

## REPEATED SEQUENCE DNA COMPARISONS BETWEEN TRITICUM AND AEGILOPS SPECIES

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### SUMMARY

The families of repeated sequences in the genomes of a range of *Triticum* and *Aegilops* species have been compared. All the genomes are very similar. However, using a DNA probe from *Aegilops speltoides* that contains the most highly repeated sequences, diploid *Aegilops* species could be distinguished from diploid *Triticum* species. Different *Aegilops* species' DNAs also hybridise to differing extents with this probe. The results are consistent with the hypothesis that speciation is accompanied by quantitative changes in the repeated sequence complements of genomes.

Most if not all of the families of repeated sequences in hexaploid wheat can be detected in *Aegilops speltoides* (related to the B genome) and in *Aegilops squarrosa* (related to the D genome). However, some families of repeated sequences of hexaploid wheat were not found in *Triticum monococcum* (related to the A genome). Some of the most highly repeated sequences of hexaploid wheat are preferentially concentrated in the B genome. These sequences are useful as probes for distinguishing the three diploid genomes of hexaploid wheat.

### 1. INTRODUCTION

THE repeated sequence complements of complex genomes can change considerably during species divergence. Easily detectable differences are often found between the repeated sequences in related species (Chooi, 1971; Goldberg *et al.*, 1972; Rice, 1972; Straus, 1972; Rice and Esposito, 1973; Stein and Thompson, 1975; Mizuno *et al.*, 1976; Barnes *et al.*, 1978). In general, the extent of repeated sequence DNA homology between species is related to their phylogenetic similarity concluded from classical taxonomic assessments, *i.e.* more closely related species have more families of repeated sequences in common. Previous studies in this laboratory (Smith and Flavell, 1974; Flavell *et al.*, 1977; Rimpau *et al.*, 1978) as well as from other laboratories (Bendich and McCarthy, 1970) have shown this to be true for oats, barley, wheat and rye, members of the Gramineae, which have diverged from a common ancestor (Bell, 1965; Flavell *et al.*, 1977). However, each of these species also possesses species-specific families of repeated sequences and we have recently illustrated how these can be used to detect rye and barley chromosome fragments in a wheat nucleus (Flavell *et al.*, 1978).

Hexaploid wheat is an allohexaploid ( $2n = 6x = 42$ ) with a *Triticum* species (*Triticum monococcum*) and two *Aegilops* species (*Ae. squarrosa* and *Ae. speltoides* or a close relative) as the three diploid ( $2n = 2x = 14$ ) parents. Some repeated sequence DNA comparisons between these diploid genomes were reported by Bendich and McCarthy in 1970. We wished to extend these comparisons paying particular attention to very high copy number repeats which frequently include species-specific families of repeated sequences. Our results, reported in this paper, show that *Triticum* and

*Aegilops* species do have small repeated sequence differences and these are useful for distinguishing the diploid parents of hexaploid wheat.

## 2. MATERIALS AND METHODS

### (i) *Plant genotypes*

The wheat (*Triticum aestivum*) variety Chinese Spring and rye (*Secale cereale*) variety Petkus were used as sources of wheat and rye DNA. Seed of the other species used for DNA isolation were from stocks kept at the Plant Breeding Institute.

### (ii) *Isolation of unlabelled and in vivo labelled DNAs*

Unlabelled DNAs were isolated from green leaf tissue of plants grown under continuous illumination. The details of the DNA purification have been described previously (Smith and Flavell, 1974). *In vivo* tritium labelled wheat DNA was purified from about 3-day-old dark grown seedlings, germinated in the presence of (Me-<sup>3</sup>H) thymidine also as described previously (Smith and Flavell, 1974). It had a specific activity of about 40,000 cpm/ $\mu$ g. All DNAs were sheared by sonication, in an ice bath, to give weight average, denatured fragment lengths between 300 and 400 nucleotides. Average fragment sizes were determined by boundary velocity sedimentation in 0.9M NaCl 0.1M NaOH as described by Studier (1965) using an MSE "Centriscan" analytical ultracentrifuge.

### (iii) *Renaturation of denatured DNA in solution and S<sub>1</sub> nuclease treatment*

In the experiments where the renatured duplexes were to be treated with S<sub>1</sub> nuclease, renaturation of the sheared, denatured labelled wheat plus unlabelled DNAs was carried out in 0.3 M NaCl 0.01 M Pipes buffer pH 6.8 at 65°C. The DNAs were denatured by heating to 100°C for 5 min. After incubation to the desired C<sub>0</sub>t (C<sub>0</sub>t = moles nucleotide per litre  $\times$  incubation time in sec.) an equal volume of 0.05 M sodium acetate buffer pH 4.3 containing 0.2 mM Zn<sup>++</sup> and 5.5 mM mercaptoethanol was added to bring the pH to 4.3. Enough S<sub>1</sub> nuclease, purified by the method of Sutton (1971), was added to be able to digest all the DNA, if it were single stranded, during a 60 min. incubation at 37°C. After incubation of the DNA with S<sub>1</sub> nuclease for 60 min. at 37°C, 100  $\mu$ g of bovine serum albumin and TCA (5 per cent final concentration), were added and the precipitate collected on GF/B glass fibre filters after standing at 3°C for at least 1 hour (Flavell and Smith, 1976). The filters were dried and their radioactivity determined in a scintillation counter.

In the experiments where the very rapidly renaturing "nick translated" *Ae. speltoides* and *T. monococcum* DNAs were incubated with unlabelled DNAs in solution, renaturation was carried out at 60°C or 68°C in 0.12 M phosphate buffer pH 6.8. After renaturation, the renatured fragments were separated from the denatured fragments by hydroxyapatite chromatography at 60°C or 68°C exactly as described previously (Flavell *et al.*, 1978). The DNAs in both hydroxyapatite fractions were precipitated with TCA and their radioactivity determined as described above.

- (iv) *Isolation and radioactive labelling of very rapidly renaturing DNA from Triticum aestivum, Aegilops speltoides and Triticum monococcum*

Aliquots of *Triticum aestivum*, *Aegilops speltoides* or *Triticum monococcum* DNA in 0.12 M phosphate buffer were sheared to give average fragment lengths of about 800 nucleotides, denatured by boiling, incubated at 60°C to a  $C_0t$  of approximately  $10^{-2}$  and applied to hydroxyapatite columns. The non-renatured DNA was eluted with 0.12 M phosphate buffer at 60°C. The DNA fragments containing renatured regions were eluted with 0.5 M phosphate buffer pH 6.8 at 60°C. This DNA was radioactively labelled *in vitro* ("nick translated") as described by Flavell *et al.* (1978).

- (v) *Hybridisation of "nick translated" Triticum aestivum and Aegilops speltoides DNAs to filter-bound unlabelled DNAs*

Unlabelled DNAs in  $0.1 \times \text{SSC}$  ( $\text{SSC} = 0.15 \text{ M sodium chloride } 0.015 \text{ M sodium citrate}$ ) were denatured with an equal volume of 1 M NaOH. After approximately 15 min., 4 volumes of a 2 : 1 : 1 mixture of 3 M NaCl, 1 M tris-HCl (pH 8.0) and 1 N HCl were added. The solutions were passed under gravity through 10 mm diameter Millipore HAWP filters and the filters washed with 5 ml of  $6 \times \text{SSC}$ . After drying in air the filters were stored at  $-20^\circ\text{C}$  until use. Immediately before hybridisation, the filters were heated at  $80^\circ\text{C}$  *in vacuo* for 2 hours. Approximately 25  $\mu\text{g}$  of DNA was bound per filter.

For the typical hybridisation reaction, filter-bound DNAs of each of the genotypes to be compared were incubated together at  $40^\circ\text{C}$  for 16 hours in  $2 \times \text{SSC}$  50 per cent formamide containing approximately 1 to  $2 \times 10^{-3}$   $\mu\text{g}$  labelled DNA per filter. Five replicate filters of each DNA were usually included and approximately four filters were incubated per ml of solution. The amount of DNA bound to each filter was determined after hybridisation and scintillation counting, by hydrolysing the DNA in HCl (Brown and Weber, 1968). Filters lacking DNA were included as controls in all experiments. All results (see tables 2 and 3) are corrected for the results obtained from these control blank filters.

### 3. RESULTS

- (i) *Comparison of the repeated sequences in Triticum monococcum (2x), Aegilops speltoides (2x), Aegilops squarrosa (2x), Triticum aestivum (6x) and Secale cereale (2x)*

Studies were first carried out to investigate if all the families of repeated sequences in hexaploid wheat, *Triticum aestivum*, are present in each of the three diploids related to the ancestral diploid progenitors of hexaploid wheat. *Secale cereale*, rye, was also included in the experiments as a control, as previous studies have shown that wheat possesses families of repeated sequences not found repeated in rye (Flavell *et al.*, 1977). An aliquot of sheared  $^3\text{H}$  labelled wheat DNA was added to a 10,000-fold excess of sheared, unlabelled DNA fragments from each of the species, in 0.3 M NaCl 0.01 M Pipes buffer pH 6.8. The DNAs were denatured and allowed to renature at  $65^\circ\text{C}$  for different  $C_0t$  values between  $10^{-2}$  and 10.

In these sorts of experiments, DNA renaturation is driven by the unlabelled DNA which is in sufficient excess over the labelled wheat DNA to virtually eliminate the renaturation of labelled DNA fragments to one another. The extent to which labelled wheat DNA fragments become incorporated into duplexes is therefore a measure of DNA sequence homology between the labelled and unlabelled DNAs. Previous studies (Smith and Flavell, 1975; Flavell and Smith, 1976) have shown that most cereal DNA fragments possessing repeated sequences have renatured by  $C_0t$  10, so most families of repeated sequences were assayed by carrying out incubations to  $C_0t$  10.

After incubation, non-renatured single stranded DNA was digested by incubating with  $S_1$  nuclease at  $37^\circ\text{C}$  for 60 min. (see Materials and Methods). The  $S_1$  nuclease resistant renatured DNA was then precipitated with TCA and collected on GF/B filters (Flavell and Smith, 1976). After the filters were dried, the  $^3\text{H}$  wheat DNA surviving the  $S_1$  nuclease digestion was estimated. The results are shown as  $C_0t$  curves in fig. 1 for the experiments where five different unlabelled DNAs were used to drive the renaturation of the labelled wheat DNA. The proportion of  $^3\text{H}$  labelled wheat DNA able to hybridise with rye DNA was clearly less than that able to hybridise to all the other unlabelled DNAs. No clearly significant differences were found between the curves for different unlabelled *Triticum* and *Aegilops* DNAs, although there was a hint that hybridisation to *T. monococcum* DNA might be less at high  $C_0t$  values. Thus most if not all of the families of repeated sequences in hexaploid wheat are also in each of the diploid *Triticum* and *Aegilops* DNAs.

To examine this more thoroughly, two different preparations of DNA from

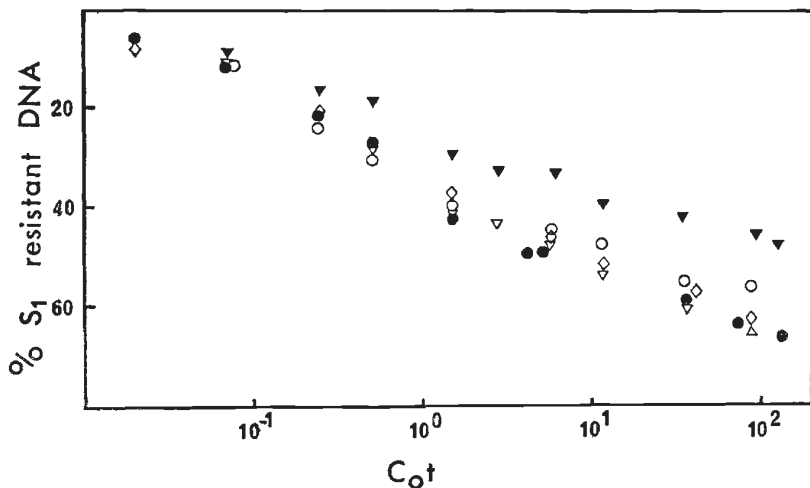


FIG. 1.—Hybridisation of  $^3\text{H}$  labelled hexaploid wheat DNA to *Ae. speltoides*, *Ae. squarrosa*, *T. monococcum*, *T. aestivum* and *Secale cereale* DNAs.  $^3\text{H}$  labelled wheat DNA was incubated with large excesses of sheared denatured unlabelled DNAs in  $0.3\text{ M NaCl } 0.01\text{ M Pipes buffer pH } 6.8$  at  $65^\circ\text{C}$  to the  $C_0t$  values shown. The proportion of  $^3\text{H}$  labelled DNA incorporated into duplex DNA and therefore  $S_1$  nuclease resistant was determined as described in Materials and Methods: ▼ *Secale cereale*, ○ *T. monococcum*, ▽ *Ae. speltoides*, ◇ *Ae. squarrosa*, ● *T. aestivum*.

*Triticum monococcum*, *Aegilops speltoides*, *Aegilops squarrosa* and *Triticum aestivum* were renatured in quadruplicate to  $C_0t$  100 with  $^3H$  labelled *Triticum aestivum* DNA. The proportion of  $^3H$  labelled wheat DNA hybridised to each unlabelled DNA was determined as described above. The mean values are shown in table 1. Significantly less labelled wheat DNA hybridised to unlabelled *Triticum monococcum* and *Secale cereale* than to unlabelled wheat, *Aegilops speltoides* or *Aegilops squarrosa* DNA. This implies that some families of repeated sequences occupying approximately 11 per cent and 18 per cent of the wheat genome are not found in the repeated sequence fractions of *T. monococcum* and *S. cereale* respectively.

TABLE 1

Hybridisation of  $^3H$  labelled wheat DNA to  $C_0t$  100 with unlabelled DNAs from *T. monococcum*, *Ae. speltoides*, *Ae. squarrosa* and *Secale cereale*

Unlabelled DNA		% $^3H$ wheat DNA $S_1$ nuclease resistant (mean of four replicates)
<i>T. aestivum</i> (6x)	(i)	60.6
	(ii)	66.5
<i>T. monococcum</i> (2x)	(i)	52.9*
	(ii)	51.5*
<i>Ae. speltoides</i> (2x)	(i)	59.7
	(ii)	65.0
<i>Ae. squarrosa</i> (2x)	(i)	61.3
	(ii)	58.5
<i>S. cereale</i> (2x)	(i)	45.3*

\* = significantly lower than hybridisation to *T. aestivum* at the 5 per cent level probability.

Denatured  $^3H$  labelled wheat DNA was incubated with a 10,000-fold excess of denatured unlabelled DNA from the species shown to  $C_0t$  100 at 65°C in 0.3 M NaCl 0.01 M Pipes pH 6.8. After  $S_1$  nuclease treatment, the  $S_1$  nuclease resistant labelled DNA was determined by precipitating with TCA, collecting on a glass fibre filter and counting in a scintillation counter.

(ii) *Comparison of the most highly repeated sequences in Triticum aestivum with those of related species*

The DNA of *Triticum aestivum* which renatured by approximately  $C_0t$   $10^{-2}$  was isolated and tritium labelled *in vitro* by "nick translation" as in Flavell *et al.* (1978). This DNA includes inverted repeats which renature rapidly due to intrastrand renaturation (Flavell and Smith, 1976), the neighbouring sequences to these inverted repeats which would be on the same fragments, the very highly repeated sequences in excess of approximately  $0.25 \times 10^6$  copies per constituent haploid genome and their neighbouring sequences.

To study the relative concentrations of the very highly repeated sequences in the species closely related to the constituent genomes of *Triticum aestivum*, four or five replicate filters loaded with DNA from each species were incubated together in the same solution with a low concentration of labelled

*Triticum aestivum*  $C_0t$   $10^{-2}$  DNA (see Materials and Methods and legend to table 2). In these experiments filter bound unlabelled DNAs are in considerable excess, so the DNAs from the different species compete for the available labelled DNA in solution. Thus, those filter-bound unlabelled DNAs possessing a higher concentration of sequences homologous to those in the  $C_0t$   $10^{-2}$  DNA hybridise with a higher proportion of the labelled  $C_0t$   $10^{-2}$  *Triticum aestivum* DNA. The labelled DNA which binds to the filters during hybridisation consists of the very highly repeated sequences of *Triticum aestivum* since the labelled inverted repeats which renature by intrastrand reassociation do not bind to the millipore filters under the hybridisation conditions. The results of the experiments, *i.e.* the amounts of labelled DNA hybridised to the filters loaded with DNA of each species

TABLE 2

*Hybridisation of very rapidly reannealing DNA from Triticum aestivum to DNAs from related species*

Species	Labelled DNA bound to filter cpm/ $\mu$ g filter bound DNA <sup>a</sup>
<i>Triticum aestivum</i>	73.6 $\pm$ 2.9
<i>Aegilops speltoides</i>	78.3 $\pm$ 5.2
<i>T. dicoccoides</i>	94.9 $\pm$ 1.5***
<i>T. timopheevi</i>	91.3 $\pm$ 2.7**
<i>Aegilops bicornis</i>	74.4 $\pm$ 0.9
<i>Aegilops squarrosa</i>	55.9 $\pm$ 3.1**
<i>Triticum boeoticum</i>	
( <i>T. Thaoudar</i> )	49.5 $\pm$ 2.2***
( <i>T. Aegilopoides</i> )	45.3 $\pm$ 2.2***

Filters loaded with 20 to 30  $\mu$ g DNA from the species listed above were incubated together for 16 hours at 40°C in  $2 \times$  SSC 50 per cent formamide containing 4 to  $8 \times 10^{-3}$   $\mu$ g ml of nick translated  $C_0t$   $10^{-2}$  DNA from *Triticum aestivum*.

<sup>a</sup> Results are mean values  $\pm$  standard error of the mean for five replicate filters.

\*\* , \*\*\* = significantly different from *T. aestivum* at the 1 per cent and 0.1 per cent levels of probability, respectively.

are shown in table 2. Significantly more  $C_0t$   $10^{-2}$  DNA hybridised to the tetraploid *T. dicoccoides* (genome description AB) and *T. timopheevi* (AS; S is closely related to B) DNAs than to hexaploid *Triticum aestivum* (ABD) DNA. This indicates that  $C_0t$   $10^{-2}$  DNA contains sequences which are preferentially located in the A and/or B genomes of hexaploid wheat. This is supported by the significantly lower hybridisation to *Ae. squarrosa* (D) DNA. The amounts of  $C_0t$   $10^{-2}$  hybridising to the *Triticum boeoticum* diploids related to the A genome of hexaploid wheat were only approximately 50 per cent that which hybridised to the tetraploids. This indicates, assuming the similarity of the A genomes of the tetraploids and the *T. boeoticum* genome, that the  $C_0t$   $10^{-2}$  highly repeated sequences of *Triticum aestivum* come preferentially from the B genome. The high hybridisation levels of  $C_0t$   $10^{-2}$  DNA to *Ae. speltoides* and *Ae. bicornis* (very closely related species) are consistent with this conclusion. To investigate further the most highly repeated sequences concentrated in chromosomes related to the B genome of hexaploid wheat, a  $C_0t$   $10^{-2}$  fraction was isolated from *Ae. speltoides* DNA.

(iii) *Comparison of the very highly repeated sequences of Ae. speltoides with those in other Triticum and Aegilops species*

The DNA of *Ae. speltoides* which renatured by  $C_0t\ 10^{-2}$  was isolated and tritium labelled *in vitro* by "nick translation" as described in Materials and Methods. This fraction was used in two kinds of hybridisation experiments: (1) Where the unlabelled DNAs were denatured and immobilised on millipore filters and (2) where the unlabelled DNAs were sheared and incubated in 0.12 M phosphate buffer pH 6.8.

To study the relative concentrations, in a range of *Triticum* and *Aegilops* species, of the sequences homologous to the very rapidly reannealing sequences of *Aegilops speltoides*, four to six replicate filters loaded with each of the DNAs were incubated together with the labelled *Ae. speltoides* DNA. The amounts of labelled DNA hybridised to DNAs from each of the species is shown in table 3.

More labelled *Ae. speltoides* DNA hybridised to unlabelled *Ae. speltoides* DNA than to any other DNA. Between 60 and 75 per cent of the labelled *Ae. speltoides* DNA which hybridised to unlabelled *Ae. speltoides* DNA hybridised with other diploid *Aegilops* species. The amount of highly repeated

TABLE 3

*Hybridisation of the very highly repeated sequences of Aegilops speltoides to DNAs from related species.*

Selection	Species	Labelled DNA bound to filters cpm/ $\mu$ g filter bound DNA	
		Expt 1	Expt 2
<i>Aegilops</i> genus			
Sitopsis	<i>Ae. speltoides</i>	105.5 $\pm$ 2.9	130 $\pm$ 3.9
	<i>Ae. longissima</i>	66.7 $\pm$ 3.0	—
	<i>Ae. sharonensis</i>	72.5 $\pm$ 2.9	—
	<i>Ae. bicornis</i>	—	102 $\pm$ 2.6
Polyeides	<i>Ae. umbellulata</i>	68.8 $\pm$ 2.1	—
	<i>Ae. triuncialis</i>	69.9 $\pm$ 5.4	—
Cylindropyrum	<i>Ae. caudata</i>	67.2 $\pm$ 1.2	—
Amblyopyrum	<i>Ae. mutica</i>	67.2 $\pm$ 2.5	—
Vertebrata	<i>Ae. squarrosa</i>	—	70.2 $\pm$ 6.5
	<i>Ae. crassa</i> (4x)	50.9 $\pm$ 1.2	—
	<i>Ae. crassa</i> (6x)	45.5 $\pm$ 1.8	—
<i>Triticum</i> genus (diploids)			
	<i>T. monococcum</i>	—	49.7 $\pm$ 5.2
	<i>T. boeoticum</i>	—	—
	<i>T. aegilopoides</i>	35.5 $\pm$ 4.0	63.0 $\pm$ 5.1
	<i>T. thaouadar</i>	—	65.0 $\pm$ 6.4
	<i>T. urartu</i>	21.9 $\pm$ 2.5	—
<i>Triticum</i> genus (polyploids)			
	<i>T. dicoccoides</i>	—	102 $\pm$ 4.6
	<i>T. timopheevi</i>	—	105 $\pm$ 4.8
	<i>T. aestivum</i>	—	100.5 $\pm$ 3.5
Calf		2.9 $\pm$ 0.4	—

Unlabelled DNAs from each species were denatured, immobilised on filters and incubated with trace amounts of  $^3H$  labelled *Ae. speltoides*  $C_0t\ 10^{-2}$  DNA in  $2 \times SSC$  50 per cent formamide at 40°C for 16 hours.

sequence DNA of *Ae. speltooides* that bound to hexaploid and tetraploid wheat DNAs was considerably more than that which bound to *Aegilops squarrosa* DNA from which the D genome descended and to *Triticum monococcum* from which the A genome descended. Thus the B genome of hexaploid wheat must be highly enriched with sequences homologous to the very highly repeated sequences in *Ae. speltooides*. This conclusion supports the notion that the B genome of hexaploid wheat is closely related to an *Ae. speltooides*-like genome. It is also consistent with the results in Results (ii) which showed that the most highly repeated sequences of hexaploid wheat are preferentially localised in the B genome.

*Ae. speltooides* labelled DNA hybridised least to DNAs from those diploids classified in the *Triticum* genus viz. *T. monococcum* and *T. boeoticum*. The lower hybridisation levels of *Ae. speltooides* very rapidly reannealing DNA to diploid *Triticum* species could be because repeated sequences homologous to those in the *Ae. speltooides* probe DNA are absent or because they are present but in lower frequency in *T. monococcum*. This was investigated by incubating labelled rapidly reannealing *Ae. speltooides* DNA with a 300,000-fold excess of *T. boeoticum* DNA (isolates *T. thaoudar* and *T. aegilopoides*) to  $C_{0t}$  10, a  $C_{0t}$  value by which most families of repeated sequences have renatured (Flavell and Smith, 1976). The proportion of labelled DNA which renatured was compared with the proportion which renatured to *Ae. speltooides*, *T. timopheevi* and *T. dicoccoides* DNAs. These latter DNAs all contain DNAs from *Aegilops* species. Calf thymus DNA was also included as a non-homologous DNA control. Twenty-five to thirty per cent of the labelled *Aegilops speltooides* DNA ended up in the duplex fraction in this control experiment (table 4). Much of this DNA probably renatured by intrastrand reassociation rather than by a second order reaction (Flavell and Smith, 1976; Smith and Flavell, 1975 (see also fig. 2)). The other results detailed in table 4 clearly indicate that approximately 20 to 30 per cent of the sequences in the labelled *Ae. speltooides* DNA which hybridise to

TABLE 4

*Hybridisation of highly repeated Aegilops speltooides DNA to repeated sequences from other species*

Unlabelled DNA	% labelled DNA in duplex fraction <sup>a</sup>	
	Expt 1	Expt 2
Calf thymus	25.6	31
<i>Ae. speltooides</i>	75	68
<i>T. timopheevi</i>	72	68
<i>T. boeoticum</i> ( <i>T. Thaoudar</i> )	64	58
( <i>T. Aegilopoides</i> )	—	55
<i>T. dicoccoides</i>	—	64
<i>Ae. squarrosa</i>	—	59

<sup>a</sup> Each figure is the mean of three replicates.

<sup>3</sup>H labelled *Aegilops speltooides* very rapidly reannealing DNA was added to sheared unlabelled DNA in a ratio of approximately 1 : 300,000. After denaturation, renaturation was to  $C_{0t}$  10 at 68°C in 0.12 M phosphate buffer. The proportion of <sup>3</sup>H labelled DNA in the duplex fraction was determined by hydroxyapatite chromatography. The failure of all the <sup>3</sup>H labelled DNA to renature to *Ae. speltooides* unlabelled DNA implies it either contains single copy or low copy number sequences or some of the fragments are very short and consequently are unable to renature.



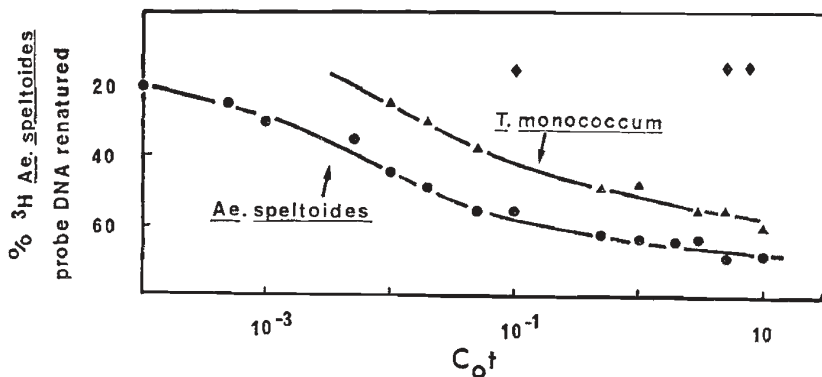


Fig. 2.—Hybridisation kinetics of *Ae. speltooides*  $C_0t$   $10^{-2}$  DNA to *Ae. speltooides* and *T. monococcum* repeated sequences. “Nick translated” *Ae. speltooides*  $C_0t$   $10^{-2}$  DNA was incubated with a large excess of denatured, sheared unlabelled *Ae. speltooides* (● — ●) or *T. monococcum* (▲ — ▲) DNA in 0.12 M phosphate buffer at 68°C. The proportion of labelled DNA in the duplex fraction was determined by hydroxyapatite chromatography. The absence of renaturation of the labelled DNA incubated without unlabelled DNA is shown by ◆.

unlabelled *Ae. speltooides* DNA do not hybridise to *T. boeoticum* DNA, *i.e.* there appears to be highly repeated sequences in *Ae. speltooides* (2 to 3 per cent of the genome) which are not repeated, or only a few times so, in *T. boeoticum*.

The kinetics of renaturation of the labelled, very rapidly reannealing DNA from *Ae. speltooides* to large excesses of unlabelled *Ae. speltooides* and *T. monococcum* DNAs were studied in more detail. The labelled plus unlabelled DNA mixtures were denatured and incubated at 68°C in 0.12 M phosphate buffer to  $C_0t$  values between  $10^{-4}$  and 10. The proportions of labelled *Ae. speltooides* DNA in the duplex fraction were determined following hydroxyapatite chromatography. The results are shown in fig. 2. The renaturation percentages at  $C_0t$  values 1 to 10, show that 17 per cent of the labelled DNA that hybridised to *Ae. speltooides* DNA (approximately 2 per cent of the *Ae. speltooides* genome) was not hybridised with *T. monococcum* DNA. These results endorse those in table 4. Renaturation of *T. monococcum* sequences homologous to the *Ae. speltooides* labelled sequences did not begin until a  $C_0t$  value of approximately  $3 \times 10^{-3}$ . However, 19 per cent of the *Ae. speltooides* labelled DNA (2 per cent of the *Ae. speltooides* genome) had renatured by the  $C_0t$  value when *Ae. speltooides* DNA was driving renaturation of the labelled DNA. This provides further evidence of the difference between the most highly repeated fractions of *T. monococcum* and *Ae. speltooides*. From the shapes of the renaturation curves in fig. 2 it cannot be unequivocally concluded that the sequences not repeated in *T. monococcum* renature between  $C_0t$   $10^{-4}$  and  $C_0t$   $10^{-2}$  in *Ae. speltooides* DNA. The alternative possibility is that the sequences which renature between  $C_0t$   $10^{-4}$  and  $C_0t$   $10^{-2}$  in *Ae. speltooides* DNA are present in a lower frequency in *T. monococcum* DNA and so renature between  $C_0t$   $10^{-2}$  and  $C_0t$  10. In this latter situation, the *Ae. speltooides* highly repeated sequences absent from *T. monococcum* would presumably renature between  $C_0t$   $10^{-2}$  and  $C_0t$  10 in *Ae. speltooides* DNA.

To test whether *T. monococcum* contains highly repeated sequences not found in *Ae. speltooides*, a highly repeated sequence fraction (15 per cent of

the genome) was isolated from *T. monococcum*, "nick translated" and incubated to a range of  $C_0t$  values with unlabelled *T. monococcum* and *Ae. speltooides* DNAs. The results are shown in fig. 3. The renaturation of the highly repeated sequences of *T. monococcum* to *Ae. speltooides* and *T. monococcum* DNAs are very similar, although it appears that a minor fraction (5 per cent) of the labelled DNA (0.5 per cent of *T. monococcum* genome) is not repeated in *Ae. speltooides* DNA. The labelled DNA that was in the duplex fraction by  $C_0t$   $10^{-4}$  consists of reverse repeats that renature by intrastrand reassociation (Flavell and Smith, 1976). Renaturation of labelled *T. monococcum* sequences did not begin until a  $C_0t$  value about  $10^{-2}$ . Thus

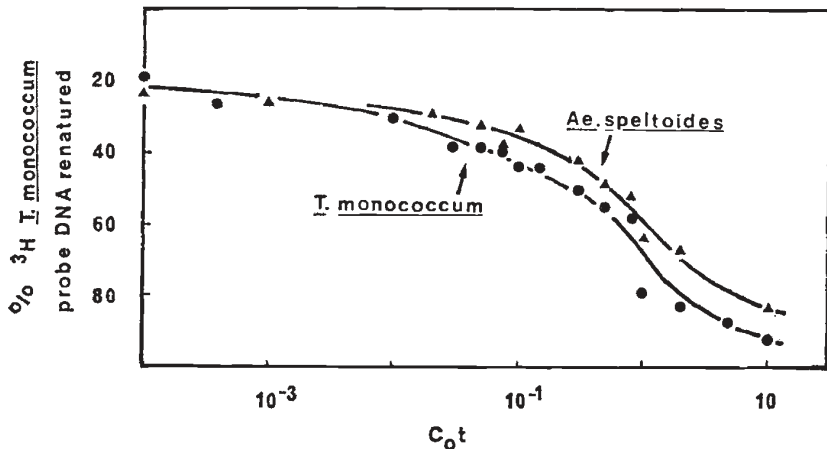


FIG. 3.—Hybridisation of *T. monococcum* highly repeated sequences to *Ae. speltooides* and *T. monococcum* repeated sequences. "Nick translated" *T. monococcum* DNA was incubated with a large excess of denatured, sheared unlabelled *Ae. speltooides* ( $\blacktriangle$ — $\blacktriangle$ ) or *T. monococcum* ( $\bullet$ — $\bullet$ ) DNA in 0.12 M phosphate buffer at 60°C. The proportion of labelled DNA in the duplex fraction was determined by hydroxyapatite chromatography.

*T. monococcum* appears not to have sequences (detectable by the methods employed here) in such a high copy number as does *Ae. speltooides* where renaturation started around  $C_0t$   $10^{-4}$  (fig. 2).

#### 4. DISCUSSION

The extent of hybridisation between DNAs from *Triticum*, *Aegilops* and *Secale* species indicates that the repeated sequence complements of these genomes are very similar. Most of the repeated sequence families in each of these species are related to families in each of the other species. This is expected from studies for such closely related species (Flavell *et al.*, 1977). The degree of similarity of repeated sequences between closely related species is an important topic for study but in this paper we have focused upon the detection and analysis of repeated sequence differences between the species. From the analysis of sequence differences it is possible to infer how chromosomes change during the evolution of distinct species.

(i) *Highly repeated sequence DNA and species divergence*

The classification of all the species studied in this paper into different genera or groups by cytogeneticists and taxonomists has not been straightforward (Mackey, 1954; Bowden, 1959). One difficulty is that within the range of *Triticum* species there are diploid, tetraploid and hexaploid forms, these polyploid forms having been derived by combination of a diploid from the *Triticum* group of species with diploids classified as *Aegilops* species. Thus separation of *Triticum* species and *Aegilops* species into different genera leads to difficulties when the polyploid forms of *Triticum* are considered.

The studies reported in this paper clearly indicate that the most highly repeated sequence DNA fraction of the diploid *Aegilops* representative we selected (*Aegilops speltoides*) is more closely related to the DNAs of *Aegilops* species than to the DNAs of diploid *Triticum* species (*Triticum monococcum* and *Triticum boeoticum* isolates *T. thaouadar*, *T. aegilopoides* and *T. urartu*, see table 3). The separation of diploid *Triticum* and *Aegilops* groups using DNA hybridisation techniques, correlates well with the separation of *Triticum* and *Aegilops* species into different genera. However, the distinction between *Aegilops* species and diploid *Triticum* species by DNA hybridisation is not complete; members of the vertebrata section of the *Aegilops* appear close to the diploid *Triticum* species (table 3). As would be expected, the polyploid *Triticum* species which have *Aegilops* species in their parentage give hybridisation results more like *Aegilops* species than *Triticum* species, using highly repeated sequences from *Aegilops speltoides* as probe DNA.

The *Aegilops* genus has been divided into six sections on the basis of morphological and cytogenetic criteria (see table 3 and Kihara and Tanaka, 1970). It is interesting to note that the diploid and tetraploid species, classified in the vertebrata section on the basis of ear morphology, appear to show lower hybridisation to the *Aegilops speltoides*  $10^{-2}$  DNA probe than all the other *Aegilops* species. This result suggests highly repeated sequence DNA comparisons may also be useful for classifying species into different sections of a genus. However, considerably more comparisons carried out in a more detailed way would be necessary to distinguish reliably all the various *Aegilops* sections.

Although the *Aegilops speltoides*  $C_{0t}$   $10^{-2}$  DNA hybridised more to *Aegilops* species' DNAs than to diploid *Triticum* species DNAs, the hybridisation to homologous *Aegilops speltoides* DNA was considerably greater (table 3). This suggests that *Aegilops speltoides* possesses a very highly repeated sequence DNA pattern which is species-specific. The *T. monococcum* and *T. boeoticum* accessions we studied differed in their hybridisation with the  $C_{0t}$   $10^{-2}$  DNA from *Ae. speltoides* (table 3). This also points to species-specific patterns of highly repeated sequences.

In a previous paper we showed that quantitative changes in families of repeated sequences occur sufficiently often during evolution to enable species from the related genera *Triticum*, *Secale*, *Hordeum* and *Avena* to be distinguished easily. Furthermore, the magnitude of the DNA differences between the species was related to the extent of the evolutionary divergence that had occurred between the genera.

The species studied in this paper were more closely related to one another than those studied previously. The magnitude of the repeated

sequence DNA differences were also considerably less, endorsing the correlation between species divergence and repeated sequence DNA divergence. Our ability to detect repeated sequence DNA differences between species within a genus supports the hypothesis that speciation is always accompanied by quantitative nucleotide sequence changes. The possibility that these chromosomal changes might drive speciation is particularly interesting. If gross chromosomal changes are major factors in causing speciation then changes such as those we have recognised here in the repeated sequence fraction of the chromosomes cannot be ignored in discussions of speciation (see Jones, 1972; Hatch *et al.*, 1976; Jones *et al.*, 1976).

(ii) *Highly repeated sequences and the diploid genomes of hexaploid wheat*

The similar hybridisation of labelled hexaploid wheat DNA to *Ae. speltooides* and *Ae. squarrosa* DNAs as to hexaploid wheat DNA (fig. 1) implies that all the families of repeated sequences in the A, B and D genomes of wheat are found in the B and D genomes, assuming *Ae. speltooides* and *Ae. squarrosa* are closely related to the B and D genomes respectively of hexaploid wheat. Some repeated sequences were detected in *T. monococcum* rapidly reannealing DNA which did not hybridise to *Ae. speltooides* DNA (fig. 3) but these accounted for about only 0.5 per cent of the *T. monococcum* genome and so would not be detectable in the experiments of fig. 1, even assuming they were present in the A genome of hexaploid wheat. The presence of the same families in different genomes does not necessarily imply that each family contains the same number of copies of a repeat in each genome. The lower hybridisation of  $C_0t\ 10^{-2}$  DNA from hexaploid wheat to *Ae. squarrosa* DNA than to *T. aestivum* and *T. dicoccoides* DNA (table 2) implies that the highly repeated sequences of hexaploid wheat are present in lower copy numbers in *Ae. squarrosa*.

Approximately 11 per cent of the hexaploid wheat genome appears to consist of repeated sequences not found substantially repeated in *T. monococcum* (table 1). How many families are in this 11 per cent and how many repeats of each kind of sequence cannot be inferred from results obtained so far. A significant fraction of the 11 per cent might be sequences repeated over  $0.25 \times 10^6$  times in the hexaploid wheat genome since the  $C_0t\ 10^{-2}$  fractions of hexaploid wheat showed low hybridisation to *T. boeoticum* DNA (table 2). However, most of the repeated sequences not detected in *T. monococcum* are probably of lower copy number, renaturing between  $C_0t\ 10^{-2}$  and  $C_0t\ 10$ . This conclusion is supported by the results in fig. 1 where lower hybridisation of wheat DNA to *T. monococcum* DNA was detected only at higher  $C_0t$  values.

It has already been argued in the results section that the ability of DNAs, from different *Triticum* and *Aegilops* species of differing ploidy, to compete for the highly repeated sequences in the  $C_0t\ 10^{-2}$  DNA fraction of hexaploid wheat (table 2) implies that a significant proportion of the very highly repeated sequences in this  $C_0t\ 10^{-2}$  fraction come predominantly from the B genome chromosomes. Some of these sequences are repeated in the A and D genomes, possibly in a lower frequency, while a small proportion are not found in the repeated sequence fractions of the A genome. The similarity of the  $C_0t\ 10^{-2}$  DNA fractions from hexaploid wheat and from *Aegilops speltooides* with respect to their hybridisation patterns to *Triticum* and *Aegilops*

DNAs (tables 2 and 3) is consistent with the B genome of hexaploid wheat coming from *Aegilops speltoides* or a closely related diploid. *Triticum urartu*, suggested as a possible B genome donor on the basis of grain proteins (Johnson, 1975), does not possess a highly repeated sequence complement similar to that of the B genome of wheat (table 3).

The concentration in the B genome of the most highly repeated sequences of hexaploid wheat suggests this fraction may be an easily isolated source of sequences to distinguish B genome chromosomes from A and D genome chromosomes following its *in situ* hybridisation to wheat metaphase chromosomes. This was indeed shown to be the case by Gerlach (personal communication). The  $C_0t$   $10^{-2}$  fraction from *Aegilops speltoides* would also be expected to be a similarly useful *in situ* hybridisation marker DNA for B genome chromosomes.

Several laboratories have shown that a satellite can be isolated from hexaploid wheat DNA after centrifugation in  $Ag^+$   $Cs_2SO_4$  gradients (Huguet and Jouanin, 1972; Ranjekar *et al.*, 1976; Peacock *et al.*, 1977). This simple sequence satellite hybridises strongly to all the B genome chromosomes and to two A genome chromosomes of hexaploid wheat but hardly at all to the remaining A and D genome chromosomes when tested by *in situ* hybridisation (Peacock *et al.*, 1977). These satellite sequences are present in the  $C_0t$   $10^{-2}$  fraction from hexaploid wheat but since they occupy only 0.1 per cent of the wheat genome they must constitute only a small proportion of all the  $C_0t$   $10^{-2}$  DNA. Nevertheless they are responsible for some of the preferential hybridisation of the  $C_0t$   $10^{-2}$  DNA to the B genome-like species. This satellite is also present in *Aegilops speltoides* DNA but is not readily detectable in  $Ag^+$   $Cs_2SO_4$  gradients of *Triticum monococcum* DNA (unpublished results). This finding substantiates the claim that the species closely related to the A and B genome donors of hexaploid wheat differ in the amounts of  $Ag^+$  binding "satellite" repeated sequences they possess.

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