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The Heterogeneity of B-Chromosome DNA: No Evidence for a B-Chromosome Specific Repetitive DNA Correlated with B-Chromosome Effects on Meiotic Pairing in the Triticinae

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Abstract. The compositional heterogeneity of DNAs of A (normal) and B (supernumerary) chromosomes of *Aegilops speltoides*, *Ae. mutica* and *Triticum aestivum* has been compared in order to elucidate the mechanism of B-chromosome disruption of meiotic pairing in interspecific hybrids. Comparisons of % heterologous association after DNA/DNA hybridation at C_0t 10^{-2} (highly repetitious DNA) and C_0t 100 (moderately repetitious DNA), and comparisons of nucleotide base divergence (ΔT_ms) and thermal elution profiles of homologous and heterologous duplexes, show that genotypes of *Aegilops* spp., having large numbers of Bs, do not carry additional families of repetitious DNA exclusive to B-chromosomes. Neither the presence of Bs nor the direction of DNA/DNA hybridisation affect the above parameters. No cryptic DNA satellites were revealed in A- and B-chromosome DNA after sedimentation in actinomycin D-CsCl gradients; and there were no significant differences in buoyant densities of main-band DNA. Mean melting temperatures (T_m); transition temperatures (ΔT) and numbers and positions of peaks of dissociating DNA fractions in profiles of differentiated melting curves of native DNAs were similar in strictly comparable denaturation conditions. One small AT-rich ($< 5\%$) DNA fraction correlated with *speltoides* Bs was revealed; however, no corresponding fraction is associated with *mutica* Bs. The overall similarity in numbers and base composition of families of DNA (repetitious and unique) of As and Bs is discussed in relation to the origin of Bs and the origin of the meiotic diploidising system in haploid *T. aestivum*.

Introduction

The genetics and interactions of B (supernumerary) chromosomes with A-chromosome loci that control the level of meiotic chromosome pairing in hexaploid wheat and its diploid relatives, are complex. F_1 hybrids between wheat (*Triticum aestivum*) and either of the two diploids, *Aegilops speltoides* and *Aegilops mutica*, fall into four classes with regard to the degree of meiotic chromosome pairing (Dover and Riley, 1972a) and the presence of B-chromosomes in some hybrids, introduced *via* the diploid parents, does not effectively alter the mean level of pairing in each class (Vardi and Dover, 1972). However, removal of wheat A-chromosome no. 5B in F_1 hybrids allows for the full pairing potential of all chromosomes to be expressed and all hybrids exhibit very high levels of pairing (Dover and Riley 1972b; Dover, 1973). This is explained by the absence of the restriction of pairing to strictly homologous chromosomes normally effected by a locus (*ph*) on the long-arm of chromosome no. 5B (for review see Riley and Law 1965). Hybrids that contain B-chromosomes and also lack chromosome no. 5B do not exhibit high levels of pairing but appear almost achiasmate (Dover and Riley, 1972b).

Functionally, the B-chromosomes are able to compensate for the absence of wheat chromosome no 5B. No other loci, on the A-chromosomes of *Aegilops* spp. that affect meiotic pairing in interspecific hybrids, are able to perform a similar compensatory role.

Similar restrictions of pairing to homologues only in interspecific hybrids of *Lolium* spp. in the presence of B-chromosomes, have been described by Evans and Macefield (1972).

The B-chromosome "diploidising" effects, to quote Rees (1974) "are of obvious, indeed profound significance in promoting regular and effective chromosome pairing" in polyploids. However, the origin of B-chromosomes in general and the origin of the major "diploidising" *ph* locus on chromosome 5B in wheat, which played a critical role in the fertility and successful evolution of the hexaploid (Riley, 1965), are still elusive. Data are presented below, characterising the nature and molecular heterogeneity of the DNA of B-chromosome carrying genotypes in *Aegilops* spp. The light thrown on the origin of Bs and 5B in this species group is discussed.

Materials and Methods

Genotypes. Triticum aestivum var. Chinese Spring, (2n=6x=42). *Aegilops speltoides* ± B-chromosomes (2n=14+Bs). *Aegilops mutica* ± B-chromosomes (2n=14+Bs). *Triticum aestivum* tetrasomic for chromosome no. 5B.

Plants were grown singly in continuous light at 20° C. The number of B-chromosomes in each plant was determined by cytological examination at first meiotic metaphase. Fig. 1 (p. 157) depicts a squash, Feulgen-stained, preparation of a pollen mother cell of *Ae. speltoides* with 6 B-chromosomes inducing irregular meiotic pairing of A-chromosomes. DNA was extracted from individual B-chromosome containing plants; and either from single or populations of plants of genotypes not containing B-chromosomes.

Isolation of DNA. Total DNA was extracted using a method based on Bolton *et al.* (1965) with the inclusion of 0.2% diethylpyrocarbonate and without the use of phenol for deproteinisation. This modification was introduced to avoid preferential extraction of d(A-T)n repetitive DNA by phenol (Morgan and Wells, 1968).

Leaf and stem tissue was crushed in liquid N_2, ground to a powder in a coffee grinder, and ground further, in a mortar in an equal volume of 0.33M NaCl, 0.1M EDTA, 1% sodium lauryl sulphate, 0.2% diethyl pyrocarbonate and acid washed sand. The mixture was shaken in 2 volumes of chloroform containing 1% 2-octanol and centrifuged for 5 minutes at 7,500 × g. The aqueous phase was heated to 70° C for 5 minutes; cooled in ice and deproteinised with chloroform/2-octanol after addition of 1M sodium perchlorate. DNA was spooled from the aqueous phase with 2 volumes of ethanol and dissolved in 1 × SSC, (0.15M NaCl+0.015M Na-citrate). The solution was treated with α-amylase (100 μg/ml), ribonuclease T1 (50 λ/100 ml), pancreatic ribonuclease (200 μg/ml), and protease (500 μg/ml). After treatment with enzymes the solution was deproteinised three times with chloroform 2-octanol (24:1) and dialysed against 0.12M phosphate buffer (PB) at pH 6.8. DNA was collected by centrifugation at 115,000 × g for 16 hours. The pellet was dissolved in 0.12M PB or 0.1 × SSC and sheared to a mean size of 300 base pairs with sonication. Fragment sizes were determined by velocity sedimentation in 1M NaCl in a Centriscan 75 analytical centrifuge, using the method of Studier (1965). DNA purity was checked by 260 mm:280 nm absorbance (2:1); hyperchromicity (33% –38%); retention on hydroxylapatite (HAP) in 0.12M PB at 60° C (> 95%) and absence of bands after sedimentation in neutral CsCl gradients using Schlieren optics.

Iodination of DNA with ^{125}I. Total DNA was iodinated as described by Mizuno and Macgregor (1974). A reaction mixture of 100 μg of heat-denatured DNA, 3mCi of ^{125}I (Radiochemical Centre, > 14Ci/mg 5mCi/50 μl of dilute NaOH, adjusted to 6×10^{-5}M Na_2SO_3 and 0.26N H_2SO_4), 4.5×10^{-4}M KI, 2.25×10^{-3}M $TlCl_2 \cdot 4H_2O$ (in 0.30MN a acetate–0.12M acetic acid, pH 5.0) was incubated for 50 minutes at 70° C. Reaction was terminated with 0.4N NaOH and

10^{-2}M Na_2SO_3 and incubated at 60° C for 20 minutes. Unattached iodine was removed on a 1.0×36 cm Sephadex G-50 (medium grade) column. The DNA-^{125}I fraction was eluted with 0.08M PB and bound to hydroxylapatite washed with 0.08M PB. The bound DNA was released with 0.5M PB, dialysed against water and radioactivity determined by liquid scintillation counting on the ^{3}H-channel using a toluene-triton X-100 based scintillation cocktail.

DNA/DNA Hybridization and Thermal Elution from Hydroxylapatite. Sheared unlabelled DNA and sheared ^{125}I-labelled DNA were mixed in 0.12M PB at a ratio of 3,000:1 respectively. The mixture was heat-denatured, cooled quickly in ice to 60° C and incubated at 60° C (T_m − 25° C) for periods of time to allow for renaturation of either highly repetitive (C_0t 10^{-2}, moles $l^{-1} \cdot sec^{-1}$) or highly repetitive plus moderately repetitive DNA (C_0t 100, moles $l^{-1} \cdot sec^{-1}$) only. Incubation times were calculated from reassociation rates published for *T. aestivum* (Bendich and McCarthy, 1970; Smith and Flavell, 1975) and *Aegilops* spp. (Dover, unpublished). After incubation, the DNA mixture was fractionated into single and double stranded DNA by passage through a waterjacketed column of 0.5 g HAP in 0.12M PB at 60° C. Single-stranded DNA was eluted with 3×2 ml washes of 0.12M PB. Homologous and heterologous duplexes of bound DNA were denatured by raising the column temperature, at 2° C intervals, from 60° C to 96° C. Denatured single-stranded DNA was eluted with 3×2 ml 0.12M PB at each temperature. Optical density and radioactivity of each eluant were determined. Thermal stability and thermal elution profiles are given below for labelled (heteroduplex) and unlabelled (homoduplex) DNA from mixtures initially incubated to C_0t 100. Mean percentage heteroduplex formation was calculated from separate experiments at which the relative percentages of bound labelled and unlabelled DNAs were determined at 60° C and 96° C only. This effectively eliminates overestimation of optical density and radioactivity of bound DNA due to accumulation of high background effects when calculated from separate 2° C intervals. At 96° C the DNA was eluted with 0.5M PB and the optical densities corrected for hypochromicity and background U.V. absorbance of the buffer and HAP particles. A check on the efficiency of renaturation at the chosen stringency of conditions was obtained by further melting and incubation to C_0t 100 of the single-stranded DNA not binding to HAP at the first incubation. 95%–97% of the unlabelled DNA (O.D.) and 93%–95% of the labelled DNA (radioactivity) eluted as single-stranded DNA after the second incubation in selected hybridisations.

Step-wise thermal elution of HAP-bound DNA of both homologous and heterologous duplexes after incubation of mixtures to C_0t 10^{-2} was not possible due to the low total amounts of highly repetitive DNA retained at this C_0t. Duplexes were denatured and eluted at 96° C with 0.5M PB and optical densities of the eluants corrected for hypochromicity and background absorbance as described above.

Isopycnic Centrifugation in Neutral CsCl Gradients Before and After Binding with Actinomycin-D. 10 μg native DNA plus 0.5 μg of marker DNA, *Pseudomonas aeruginosa* (buoyant density 1.726 g cm^{-3}) in 10 mM Tris-HCl, pH 7.5, brought to an initial density of 1.700 g cm^{-3} with CsCl, was centrifuged at 44,000 r.p.m. for 20 hours at 25° C in an M.S.E. Centriscan analytical ultracentrifuge. Tracings of the U.V. absorbance patterns at equilibrium were recorded directly. Buoyant densities were calculated relative to the marker according to Mandel *et al.* (1968).

Similar samples of DNA in 10 mM Tris-HCl pH 7.5 and 1 mM EDTA were mixed with actinomycin-D in a ratio of 1:2 (DNA: actinomycin-D) (Peacock *et al.*, 1973). CsCl was added to an initial density of 1.66 g cm^{-3} and the mixture spun at 44,000 r.p.m. for 20 hours at 25° C. The efficiency of the method was verified using total DNA of 20 hour old eggs of *Drosophila melanogaster* which was successfully fractionated into main-band and satellites. All U.V. absorbance bands at equilibrium were checked for protein and polysaccharide contamination using Schlieren optics.

Thermal Denaturation of Native DNA: Separation of Genotypic and Environmental Variation. Native sheared DNA samples of average size 300 base pairs were melted in 0.1×SSC or 0.12M PB in an electrically heated block at a constant rise in temperature of 0.5° C min^{-1}. Experiments were designed to minimise environmental fluctuations between successive melts. Three samples of DNA of a genotype were melted simultaneously and changes in O.D. (hyperchromicity) and temperature were monitored continuously. Thermal stability profiles of the mean change in O.D. and standard errors as a percentage of the total change were plotted at 0.5° C intervals. Differentiation of these profiles, giving the mean percentage change and

standard errors in O.D. between consecutive temperatures were derived and plotted by computer, according to the relation $(A_{(t1)}-A_{(t2)})/(A_{(tn)}-A_{(t1)})\times 100$. $A_{(t1)\,(t2)\cdots(tn)}$ are the absorbance values measured at temperatures t_1 and t_2 etc. The temperature interval t_1-t_2 was 0.5° C. Differences between genotypes in melting characteristics could be confounded with environmental differences between melts. To eliminate this possibility samples of each genotype were also melted simultaneously in three or more consecutive experiments and mean derived curves and standard errors were plotted for each genotype. This method allowed for the separation of experimental and genotype variation. Consistent differences between samples melted in this way could therefore be assigned to genotypic differences.

Results

It is not possible to extract the DNA of B-chromosomes in isolation. DNA was extracted from single plants with a determined number of B-chromosomes in addition to the normal complement of A-chromosomes. The amount of nuclear DNA in *Ae. speltoides* and *Ae. mutica* has been measured as 12.14 pg and 13.14 pg respectively at the 2C level (M. D. Bennett; personal communication). Each B-chromosome, just over half the size of an A-chromosome, represents the addition of an extra 5% of DNA. An increase of 30% in the DNA of individuals with 6 Bs, (Fig. 1) would be detected in experiments aimed at characterising the molecular heterogeneity of the DNA, if the B-DNA represented a separate molecular species. B-chromosomes accumulate rapidly within one or two generations (Vardi and Dover, 1972) and their pairing behaviour at meiosis supports the assumption that they are identical, having a common origin. On this basis, a graded difference in DNA type in a range of plants having 0 to 6 Bs could safely be ascribed to the Bs themselves. Low numbers of Bs do not disrupt the regular A-chromosome meiosis (Vardi and Dover, 1972); however, the presence of 6 Bs affects meiosis in the diploids themselves (Fig. 1). The 2C DNA content per nucleus of *T. aestivum* is 36.2 pg (Bennett, 1971).

DNA/DNA Hybridisation

The mean percentage and standard errors of the degree of reassociation of excess sheared unlabelled DNA of *T. aestivum* and shared labelled DNA of *Ae. speltoides* ± Bs, incubated to C_0t 10^{-2} and C_0t 100 are given in Tables 1 and 2. A concentration ratio of 3,000:1 of unlabelled: labelled DNA effectively ensures that at a given C_0t, reassociation of labelled molecules is on the basis of the number and type of shared sequences between the labelled and unlabelled molecules (Rice, 1972); the fidelity of base matching depending on the stringency of the incubation conditions (Ullman and McCarthy, 1973). At C_0t 10^{-2} about 10% of the total genome, representing the highly repetitive sequences and the "zero-time binding fraction" (Davidson *et al.*, 1973; Smith and Flavell, 1975) were retained on HAP. At C_0t 100 HAP-bound DNA accounted for 80% of the genomes of all genotypes (Tables 1 and 3) consisting of highly repetitive and moderately repetitive DNA families within *T. aestivum* (Smith and Flavell, 1975) and *Aegilops* spp. (Dover, unpublished).

The percentage heteroduplex formation of excess of *T. aestivum* × *Ae. speltoides* ± Bs was not significantly altered with the addition of 30% B-DNA at either C_0t value. At C_0t 100 (Table 1) the level of heterologous association was as high as

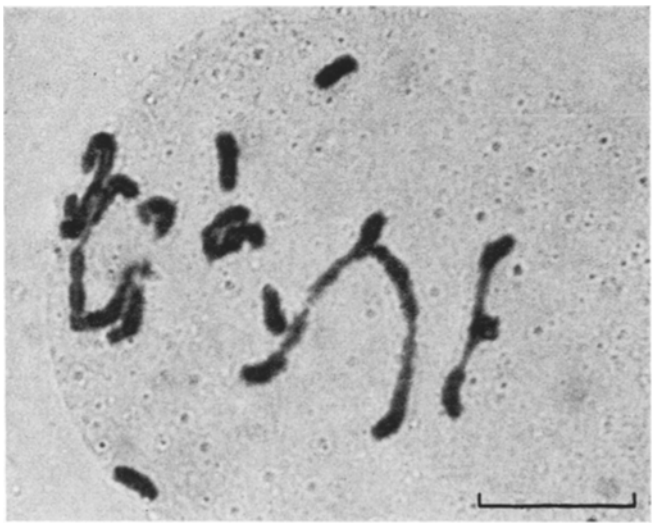

Fig. 1. Pollen mother cell of *Aegilops speltoides* (2n=14+6 B-chromosomes) at first meiotic metaphase: 4 univalents (including 3 Bs); 5 bivalents; 2 trivalents (including one B-chromosome trivalent). Bar=10 μm

Table 1. Percentage heteroduplex formation between repeated nucleotide sequences of *T. aestivum* DNA and *Ae. speltoides* DNA with or without B-chromosomes, incubated to C_0t 100 moles l^{-1} sec^{-1}. (Figures in brackets represent homoduplexes of unlabelled DNA.) Standard errors are indicated. Excess unlabelled DNA and ^{125}I-labelled DNA were sonicated, heat-denatured and incubated together to C_0t 100 at 60° C. Percentage heteroduplex formation was determined by number of counts retained on hydroxylapatite at 60° C in 0.12 M phosphate buffer. Percentage homoduplex formation was determined by optical density of HAP retained DNA

C_0t 100	^{125}I-labelled DNA		
Unlabelled DNA	*T. aestivum*	*Ae. speltoides*/0B	*Ae. speltoides*/6B
T. aestivum	62.6 ± 1.2 (79.1 ± 0.1)	65.9 ± 0.5 (80.4 ± 0.9)	66.3 ± 0.8 (82.3 ± 0.4)
Ae. speltoides/0B	68.7 ± 0.1 (78.1 ± 0.1)	66.6 ± 4.3 (77.5 ± 0.9)	65.5 ± 3.7 (76.3 ± 0.7)
Ae. speltoides/6B	71.59 (80.35)	68.98 (79.78)	

homologous association of excess *T. aestivum* × labelled *T. aestivum* and excess *Ae. speltoides* ± Bs × labelled *Ae. speltoides* ± Bs. At C_0t 10^{-2} heterologous association was just over half that of the relative homologous association (Table 2).

Thermal elution profiles (Fig. 3a and b) show that dissociation of homoduplexes and heteroduplexes occurred over a wide range of temperatures; individual peaks indicate several species of DNA melting at optimum temperatures

Table 2. Percentage heteroduplex formation between repeated nucleotide sequences of *T. aestivum* and *Ae. speltoides* DNA with or without B-chromosomes and between *T. aestivum* and *Ae. mutica* DNA, with or without B-chromosomes, incubated to C_0t 10^{-2} moles l^{-1} sec^{-1}. (Figures in brackets represent homoduplexes of unlabelled DNA.) Standard errors are indicated. Excess unlabelled DNA and ^{125}I-labelled DNA were sonicated, heat-denatured and incubated together to C_0t 10^{-2} at 60° C. Percentage heteroduplex formation was determined by number of counts retained on hydroxylapatite at 60° C in 0.12 M phosphate buffer. Percentage homoduplex formation determined by optical density of HAP retained DNA

C_0t 10^{-2}	^{125}I-labelled DNA				
Unlabelled DNA	*T. eastivum*	*Ae. speltoides*/0B	*Ae. speltoides*/6B	*Ae. mutica*/0B	*Ae. mutica*/4B
T. aestivum	5.5±0.6 11.5±0.6)	2.9±0.2 (11.3±0.6)	2.2±1.1 (10.5±0.3)	1.87 (8.90)	0.9±0.6 (9.7±1.1)
Ae. speltoides/0B		4.0 (16.6)	3.8 (15.7)		

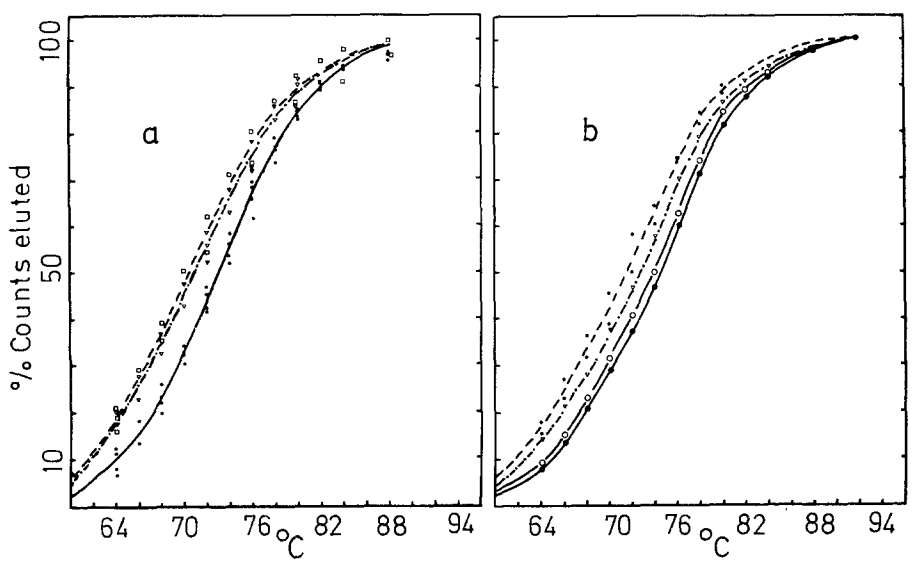

Fig. 2a and b. Thermal stability profiles of homologous and heterologous repeated sequence DNA duplexes of *T. aestivum* and *Ae. speltoides*±B-chromosomes. A mixture of excess sheared denatured DNA and sheared denatured ^{125}I-labelled DNA (3,000:1) was incubated at 60°C in 0.12M PB to C_0t 100. Duplexes formed were adsorbed on hydroxylapatite and eluted with 0.12M PB at 2°C intervals from 60°C to 96°C. Mean curves of several replicate experiments are drawn where appropriate. (a) •—• homoduplex of *T. aestivum*×*T. aestivum*, ▵-----▵ heteroduplex of *T. aestivum*×^{125}I-*Ae. speltoides*/0B, ▫---▫ heteroduplex of *T. aestivum* ×^{125}I-*Ae. speltoides*/6B. (b) •—• homoduplex of *Ae. speltoides*/4B×*Ae. speltoides*/4B, ○—○ homoduplex of *Ae. speltoides*/0B×*Ae. speltoides*/0B, ▵-----▵ heteroduplex of *Ae. speltoides*/ 4B×^{125}I-*T. aestivum*, •---• heteroduplex of *Ae. speltoides*/0B×^{125}I-*T. aestivum*

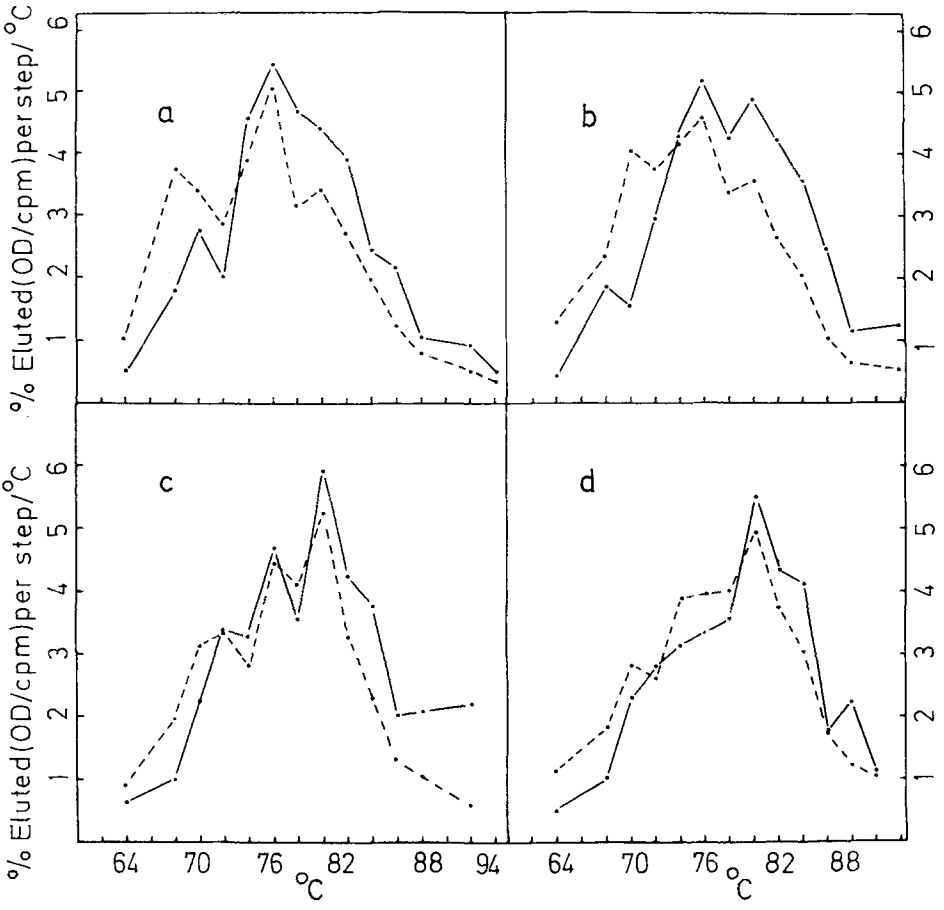

Fig. 3a—d. Thermal elution profiles of homologous and heterologous repeated sequence DNA duplexes retained on hydroxylapatite at 60°C in 0.12M PB. Conditions of incubation and elution are given in legend to Fig. 2. (a) •—• homoduplex *T. aestivum* × *T. aestivum*, •---• heteroduplex *T. aestivum* × ^{125}I-*Ae. speltoides*/0B. (b) •—• homoduplex *T. aestivum* × *T. aestivum* •---• heteroduplex *T. aestivum* × ^{125}I-*Ae. speltoides*/4B. (c) •—• homoduplex *Ae. speltoides*/0B × *Ae. speltoides*/0B, •---• heteroduplex *Ae. speltoides*/0B × ^{125}I-*T. aestivum*. (d) •—• homoduplex *Ae. speltoides*/4B × *Ae. speltoides*/4B, •---• heteroduplex *Ae. speltoides*/4B × ^{125}I-*T. aestivum*

that were determined by base composition and degree of base mismatching. The number and positions of peaks remained constant for the dissociation of homoduplexes and heteroduplexes in any one experiment irrespective of the presence of B-DNA of *Ae. speltoides*.

Mean thermal stability profiles (Fig. 2a) indicate a fall in melting temperature (ΔT_m) (Table 4) between homoduplexes and heteroduplexes; however, the magnitude of this ΔT_m was not significantly affected by the presence of B-DNA.

Table 3. Percentage heteroduplex formation between repeated nucleotide sequences of *T. aestivum* DNA and *Ae. mutica* DNA with or without B-chromosomes, incubated to C_0t 100 moles l^{-1} sec^{-1}. (Figures in brackets represent homoduplexes of unlabelled DNA.) Standard errors are indicated. For experimental conditions see legend to Table 1

C_0t 100	^{125}I-labelled DNA		
Unlabelled DNA	*T. aestivum*	*Ae. mutica*/0B	*Ae. mutica*/4B
T. aestivum	62.6±1.2 (79.1±0.1)	63.2±1.4 (77.9±0.3)	14.2±1.2[a] (78.6±0.1)
Ae. mutica/0B		58.3 (77.4)	18.2[a] (67.2)
Ae. mutica/4B			23.5±0.4[a] (75.5±1.1)

[a] See text for discussion.

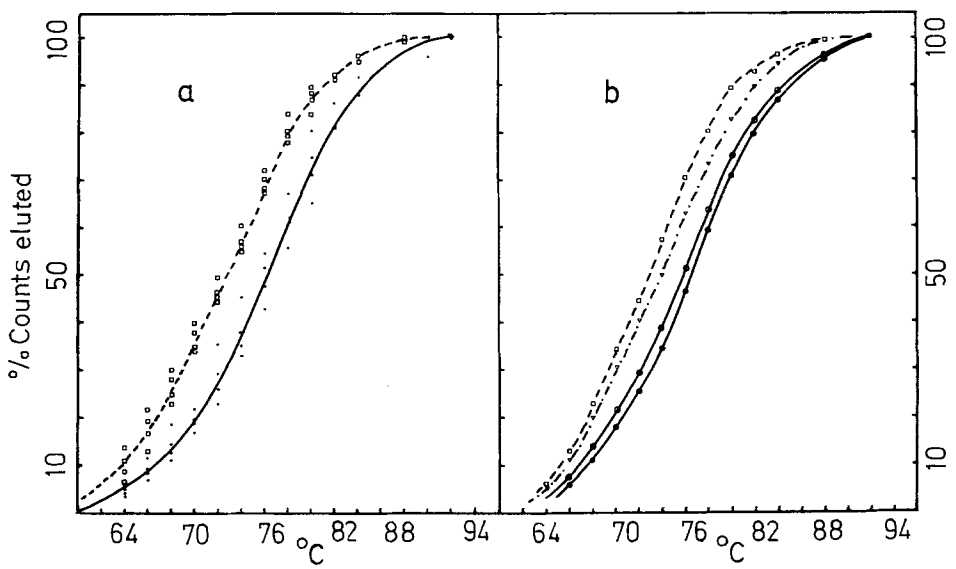

Fig. 4a and b. Thermal stability profiles of homologous and heterologous repeated sequence DNA duplexes of *T. aestivum*×*Ae. mutica* and *T. aestivum*×*Ae. speltoides*. In (b) mixtures were melted, incubated and simultaneously eluted from hydroxylapatite under similar experimental conditions, (see text). Conditions of incubation and elution are given in legend to Fig. 2. (a) •—• homoduplex of *T. aestivum*×*T. aestivum*, ▫---▫ heteroduplex of *T. aestivum*×^{125}I-*Ae. mutica*/0B. (b) •—• homoduplex of *T. aestivum*×*T. aestivum* (from incubation mixture of *T. aestivum* ×^{125}I-*Ae. speltoides*/0B), ○—○ homoduplex of *T. aestivum*×*T. aestivum* (from incubation mixture of *T. aestivum*×^{125}I-*Ae. mutica*/0B), ▵----▵ heteroduplex of *T. aestivum*×^{125}I-*Ae. speltoides*/0B, ▫---▫ heteroduplex of *T. aestivum*×^{125}I-*Ae. mutica*/0B

Table 4. Mean differences and standard errors between thermal stabilities (ΔT_m) of hetero- and homoduplexes of labelled and unlabelled repeated sequence DNA incubated to C_0t 100, of *T. aestivum*, *Ae. speltoides* ± B-chromosomes and *Ae. mutica* ± B-chromosomes. For experimental conditions see legend to Fig. 2. Selected comparisons are taken from the literature

Source of unlabelled DNA	Source of labelled DNA	Mean ΔT_m° C	Mean % nucleotide mismatching[a]	References
T. aestivum	*Ae. speltoides*/0B	2.1±0.7[b]	1.31±0.44	
T. aestivum	*Ae. speltoides*/6B	2.5±0.5	1.56±0.31	
T. aestivum	*Ae. mutica*/0B	3.61±0.11[b]	2.26±0.07	
Ae. speltoides/0B	*T. aestivum*	2.5±0.5	1.56±0.31	
Ae. speltoides/4B	*T. aestivum*	1.9	1.19	
T. monococcum	*T. aestivum*	1.7	1.06	Bendich and McCarthy (1970)
T. monococcum	*Ae. squarrosa*	2.2	1.37	Bendich and McCarthy (1970)
T. aestivum	*Secale cereale*	6	3.75	Smith and Flavell (1974)
S. cereale	*T. aestivum*	7	4.37	Smith and Flavell (1974)

[a] Calculated as 1.6° C $\Delta T_m = 1\%$ nucleotide mismatching (Ullman and McCarthy, 1973).
[b] Significantly different at $p = 0.05$.

Reciprocal hybridization, *i.e.*, excess *Ae. speltoides*±Bs × labelled *T. aestivum* revealed similar percentage heteroduplex formation at C_0t 100 (Table 1), also irrespective of the presence of B-DNA. The homoduplexes and heteroduplexes eluted over the same temperature range and at the same temperature optima (Fig. 3c and d); and the ΔT_m between duplexes is of the same magnitude as the previous direction of hybridisation (Table 4) and is similarly not affected by the presence of B-DNA (Fig. 2b).

In conclusion the % heteroduplex formation at C_0t 100 (under given conditions of stringency), the stability of the duplexes and the thermal dissociation profiles of the duplexes are not affected by the direction of the hybridisation nor by the presence of B-chromosome DNA of *Ae. speltoides*.

At C_0t 10^{-2} the % heteroduplex formation of excess *T. aestivum* × labelled *Ae. speltoides*±Bs was just over half that found in excess *T. aestivum* × labelled *T. aestivum* at the same C_0t and about two-thirds that found in excess *Ae. speltoides*/0B × labelled *Ae. speltoides*±Bs, (Table 2). However, the % heteroduplex formation is not affected by the presence of B-DNA at this C_0t.

Results obtained from DNA/DNA hybridisations using DNA of *Ae. mutica*± Bs are shown in Tables 2 and 3 and Figs. 4 and 5. There was a relatively large difference in the level of heterologous reassociation at C_0t 100 between excess *T. aestivum* × labelled *Ae. mutica*/0B (63%) and excess *T. aestivum* × *Ae. mutica*/4B

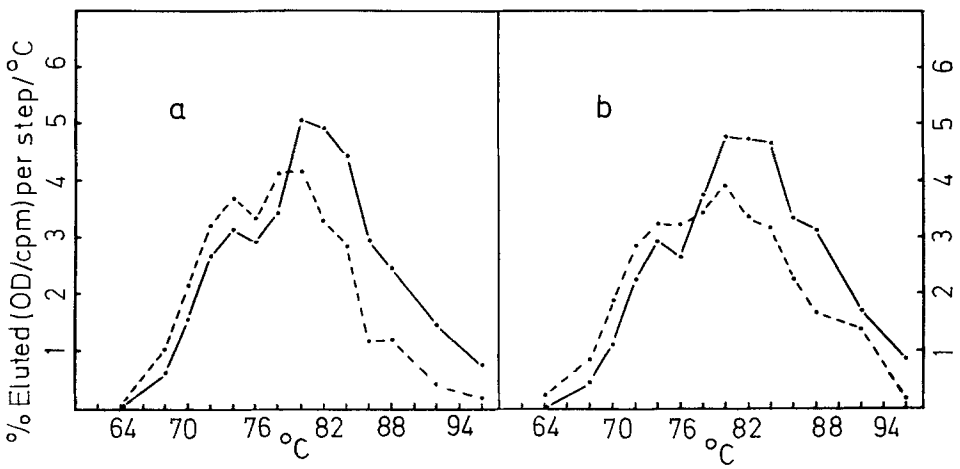

Fig. 5a and b. Thermal elution profiles of homologous and heterologous repeated sequence DNA duplexes retained on hydroxylapatite at 60° C in 0.12M PB. Species of DNA, conditions of incubation and elution are given in legends to Figs. 2 and 4(b). (a) •—• homoduplex of *T. aestivum* × *T. aestivum*, •---• heteroduplex of *T. aestivum* × ^{125}I-*Ae. mutica*/0B. (b) •—• homoduplex of *T. aestivum* × *T. aestivum*, •---• heteroduplex of *T. aestivum* × ^{125}I-*Ae. speltoides*/0B

(14%). However, the low level of homologous association of labelled *Ae. mutica*/4B to excess *Ae. mutica*/4B (28%) and to excess *Ae. mutica*/0B (18%) calls for caution in the interpretation of these results. It is possible that not all the free ^{125}iodine was successfully removed during iodination and this has caused an overestimation of the amount of labelled DNA eluting at 60° C from HAP in 0.12M PB.

Elution profiles of the thermal dissociation of homologous and heterologous duplexes from hybridization to C_0t 100 of excess *T. aestivum* × labelled. *Ae. mutica*/0B (Fig. 5a) show dissociation occurring over a similar range of temperature and at similar temperature optima. The thermal stabilities of these duplexes (Fig. 4a) indicate a mean ΔT_m of 3.61 ± 0.11° C (Table 4). This is significantly different (at $p=0.05$) from the mean ΔT_m of 2.1 ± 0.7° C between homologous and heterologous associations of excess *T. aestivum* × labelled *Ae. speltoides*/0B. A check on the genotypic basis of this difference between ΔT_ms was made by simultaneously melting, incubating and thermally dissociating in HAP-columns, connected in series, two mixtures of DNA: one excess *T. aestivum* × labelled *Ae. speltoides*/0B; the other excess *T. aestivum* × labelled *Ae. mutica*/0B. Elution profiles (Fig. 5a and b) and duplex stabilities (Fig. 4b) show that this difference in ΔT_ms is maintained.

Percent heteroduplex formation at C_0t 10^{-2} between excess *T. aestivum* × labelled *Ae. mutica* ± Bs (Table 2) was about half that found in the hybridisation of excess *T. aestivum* × labelled *T. aestivum*. The results tentatively indicate that the presence of B-DNA has not affected the % duplex formation; however, no standard errors can be put on the single experiment reported in one of the hybridisations concerned.

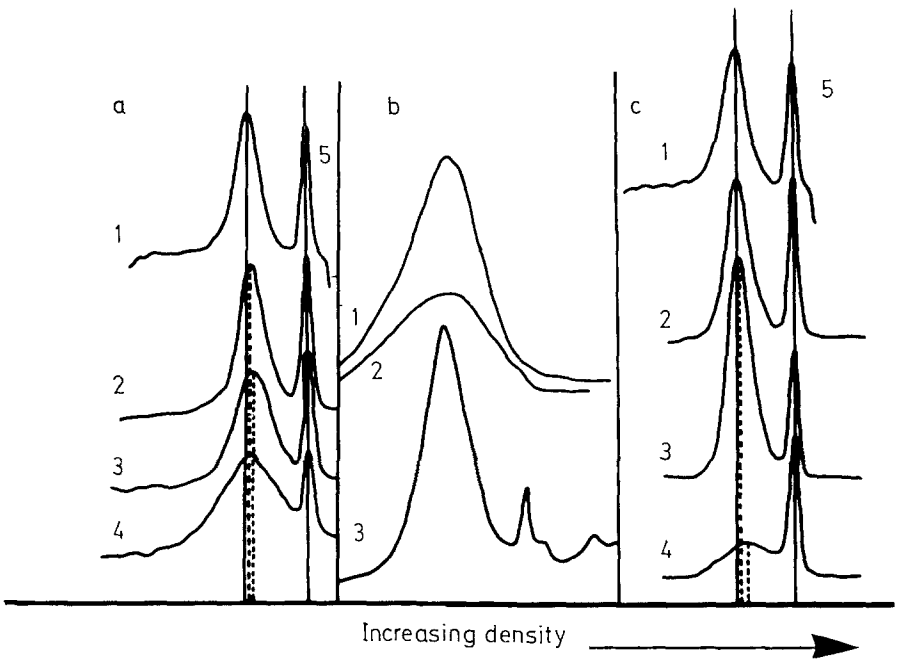

Fig. 6a—c. (a) and (c) Buoyant density patterns after analytical centrifugation in neutral CsCl of native DNA of *T. aestivum*, *Ae. speltoides*±Bs and *Ae. mutica*±Bs. 10 μg DNA in 10 mM Tris-HCl, pH 7.5 plus 0.5 μg of marker DNA, *Pseudomonas aeruginosa* (buoyant density 1.726 gm cm^{-3}) were loaded and spun in CsCl (initial density 1.700 gm cm^{-3}) at 44,000 r.p.m. for 20 hours at 25° C. Buoyant densities calculated relative to the marker according to Mandel *et al.* (1968). (a) 1 *T. aestivum*, 2 *Ae. speltoides*/6B, 3 *Ae. speltoides*/3B, 4 *Ae. speltoides*/0B, 5 *Pseudomonas aeruginosa*, (c) 1 *T. aestivum*, 2 *Ae. mutica*/0B, 3 *Ae. mutica*/3B, 4 *Ae. mutica*/4B, 5 *Pseudomonas aeruginosa*. (b) Buoyant density patterns in neutral CsCl of native DNA of *Ae. speltoides*/6B and *Ae. mutica*/4B after binding to actinomycin-D. Native unsheared main-band and satellite DNA of *Drosophila melanogaster* were similarly bound to actinomycin-D as a check on the success of the method. 10 μg DNA were bound to 20 μg actinomycin-D in 10 mM Tris-HCl, pH 7.5 and 1 mM EDTA and spun in CsCl (initial density 1.66 gm cm^{-3}) at 44,000 r.p.m. for 24 hours at 25° C. 1 *Ae. speltoides*/6B, 2 *Ae. mutica*/4B, 3 *Drosophila melanogaster* (main-band DNA and satellites). (c) 1 *T. aestivum*, 2 *Ae. mutica*/0B, 3 *Ae. mutica*/3B, 4 *Ae. mutica*/4B, 5 *Pseudomonas aeruginosa*

Centrifugation in CsCl

Samples of DNA of *T. aestivum*, *Ae. speltoides*±Bs and *Ae. mutica*±Bs sedimented to equilibrium in neutral CsCl solution, did not reveal bands of satellite DNA in addition to the main-band (Fig. 6a and c). The buoyant densities of DNA of *Ae. speltoides*/4B–6B are lighter than the main-band density of *Ae. speltoides*/0B; 1.705—1.703 g cm^{-3} in *Ae. speltoides*/0B–6B respectively. The B-chromosomes of *Ae. mutica* induce a slight increase in main-band density: 1.702—1.705 g cm^{-3} in *Ae. mutica*/0B–4B. Main-band density of *T. aestivum* is 1.702 g cm^{-3}.

Actinomycin-D has the useful property of binding to high GC-rich main-band DNA, making it lighter in density and thus revealing cryptic satellites of similar

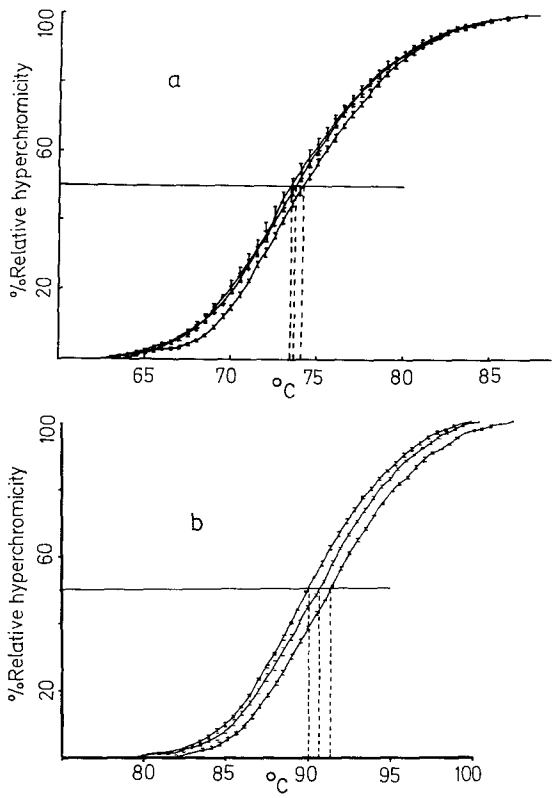

Fig. 7a and b. Mean melting curves and standard errors of native sheared DNA of *Ae. speltoides*±Bs and *Ae. mutica*±Bs. For each experiment, genotypes were melted simultaneously at a temperature rise of 0.5° C min^{-1} in either 0.1 × SSC or 0.12M PB for three successive melts; (see methods). Mean % relative change in hyperchromicity (O.D.) and standard errors are plotted by computer at half degree intervals. Temperatures (T_m), at which 50% change in optical absorbance are indicated. (a) DNA extractions of single plants of 1. *Ae. speltoides*/0B, 2. *Ae. speltoides*/3B and 3. *Ae. speltoides*/6B melted in 0.1 × SSC. (b) DNA extractions of single plants of 1. *Ae. mutica*/0B, 2. *Ae. mutica*/2B and 3. *Ae. mutica*/4B melted in 0.12M PB

GC content as main-band DNA (Peacock *et al.*, 1973). U.V. absorbance bands of native DNA of *Ae. speltoides*/6B, *Ae. mutica*/4B and *Drosophila melanogaster*, after binding to actinomycin-D (10 μg DNA: 20 μg actinomycin-D) are depicted in Fig. 6b. The nuclear DNA of 20 hour embryos of *D. melanogaster*, used as a check on the successful binding of the antibiotic to DNA, has been fractionated into main-band and satellite DNAs. The main-band DNAs of *Ae. speltoides*/6B and *Ae. mutica*/0B show considerable spread at equilibrium in actinomycin-D CsCl gradients: a reflection of the differential binding of actinomycin-D to molecular species of DNA that make up the total main-band heterogeneity of A and B chromosomes (see Discussion). No clear-cut main-band cryptic satellites were revealed in either genotype.

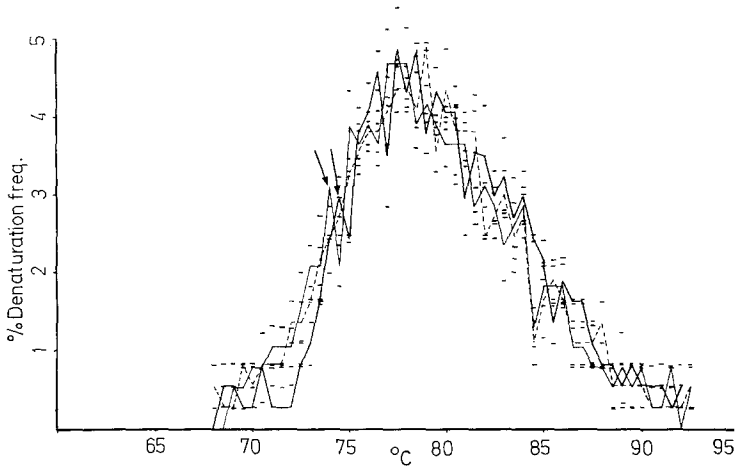

Fig. 8. Mean derived curves and standard errors from thermal denaturation profiles of DNA of *Ae. speltoides*±Bs. Three samples of each genotype were melted simultaneously at a temperature rise of 0.5° C min^{-1} in 0.1 × SSC, (see methods). The frequence of dissociating DNA molecules between temperatures t_2 and t_1 were calculated with the relation $(A_{(t1)}-A_{(t2)})/A_{(tn)}-A_{(t1)})\times 100$. $A_{(t1)\,(t2)\cdots(tn)}$ are the absorbance values measured at temperatures t_1 and t_2 *etc.* Interval (t_2-t_1) was 0.5° C. Arrows indicate small (A-T)n rich DNA fraction associated with genotypes carrying 3B- and 6B-chromosomes (continuous lines). *Ae. speltoides*/0B (broken line)

Thermal Denaturation of Native DNAs

Precise comparisons and characterisation of the molecular heterogeneity of native DNA from differing genotypes can be made by melting DNA in controlled experiments designed to eliminate the confounding of environmental with genotypic differences. Several parameters of melting curves of DNA reflect the intramolecular composition of DNA. The temperature at 50% change in absorbance (T_m° C) is related to average % GC content. The transition temperature (ΔT° C) between 17%–83% absorbance is a measure of the compositional heterogeneity of a DNA sample which, for Gaussian distributions, is related to the % GC at two standard deviations about the mean (Mandel and Marmur, 1968), (see legend to Table 5). Differentiation of melting curves reveals regions of DNA of differing average base composition melting at specific temperature optima. Narrow Gaussian peaks in derived melting curves (Figs. 8–10) are indicative of discrete homogenous fractions of DNA dissociating over a range of temperatures relative to their % GC content (Huguet and Jouanin, 1972; Michel, 1974; Dover, 1974).

The randomisation of genotypes over successive melts is described in Materials and Methods, and the experimental conditions of individual sets of experiments are given in the legends to Figs. 7–10 and Table 5. Mean melting curves and standard errors of native DNA of *Ae. speltoides*±Bs and *Ae. mutica*±Bs (Fig. 7a and b) show very close proximation and the mean T_ms (Table 5) are not significantly different. Samples of DNA of *Ae. speltoides*/0B and *Ae. mutica*/0B melted simultaneously with DNA of *T. aestivum* over three consecutive melts revealed no

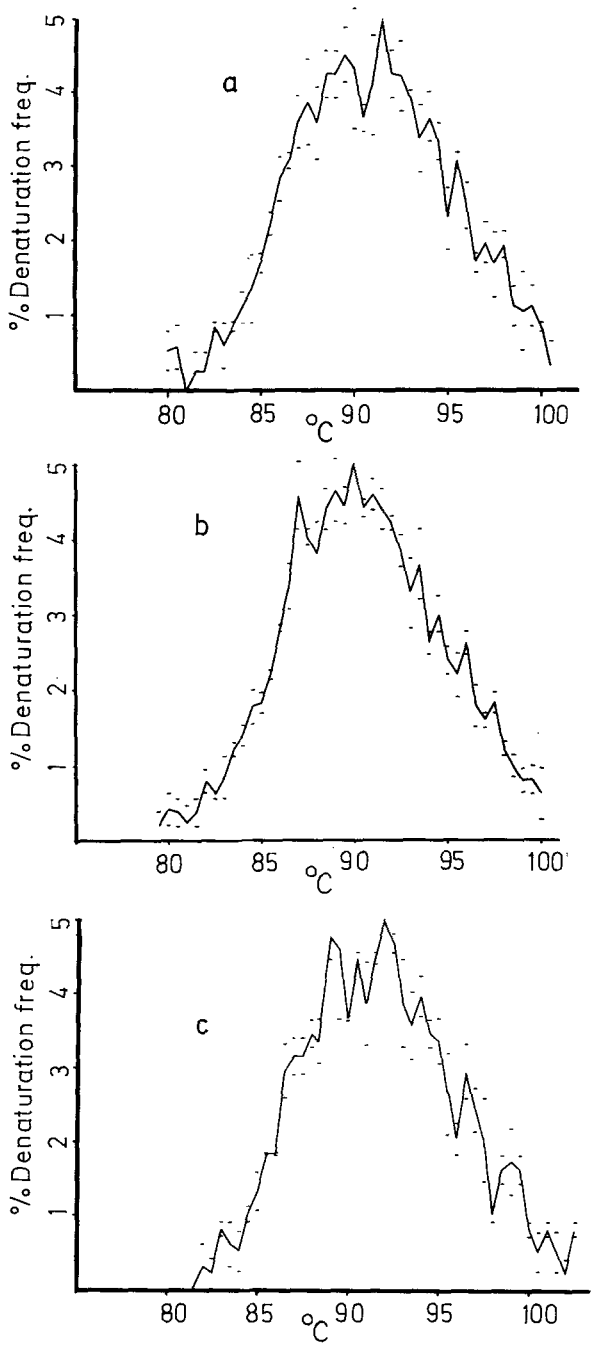

Fig. 9a—c. Mean derived curves and standard errors from thermal denaturation profiles of DNA from *Ae. mutica*±Bs. For conditions of melting and randomisation of genotypes see legend to Fig. 7b. For derivation of curves see legend to Fig. 8. (a) *Ae. mutica*/0B, (b) *Ae. mutica*/3B, (c) *Ae. mutica*/4B

Table 5. Mean melting temperatures (T_m° C); mean transition temperature (ΔT_m° C) between 17% to 83% of absorbance rise; mean compositional heterogeneity; and untransformed standard errors of native DNAs of *T. aestivum*, *T. aestivum* tetrasomic 5B *Ae. speltoides* ± Bs and *Ae. mutica* ± Bs. For randomisation of experiments and conditions of melting see legend to Fig. 7 and 10

Source of DNA	Solvent	Mean melting temperature (T_m° C)	Mean transition temperature at 17–83% absorbance (ΔT_m° C)	Compositional heterogeneity (% GC) at two standard deviations about the mean[a]
Ae. speltoides/0B	0.1 × SSC	73.7 ± 0.15	9.0 ± 0.20	14.6 ± 0.49
Ae. speltoides/4B	0.1 × SSC	73.5 ± 0.40	9.1 ± 0.30	14.9 ± 0.73
Ae. speltoides/6B	0.1 × SSC	74.18 ± 0.25	8.78 ± 0.30	14.1 ± 0.73
Ae. mutica/0B	0.12 M PB	90.7 ± 0.20	8.4 ± 0.20	13.2 ± 0.49
Ae. mutica/2B	0.12 M PB	90.1 ± 0.10	8.1 ± 0.10	12.4 ± 0.24
Ae. mutica/4B	0.12 M PB	91.4 ± 0.14	8.7 ± 0.15	13.9 ± 0.37
T. aestivum	0.12 M PB	87.3 ± 0.21	8.6 ± 0.20	13.7 ± 0.40
Ae. speltoides/0B	0.12 M PB	88.0 ± 0.35	8.9 ± 0.30	14.4 ± 0.73
Ae. mutica/0B	0.12 M PB	88.6 ± 0.10	9.0 ± 0.25	14.6 ± 0.61
T. aestivum	0.12 M PB	89.40 ± 0.75	8.38 ± 0.28	13.13 ± 0.68
T. aestivum/tetrasomic chromosome no. 5B	0.12 M PB	88.47 ± 0.76	8.99 ± 0.11	14.61 ± 0.27

[a] Calculated according to Mandel and Marmur (1968) $2\sigma = (\Delta T - 3.0)$ 2.44 on the assumption that distributions are Gaussian. (However see also Figs. 8–10.)

significant differences in mean T_m. Similarly no significant differences were found in compositional heterogeneity derived from ΔTs at 17%–83% absorbance between any of the samples of DNA melted under strictly comparable conditions (Table 5). DNA of *T. aestivum* tetrasomic for wheat chromosome no. 5B showed no significant differences in T_m and heterogeneity as measured by ΔT or in the overall profile of derived melting curves (Fig. 10), when melted simultaneously and consecutively with euploid *T. aestivum*.

Mean derived melting profiles of *Ae. speltoides*/3B–6B show a small AT-rich DNA fraction (<5%) with a characteristic melting peak (arrowed in Fig. 8). No equivalent peak is observed in the profile of DNA of *Ae. speltoides*/0B. Three samples of each genotype were melted simultaneously in this experiment. However, the peak persisted after randomisation of genotypes over successive melts (Dover, 1974). The remainder of the melting profile of DNA of genotypes with up to 6B-chromosomes was similar to that of 0B individuals. All profiles show the same characteristic skewed distribution having an initial early melt of relatively fewer AT-rich DNA fractions followed by substantial fractions of late melting GC-rich DNA.

No major differences were observed in the profiles of derived melting curves of *Ae. mutica*±Bs (Fig. 9). The presence of B-DNA in genotypes of *Ae. mutica* did not introduce any separate melting peaks similar to the small AT-fraction correlated with the B-DNA of *Ae. speltoides* described above.

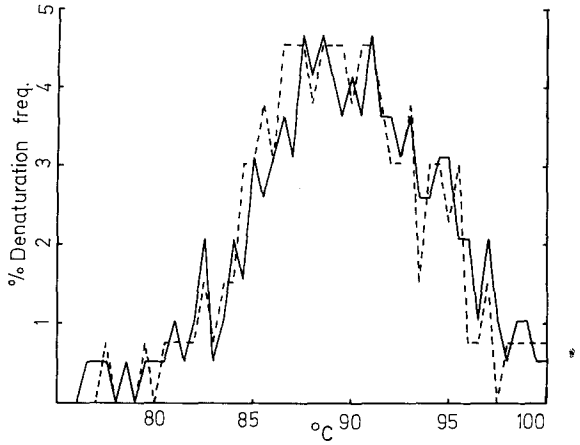

Fig. 10. Derived curves from thermal denaturation profiles of DNA from *T. aestivum* and *T. aestivum* tetrasomic chromosome no. 5B, melted simultaneously in 0.12M PB at temperature rise of 0.5°C min^{-1}. Mean T_ms and standard errors from three replicate melts are given in Table 5. For derivations of curves see legend Fig. 8. — *T. aestivum* tetrasomic 5B, --- *T. aestivum* euploid

Discussion

Accessory (B) chromosomes to the main A-chromosome complement of higher organisms enjoy the dubious status of being considered biological curios, despite their reported presence in 700 species and their multitude of effects. Some contributory factors to this situation are due to effects that are indeed curious, such as the quantitative zig-zag changes in plant growth with odds-and-evens B frequency (Rees, 1974; Jones, 1975), and the increase in hybridisable ribosomal RNA genes of wheat in the presence of rye Bs, (Flavell and Rimpau, 1975). They carry no proper functional cistrons; their mode of inheritance is often non-Mendelian; they can have far-reaching implications for the genetic systems of B-carrying organisms by their diverse effects on A-chromosome meiosis; and their origin is enigmatic.

It is through the effects of Bs on meiosis, in particular in the wheat complex of species, that some insight might be gained on another perplexing problem—the mechanism of homologous chromosome recognition *prior* to meiotic synapsis.

Meiotic pairing in hexaploid wheat is restricted to homologous chromosomes by the activity of a gene (*ph*) on wheat A-chromosome no. 5B, despite the genetic similarity of all three genomes (Riley and Law, 1965; Sears, 1969). The absence of 5B induces high levels of homoeologous pairing: for example, all F_1 hybrids of *T. aestivum* × *Ae. mutica* fall into a "super-high" class of pairing when 5B is absent (Dover and Riley, 1972b; Dover, 1973). The B-chromosomes of *Ae. speltoides* and *Ae. mutica* are found to compensate for the absence of 5B, in that a restriction in pairing is re-imposed in their presence in interspecific hybrids of *T. aestivum*/5B × *Ae. speltoides* + Bs and *T. aestivum*/5B × *Ae. mutica* + Bs (Dover and Riley, 1972b; Vardi and Dover, 1972). The B-chromosomes have no effect on

meiosis in the two diploids except when present in high numbers (Fig. 1). Furthermore, investigations have shown that the Bs disrupt mitotic and meiotic spindles in interspecific hybrids and that hybrids with Bs show much reduced levels of pairing when subjected to low temperatures during the premeiotic interphase (Vardi and Dover, 1972). These last two effects make sense when considered in the light of possible spindle participation in chromosome readiness for pairing (Dover and Riley, 1973), and the locating of the cold temperature sensitive stage and other events in early premeiotic interphase in wheat (Bayliss and Riley, 1972; Bennett *et al.*, 1974).

It has been suggested that one possible role of highly repetitious DNA (satellite), in particular that located in contiguous blocks around the centromeres, might be in chromosome pairing or chromosome movement during division (Walker, 1971; Yunis and Yasmineh, 1971; Flamm, 1972). The 5B-like diploidising effect of B-chromosomes of *Aegilops* spp. and of *Lolium* spp. (Evans and Macefield 1972) and the B-effect on spindles in *Triticum/Aegilops* hybrids might be the result of B-chromosome satellite DNA interfering with chromosome behaviour controlled by A-chromosome satellite DNA. The present results of comparative experiments testing for DNA compositional similarities and differences, make it clear that the mechanism of interference is not due to an exclusive B-specific repetitive DNA. The Bs apparently contain representatives of all the families of repetitive DNA of As: the range and nature of the molecular heterogeneity of the DNA is similar in the two types of chromosomes.

High % levels of heterologous DNA associations in all DNA/DNA combinations (Tables 1 and 3), at incubation conditions allowing for the reassociation of all the highly repetitive and moderately repetitive DNA (C_0t 100) in *T. aestivum* (Smith and Flavell, 1975) and *Aegilops* spp. (Dover, unpublished) were found to be similar irrespective of the presence of B-DNA and irrespective of the direction of hybridisation. (In one instance, the low levels of hybridisation in combinations involving ^{125}I-labelled *Ae. mutica*/4B must be treated with caution.)

No major family of moderately repetitive DNA is present in the DNA of wheat that is not also present in the DNA of A-chromosomes of *Aegilops* spp.; and similarly the DNA of *Aegilops* spp. does not contain a repetitive DNA family exclusive to this genus. Furthermore, the addition of up to 30% B-DNA in 6-B genotypes does not add any family of repetitive DNA not already present in the DNA of A-chromosomes. This conclusion is supported by the similarities in elution profiles of dissociation of homologous and heterologous duplexes (Figs. 3 and 5). There are no thermally stable repetitive fractions of DNA specific to either genus as are reported in several genera of rodents using similar assays (Rice, 1972).

At incubation times allowing for the reassociation of only highly repetitive DNA (C_0t 10^{-2}), the % heterologous association between *T. aestivum* and either *Ae. speltoides*$\pm$Bs or *Ae. mutica*$\pm$Bs is half that found in the control hybridisation of excess *T. aestivum* with labelled *T. aestivum* (Table 2). This indicates that there are some families of highly repetitious DNA in wheat not present in *Aegilops* spp. The presence of B-DNA does not significantly alter the % level of heterologous association at this C_0t.

Thermal stability profiles (Figs. 2 and 4) and ΔT_ms (Table 4) between homologous and heterologous duplexes indicate that the precision of complementary

base pairing is not the same in the two types of duplex and that divergence in nucleotide sequence within similar repetitive DNA families has occurred. The presence of B-DNA in heterologous combinations does not alter the relative stability of the duplex.

It is of interest to note that the degree of divergence between the repetitive DNA of *Ae. speltoides*/0B and the repetitive DNA of *T. aestivum* is no greater than estimates of divergence of the DNAs of *T. aestivum*, *T. monococcum* and *Ae. squarrosa* made under similar stringencies (Bendich and McCarthy, 1970) (Table 4). *T. monococcum* and *Ae. squarrosa* are the diploid donors of the A- and D-genomes of the hexaploid respectively (Sears 1969). A relatively small divergence in DNA base composition supports the candidature of *Ae. speltoides* as the B-genome donor; although there are wide discrepancies in the reports on Giemsa banding patterns of *speltoides* chromosomes and B-genome chromosomes (Natarajan and Sarma, 1974; Gill and Kimber, 1974). The repetitive DNAs of *Ae. mutica* and *T. aestivum* have diverged to a significantly greater extent and place *Ae. mutica* between *Ae. speltoides* and rye (Smith and Flavell 1974) (Table 4) in terms of relative DNA base divergence.

Buoyant density patterns in neutral CsCl and in actinomycin D-CsCl gradients have not revealed DNA satellites in addition to main-band DNA in the presence of B-DNA of either species (Fig. 6). Main-band DNA buoyant densities are very similar to main-band DNA *T. aestivum* indicating the same average % GC content. The presence of B-DNA induces small shifts in density, although in opposite directions in the two species. Mean melting temperatures (T_ms) of DNA of *Ae. speltoides*/0B–6B and *Ae. mutica*/0B–4B (Fig. 7, Table 5) are not significantly different and suggest that the shifts in buoyant density are not genotypically determined. The large spread of main-band DNA (Fig. 6b) suggests considerable heterogeneity in base composition of families of DNA binding differentially to actinomycin-D.

Comparisons of dissociation peaks of separate homogenous families of native DNA of differing average base composition in mean derived melting curves of *Ae. speltoides*±Bs (Fig. 8) and *Ae. mutica*±Bs (Fig. 9) support the conclusions drawn from thermal elution of renatured heterologous duplexes of repetitive DNA and fractionation of total DNA in actinomycin D-CsCl gradients. The overall molecular heterogeneity of total DNA (repetitive and unique) of genotypes of *Aegilops* spp., having substantial % increases of total DNA with the addition of B-DNA, is no different in range or composition than the total DNA of genotypes having A-DNA only. There does not appear to be a major fraction of B-DNA that is exclusive to the B-chromosomes. There are no significant differences in mean T_m (average % GC) or compositional heterogeneity (derived from ΔT) after thermal denaturation of any of the DNAs, listed in Table 5, in which legitimate comparisons may be made. The lack of resolvable differences in profiles of derived melting curves after simultaneous denaturation of DNAs of *T. aestivum*, *Ae. speltoides*/0B and *Ae. mutica*/0B points to the similarity in number and composition of families of DNA in these species (Dover, 1974). The melting of DNA of *T. aestivum* tetrasomic for A-chromosome no. 5B, that carries the region controlling meiotic pairing in the hexaploid, has not revealed any differences in the overall heterogeneity of total DNA when simultaneously denatured with the DNA of

euploid *T. aestivum* (Table 5, Fig. 10). Whatever the nature of this region, it does not appear to consist of a molecular species of DNA sufficiently distinctive in base composition or size to be resolvable as a separate dissociating peak in derived melting curves.

There is a small AT-rich peak of dissociating DNA correlated with the presence of B-chromosome genotypes of *Ae.speltoides* (Fig. 8); a peak that persisted after randomisation of genotypes over successive melts (Dover, 1974). It is possible that the one observable difference between the DNA of *Ae. speltoides* ± Bs is the fraction that is responsible for the B-chromosome disruption of meiotic pairing and formation of spindles. However, these effects are shared by the Bs of *Ae. mutica* in which no corresponding fraction of DNA was resolved (Fig. 9).

Additional differences in A-chromosome and B-chromosome DNA might be resolvable after suitable weighting with Ag^+ or Hg^+ ions and sedimentation to equilibrium in Cs_2SO_4 or after centrifugation in alkaline CsCl. Detailed analysis of nucleotide base sequences of A- and B-chromosome DNA or analysis of differences in the distribution of target sequences to restriction nucleases would need to wait for the successful separation of the two DNAs.

Comparisons of DNA heterogeneity in terms of numbers and composition of molecular species support the interpretation that the B-chromosomes contain representatives of all the families of repeated and unique sequence DNA found in the A-chromosomes of *Aegilops* spp. and *T. aestivum*. There are small differences in the composition of families of highly repetitive DNA ($C_0t\ 10^{-2}$) and in the extent of base divergence of moderately repeated sequences between the A-DNA of the three species. However, the presence of B-DNA does not magnify nor diminish these differences. The lack of a resolvable difference, underpinning a DNA exclusive to Bs does not entirely support the suggestion (Walker, 1971; Yunis and Yasmineh, 1971; Flamm, 1972) that satellite DNAs located around the centromeres in many species might be responsible for chromosome behaviour. At the least, in the wheat group of species, the complex set of interactions of A and B-chromosomes affecting meiotic pairing and spindles (Dover, 1973), might not be explained so simply. "That other links exist, on other levels, between these two affairs, is not impossible," (Samuel Beckett).

Some light is shed on the origin of Bs and a possible origin of the pairing-control system of 5B. The apparent similarities in base composition of all DNA families between As and Bs would indicate an origin of Bs from As and would suggest an interspersion of families of DNA on the A-chromosomes of *Aegilops* spp. similar to that shown for wheat (Smith and Flavell, 1975) and *Xenopus* (Davidson *et al.*, 1973). Similarities in DNA heterogeneity of As and Bs of maize (Chilton and McCarthy, 1973) and rye (Rimpau and Flavell, 1975) would point to the same origin for B-chromosomes.

If this interpretation is correct then it is possible that the successful survival of Bs in a population in which they arise would depend on an immediate ban on A/B-chromosome pairing at meisois. Such a control "locus" might first arise on the Bs; and so explain the ability of Bs, in preventing high levels of homeologous pairing, to compensate for the absence of 5B in interspecific hybrids (Dover and Riley, 1972b). Translocation of the putative control region to A-chromosomes of *Aegilops* would form a basis for the varying ability of A-chromosome loci to control

the level of pairing in hybrids with wheat (Vardi and Dover, 1972; Dover and Riley, 1972a). It might be of more than passing coincidence that the only two species of *Aegilops* (*speltoides* and *mutica*) that have A-chromosome loci interacting with the 5B-system of wheat also carry B-chromosomes.

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