wheats, related cereals, and in the genomes of wild diploid wheat species (Flavell *et al.*, 1977, 1979). The fact that these DNA sequences are identifiable over a wide range of species suggests that it should be possible to use the chromosomal distribution pattern of a sequence to trace the evolutionary history of chromosomes and chromosome segments.

We have isolated highly repeated sequences from hexaploid wheat and mapped their chromosomal locations (Gerlach and Peacock, 1980). The sequences were isolated as rapidly renaturing DNA, used as templates for the production of radioactive complementary RNA which was hybridized *in situ* to metaphase chromosome preparations. Autoradiographs showed that these sequences are located primarily on the seven chromosomes of the B genome, with major sites also on chromosomes 4 and 7 of the A genome. Each of these nine chromosomes has a distinctive pattern of sites and can be recognised in both tetraploid and hexaploid wheats, suggesting that these chromosomes might be recognisable in diploid wheat species. The concentration of highly repeated DNA in the B genome of hexaploid wheat also suggested that any B genome donor would be readily differentiated from A or D genome ancestors. The diagnostic patterns of sites on two chromosomes of the A genome might provide a powerful tool for identifying the A genome donor.

Analysis with a single highly repeated DNA sequence

A limitation in the use of total highly repeated DNA is that it consists of a population of different sequences so that significant changes in component sequences in different wheat species might not necessarily be disclosed by the hybridization analyses. If, on the other hand, the radioactive probe used for chromosome mapping was a single, pure, highly repeated sequence, then changes in chromosomal patterns would be readily detectable. A buoyant density satellite DNA provided such a probe. When the total nuclear DNA of Chinese Spring wheat is centrifuged in Cs_2SO_4 gradients containing Ag^+, a small proportion of the DNA is separated from the remainder of the nuclear DNA because its density is increased by preferential binding of Ag^+ (Figure 3.1). Reassociation kinetics, restriction enzyme analysis and sequencing of complementary RNA transcripts of this DNA showed it to be composed of tandem repeats of a simple sequence (Dennis *et al.*, 1980). Direct sequencing of cloned segments of this satellite confirmed this conclusion, showing the sequence to be composed of combinations of the triplets $\frac{GAA}{CTT}$ and $\frac{GAG}{CTC}$ (Figure 3.2).

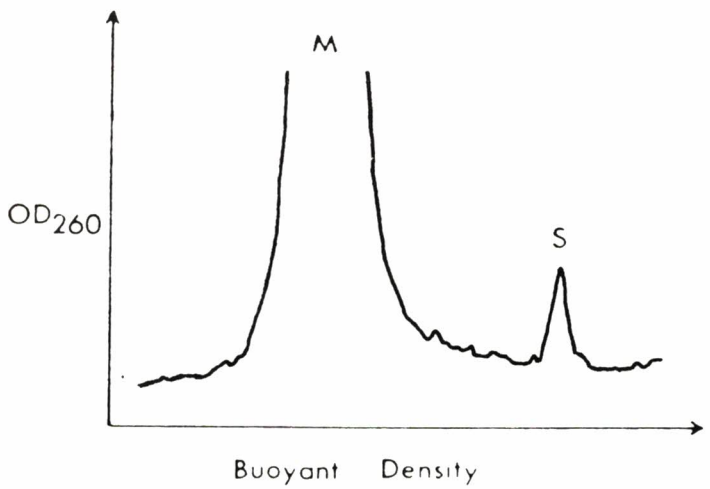

Fig. 3.1. Isolation of a satellite DNA from hexaploid wheat. Total DNA from wheat (Chinese Spring) was centrifuged to equilibrium in a Ag^+/Cs_2SO_4 gradient. A satellite DNA (S) separates from the mainband DNA (M).

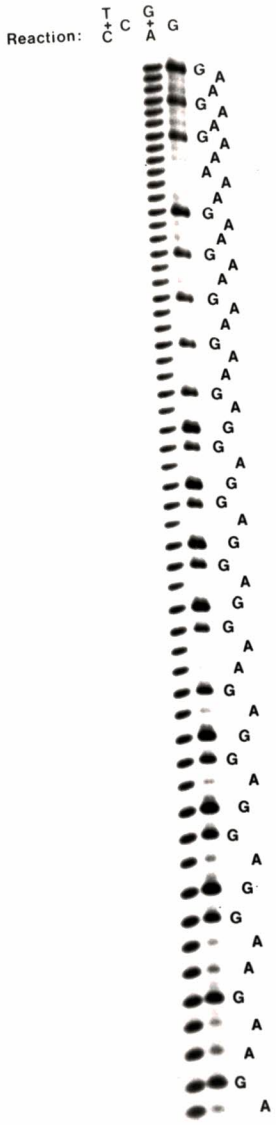

Fig. 3.2. Nucleotide sequence of a cloned example of Ag^+-satellite DNA from wheat. The base sequence was determined chemically using rapid sequencing techniques (Maxam and Gilbert, 1977). A resultant autoradiograph is shown with the deduced base sequence of the satellite DNA.

In situ hybridization with this sequence gave precisely the same pattern as did the total highly repeated DNA (Figure 3.3). All seven chromosomes of the B genome and chromosomes 4 and 7 of the A genome contained numbers of major sites, with some other chromosomes of the A and D genomes having minor sites (Figure 3.3a). The identity of each chromosome was checked by *in situ* hybridization to the ditelocentric marker chromosomes generated by Sears (1954). The major sites of tandem repeats of the triplet sequences are in centromeric, interstitial and terminal locations. This enables specific identification of many of the chromosome arms, so that even if introgression of chromosomes from different genomes or rearrangements of segments of chromosomes have occurred, we have some chance of recognising these events. The congruence of the sites of the Ag^+ satellite and total repeated DNA in hexaploid and tetraploid wheats implies that since the time of origin of hexaploid wheats there has been no change in the representation or distribution of highly repeated sequence DNA in the chromosome complements. Archaeological evidence places the time of emergence of hexaploids to be about 10 000 years ago (Harlan, Chapter 1). In addition, the primitive and cultivated tetraploids have identical patterns.

In situ hybridization has identified a distinction between the Russian (*T. timopheevi*) and Mediterranean tetraploids. The presumptive chromosome 4B of *T. timopheevi* differs from its counterpart in the Mediterranean wheats by a possible pericentric rearrangement as detected by a shift in the location of a major site of the Ag^+-satellite sequence (Gerlach *et al.*, 1978). The A genome of the Russian species contains a chromosome with a pattern that is not represented in the Mediterranean tetraploids. Historically it has been assumed that the B genome ancestors were not the same for these two groups of wheat species. Our observations suggest that this may not have been so, since the B genome patterns differ by only a single site. The A genomes show more substantial differences.

We have argued that because the basic pattern of the major sites has remained constant in the polyploid wheats, the Ag^+-satellite may provide a way of identifying the donors of both the A and B genomes among wild diploid wheats. The donor of the B genome would be expected to have these sequences accounting for approximately 3% of its DNA and to have major locations of the sequences on all chromosomes. *T. urartu* does not fulfil either of these criteria and can be definitely ruled out. In common with *T. monococcum* and *T. boeoticum*, *T. urartu* has only a small amount of the sequence and does not contain any major chromosomal locations (Figure 3.4). On the other hand, there are several *Aegilops* species which have a significant concentration of these satellite sequences on every chromosome of the complement (Figure 3.5). All species are in the Sitopsis section of the genus. *Ae. longissima* most closely resembles the B genome in its pattern of sites. Chromosomes 2B, 5B and 7B have direct

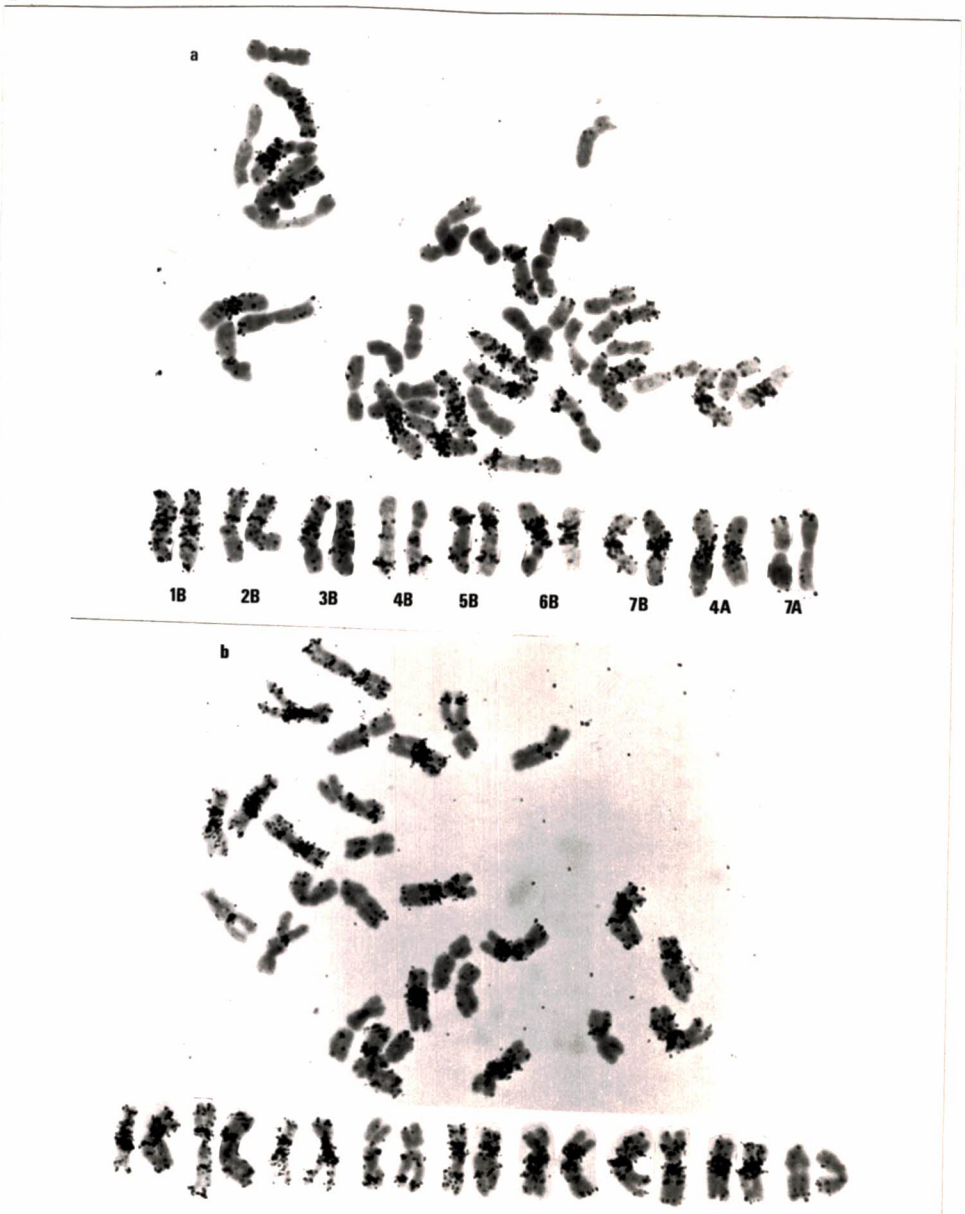

Fig. 3.3. Chromosome localisation of the Ag^{+}-satellite DNA from wheat. *In situ* hybridization of ^{3}HcRNA to metaphase chromosomes from:

(a) hexaploid *T. aestivum* cv. Chinese Spring; and

(b) tetraploid *T. dicoccoides*.

Identities of labelled chromosomes are shown.

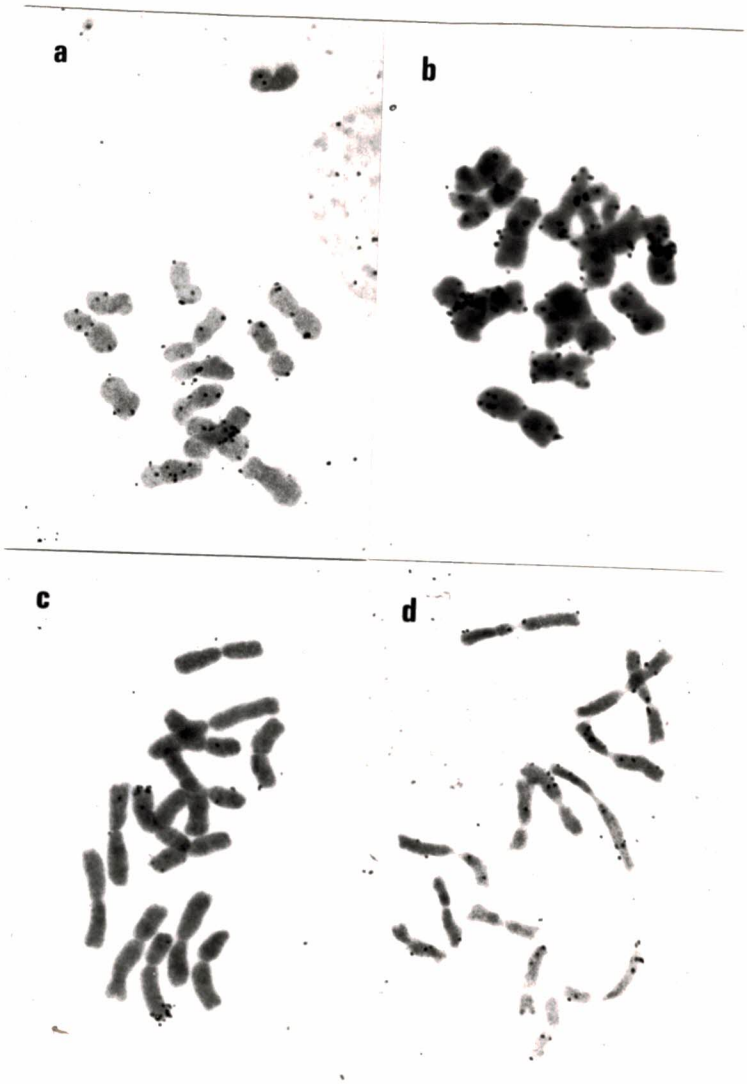

Fig. 3.4. Chromosomal location of the Ag^+-satellite DNA of wheat in the diploid species:
(a) *T. urartu*
(b) *T. monococcum*
(c) *T. boeoticum*
(d) *Ae. squarrosa*
by *in situ* hybridization to root tip metaphase chromosomes.

counterparts in *Ae. longissima* and 1B is also similar. *Ae. speltoides*, long favoured as the B genome donor (Sarkar and Stebbins, 1956; Riley *et al.*, 1958), does have major sites on all chromosomes but the pattern does not resemble that found in the polyploids. A species collected only recently, *Ae. searsii*, has been proposed as the donor of the B genome (Feldman, 1978), but its pattern of sites also fails to correspond to that of the B genome of the modern species.

None of the proposed B genome donors has a pattern of satellite sites identical to that in the polyploids. It is possible that other wild species will show greater correspondence to the B genome pattern. Since a characteristic feature of highly repeated DNA is that changes of repetition frequency can occur between species (Peacock *et al.*, 1980) it is also possible that alterations of the pattern of sites have occurred since the formation of the tetraploid wheats. The stability of pattern throughout the range of primitive and cultivated polyploids makes this unlikely. It is clear that the donor(s) of the B genome must be closely related to the species in the Sitopsis section of *Aegilops*.

The Ag^+-satellite probe also provides information relating to the origin of the A genome. One accession of *T. boeoticum* has the small telomeric block of sequences on one chromosome similar to that found on chromosome 7A of some polyploid wheats (Figure 3.4). Neither *T. boeoticum* nor any other of the diploid species contains a chromosome with the distinctive pattern of sites characteristic of chromosome 4A. Perhaps this is not surprising since other data suggest that 4A may have had a complex evolutionary history. In crosses of hexaploid wheat with *T. boeoticum*, chromosome 4A pairs with a *boeoticum* homoeologue (Chapman and Riley, 1966), but when the hexaploid wheat is crossed to *T. urartu* it is the only chromosome of the A genome which fails to pair with a chromosome from the diploid species (Chapman *et al.*, 1976; Dvorak, 1976). Furthermore, in substitution analysis chromosome 4A can be replaced by a chromosome from *Ae. sharonensis* (Miller and Chapman, pers. comm.), a species from the Sitopsis section and one of the contenders for B genome ancestry.

But, Ken Shepherd has recently crossed Chinese Spring with six other diploid wheats, including his line of T. boeoticum and he never saw 4A pairing!!!

Can the ribosomal RNA genes define the ancestry of modern wheat?

In higher organisms, the genes coding for the major ribosomal RNAs are reiterated and organised in tandem arrays. The repeating unit contains the coding sequences for the 18S and 26S RNAs together with spacer sequences. Characteristically the coding sequences are conserved but the sequences within spacer regions can show considerable diversity between species. These genes, therefore, provide another possible tool for the identification of the diploid progenitors of polyploid wheats.

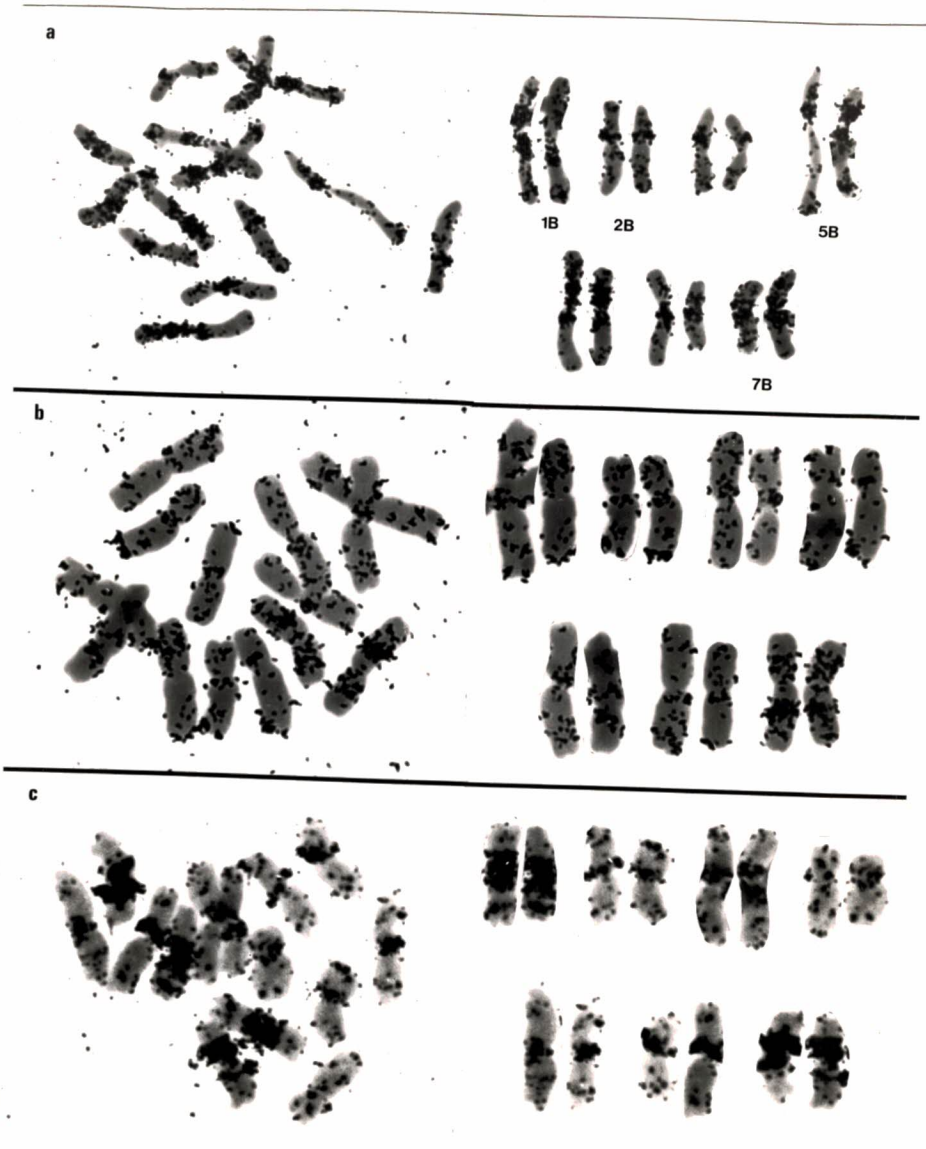

Fig. 3.5. Chromosomal localisation of the wheat Ag^+-satellite in species in the Sitopsis section of *Aegilops*. Metaphase spreads and karyotypes are shown for each species. Chromosomes of *Ae. longissima* which have patterns similar to B genome chromosomes from polyploid wheat are marked: (a) *Ae. longissima*, (b) *Ae. searsii*, and (c) *Ae. speltoides*.

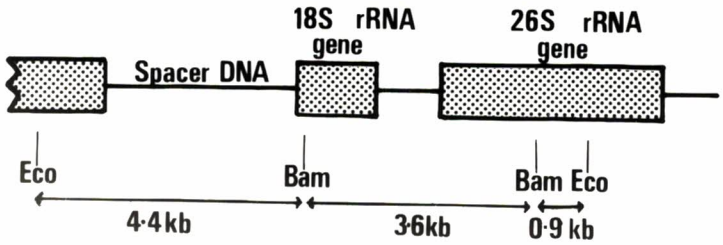

Fig. 3.6. Diagram of the ribosomal RNA gene repeating unit from wheat. Sites for the restriction enzymes BamH1 and EcoR1 are indicated. The fragment lengths obtained following simultaneous digestion with these two enzymes are shown.

In hexaploid wheat (cv. Chinese Spring) there are approximately 2300 copies of the repeating ribosomal gene unit per haploid genome (Appels *et al.*, 1980). The repeating unit is 9 kb and contains both EcoR1 and BamH1 restriction enzyme recognition sites (Figure 3.6). *In situ* hybridization has shown that in the cultivar Chinese Spring, 90% of the ribosomal RNA genes are on chromosomes 1B and 6B with the remaining repeat units being located on chromosome 5D (Figure 3.7). This is a surprising result since we would have expected ribosomal RNA genes to be present on each of the component genomes of the hexaploid. For example, all diploid wheat species being considered as A genome donors have two chromosomal sites for the ribosomal RNA genes (Gerlach *et al.*, 1980). This argues that the genome of the hexaploid wheat Chinese Spring cannot be ascribed simply to two successive hybridization events, without any changes in the chromosomes. Either some A genome chromosomes or segments of the chromosomes carrying the ribosomal genes have been lost in chromosome rearrangements or introgression, or else there has been a diminution of the total numbers of ribosomal gene repeats, to such an extent on the A genome chromosomes that they are no longer detectable. The numbers of ribosomal repeats in hexaploid, tetraploid and diploid wheat species vary, but are of a comparable order of magnitude (Mohan and Flavell, 1974; Flavell and Smith, 1974; Liang *et al.*, 1978). The B genome sites are preferentially retained in the hexaploid.

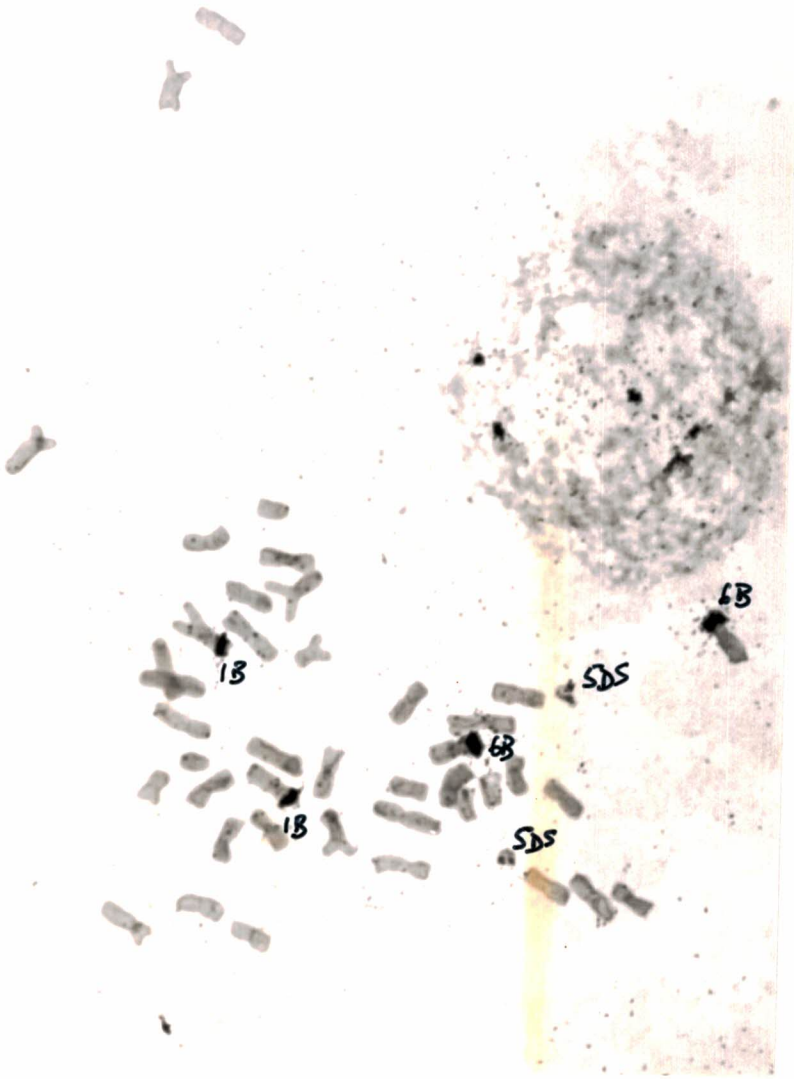

Fig. 3.7. Localisation of the ribosomal RNA genes in hexaploid wheat *T. aestivum* cv. Chinese Spring. *In situ* hybridization of ^{125}I-18S and 26S RNA to chromosomes of a 5D double ditelocentric tester stock. Chromosomes containing ribosomal RNA sites are indicated. The sites are also visible in the early prophase nucleus.

The majority of repeat units have identical restriction maps, and since they are primarily in two B chromosome sites they could provide another means of identifying the B genome ancestor. We have therefore looked at the molecular organisation of the ribosomal genes. EcoR1 and BamH1 digests of several diploid and polyploid species are shown in Figure 3.8.

In Chinese Spring, the restriction enzymes produce a characteristic pattern of molecular fragments. Two segments, 0.9 and 3.6 kb long, are derived from the 18S and 26S coding regions and a 4.4 kb segment primarily from the spacer regions (Figure 3.6). There are two other minor variants of this spacer segment, the molecules being 4.5 and 4.6 kb long respectively. Another hexaploid wheat, *Gabo*, has precisely the same coding region segments and the same major spacer segment but lacks the shorter minor spacer variant (Figure 3.8). The tetraploid wheats show a markedly different pattern for the spacer region. Two accessions of the primitive tetraploid *T. dicoccoides* have different spacer bands. Neither of these is identical to the patterns in two accessions of the cultivated tetraploid, *T. dicoccum*. Another tetraploid, *T. durum*, has a pattern identical to one of the *T. dicoccum* patterns. The spacer variability does not necessarily mean that each polyploid has had a unique origin, since localised amplification events can occur in tandem arrays of repeating sequences resulting in a particular molecular variant being expanded.

One spacer length that occurs in all polyploids, other than one accession of *T. dicoccoides*, is the 4.4 kb segment, which we know to be located on chromosome 1B and 6B in Chinese Spring (Appels *et al.*, 1980). We might expect A genome donors not to have this 4.4 kb segment and this is the case. They do contain a 4.8 kb segment comparable to that seen in the tetraploid. Only *T. monococcum* has a 4.0 kb segment. When the presumptive B genome donors are examined, no species contains the 4.4 kb band! Instead they all contain the 4.8 kb band and, in addition, each species has its own characteristic set of other fragment lengths. Another perplexing result is that the only diploid species to show a band of length 4.4 kb is *Ae. squarrosa* which is regarded as a DD diploid. *Ae. squarrosa* also contains both of the 4.8 kb and 4.0 kb bands present in most tetraploid wheats. Does this mean that the ribosomal RNA genes from *Ae. squarrosa* have become incorporated into the B genome of polyploid wheats?

5S ribosomal RNA genes as probes

The third probe we have used for investigating the phylogeny of wheats is the 5S ribosomal RNA gene. The units coding for the 5S RNA genes of Chinese Spring are of two different sizes, each organised in tandem arrays (Appels *et al.*, 1980).

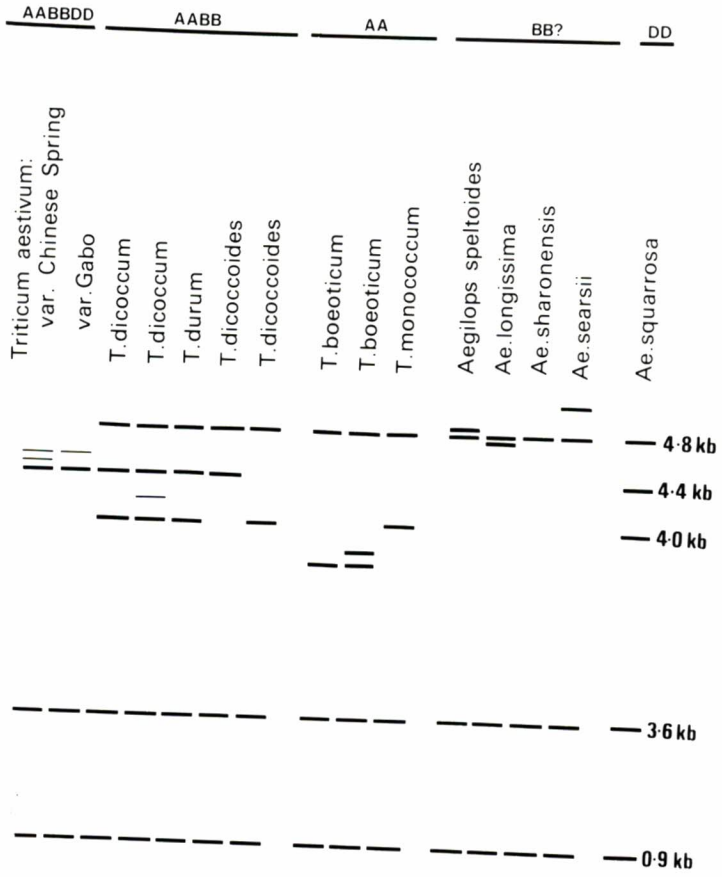

Fig. 3.8. Ribosomal RNA gene fragment lengths in a number of wheat species. DNA was digested with restriction enzymes EcoRI and BamHI, separated according to size by electrophoresis and hybridized with radioactive probe for the ribosomal RNA ~~genes~~. repeating unit. A 0.9kb fragment (not shown) is common to all species. Where a species has been listed twice different accessions have been tested.

The restriction enzyme BamH1 defines two fragments of length 420 bp and 500 bp which hybridizes radioactive 5S RNA probe. Each segment contains one 5S RNA gene, 120 bp long, and spacer DNA. The tandem arrays of the 420 bp repeating unit have a major location on chromosome 1B, so this 420 bp segment could be definitive for this particular chromosome region.

We have examined a range of wheats for the presence of the 420 bp unit (Figure 3.9). The hexaploid and tetraploid wheats are identical, containing both 500 and 420 bp length fragments. This demonstrates stability in this character since the formation of tetraploid wheats, and encouraged us to probe the diploid species. The three AA diploids examined do not contain the 420 bp segment. This is consistent with the 420 bp gene having its location in the B genome. *Ae. speltoides* has only the 500 bp repeat. Other *Aegilops* species, *Ae. longissima*, *sharonensis* and *searsii*, all contain the 420 bp repeat unit in addition to the 500 bp repeat, consistent with them being closely related to the source of the B genome. *Ae. squarrosa* contains both the 420 bp and 500 bp repeat unit.

The 420 bp repeat in the diploids could not be differentiated from the 420 bp repeat in the polyploid wheats by restriction enzyme analysis. A particular HaeIII recognition site is present in all 420 bp repeats.

Discussion

The three repeated DNA probes, the Ag^+-satellite DNA, the ribosomal RNA and 5S RNA gene repeating units have all yielded information on the relationship of the genomes of the modern polyploid wheats and the genomes of wild diploid grass species.The Ag^+-satellite has its major concentration in the B genome and we found that several *Aegilops* species had comparable contents of this highly repeated sequence, thus giving support to the view that the B genome donor might well be one of the wild diploid *Aegilops* species. The pattern of distribution of the Ag^+-satellite DNA sequences in hexaploid wheat was also consistent with the D genome being derived from *Ae. squarrosa* – neither complement of chromosomes contained any major sites of the sequence (Figures 3.3,3.4).

The diploid *Triticum* species which have been proposed as likely progenitors for the A genome also lack major sites for this sequence and differ from the polyploids' A genome. In both tetraploid and hexaploid wheats chromosome 4A has heavy concentrations of the sequence and chromosome 7A has a major site on one or both arms. By this criterion none of the diploid *Triticum* species can be identified as the participant in a hybridization event with an *Aegilops* species in the formation of tetraploid wheat. The pattern and content of the sequences in chromosome 4A are suggestive of an origin for this chromosome different from that of the remaining six chromosomes of the A genome. Chromosome 4A may have been derived by introgression from a species more closely related to the diploids of the Sitopsis section of *Aegilops*.

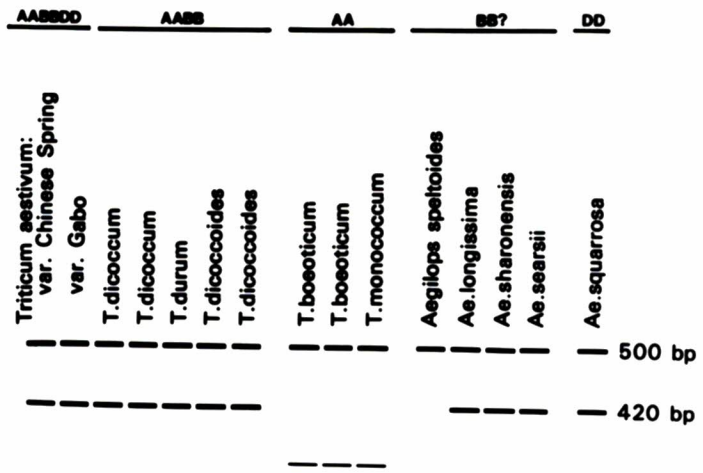

Fig. 3.9. The lengths of the 5S ribosomal RNA gene repeats in a number of hexaploid, tetraploid and diploid wheat and *Aegilops* species. DNA was digested with restriction enzyme BamH1, separated according to size by electrophoresis and hybridized with radioactive probe for the 5S RNA genes.

The Ag^+-satellite DNA was also important in demonstrating the close relatedness of the tetraploid and hexaploid wheats and supports the accepted notions of their successive formation. The patterns of distribution on the seven chromosomes of the B genome and chromosomes 4A and 7A were identical in a number of hexaploids and tetraploids we examined. There were minor differences at the tetraploid level between *T. timopheevi* and *T. dicoccum*. Another minor difference, the loss of one site on an arm of 7A, occurs within the polyploid wheats. This latter difference is explicable in terms of modulation of the number of repeats, a property well documented for highly repeated DNA (Peacock *et al.*, 1980).

The ribosomal RNA genes were also largely contained in the B genome of Chinese Spring. There must have been selective loss of the ribosomal genes from the A and D genomes. The plasticity of this particular component of the genome is also shown by the dramatic differences in their spacer DNA restriction patterns. This applies to the hexaploids and tetraploids which were so similar when examined by the Ag^+-satellite probe. Unfortunately, we lack *in situ* hybridization data for tetraploid wheats. Restriction enzyme analyses show most of them to contain DNA segments characteristic of the diploid *Triticum* species thought to be related to the A genome, whereas the hexaploids we have examined do not have any representatives of this particular segment class. Thus, tetraploid wheats should also have A genome locations for these genes.

The 5S RNA genes have one major site on chromosome 1B and the 420 bp repeat unit is restricted to this site (Appels *et al.*, 1980). The 500 bp unit length repeats occur in other sites in the genome which have not been mapped. All hexaploids and tetraploid species have these two different repeat units. So too do all of the diploid *Aegilops* species, other than *Ae. speltoides*. The proposed D genome donor, *Ae. squarrosa* has approximately equal numbers of the two units even though in the hexaploid wheat the 420 bp unit is certainly restricted to chromosome 1B and is not detectable on any of the D genome chromosomes.

Conclusions

With these three repeated DNA probes, although they represent a minor proportion of the total population of different repeated DNAs in the wheat genome, we have been able to come to some conclusions about the A, B and D genomes of wheat and place some restrictions on their potential origins. Previously suggested donors of the A genome have included *T. boeoticum* and *T. urartu*. However, the patterns we have observed with the Ag^+-satellite probe excludes these proposals in their simplest form. Either there have been events

more complex than a straightforward hybridization, or the appropriate diploid species has not yet been identified. The ribosomal RNA probe also excludes any simple equation of the diploid *Triticum* species and the A genome of the polyploid wheats. The restriction enzyme generated bands of the spacer regions in the diploid species are not present at all in some hexaploids and are only partially represented among tetraploids. The 5S RNA probe consolidates this argument since both *T. boeoticum* and *T. monococcum* have a restriction band which is not found in any of the polyploid wheats.

The Ag^+-satellite pattern has confirmed that the diploid ancestor of the B genome almost certainly is an *Aegilops* species of the Sitopsis section of that genus. None of the previously favoured diploid species has precisely the same pattern of sites as the polyploid wheats. The diploid species with greatest similarity to the polyploid B genome pattern is *Ae. longissima*. However, this species does not have the same ribosomal RNA gene organisation as any of the B genomes of the polyploid wheats. Nor, for that matter, do any of the other diploid species. The 5S gene patterns exclude *Ae. speltoides* but are consistent with the other species of the Sitopsis section being closely related to the B genome.

The D genome presents a puzzling situation. The Ag^+-satellite result is consistent with *Ae. squarrosa* as the D genome donor. The 5S RNA probe shows an identity of the *Ae. squarrosa* pattern with the B genome pattern. The 120 bp repeat, which represents approximately 50% of the 5S genes in *Ae. squarrosa*, is not detectable in the hexaploid wheat D genome. The ribosomal RNA gene probe also poses problems for the *Ae. squarrosa*-D genome equivalence, because we were not able to detect even small numbers of ribosomal genes on the D genome. Furthermore, *Ae. squarrosa* is the only diploid species which contains a ribosomal segment which is characteristic of the B genome of both tetraploid and hexaploid wheats. *Ae. squarrosa* may have had an involvement with both the B and D genomes of polyploid wheats!

References

Appels, R., Gerlach, W.L., Dennis, E.S., Swift, H. and Peacock, W.J. (1980) Molecular and chromosomal organization of DNA sequences coding for the ribosomal RNAs of cereals. *Chromosoma* 78, 293–311.

Chapman, V. and Riley, R. (1966) The allocation of the chromosomes of *Triticum aestivum* to the A and B genomes and evidence of genome structure. *Can. J. Genet. Cytol.* 8, 57–63.

Chapman, V., Miller, T.E. and Riley, R. (1976) Equivalence of the A genome of bread wheat and that of *Triticum urartu*. *Genet. Res.* 27, 69–76.

Dennis, E.S., Gerlach, W.L. and Peacock, W.J. (1980) Identical polypyrimidine-polypurine satellite DNA in wheat and barley. *Heredity* 44, 345–366.

Dhaliwal, H.S. (1976) Fertility and morphology of synthetic amphiploids and the origin of tetraploid wheats. *Cereal Res. Comm.* 4, 411–418.

Dhaliwal, H.S. and Johnson, B.L. (1976) Anther morphology and the origin of the tetraploid wheats. *Am. J. Bot.* 63, 363–368.

Dover, G.A. and Riley, R. (1972) Prevention of pairing of homoeologous meiotic chromosomes of wheat by an activity of supernumerary chromosomes of *Aegilops*. *Nature* 240, 159–161.

Dvorak, J. (1976) The relationship between the genome of *Triticum urartu* and the A and B genomes of *Triticum aestivum*. *Can. J. Genet. Cytol.* 18, 371–377.

Feldman, M. (1978) New evidence on the origin of the B genome of wheat. Proc. 5th Int. Wheat Genetics Symp., New Delhi, 1978. Vol. 1, pp. 120–132.

Flavell, R.B. and Smith, D.B. (1974) Variation in nucleolar organizer rRNA gene multiplicity in wheat and rye. *Chromosoma* 47, 327–334.

Flavell, R.B., Rimpau, J. and Smith, D.B. (1977) Repeated sequence DNA relationships in four cereal genomes. *Chromosoma* 63, 205–222.

Flavell, R.B., O'Dell, M. and Smith, D.B. (1979) Repeated sequence DNA comparisons between *Triticum* and *Aegilops* species. *Heredity* 42, 309–322.

Gerlach, W.L. and Peacock, W.J. (1980) Chromosomal locations of highly repeated DNA sequences in wheat. *Heredity* 44, 269–276.

Gerlach, W.L., Appels, R., Dennis, E.S. and Peacock, W.J. (1978) Evolution and analysis of wheat genomes using highly repeated DNA sequences. Proc. 5th Int. Wheat Genetics Symp., New Delhi, 1978, Vol. 1, pp. 81–91.

Gerlach, W.L., Miller, T.E. and Flavell, R.B. (1980) The nucleolus organizers of diploid wheat revealed by *in situ* hybridization. *Theor. Appl. Genet.* (in press).

Jaaska, V. (1980) Electrophoretic survey of seedling esterases in wheats in relation to their phylogeny. *Theor. Appl. Genet.* 56, 273–284.

Johnson, B.L. (1975) Identification of the apparent B-genome donor of wheat. *Can. J. Genet. Cytol.* 17, 21–39.

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

Liang, G.H., Wang, A.S. and Phillips, R.L. (1978) Control of ribosomal RNA gene multiplicity in wheat. *Can. J. Genet. Cytol.* 19, 425–435.

Maxam, A. and Gilbert, W. (1977) A new method for sequencing DNA. *Proc. Nat. Acad. Sci. U.S.A.* 74, 560–564. M

McFadden, E.S. and Sears, E.R. (1946) The origin of *Triticum spelta* and its free threshing hexaploid relatives. *J. Hered.* 37, 81–89, 107–116.

Mohan, J. and Flavell, R.B. (1974) Ribosomal RNA cistron multiplicity and nucleolar organizers in hexaploid wheat. *Genetics* **76**, 33–44.

Natarajan, A.T. and Sharma, N.P. (1974) Chromosome banding patterns and the origin of the B genome in wheat. *Genet. Res.* **21**, 103–8.

Nishikawa, K. and Furuta, Y. (1978) DNA content of nucleus and individual chromosomes and its evolutionary significance. Proc. 5th Int. Wheat Genetics Symp., New Delhi, 1978. Vol. **1**, pp. 133–140.

Peacock, W.J., Dennis, E.S. and Gerlach, W.L. (1980) Satellite DNA – change and stability. *Chromosomes Today* **7** (in press).

Rees, H. and Walters, M.R. (1965) Nuclear DNA and the evolution of wheat. *Heredity* **20**, 73–82.

Riley, R. and Chapman, V. (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* **182**, 713–715.

Riley, R., Unrau, J. and Chapman, V. (1958) Evidence on the origin of the B genome of wheat. *J. Heredity* **49**, 91–98.

Sarkar, P. and Stebbins, G.L. (1956) Morphological evidence concerning the origin of the B genome in wheat. *Am. J. Bot.* **42**, 297–304.

Sears, E.R. (1954) The aneuploids of common wheat. *Mo. Agric. Exp. Stn. Res. Bull.* **572**, 1–58.

Sears, E.R. (1956) The B genome of *Triticum*. *Wheat Inf. Serv.* **4**, 8–10.

Sears, E.R. and Okamoto, M. (1958) Intergenomic relationships in hexaploid wheat. *Proc. 10th Int. Congr. Genet.* **2**, 258–259.

Suemoto, H. (1978) The origin of the cytoplasm of tetraploid wheat – III. Proc. 5th Int. Wheat Genetics Symp., New Delhi, 1978. Vol. **1**, pp. 273–281.

Vitozzi, L. and Silano, V. (1976) The phylogenesis of protein α-amylase inhibitors from wheat seed and the speciation of polyploid wheat. *Theor. Appl. Genet.* **48**, 279–284.

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