Pl. Syst Evol. 160, 39 – 59

Plant Systematics and Evolution

Amplification and dispersion of repeated DNA sequences in the *Triticeae*

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Received August 12, 1987

Key words: Angiosperms, *Poaceae, Triticeae.* – Evolution, dispersed repeated sequences.

Abstract: Four representatives of a family of dispersed repetitive sequences which were prominent and dispersed in the E genome of *Thinopyrum elongatum* but poorly represented in wheat, were studied in detail. The 1.4 kb sequences were present both as part of tandem and more complex arrays and appeared to have resulted from repeated amplification of the sequence and their dispersion throughout the genome. Subcloning of sections of the 1.4 kb sequences resulted in probes which improved the resolution of the E genome from the genomes in wheat and enabled identification of single E genome chromosomes introduced into wheat. The generality of these types of sequences in the tribe *Triticeae* was confirmed by isolating analogous sequences from the R (rye, *Secale cereale*), V (*Dasypyrum villosum*), and N (*Psathyrostachys juncea*) genomes. – The cloned repetitive sequences from the R, V, and N genomes each showed characteristic fluctuations in amount within the grasses examined in addition to being virtually absent from wheat. It is thus possible that these sequences may provide useful taxonomic indicators for establishing relationships within the *Triticeae*, as well as valuable probes for tracing alien chromatin introduced into wheat.

The grasses of the tribe *Triticeae* (*Hordeae*) provide a useful source of new genetic variation for the improvement of wheat. The increasing use of these grasses in wheat breeding has led to the need to understand the genomic structure of these grasses in more detail. Dewey (1982, 1984) has reviewed a classification of the perennial *Triticeae* grasses based on both genomic relationships, as defined by meiotic-pairing in interspecific and intergeneric hybrids, and more traditional taxonomic data, including morphology, geographical distribution and ecological adaptation. This system of classification has been used in the present study as a starting point for the DNA analysis of grasses in the *Triticeae*. The aims were to provide new DNA markers which can be used to trace alien chromatin following its introduction into wheat, as well as to initiate characterization of the genomes.

Eukaryotic genomes are characterized by the large variation in nuclear DNA content, even within closely related species possessing the same chromosome number; *Vicia* spp. with the same chromosome number, for example, can exhibit a 6-fold range in nuclear DNA content (Chooi 1971, Straus 1972). A major proportion of the observed variation can be explained by differences in the moderately repeated

class of sequences (Chooi 1971, Straus 1972, Miksche & Hotta 1973, Mizuno & MacGregor 1974, Baldari & Amaldi 1976, Narayan & Rees 1976, 1977, Hutchinson & al. 1980).

Moderately repeated DNA sequences (reiteration frequency 10^1 to 10^5) are usually interspersed between unrelated, often single copy, DNA and rarely occur in long tandem arrays. The component generally has a very similar G-C content to the entire genome and many sequence elements are transciptionally active. Individual members of a family may show considerable (3 – 20%) sequence divergence and, in general, both the consensus sequence and copy number of a family are conserved among closely related genomes. Such features distinguish the moderately repeated sequence families from the satellite or simple sequence class of DNA (Lewin 1974, Bouchard 1982).

Detailed studies on some organisms possessing large genomes with a large moderately repeated component, as found in Salamander, cereals and *Lilium* spp., have shown that the increase in amount of this class of sequence has occurred predominantly by the addition of new sequence families. These arise from the amplification of sequences not detectably repetitive in other related lineages of the respective organisms (Salamander: Mizuno & MacGregor 1974, Mizuno & al. 1976; cereals: Flavell & al. 1977, Rimpau & al. 1978, 1980; *Lilium* spp.: Bouchard & Stern 1980). In other organisms with a smaller moderately repeated component, such as chicken (Eden & al. 1978) and sea urchins (Harpold & Craig 1977, Moore & al. 1978, Klein & al. 1978), the new families appear to arise from sequences already somewhat repeated in related organisms, but at a considerably lower copy number.

The large size of cereal genomes (5 – 18 pg, Bennett 1972) initially led to their characterization in terms of families of sequences as defined by renaturation analyses (Flavell & Smith 1976, Smith & Flavell 1977, Appels & al. 1978, Ranjekar & al. 1978, Rimpau & al. 1978, Flavell & al. 1980 a, b, Hake & Walbot 1980). Several important features of these early analyses include the observations that >60% of the genome of maize, and >70% of the genomes of wheat, rye, barley and oats were in the moderately repeated class. Secondly, approximately $^2/_3$ of the genomes were organized such that repeats were interspersed with other repeats or single-copy DNA. Thirdly, families of repeated sequences are present in closely related species. For example, maize, teosinte and *Tripsacum* share a 180 bp repeat family that is a major constituent of knob heterochromatin (Peacock & al. 1981). At least 23% of the moderately repeated component of wheat, rye, barley and oats, or approx. 17% of the total genome is common to all four genomes. The more closely related species (e.g., wheat and rye) share a greater proportion of the repeated sequence component than do more distantly related species (e.g., wheat and oats). Fourthly, a substantial proportion of each genome consisted of moderately repeated sequences in a category classified as species-specific; 21.6% (16%), 29.7% (22%), 39.4% (28%) and 77.3% (58%) of the moderately repeated sequence component (and of the total genomes) of wheat, rye, barley and oats, respectively. The potential for cloned sequences in the "species-specific" category to assay alien chromatin in wheat has been discussed in Appels & Moran (1984) and provide, for example, a specific assay for rye chromosomes in *Triticale*, using the in situ hybridization technique. In addition, the distribution and amount of species-specific and/or shared families of repeated sequences can be used as a phylogenetic or

taxonomic tool (for examples see JONES & FLAVELL 1982, DVORAK & APPELS 1982, ARNOLD & al. 1985). In the present paper, the properties of four different repeated sequence families from the R (*Secale cereale*), V (*Dasypyrum villosum*), N (*Psathyrostachys juncea*), and E (*Thinopyrum elongatum*) genomes are presented.

Material and methods

Plant material. Information on the various grasses used in experiments is given in Table 1.

Cloning. The isolation of DNA clones from the V, E, and R genomes in λ 1059 has been described in APPELS & MORAN (1984). The N genome was cloned, by similar procedures, into the λ vector EMBL 4 (Amersham). Subcloning of portions of the cloned DNA which were responsible for the "species-specific" hybridization was carried out by restriction enzyme digestion of the E, N, and V genome λ clones with *Eco* R 1, and of the R genome λ clone with *Hind* 111. The restriction fragments were electroeluted from agarose gels and ligated into the plasmid pBR 322. The plasmid was linearized with the appropriate restriction enzyme (*Eco* R 1 or *Hind* 111) and treated with alkaline phosphatase (Boehringer) before use. The ligated plasmids were used to transform *E. coli* RR 1 cells. Colonies were selected and screened with the respective radioactively labelled λ clones for plasmids with genomic insert DNA.

DNA sequencing. Sequencing of DNA was achieved by transferring the respective DNA segment into the vector M 13 mp 10 and using the "dideoxy" procedure of SANGER & al. (1977); sequencing kits were obtained from BRESA (South Australia). Reactions were

Table 1. Grasses used in this study

Species	Genome	Source
Agropryon cristatum (L.) GAERTNER	P	D. R. DEWEY PI 314599 U.S.S.R.
Dasypyrum villosum (L.) SCHUR.	V	Aust. Wheat Coll. 17953
Psathyrostachys juncea (FISCH.) NEVSKI	N	D. R. DEWEY PI 314082 U.S.S.R.
Pseudoroegneria spicata (PURSH) LÖVE	S	D. R. DEWEY cv. "Whitmar"
Secale cereale L.	R	C. DRISCOLL cv. "Imperial"
Thinopyrum elongatum (HOST) D. R. DEWEY	E	J. DVORAK
Thinopyrum junceiforme (L. + L.) LÖVE	J_1J_2	D. R. DEWEY CS-6-61..70 Fr.
Triticum aestivum L.	ABD	cv. "Chinese Spring"
Th. elongatum "Chinese Spring" addition lines		J. DVORAK
D. villosum "Chinese Spring" addition lines		E. R. SEARS

analyzed on 4, 6 or 8% polyacrylamide gels and exposed to X-ray film either after drying the gel onto one of the glass plates used to support the gel or transferring the gel to Whatman 3 mm paper.

DNA-DNA hybridization. Genomic DNA samples were analyzed, after restriction endonuclease digestion and electrophoresis in 1 or 1.5% agarose gels, by transferring them to Gene-screen (NEN), following manufacturers instructions. The membrane filters to which the DNA was bound were baked at 80 °C under vacuum for 2 – 3 h followed by pretreatment in PH buffer [50% formamide, 3X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na citrate), 0.01 M Tris-HCl pH 8.0, 0.001 M EDTA, 0.1% SDS, 0.1% polyvinylpyrrolidone, 0.1% BSA, 0.1% ficoll] for 1 – 2 h. They were then transferred to PH plus probe (heated to 95 °C to denature the probe). ^{32}P probes were prepared using a nick-translation kit (BRESA, South Australia). Hybridization reactions were carried out at 37 °C for approx. 12 h before washing in four changes of 2X SSC/0.1% SDS solution (initially at 65 °C) over a period of 1 – 3 h. The washed filters were wrapped in food wrap and exposed to flashed X-ray film at – 80 °C.

In situ hybridization. In situ hybridizations of ^{3}H-cRNA to root tip mitotic chromosomes were carried out as previously described (Appels & al. 1978), except that 24 h iced water treatment was used instead of colchicine as the prefixative. ^{3}H-cRNA copies of the 1.4 kb sequence, pEleAcc 2 and pPjE 2-T 1 were obtained using *E. coli* RNA polymerase I (Boehringer) as per manufacturers instructions.

Determination of genomic proportions of 1.4 kb family. 0, 0.5, 1, 2, 4, 7, and 10 µg quantities of DNA from *Secale cereale, Triticum aestivum* cv. "Chinese Spring", *Dasypyrum villosum, Pseudoroegneria spicata, Psathyrostachys juncea, Agropyron cristatum, Thinopyrum junceiforme* and *Thinopyrum elongatum,* and 0, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.3 µg amounts of λ Ele 145 were loaded onto a nitro-cellulose filter in a dot-blot apparatus (Bethesda) under vacuum. After alkaline denaturation (in 1.5 M NaCl, 0.5 M NaOH) and neutralization in 0.025 M phosphate buffer pH 6.5 (1 M buffer contains 680 mls Na_2HPO_4 and 320 mls NaH_2PO_4), the filter was baked as per manufacturers instructions. Prior to hybridization, the filter was washed in the hybridization solution without probe for 1 – 2 h (see above). The filter was hybridized to ^{32}P- pEleAcc 2 (using a nick translation kit from BRESA). Differences in dot intensity were quantified through densitometry tracings as previously described by Kafatos & al. (1979).

Results

The 1.4 kb family of repeated sequences from the E genome

Two λ clones containing E genome DNA which strongly hybridized to genomic *Th. elongatum* DNA but exhibited little cross hybridization to wheat had previously been identified by Appels & Moran (1984). Restriction enzyme maps of the two clones were determined using double digestions with two restriction enzymes (Fig. 1); regions that cross hybridized within and between parts of the clones are shown. λ Ele 145 contains part of a tandem array of unit length 7.7 kb. Subcloning of parts of the tandem repeat using *Eco*R 1 revealed that the 1.4 kb segment (black box) was responsible for the major proportion of the hybridization to genomic DNA (data not shown). This sequence comprises approx. 0.02% of the genome (see variation section) and we designate this family the 1.4 kb family.

The second λ clone, λ Ele 143, cross hybridized strongly to λ Ele 145 and also contained representatives of the 1.4 kb family of repeated sequences (black boxes).

The degree of relatedness among 1.4 kb segments. The four 1.4 kb units present in the two λ clones (Fig. 1) were isolated and sequenced. Units A and B derive

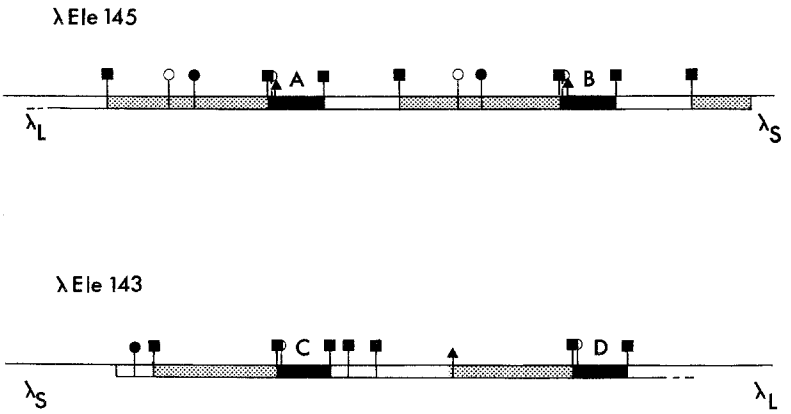

Fig. 1. Restriction maps of λ Ele 143 and λ Ele 145. The restriction sites for *Eco* R 1 (■), *Xho* 1 (○), *Hind* 111 (●), and *Bgl* 11 (▲) are illustrated for both E genome λ clones (APPELS & MORAN 1984). Fragments similarly shaded cross hybridized strongly within and between clones. The black boxes, labelled A, B, C, D, are the four 1.4 kb subclones responsible for the "E genome specific" hybridization. λ S and λ L refer to the short and the long arms of λ 1059, respectively

Table 2. Number of variable positions between 1.4 kb segments from λ Ele 145 and λ Ele 143

	143 D	143 C	145 B	145 A
145 A	78	80	1	–
145 B	79	81	–	
143 C	3	–		
143 D	–			

Table 3. Number of base pair differences between internal 186 bp duplications in the 1.4 kb units from λ Ele 145 and λ Ele 143

	λ 143		λ 145		λ 143		λ145	
	D 2	C 2	B 2	A 2	D 1	C 1	B 1	A 1
A 1	40	39	41	41	16	16	0	–
λ 145								
B 1	40	39	41	41	16	16	–	
C 1	37	36	37	37	2	–		
λ 143								
D 1	36	35	36	36	–			
A 2	11	10	0	–				
λ 145								
B 2	11	10	–					
C 2	1	–						
λ 143								
D 2	–							

from λ Ele 145, and C and D from λ Ele 143. The units were compared in a matrix to assess the number of base pair differences in each pairwise comparison. The results are summarized in Table 2 and show that 1.4 kb units from the same λ clone are more like each other than those from different clones, i.e., A and B (1 base difference), and C and D (3 bases different) are more like each other than A with C (80 variable positions) or D (78 variable positions), or B with C (81 different bases) or D (79 different bases).

Each 1.4 kb unit contains a duplication of 186 bp. The sequence of one of the 1.4 kb units from λEle145 is shown in Fig. 2. The position of a 186 bp duplication is indicated and pairwise comparisons of all 8 duplications from both λ Ele 143 and λ Ele 145 are summarized in the matrix of Table 3. Each duplication was numbered 1 and 2 from the 5′ end of the 1.4 kb unit and identified as originating from 1.4 kb unit A (A 1, A 2), B (B 1, B 2), C (C 1, C 2) or D (D 1, D 2) (see Fig. 1). The duplications fall into obvious groups on the basis of the number of base pair differences in the pairwise comparisons. The lowest numbers of differences are

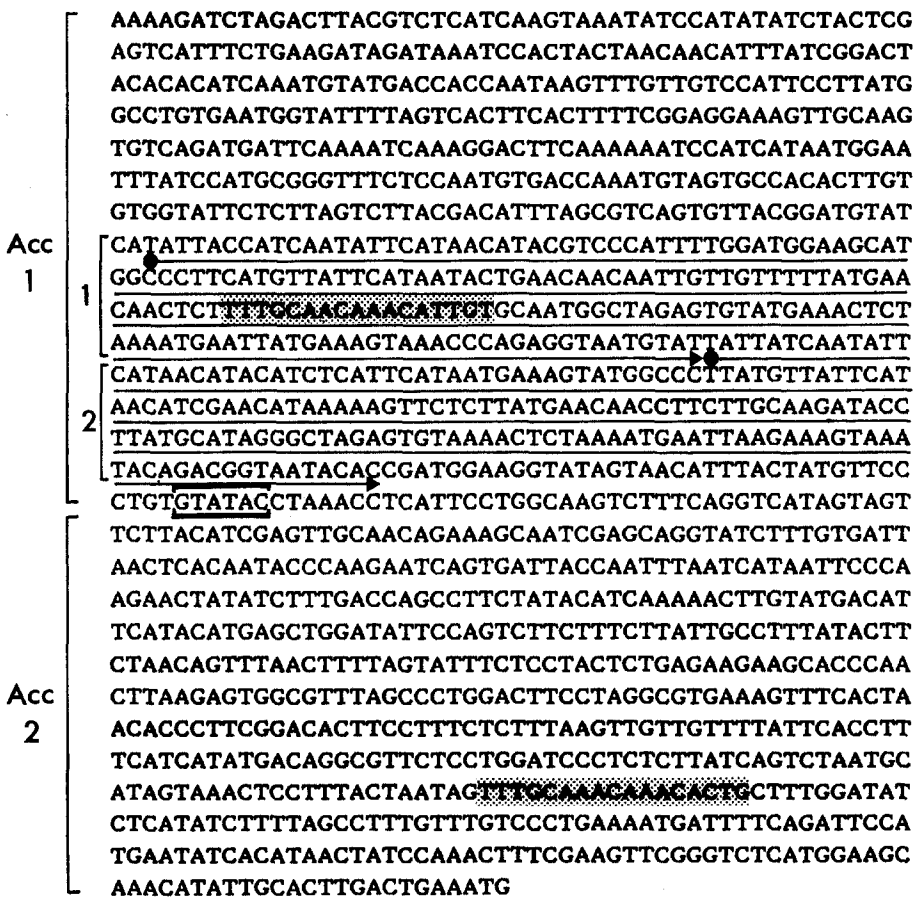

Fig. 2. Nucleotide sequence of a 1.4 kb unit. The DNA sequence of the 1.4 kb unit designated A from λ Ele 145 is illustrated. The arrows mark the position of the 186 bp direct duplication; the repeats were numbered 1 and 2, in each 1.4 kb unit, as shown. The stippled region represents a 17 bp duplication, with a single base mismatch. The *Acc* 1 site, GTATAC, used to generate the two subclones pEleAcc 1 and pEleAcc 2, is also shown

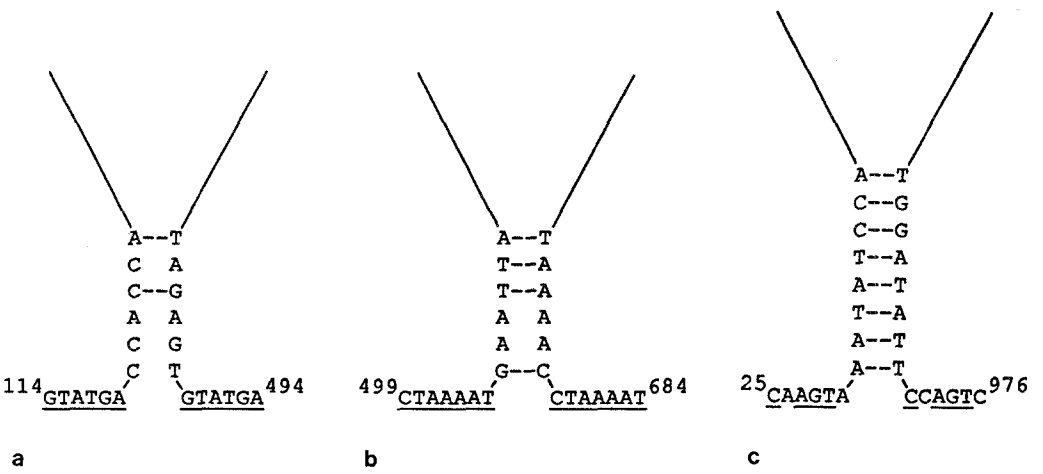

Fig. 3. *a, b* Direct repeats flank imperfect inverted repeats. *a* A 7 base pair direct repeat, flanking an imperfect (4 out of 6 bases) inverted repeat, is shown; the loop is 162 bases in length. The two direct repeats occur such that one is in each 186 bp larger direct repeat. *b* A 6 base pair direct repeat flanks an imperfect (3 out of 6 bases) inverted repeat producing a 357 bp loop. The second 6 bp repeat occurs in the number 1 186 bp repeat. *c* Inverted repeats flanked by imperfect direct repeats. An 8 bp inverted repeat, flanked by an imperfect (4 out of 6 bases) direct repeat is illustrated. The resulting loop is 926 bp in length

observed between A 1 and B 1, C 1 and D 1, A 2 and B 2, C 2 and D 2. The next lowest group with 10 – 16 base pair differences comprises A 1 with D 1, A 2 with C 2 etc. and represent pairwise comparisons between two number 1 or two number 2 duplications from any two of the four 1.4 kb units. The highest number of differences are observed in pairwise comparisons of a number 1 and a number 2 duplication regardless of whether the duplications originated from the same or different λ clones. These comparisons suggest an evolutionary order of assembly of various components in the λ clones illustrated in Fig. 1. The duplication of the 186 bp region must have preceded the amplification and dispersal of the 1.4 kb units because the number 1 duplications in each 1.4 kb unit are more alike than any number 1 and number 2 duplication from the same 1.4 kb unit. The 186 bp region is envisaged to have been duplicated first, the two duplications accumulated random changes in nucleotide sequence and the 1.4 kb unit, with the two diverged 186 bp duplications, amplified and dispersed throughout the genome.

The 1.4 kb units also contain smaller duplications. A small duplication of 17 bp has been identified in the 1.4 kb units and is marked in Fig. 2. The 17 bp duplications have only two base-pair differences in the unit illustrated, and one of the duplications is located in the number 1 186 bp unit discussed above. Numerous other smaller direct repeats are also present in the unit. Two such repeats flank imperfect inverted repeats and are illustrated in Fig. 3 a and b. In addition, an 8 bp inverted repeat is flanked by imperfect direct repeats (Fig. 3 c). Such contiguous direct and inverted repeats may represent the remnants of transposable elements (reviewed in NEVERS & al. 1986).

Several regions of dyad symmetry have been identified in the 1.4 kb units; for example ^{427}AACAACAATTGTTGTT^{443} and ^{1273}CTGAAAATGATTTTCAG^{1289}.

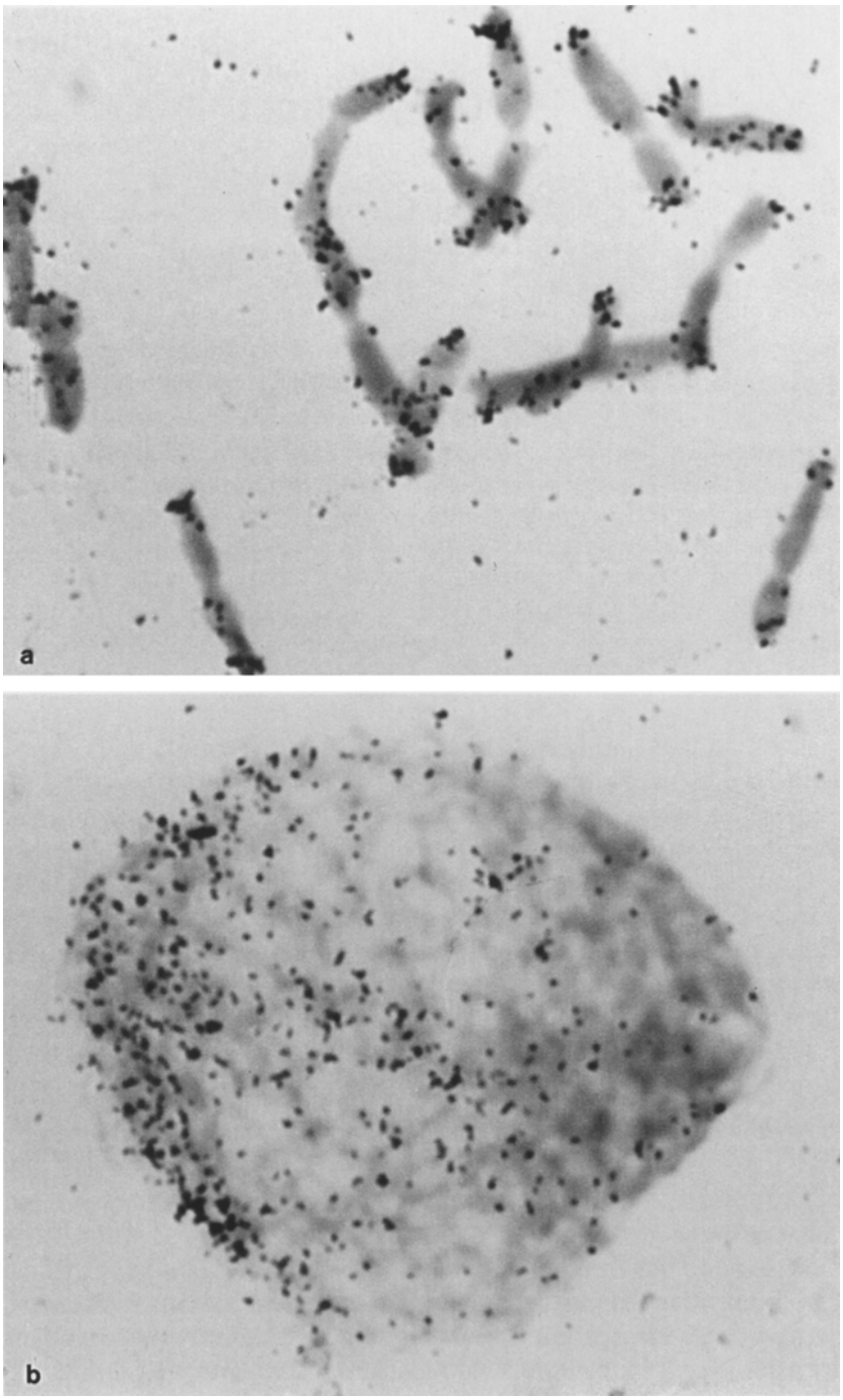

Fig. 4. In situ hybridization of the 1.4 kb family to the E genome of *Th. elongatum*. In situ hybridization to E genome chromosomes at interphase (*b*) and metaphase (*a*) demonstrates the dispersed nature of this family of repeated sequence. The probe is dispersed throughout the genome, on all 14 chromosome arms. There is some localization of silver grains at the telomeres of some chromosomes suggesting that blocks of 1.4 kb sequences occur here

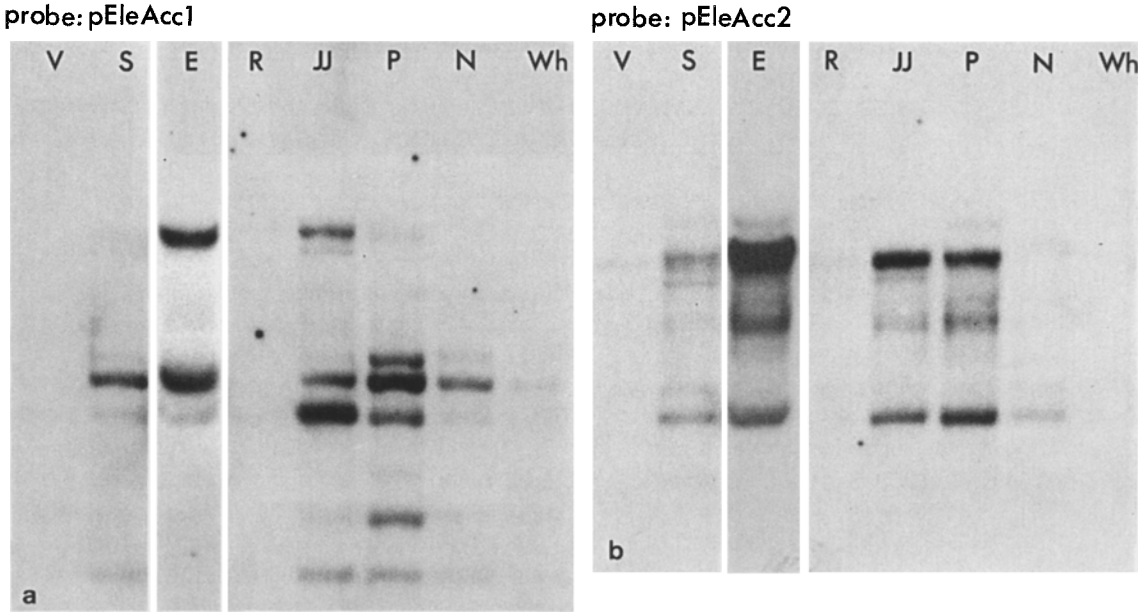

Fig. 5. *Hae* 111 digests of DNA from the V, S, E, R, J_1J_2, P, N, and Wh (wheat, ABD) genomes. *a* Probed with pEleAcc 1, *b* probed with pEleAcc 2. 20 µg quantities of DNA from each of the V, S, E, R, J_1J_2, P, N, and Wh (wheat, ABD) genomes were digested with the restriction endonuclease *Hae* 111. The digests were halved and loaded on two 1.5% agarose gels. The gels were blotted (see Materials and methods) and the filters hybridized with either pEleAcc 1 or pEleAcc 2

Chromosomal distribution of the 1.4 kb family in the E genome of *Th. elongatum*. In situ hybridization experiments using the subclone pEleAcc 2 (see Fig. 2) as a probe revealed that the 1.4 kb units are dispersed throughout much of the E genome of *Th. elongatum* (Fig. 4). The concentration of silver grains at the telomeres of some chromosomes suggests that blocks of the 1.4 kb units are located at these sites, in addition to being distributed along the rest of the chromosome.

Variation in the amount of the 1.4 kb family among a selection of related grasses in the tribe *Triticeae*. The probes pEleAcc 1 and pEleAcc 2, (see Fig. 2), were used to assay the variation in copy number of the 1.4 kb family in a selection of perennial grasses (listed in Table 1) representing different genomes in the tribe *Triticeae* (Fig. 5 a and b). Similar hybridization intensities are observed for the E, S, P, and J_1J_2 genomes, with considerably less hybridization to the V, N, R, and ABD (wheat) genomes.

Dot blot analysis of the same grasses, using pEleAcc 2, supports the variation seen in Fig. 5. The subclone pEleAcc 2 represents 0.02% of the E and P genomes, 0.01% of the J_1J_2 genomes, 0.007% of the S genomes and a much smaller proportion of the V, N, R and ABD genomes (data not shown).

An important feature of the variation shown in Fig. 5 is the very low levels of the 1.4 kb family present in wheat, as detected using either of these 1.4 kb unit derived probes; this was the initial criterion used to select the E genome λ clones, λ Ele 145 and λ Ele 143 (Appels & Moran 1984) and the specificity of the probe for the E genome was much improved by the subcloning of small sections of the

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pPj8E3      CATCACGGTCAAGTGCAATATGCTTGTTTCCATGAGACCCAAACTTCGAA
1.4kb unit  CATTTCAGTCAAGTGCAATATGTTTGCTTCCATGAGACCCAA.CTTCGAA

            AGTTTGGGATAGTTATGTGATATTCATGGAA.CTGAAAATTAGTTTCGAC
            AGTTT.GGATAGTTATGTGATATTCATGGAATCTGAAAATCATTTTCAGG

            TGCAGCCCAAGCTTGGCGTAATCATCGTCATAGCTGTTTCCTGTGTGAAA
            GACAAACAAAGGCTAAAAGATATGAGATATCCAAAGCAGTGTTTGTTTGC
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Fig. 6. Sequence homology between pPj 8.E 3 (N genome) and the 1.4 kb family (E genome). The partial nucleotide sequence of pPj 8.E 3, a subclone from an N genome λ clone, and 145 A, the 1.4 kb unit designated A from λ Ele 145, is shown; the homologies are underlined. The region of homology begins with one of the *Eco* R 1 sites, the enzyme used to derive both fragments, before terminating abruptly 100 bp further along. 145 A has been inverted relative to the sequence shown in Fig. 2

original clone. The subclone pEleAcc 2 is particularly noteworthy in its extremely low levels in wheat.

The presence of the 1.4 kb family of DNA from the E genome in several other grasses from the tribe *Triticeae* provided an opportunity for examining the family in a genome well separated from the E genome at an evolutionary level. A sequence was recovered from the N genome, in a routine screen, which showed homology to the E genome 1.4 kb family. The clone, pPj 8.E 3, was recovered in the clone λ Pj 8.1, and is discussed in more detail in the following section. Partial sequencing of pPj 8.E 3 showed that its homology with the 1.4 kb family began from one *Eco*R 1 site (3′ end, Fig. 2) and extended approx. 100 bp before terminating abruptly, as shown in Fig. 6. This observation is consistent with the dispersed nature of the 1.4 kb family of DNA in that many different DNA sequences would be expected to flank and form junctions with the 1.4 kb family. The existence of a clear junction of the type shown in Fig. 6 suggests that a particular portion of an ancient DNA sequence plus its adjoining DNA were amplified to form the present day 1.4 kb family in the E genome. The portions of the 1.4 kb family in the N genome may represent sequences which have not been amplified in the N genome, as has occurred in the E genome. An alternative proposal is that the abrupt junction observed in the N genome clone may have resulted from an insertion of an unrelated DNA sequence into the 1.4 kb unit.

A repetitive sequence family from the N genome

A λ clone, λ Pj 8.1, was isolated from *Psathyrostachys juncea* as an N genome specific sequence; it hybridized strongly to the N genome with very little detectable hybridization to wheat DNA. Restriction endonuclease digestion with *Eco* R 1 indicated that within this clone there were two cross-hybridizing sequences, designated E 1 and E 2. The restriction endonuclease *Taq* 1 was used to subclone portions of E 1 and E 2 to isolate the fragments primarily responsible for the N genome "specific" hybridization. The fragments, designated E 1 − T 1 and E 2 − T 1, are shown in Fig. 7 with the region of homology between them underlined. These sequences, plus others in the genome with which they hybridize, will be referred to as the N 8-family of sequences. Another fragment from the λ Pj 8.1 clone,

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E1-T1 GTCGAACTAAGATTGAAACAACAATCTCTCTAC.TGGGTTGCACACATACGGTGTCAGCTATCCGG
E2-T1 GTCGAACTAAGATCGAAACAACAATCTCTCTACATGGGTTGCACACATACGGTGTCAGCTATCCGG

      TAGGGCTTCGCCGTCCAGAACTATTTCCTATCGGAGACTAGACGGCCTTTCGGCAGTACGAAACTA
      CAGGGCTTCGC.GTCCAGAACTATTTCCTATCGGAGACTAGATGGCCTTTCGGCAGTACGAAACTA

      TTTC.GTGTGAGGGAGAGAAAGCAAGCCAAATAATTGCATGGCATATGTGTTGAGAGAAATCTGAA
      TTTTTGTGTGAGGGAGAGAAAGCAAGCCAGATAATTGCATGGCATATGTGTTGAGAGAAATCTGAT

      TTTGGAGAGCTCTCACTACCTCTATTTATAGGAGAAGCAAGGGGTAGAGGCTATGGGGAAATTACC
      TTTGGAGAGCTCTCTCCACCTCTATTTATAGGAGAAGCAAGGGGTAGAGGCTATGGGAGAATTACC

      AAAAAAACCCATAACTTAGCCACCAAGTAACTTAGCCACTAAGTCACATAAAGGTGGCAAGTGGGA
      AAAAAAACCCATAACTTAGCCACCAAGTAACTTAGCCACTAAGTCACATAAAGGTGGCAAGTGGGA

      GCTCCACTCCAAACGTCCAAGCATGTGTGGGGCCCCCGATTTGGGGCCCCCACCACCCCATGTGCT
      GCTCCACTCCAAATGTCAAAGCATGTGTGGGTCCCCCTATTTGGGGCCCCCACCACCCCATGTGCT

      TCTCCATAAAGGAAAATTAGTTCT.AGAAGAAAAACCCAAAAAGTGGCTTTTCCAAAAAGTCACCA
      TCTCCATAAAGGGAAATTCCTTCTTAGAAGAGAAACCCAAAA.GTGGCTTTTCCAAAAAGTCACCA

      AATCATGCTTTTCTTCCTAAAAGTGATTCCACTTGTCATTCACTATTCATGAGAACTTTTCATGAA
      AATCATGTTTTTCTTCCTAAAAGTGATTCCACTTGTCATTCACTATTCATGAGAACTTTTCATGAA

      ATATTTTTCCGTGACCATTTTTATTCGGAAAAGATTTCTGAAGGTCC.AGAACATTTCCGGCACCC
      ATATTTTTCCGTGACCATTTTTATCCGGAAAAGATTTTTGAAGGTCCTTGAACATTTTTGGCACCC

      CTTAAAATATTTCTGGACGTGTTCCAAAACATTTTTGGTAAGATGACTTTTATCTCCGAACCATTT
      CTTAAAATAATTCTGGACGCGTTCCGAAACATTTTCGGTAAGATGAATTTTATCTCCGAAACATTT

      TCGGTAACTCCGAAACACTTCCGATTTTCTCTCTGAAACTTTTT.CGTTGTCTCTAAAACTTTTCC
      .CGGTAACTCCGAAACATTTCGG.TTTTCTCTCTAAAACTTTTTTTGTTGTCTCTGAAACTTTTCC

      GGTGACTTTCTCTCAGACTCCTTGTCTAGTACTCAATAGATAGAAGACCCTTAAGTGTGTGACCCT
      AGTGACTTTCTCTCGGACTCCT.GTCTAGTACTCA.TAGATAGA.GACC.TTAAGTGTGTGACC.T

      ATAGGTTCGGTGAAATATAGACATGATCGGAACACTTTTCGATCAATGATCAATAGCGGAACCGTG
      GTAGGTTGGGTGAA.TATAGACATGACCGGA.CACATATCGATCAATGATCGATAGCG.AACTGTG

      GACATCCATATTGATCCCTATACCCACAAGAATAATTATTCGAC
      GA.ACTAATAT.GATCC.TATAGATATGGGTGTGTTTATTAATAAACTCACTTGGAGGTCAATGGC

      TTACGATAAGGACAATGAAACACCATACAATGTTTTTGGGCTCCACTCTAAATTACCGTAAGGGCA

      CTTTGTTGTTGAACGAGTGATACGGTCAATGGAGCAATGGCCAAAACAAGAGAAAAGAGCAAGAGC

      ACAAGGCCGTAGGGACACTAGTTTAGTGTGACACAGACCGGTCTGCTACTACCTATGGCATTGTGG

      TTCTCCCGGGTTTCTTATAGAGAGGTAGCTG
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Fig. 7. Sequence homologies between E 1 – T 1 and E 2 – T 1. The nucleotide sequence of the central *Taq* 1 fragments isolated from the two *Eco* R 1 subclones of λ Pj 8.1 are shown; the homologies are underlined. The homologous region extends from one end of both fragments almost to the end of the shorter fragment, E 1 – T 1, before disintegrating abruptly. E 2 – T 1 is 250 bp longer than E 1 – T 1

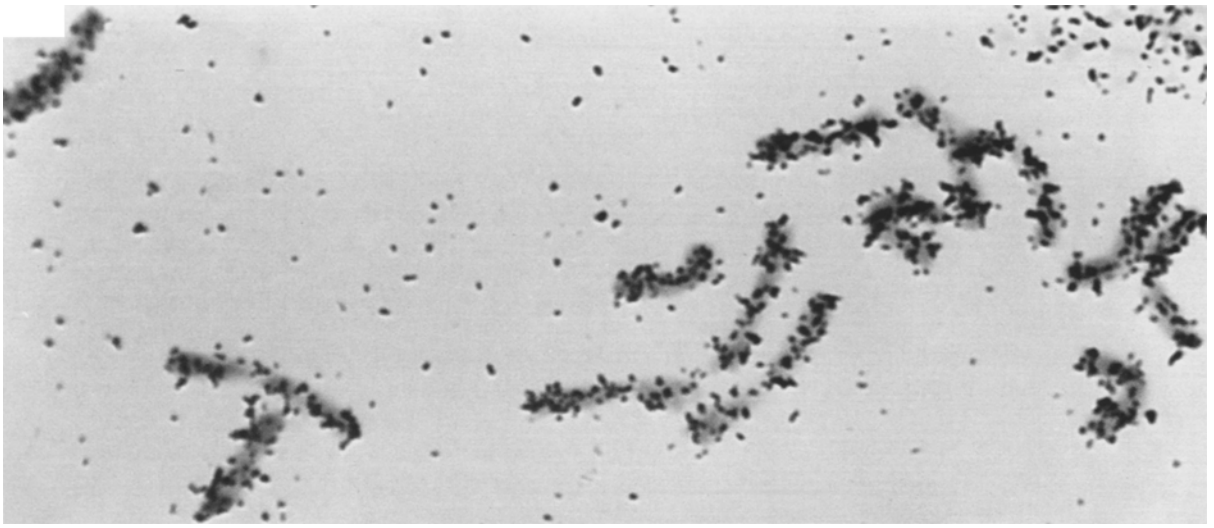

Fig. 8. In situ hybridization of the N 8 family to the N genome of *Psa. juncea*. The N 8 family of repeated sequences is present in high copy number in *Psa. juncea* and is dispersed extensively onto all 7 pairs of chromosomes

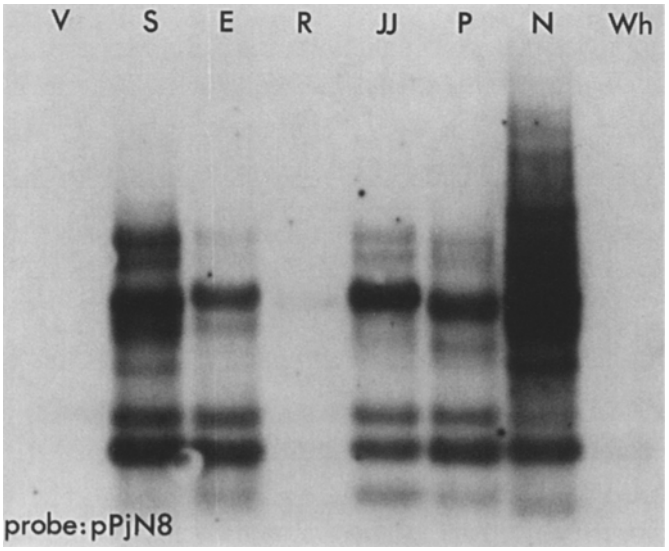

Fig. 9. N 8 family probed to *Taq* 1 digests of DNA from the V, S, E, R, J_1J_2, P, N, and ABD genome grasses. 10 µg DNA aliquots of the grasses (Table 1) were digested with *Taq* 1, Southern transferred, and hybridized with ^{32}P-pPjN 8

E 3, showed homology to the E genome 1.4 kb family and was discussed in the preceding section.

In situ hybridization experiments were also performed to determine the chromosomal distribution of the N 8-family of sequences in *Psa. juncea*. The N 8-family is evenly dispersed throughout the genome and is present on all 7 chromosome pairs (Fig. 8). The highly dispersed nature of this family of sequence is similar to that seen for the 5.3 family of sequence from rye (Appels & al. 1986).

Assaying the N 8-family of sequences in the genomes of the various grasses shows that these sequences exhibit extensive variation in copy number (Fig. 9) as was seen in the preceding sections for the E genome 1.4 kb DNA family. The N 8-family represents 1 – 2% of the N genome of *Psathyrostachys juncea*, is virtually absent from wheat, the R (*Secale cereale*) and V (*Dasypyrum villosum*) genomes and is present at an intermediate level in the other grasses tested (Table 1).

Repetitive sequence families from the V and R genomes

The 5.3 sequence from the R genome of rye (*Secale cereale*). The 5.3 dispersed repetitive sequence family was recovered from a λ clone, λ het 5, containing R genome DNA (*Secale cereale*), as described in APPELS & al. (1986). The 5.3 sequence can be subcloned using the restriction enzyme *Hind* III, generating three fragments designated 5.3 H 1, 5.3 H 2 and 5.3 H 3 (APPELS & al. 1986). As found in the 1.4 kb family and N 8-family, the 5.3 H 2-family and 5.3 H 3-family of sequences show major quantitative variation within the *Triticeae* genomes examined (Table 1). Figure 10 a and b shows restriction endonuclease *Taq I* digests of the genomes analyzed previously probed with 5.3 H 2 and 5.3 H 3, respectively. All the genomes analyzed, with the exception of the V genome, contain appreciable quantities of the 5.3 H 2 sequence. In contrast, the 5.3 H 3 family of sequence is very specific to the R and P genomes only. None of the other genomes analyzed contain detectable quantities of this sequence.

The 8.4-family from the V genome of *Dasypyrum villosum*. The 8.4 repetitive sequence family was subcloned from a λ clone containing *D. villosum* DNA, λ Hav 8.

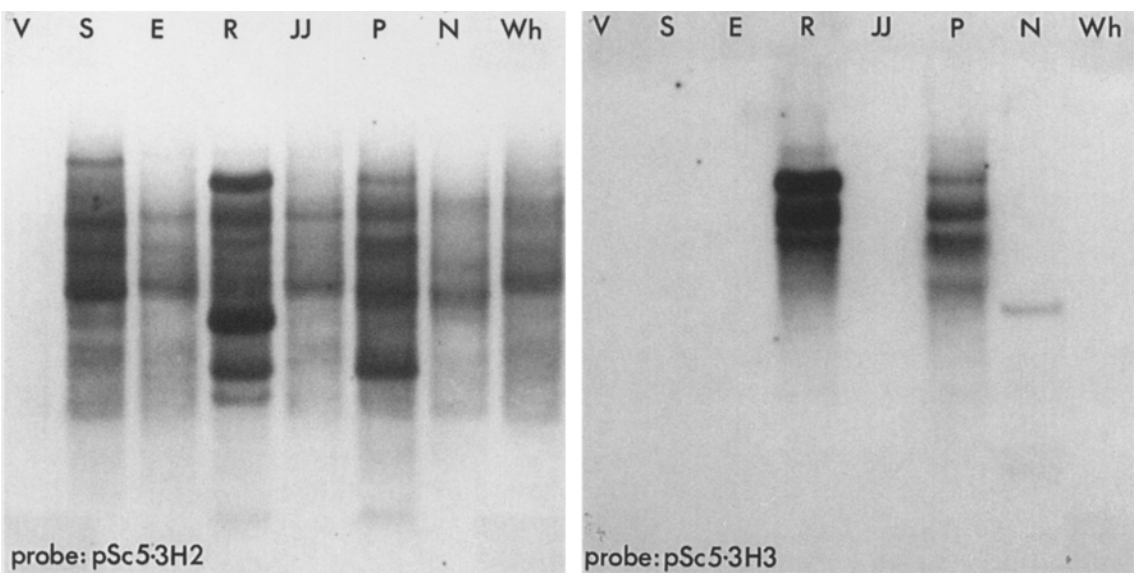

Fig. 10. 5.3 family probed to *Taq* 1 digests of DNA from V, S, E, R, J_1J_2, P, N, and ABD genome grasses. 20 µg quantities of DNA from each genome were digested with *Taq* 1. The reactions were halved and electrophoresed on two 1.5% agarose gels. The gels were Southern blotted and probed with subclone 5.3 H 2, or subclone 5.3 H 3. While 5.3 H 2 is present at intermediate levels in all genomes but V, the 5.3 H 3 sequence is only detectable in the R and P genomes

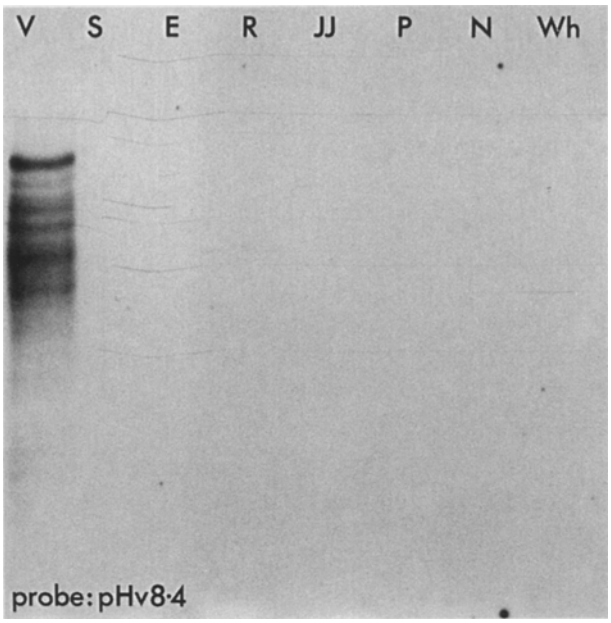

Fig. 11. 8.4 family probed to *Taq* 1 digests of DNA from V, S, E, R, J_1J_2, P, N, and ABD genome grasses. 10 µg quantities of DNA from each genome (grasses listed in Table 1) were digested with *Taq* 1, electrophoresed on 1.5% agarose gels, Southern transferred and hybridized with ^{32}P-pHv 8.4. The 8.4 family is highly specific to the V genome

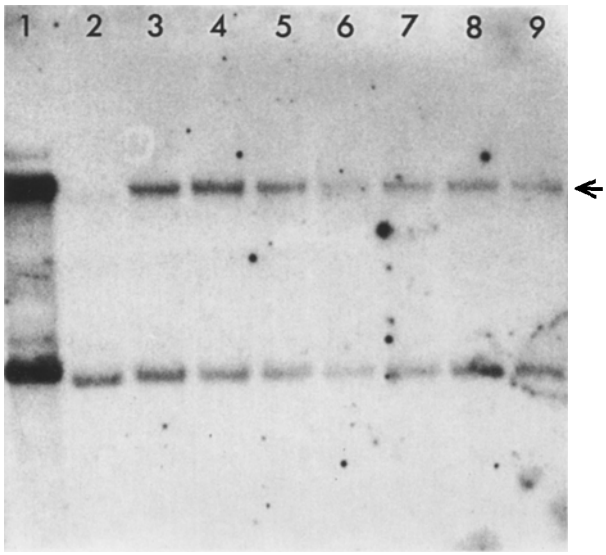

Fig. 12. *Hae* 111 digests of DNA from the E genome/"Chinese Spring" substitution lines probed with the 1.4 kb family. 1 2 µg of DNA from *Th. elongatum* (E genome); 2–9 10 µg of DNA from "Chinese Spring" (wheat), disomic substitution 7 E (7 B), disomic substitution 6 E (6 B), disomic addition 5 E, disomic substitution 4 E (4 D), disomic substitution 3 E (3 D), disomic substitution 2 E (2 B), disomic substitution 1 E (1 B). The arrow indicates the position of the "E genome-specific" band. The probe is pEleAcc 2

λ Hav 8 was originally isolated as a clone possessing *D. villosum* DNA which showed low levels of hybridization to wheat DNA (APPELS & MORAN 1984) in the same way as λ Ele 143 and λ Ele 145 (see p. 43) were obtained. The 8.4 sequence was responsible for the "specific" hybridization observed and its distribution in the *Triticeae* tribe is illustrated in Fig. 11. It is extremely specific to the V genome of *D. villosum* with very little detectable hybridization to wheat or to any of the other genomes tested.

Assaying alien chromatin in wheat with repetitive sequence family probes

The repetitive sequence families described in the preceding sections demonstrate large variations in copy number in the various genomes assayed, and virtual absence from the genomes of wheat. To test if these quantitative changes were sufficient to unambiguously identify alien chromatin such as from these grasses when introduced into wheat, a number of wheat lines carrying E genome chromatin were examined using the 1.4 kb family as a probe (Fig. 12). Although the 1.4 kb family from the E genome cross hybridizes with wheat, the hybridization band indicated with an arrow, in Fig. 12, is only present in *Th. elongatum* DNA and the E genome/wheat addition lines, and not in wheat itself. Thus the 1.4 kb family can detect E genome chromosomes present in wheat. Interestingly the 8.4 kb sequence isolated from *D. villosum* does not assay any specific sequence in the *D. villosum*/wheat addition lines produced by Dr E. R. SEARS. This is being investigated further since it suggests different accessions of *D. villosum* may have varying amounts of the 8.4 sequence.

Discussion

In this paper we have described the properties of four different families of repeated sequences from the E (1.4 kb family), N (N 8-family), R (5.3 family), and V (8.4 family) genomes. In all cases the families are characterized by their dispersed nature in the genome of origin, their low to intermediate presence in other grasses of the tribe *Triticeae* and their virtual absence from wheat. The large variation in copy number and distribution of these families of sequences in a group of related grasses suggests the evolution of these grasses involves processes of amplification, dispersal to non-homologous chromosomes and reamplification and/or deletion, consistent with the proposals of FLAVELL (1980) and THOMPSON & MURRAY (1980).

Various mechanisms have been proposed for the processes of repeated amplification, sequence divergence and dispersal essential for the evolution of such families of sequences. Large scale amplification (reviewed in FLAVELL & al. 1979) of relatively short (3 − 3 000 bp) pieces of DNA is thought to occur frequently during evolution (for example see: STRAUS 1972, MIZUNO & MACGREGOR 1974, FLAVELL & al. 1977, 1980 a, b, MOORE & al. 1978, DENNIS & PEACOCK 1984). Two major models have been suggested. TARTOF (1973) and SMITH (1973, 1976) proposed that sequences can be amplified and deleted over a long period of time by unequal crossing-over, if duplicated regions of sufficient homology are close enough to one another. A second model allows for rapid increase in copy number, perhaps in one molecular event, (WALKER 1971, SOUTHERN 1970), from an error in DNA replication (WELLS & al. 1967) or from a recent event resulting in a piece of DNA being excized from the chromosome creating a circular DNA molecule which can then

be replicated by the rolling circle mechanism and then reinserted. Amplification of rDNA in amphibian oocytes has been shown to occur by this latter procedure (Hourcade & al. 1973). The rolling circle model also requires a mechanism of integration of the amplified sequence into the chromosome. The existence of a well defined boundary of the type shown in Fig. 6 would tend to argue in favour of the second model. The present-day E genome 1.4 kb DNA family would, in this case, be envisaged to have evolved from the amplification of an ancient sequence (common to the E and N genomes) plus its adjoining DNA to form the tandem arrays in the E genome. It will be of interest to attempt to recover the 186 bp duplication from the N genome to see if portions of or the entire 1.4 kb unit is present in the N genome. If the latter, the 186 bp duplication may provide a relative time-scale for the various amplification events in the history of the 1.4 kb DNA family.

Mechanisms that may facilitate the dispersal of repeated sequences to new chromosomal locations include the transposition of mobile elements (prokaryotes: Bukhari & al. 1977; yeast: Cameron & al. 1979; *Drosophila*: Potter & al. 1979; Strobel & al. 1979; maize: Fincham & Sastry 1974; reviewed in Federoff 1984), excision and reintegration, such as has been postulated to occur with the rolling circle mechanism of amplification, and translocations, inversions and chromosome fusions. Single cross-over events may be sufficient to disperse families of repeated sequences located in telomeric regions, such as rye (Holmquist & Dancis 1979, Bedbrook & al. 1980, Appels & al. 1981), when the regions are in close proximity as in early zygotene of meiosis (Appels & al. 1981). Following dispersal to new sites, these sequences are then available for amplification and this second round of amplification explains the observed clustering of repeated sequence variants. It is worth noting that while transposable elements per se have not been found in these grasses, many of these repeated sequence families display structural characteristics of transposable elements, such as direct and inverted repeats, as illustrated in Fig. 3.

Many studies have shown that not all members of a repeated sequence family are identical (for examples see: Houck & al. 1978, 1979, Deininger & Schmid 1979, Gerlach & Dyer 1980, Rubin & al. 1980, Anderson & al. 1981, Scheller & al. 1981, Dennis & Peacock 1984, Johnson & al. 1984, Trick & Dover 1984). The 1.4 kb units from the E genome detailed in the present paper, exhibit between 1 and 80 variant sites (0.1 – 5.8%). Divergence occurs principally by accumulation of base changes, small insertions or deletions and by sequence rearrangements within a segment (Posakony & al. 1981).

The quantity of a repeated sequence family and the nature of its dispersal and divergence may be useful as taxonomic tools (Jones & Flavell 1982, Arnold & al. 1985) if the within species variation is less than the between species variation. On the basis of the distribution of the 1.4 kb family from the E genome in the E, S, N, J_1J_2, P, V, R, and wheat genomes (Fig. 5 a and b), we would suggest that the P and J_1J_2 genomes are more closely related to the E genome than are the other genomes. However, the distribution of the 5.3 H 3 sequence from the R genome suggests that the R and P genomes are closely related. The adjoining papers utilize different parameters to study relationships between genomes of the *Triticeae* and the suggested relationships are summarized in McIntyre (1988).

The quantitative nature of the genome specificity of the families of repeated sequences with respect to wheat, suggests they may be able to detect introgression

of alien chromatin into wheat (see Fig. 12). Many of the grasses in the *Triticeae* contain agronomic traits of interest to wheat breeders. Several *Thinopyrum* species (E – J genome) possess rust resistance genes (KNOTT 1961, 1964, KNOTT & al. 1977) as well as exhibiting high salt tolerance (MCGUIRE & DVORAK 1981) and drought tolerance (SHIMSI & al. 1982). Populations of *Dasypyrum villosum* (V genome) are resistant to Barley Yellow Dwarf Virus, Take-all (*Gaeumannomyces graminis*), Powdery Mildew (*Erysiphe graminis*), and leaf (*Puccinia graminis*) and stem (*Puccinia recondita*) rust (JAN & al. 1986), while some *Psathyrostachys* species (N genome) possess dwarfing characters (LAWRENCE 1967). In addition, many *Secale* species contain other useful genes including disease resistance genes (RILEY & MACER 1966, RILEY & EWART 1970). If the repeated sequence families, isolated as being species specific relative to wheat, can assay successful introductions of alien chromatin carrying potentially useful genes into wheat, then they may provide valuable new markers to allow chromosomes to be manipulated further. This has been recently demonstrated by RAYBURN & GILL (1986) who have isolated a repeated sequence from *Triticum tauschii* specific to the D genome. The cloned sequence is located in specific areas on telomeres and certain interstitial sites along the chromosomes of *T. tauschii*. In situ hybridization to double ditelosomic stocks of "Chinese Spring" (AABBDD) has confirmed that the sequence is restricted to the D genome and thus hybridization fingerprints of individual D genome chromosomes can be obtained.

The isolation and characterization of families of repeated sequences such as the 1.4 kb (E genome), N 8 (N genome), 5.3 (R genome), and 8.4 (V genome) families, provide information on genome organization in the *Triticeae* and the relationships between the different genomes. In addition, the families could provide valuable chromosome markers for use in plant breeding.

The authors are grateful to Dr BIKRAM GILL for his comments on the manuscript. CLM is supported by a Commonwealth Postgraduate Research Award.

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