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Characterization of a family of tandemly repeated DNA sequences in Triticeae

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Abstract The recombinant plasmid dpTa1 has an insert of relic wheat DNA that represents a family of tandemly organized DNA sequences with a monomeric length of approximately 340 bp. This insert was used to investigate the structural organization of this element in the genomes of 58 species within the tribe Triticeae and in 7 species representing other tribes of the Poaceae. The main characteristic of the genomic organization of dpTa1 is a classical ladder-type pattern which is typical for tandemly organized sequences. The dpTa1 sequence is present in all of the genomes of the Triticeae species examined and in 1 species from a closely related tribe (*Bromus inermis*, Bromeae). DNA from *Hordelymus europaeus* (Triticeae) did not hybridize under the standard conditions used in this study. Prolonged exposure was necessary to obtain a weak signal. Our data suggest that the dpTa1 family is quite old in evolutionary terms, probably more ancient than the tribe Triticeae. The dpTa1 sequence is more abundant in the D-genome of wheat than in other genomes in Triticeae. DNA from several species also have bands in addition to the tandem repeats. The dpTa1 sequence contains short direct and inverted subrepeats and is homologous to a tandemly repeated DNA sequence from *Hordeum chilense*.

Key words Evolution · Tandemly repeated DNA sequences · Phylogenetic relationships · RFLP · Poaceae

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Introduction

Tandemly organized DNA sequences have been investigated during the last two decades and much progress has been achieved, particularly with respect to the human genome (for review see Willard and Waye 1987). Our knowledge of their occurrence, structural organization and distribution in plants is, however, still limited. The presence of tandemly repeated DNA sequences in cereals was first demonstrated in rye by Bedbrook et al. (1980). They cloned four classes of repeated sequences, three of which were complex tandem repeats containing interspersed, unrelated sequences of short length. Their distribution in related species was later investigated by Jones and Flavell (1982). The fourth class consisted of short (120 bp), tandemly organized sequences. Another tandem repeat in rye, with a monomeric length of 350 bp, was discovered by Appels et al. (1981) and later, tandemly organized DNA sequences were cloned from knob heterochromatin of maize (Dennis and Peacock 1984). Recently, tandemly repeated DNA sequences in cereals have been described in *Hordeum vulgare* (Belostotsky and Ananiev 1990), *H. chilense* (Anamthavat-Jónsson and Heslop-Harrison 1993; Hueros et al. 1993) and rice (Zhao et al. 1989; De Kochko et al. 1991).

Highly repeated sequences organized in tandem arrays are generally assumed to have originated as homogeneous families of sequences in the genome by mechanisms such as rolling circle replication or unequal crossing-over. They may diverge by the accumulation of mutations or through processes such as conversion and crossing-over. Consequently, from an evolutionary point of view tandemly organized sequences have attracted considerable attention, as individual members of a family of repeated sequences are generally more similar within a species than between related species, a characteristic feature of concerted evolution (Dover 1982). Tandemly repeated sequences are scattered throughout the eukaryotic genome and they diverge at a relatively high rate. Together, these properties make

them suitable as tools for investigations of the evolution of genomes, chromosomes, and separate regions of chromosomes. The size of the monomeric unit is usually constant, even in distantly related species, and the tandem arrays are predominantly located at strategic chromosomal positions such as telomeric and centromeric regions. Another advantage of their use is the possibility to identify species-specific sequences and markers for separate chromosomes. This is the case for the human alphoid DNA family, present also in other primates, which has evolved into several distinct subfamilies with a common basic length of 171 bp (Willard and Waye 1987).

The tribe Triticeae is an economically important group of grasses that includes three cereal crops: wheat, barley and rye. The main cytogenetic characteristics of species in this tribe are their common basic chromosome number (x = 7), large chromosomes, frequent polyploidy and the complicated reticulate pattern of relationships due to repetitive intergeneric hybridizations. The classification of Triticeae proposed by Löve (1982; 1984) and Dewey (1984) is based on genomic relationships, as defined by chromosome pairing at meiotic metaphase I in interspecific and intergeneric hybrids (genome analysis), together with more traditional taxonomic data. Several research groups have utilized the variation among repetitive DNA sequences to reconstruct the phylogeny of selected groups of taxa in the tribe, e.g. *Triticum* (Dvorák and Zhang 1992), *Hordeum* (Gupta et al. 1989), *Secale* (Jones and Flavell 1982) and other genera (McIntyre et al. 1988; Xin and Appels 1988). A description of phylogenetic relationships among and within 16 species of Triticeae, based on 21 cDNA clones from wheat, was recently published by Monte et al. (1993).

The purpose of the investigation presented here was to study the distribution of a tandemly repeated DNA family in the tribe Triticeae and use these data for studies of phylogenetic relationships. We have characterized such a family from wheat and analyzed its structural organization in genomes representing 19 genera of Triticeae and from a few other tribes.

Materials and methods

Plant material

The selected plant species, including accession numbers from the collection of the Department of Plant Breeding Research (Svalöv, Sweden), are presented in Table 1.

DNA isolation, digestion and hybridization

Total genomic DNA was extracted from leaves of greenhouse-grown plants according to Ausubel et al. (1987). The DNA was digested with

Table 1 Plant species used, including accession numbers (or cultivars) from the collection of the Department of Plant Breeding Research (Svalöv, Sweden), genomic constitution (Dewey 1984; von Bothmer et al. 1991) and ploidy level for the Triticeae species

Tribe	Genus	Species	Ploidy	Genome	Accession code	Origin
Andropogoneae	*Zea*	*mays* L.			H4080	Pakistan, Gilgit, Babusar valley
Bromeae	*Bromus*	*inermis* Leyss.			cv. Kesto	Obtained from Svalöf-Weibull AB
Brachypodieae	*Brachypodium*	*sylvaticum* (Huds.) PB			H3716	Iran, Lorestan, Doroud, Oshtorankuh
Phleeae	*Phleum*	*pratense* L.			cv. Kämpe II	Obtained from Svalöf-Weibull AB
Poeae	*Avena*	*sativa* Huds.			H8021	China, Sichuan, Mian Ning Co
	Festuca	*pratensis* L.			cv. Mimer	Obtained from Svalöf-Weibull AB
	Poa	*pratensis* L.			cv. Primo	Obtained from Svalöf-Weibull AB
Triticeae	*Aegilops*	*tauschii* Coss. [= *Aegilops squarrosa* auct. non L.]	2x	D	H8467	China, Xinjiang, Xinyuan Co
	Agropyron	*cristatum* (L.) Beauv.	2x	P	H10154	Russia, Altai, W of Kuraj
	Amblyopyrum	*muticum* (Boiss.) Eig	2x	Z	160AE	Obtained from Gatersleben
	Australopyrum	*velutinum* (Nees) Simon	2x	W	H4200	Australia
	Dasypyrum	*villosum* (L.) Cand.	2x	V	H3025	Greece, Peloponnesos, Lakonia
	Elymus	*semicostatus* (Nees ex Steud.) Meld.	4x	SY	H4100	Pakistan, Hazara, Naran village
		sibiricus L.	4x	SH	H10018	Russia, Altai, Seminski mts.
	Elytrigia	*repens* Desv.	6x	SS?	H3375	Sweden, Södermanland, Södertälje
	Eremopyrum	*distans* (Koch) Nevski	2x	F	H3130	Afghanistan, Farah, Jamal Ghazi
	Henrardia	*persica* (Boiss.) Hubb.	2x	O	H5556	Iran, SE of Maku
	Hordelymus	*europaeus* (L.) Harz	4x	??	H5029	Denmark, Sorø
	Hordeum	*arizonicum* Covas	6x	HHH	H3253	USA, Arizona, Picacho
		bogdanii Wil.	2x	H	H8700	China, Xinjiang, Altai Co
		brachyantherum ssp. *brachyantherum* Nevski	4x	HH	H4216	USA, Wyoming, Teton Co
		brachyantherum ssp. *brachyantherum* Nevski	6x	HHH	H2001	USA, California, Louis Obispo Co
		brachyantherum ssp. *californicum* (Covas et Stebb.) Bothm. et al.	2x	H	H2401	USA, California, San Diego Co, Julian
		brevisubulatum ssp. *violaceum* (Boiss. et Hohen.) Tzvel.	4x	HH	H306	Turkey, Vilaj Erzurum
		bulbosum L.	2x	I	H3023	Greece, Peloponnesos, Lakonia
		capense Thunb.	4x	HH	H334	South Africa, Ventersted, Oviston
		chilense Brongn.	2x	H	H1816	Chile, Coquimbo, Los Vilos

Table 1 (Continued)

Tribe	Genus	Species	Ploidy	Genome	Accession code	Origin
		comosum Presl.	2x	H	H1181	Argentina, Rio Negro, S. C. Bariloche
		cordobense Bothm. et al.	2x	H	H1711	Argentina, Cordoba, Bialet Massé
		depressum (Scribn. et Smith) Rydb.	4x	HH	H2308	USA, California, Kings Co
		erectifolium Bothm. et al.	2x	H	H1150	Argentina, Buenos Aires, Bahia Blanca
		euclaston Steud.	2x	H	H2148	Uruguay, Paysandu
		flexuosum Steud.	2x	H	H1116	Argentina, Buenos Aires, Juares
		fuegianum Bothm. et al.	4x	HH	H6156	Argentina, Tierra del Fuego, Rio Grande
		guatemalense Bothm. et al.	4x	HH	H2299	Guatemala, Sierra de los Cuchumatanes
		intercedens Nevski	2x	H	H1941	USA, California, Lakeview
		jubatum L.	4x	HH	H1162	Argentina, Buenos Aires, Guanini
		lechleri (Steud.) Schenck	6x	HHH	H6344	Argentina, Neuquen, Carrei
		marinum ssp. *marinum* Huds.	2x	"X"	H759	Greece, Crete, Ag. Nikolaos
		murinum ssp. *murinum* L.	4x	"YY"	H217	Germany, Berlin, Dahlem
		muticum Presl.	2x	H	H6468	Argentina, Jujuy, Senador Perez
		parodii Covas	6x	HHH	H6255	Argentina, Chubut, Alto Rio Senguerr
		patagonicum ssp. *patagonicum* (Haumann) Covas	2x	H	H1520	Argentina, Santa Cruz, Rio Deseado
		procerum Nevski	6x	HHH	H1781	Argentina, San Juan, San José
		pubiflorum Hook. f.	2x	H	H6360	Argentina, Neuquen, Lago Tromen
		roshevitzii Bowd.	2x	H	H8787	China, Xinjiang, Jeminay Co
		secalinum Schreb.	4x	HH	H296	Spain, 14 km E Huelva
		stenostachys Godr.	2x	H	H6437	Argentina, La Rioja, El Potrerillo
		tetraploidum Covas	4x	HH	H6364	Argentina, Mendoza, Laguana Coipillauqen
		vulgare L.	2x	I	cv. Betzes	Obtained from Svalöf-Weibull AB
	Leymus	*arenarius* (L.) Hochst.	8x	NN??	H3417	Sweden, Gotland, N of Visby
	Peridictyon	*sanctum* (Janka) Seberg et al.	2x	?	H3841	Greece, Pangeon mtn.
	Psathyrostachys	*stoloniformis* Baden	2x	N	H7031	China, Qinghai, Gonghe
	Pseudoroegneria	*cognata* (Hackel) Löve	2x	S	H4033	Pakistan, Gilgit, Bathura glacier
	Secale	*cereale* L.	2x	R	cv. Danko	Obtained from Svalöf-Weibull AB
	Taeniatherum	*caput-medusae* ssp. *crinitum* (Schreb.) Meld.	2x	T	H5011	Iran, Markazi
	Thinopyrum	*junceum* (L.) Löve	6x	JJJ	H3540	Spain, Mallorca, Alcudia
	Triticum	*aestivum* L.	6x	ABD	004VE	Svalöf-Weibull AB, breeding line
		durum Desf.	4x	AB	009VE	Svalöf-Weibull AB, breeding line
		monococcum L.	2x	A	035VE	Basel Botanical Garden, CH-4056

restriction endonucleases under the conditions specified by the manufacturer (Boehringer Mannheim), and the resulting DNA fragments were separated on 1.5% agarose gels and transferred to GeneScreen membranes (DuPont). DNA was labelled by random priming using either $\alpha[^{32}P]$-dCTP (Amersham) or digoxigenin-dUTP (Boehringer Mannheim) as recommended by the supplier. Hybridization was carried out in 5 × SSC, 0.5% Blocking Reagent (Boehringer Mannheim), 0.1% *N*-laurylsarcosine and 0.02% SDS at 65 °C (high stringency) or 55 °C (low stringency) for 16 h. The membranes were washed with two changes of 1 × SSC, 0.1% SDS at room temperature and then washed in 0.1 × SSC, 1% SDS for 1 h at the hybridization temperature. Hybridized, non-radioactively labelled probe was visualized with the chemiluminescent substrate Lumigen-PPD (Boehringer Mannheim).

The copy number of dpTa1 was determined by dot hybridization at low stringency. The radioactivity in each spot was determined by liquid scintillation counting, and the copy number was calculated as described by Rivin et al. (1986).

Cloning and sequencing

We have used a cloned DNA sequence from *Triticum aestivum*, originally described by Metzlaff et al. (1986), as probe. The plasmid pTa1, which has an insert of about 700 bp of "relic" DNA in vector pEMBL 8, was obtained from Dr. M. Metzlaff. During transportation or storage in our laboratory a part of the insert has spontaneously deleted. Such deletions are not uncommon in plasmids, and the mechanism has been studied in detail (Dianov et al. 1991). The remaining insert, dpTa1, was recloned into pBluescript II KS+ and pBluescript II SK+, and the plasmids were named dpTa1KS+ and dpTa1SK+, respectively. Both clones were sequenced by double-stranded sequencing using $[^{35}S]$-dATP (Sanger et al. 1977). Computer-assisted analysis of the sequence data was performed using either Gene Works v. 2.2 (Intelligenetics) or the GCG package (Devereux et al. 1984).

The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X76300.

Results

When DNA from *Triticum aestivum* and *Hordeum vulgare* was cut with various restriction enzymes and hybridized to dpTa1, a pattern typical of a tandemly

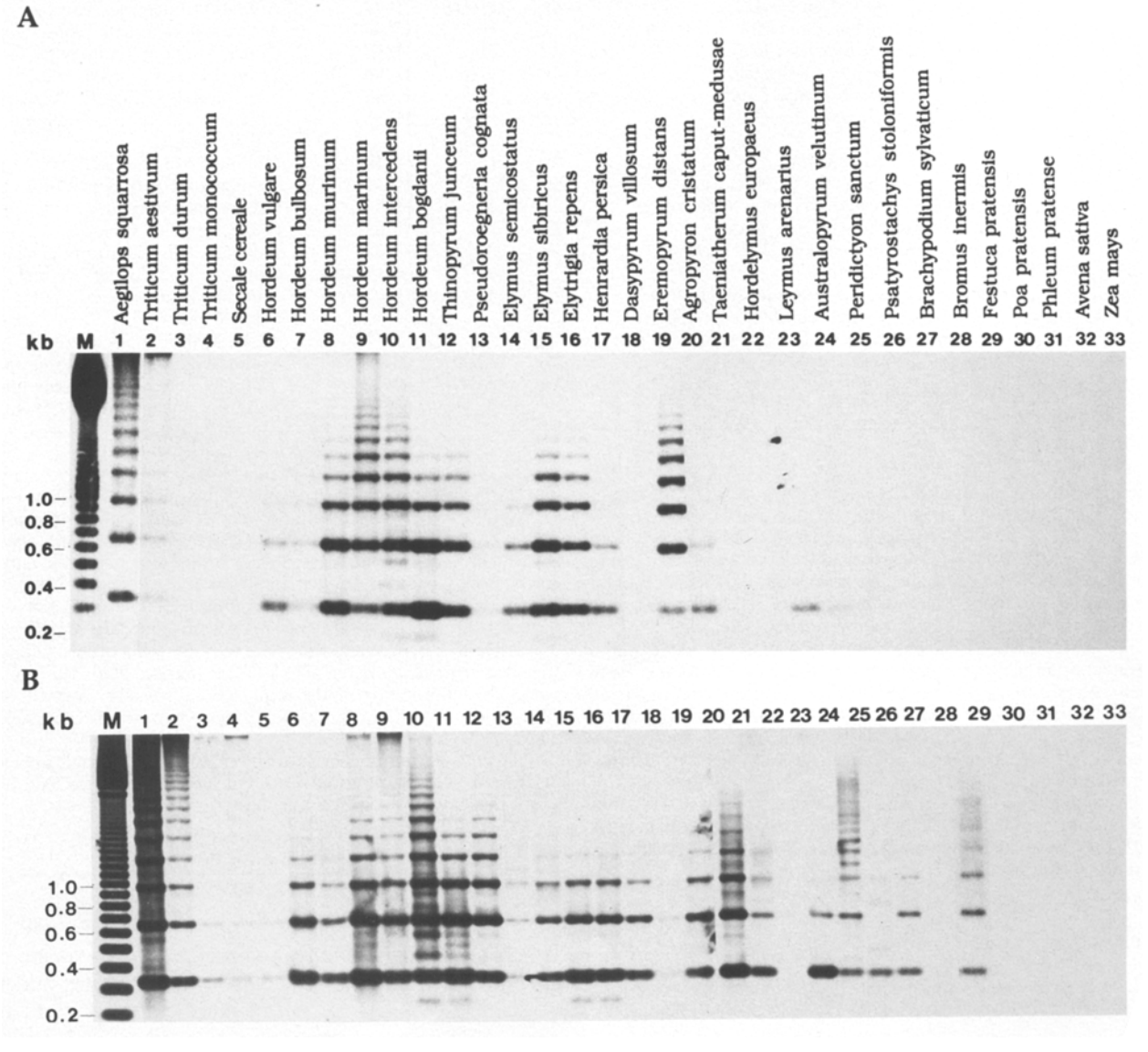
repeated sequence family, where base changes have accumulated at the restriction sites, was revealed. The size of the monomeric unit is approximately 340 bp. A similar type of pattern appeared with the 15 different restriction enzymes that were tested: *Alu*I, *Ava*II, *Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Hin*fI, *Msp*I, *Rsa*I, *Sac*I, *Sau*3A, *Taq*I and *Xba*I (data not shown). *Taq*I was chosen as the restriction enzyme for this investigation as it gave a full ladder-pattern with these species.

Genomic organization of dpTa1 in Poaceae

The genomic organization of dpTa1 was initially studied in 26 species of Triticeae and in 7 species representing other tribes of Poaceae (Table 1). In Triticeae we selected all of the species available that could represent the basic genomes according to Löve's (1984) and Dewey's (1984) classification, except for some genomes in the *Triticum-Aegilops* complex. The probe dpTa1 was hybridized to Southern blots at two different stringencies in order to be able to detect sequence divergence (Fig. 1). At high stringency (65 °C) the ladder-pattern is clearly visible in *Aegilops tauschii* (= *Aegilops squarrosa*), *Agropyron cristatum*, *Australopyrum velutinum*, *Elymus semicostatus*, *Elymus sibiricus*, *Elytrigia repens*, *Eremopyrum distans*, *Henrardia persica*, *Leymus arenarius*, *Thinopyrum junceum*, *Triticum aestivum* and in all 6 species of *Hordeum*. In addition to the ladder-pattern, a fragment of about 240 bp is present in *Elymus sibiricus*, *Hordeum bogdanii* and *Hordeum intercedens*. This fragment is, however, less intense than the ladder fragments.

Hybridization at low stringency (55 °C) also allowed *Bromus inermis*, *Dasypyrum villosum*, *Peridictyon sanctum*, *Psathyrostachys stoloniformis*, *Pseudoroegneria cognata*, *Secale cereale*, *Taeniatherum caput-hmedusae* ssp. *crinitum*, *Triticum durum* and *Triticum monococcum* to hybridize to tandem repeats, indicating diverged

Fig. 1A, B Hybridization of dpTa1 to *Taq*I-digested plant DNA at high stringency (**A**) and low stringency (**B**)



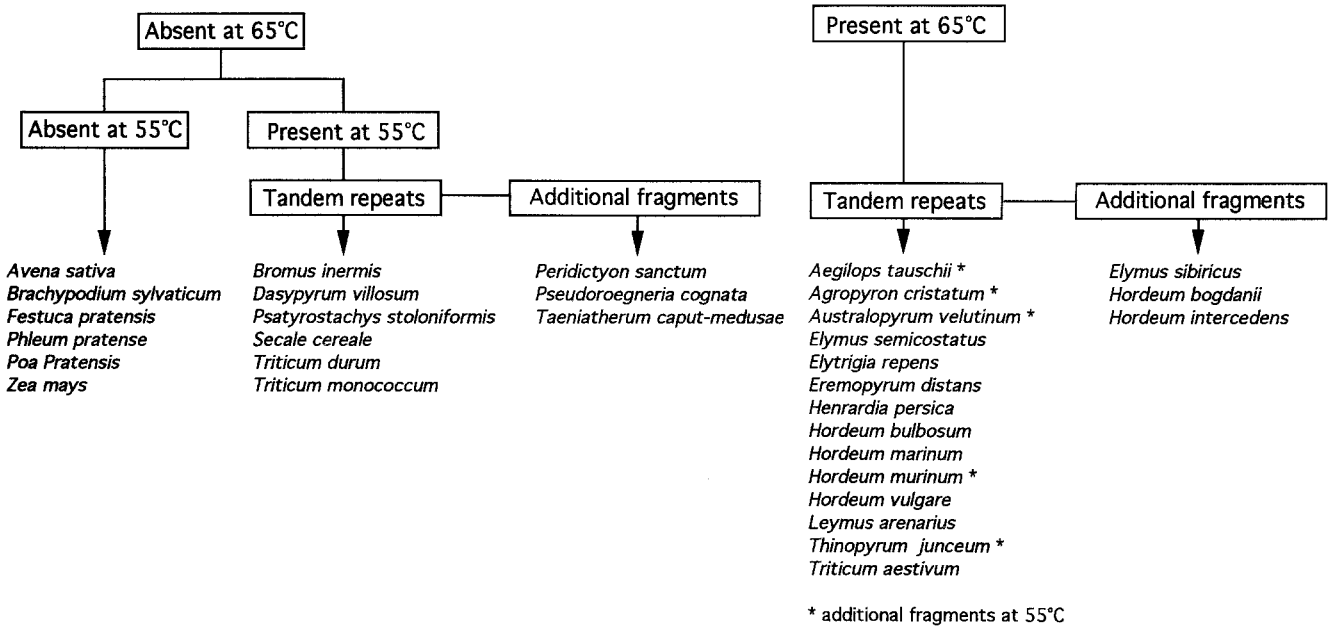


Fig. 2 Distribution of the dpTa1 family of repetitive sequences in Triticeae and related tribes at low and high hybridization stringency conditions

sequences (Fig. 1B). Under these conditions new fragments were also revealed in the genomes of some species (Fig. 2). The predominant sequences among these are approximately 430 bp, 560 bp, 760 bp and 870 bp long. These additional fragments demonstrate the presence of interspecies variation both in intensity and in length. *Peridictyon sanctum* is unique as it only has the monomeric unit of the tandem repeat in addition to 460-bp, 735-bp, and 960-bp-long fragments.

Several species did not hybridize to dpTa1 even at low stringency. All of these species belong to tribes other than Triticeae except for *Hordelymus europaeus*. A weak signal to the monomeric band appeared, however, after hybridization at 65 °C, washing at room temperature and prolonged exposure (data not shown). The only representative of the other tribes that hybridized to dpTa1 was *Bromus inermis* (Bromeae).

In addition to the ladder-pattern which is typical of tandemly organized DNA sequences, other bands were also present in many species, particularly within the genus *Hordeum* (Figs. 1 and 2). The genomic organization of dpTa1 was therefore studied more thoroughly in 32 species of this genus (Table 1; Fig. 3). The data demonstrate that all species carrying the H-genome are distinct from the remaining species by the presence of a dpTa1 subfamily. This additional band is not present in *H. vulgare* (I), *H. bulbosum* (I), *H. marinum* ("X") and *H. murinum* ("YY").

Fig. 3 Hybridization of dpTa1 to *Taq*I-digested DNA from *Hordeum* species at low stringency

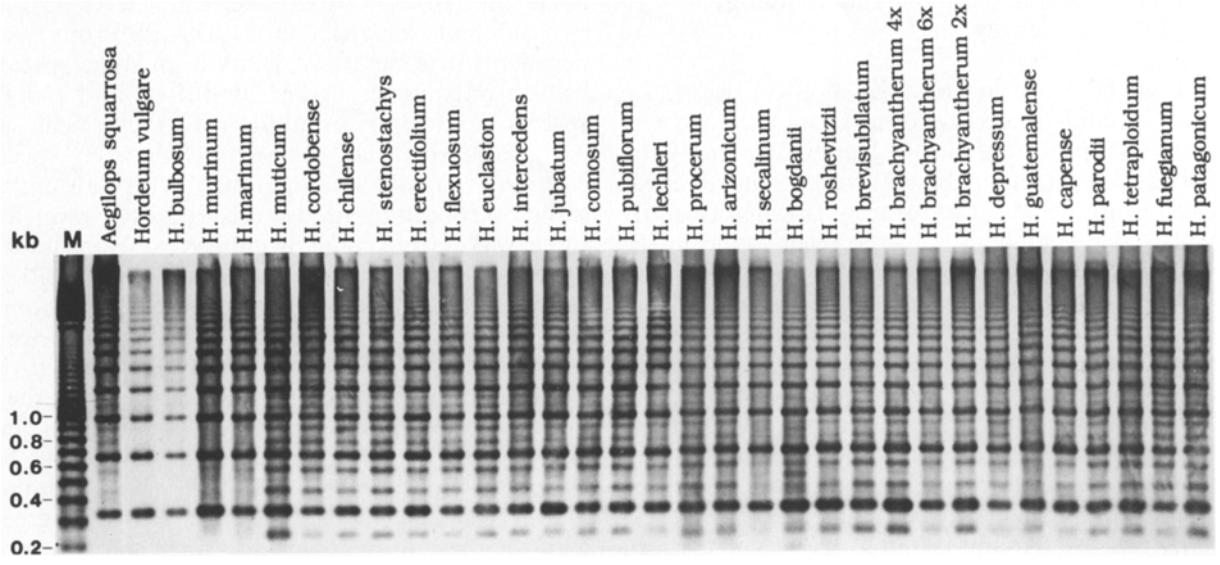


Table 2 Copy number of dpTa1 as estimated by dot-blot hybridization at low stringency in some Triticeae species

Species	Genome	Genome size bp × 10^9	Copy number per genome
Aegilops tauschii	D	4.0	11×10^4
Triticum aestivum	ABD	5.3	3.0×10^4
Thinopyrum junceum	JJJ	5.0	2.7×10^4
Elymus sibiricus	SH	5.0	2.4×10^4
Elytrigia repens	SS?	5.0	2.0×10^4
Hordeum intercedens	H	4.9	1.8×10^4
Hordeum vulgare	I	4.9	1.0×10^4
Hordeum bulbosum	I	4.9	0.7×10^4
Elymus semicostatus	SY	5.0	0.6×10^4

Variation in copy number

The copy number of dpTa1 was determined by dot-blot hybridizations at low stringency in those species that gave the strongest signal on Southern blots (Table 2). The genome size for the various species was taken from Bennett and Smith (1971) and Arumuganathan and Earle (1991). As there was no information available on genome size for *Elymus semicostatus*, *Elymus sibiricus*, *Elytrigia repens* and *Thinopyrum junceum*, we used a value that is an approximate average of the genome size for species within Triticeae, i.e., 5×10^9 bp. The results show that the sequence is most abundant in *Aegilops tauschii*, indicating that this sequence is very frequent in the D-genome. This is verified by the high copy number in *Triticum aestivum* (ABD) and the low hybridization level to Southern blots of *Triticum durum* (AB) and *Triticum monococcum* (A). *Thinopyrum junceum* (J) as well as species with the H-genome also have relatively high copy numbers. However, only a few species have been analyzed, and the data should be interpreted with care.

The primary structure of dpTa1 and homology to other DNA sequences

The size of the insert in dpTa1KS+/SK+ is 393 bp with a G + C content of 37%. Many short, perfect, direct and inverted repeats, the longest being 10 bp and 9 bp, respectively, are present in the sequence. As the sequence is derived from the wheat sequence pTa1, described by Metzlaff et al. (1986), high homology was found to the 150 bp that had been sequenced by this group (Fig. 4A). The pTa1 sequence is identical to position 1–152 of dpTa1 except for position 9 and a 2-bp gap in pTa1.

Sequence comparison revealed 62% similarity between a 87-bp overlap at the 3′-end of dpTa1 and a family of dispersed repetitive sequences (Hch1) from *Hordeum chilense* (Hueros et al. 1993; see Fig. 4A). In addition, high homology was found to the complement of a tandemly repeated sequence (cpHcKB6), also present in *H. chilense* (Anamthawat-Jónsson and Heslop-Harrison 1993). The pHcKB6 sequence contains the monomeric unit (339 bp) of a tandemly repeated sequence, which has 82% homology to dpTa1 in a 208-bp overlap (pos. 186-393 in dpTa1 and 1–208 in cpHcKB6; see Fig. 4B). The analysis also demonstrated 68% homology in position 58–185 of dpTa1 and position 209–336 of cpHcKB6 (Fig. 4C). Combined, our data demonstrate 77% identity between dpTa1 and the *H. chilense* tandem repeat and show that the complete sequence of the Triticeae monomer is present in dpTa1.

Discussion

We have described a family of tandemly repeated DNA sequences, the dpTa1 family, which is present in 19 investigated genera (53 species) of the tribe Triticeae. The sequence is also present in the genome of *Bromus inermis* (Bromeae), but not in 6 species representing the tribes Andropogoneae, Brachypodieae, Phleeae and Poeae. DNA from *B. inermis* hybridizes weakly to dpTa1 and only at low stringency conditions, demonstrating the presence of highly diverged dpTa1 sequences in low copy numbers. These data are in agreement with the consensus tree model of Frederiksen and Seberg (1992), which was based on morphological data. According to this tree, *Brachypodium* is the sister-group of the clade including both *Bromus* and the Triticeae. Thus, our data do not contradict their hypothesis that the Triticeae may possibly be a non-monophyletic group. However, it is more probable that the dpTa1 family is more ancient than Triticeae and constitutes a synapomorphy for a clade including both Triticeae and Bromeae. Soreng et al. (1990) and Kellogg (1991, 1992) suggested that Bromeae is the sister group (closest relative) of Triticeae based on chloroplast DNA variation. These results further strengthen arguments against a placement of *Brachypodium* within the Triticeae as done by Clayton and Renvoize (1986).

We demonstrated the heterogeneity of the dpTa1 family in Triticeae by Southern blot hybridization at two different stringency levels (Fig. 2). From these experiments it is clear that DNA from some species hybridize only under gentle conditions and that some species have bands in addition to the typical ladder-pattern. *Hordelymus europaeus* is the only species in Triticeae that did not hybridize to dpTa1 under the standard conditions used in this study. However, a weak signal was detected after prolonged exposure, indicating the presence of a low copy sequence. Löve (1982) postulated that *H. europaeus* possesses the genomic combination HT, where the H-genome would come from *Hordeum* and the T-genome from the annual genus *Taeniatherum*. However, recent data show that the position of *Hordelymus* is quite unclear. Based on genome analysis and C-banding patterns, Bothmer and Jacobsen (1989) and Bothmer et al. (1993) found no homology to other studied genera of the tribe. This is in agreement with the present investigation, which shows a separate

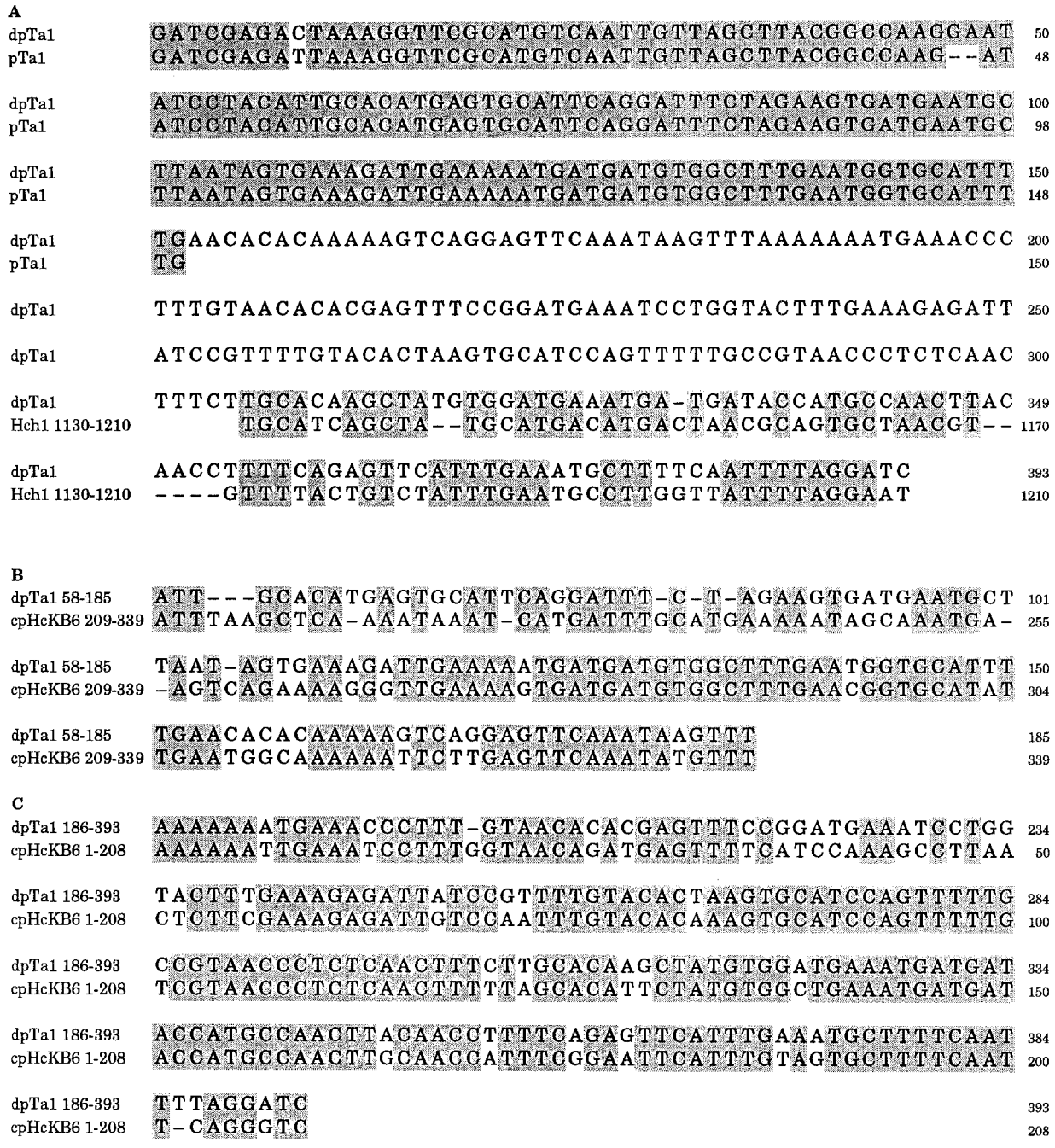


Fig. 4A–C **A** Alignment of dpTa1 to the sequenced portion of pTa1 (Metzlaff et al. 1986) and the Hchl family of dispersed repetitive sequences (pos. 1130–1210) from *Hordeum chilense* (Hueros et al. 1993). **B** and **C** Alignment of dpTa1 and the complement of pHcKB6 (cpHcKB6) from *Hordeum chilense* (Anamthawat-Jónsson and Heslop-Harrison 1993). Identical nucleotides are *shaded*

state of *Hordelymus europaeus* in the Triticeae. Neither do our results support the amalgamation of *Taeniatherum* and *Hordelymus* into *Hordeum* as suggested by Kellogg (1989).

The genomic organization of dpTa1 is conserved in all species as shown by the presence of the ladder-pattern. *Peridictyon sanctum* deviates to some extent from the general pattern since it has only the monomeric form. The isolated position of *P. sanctum* in Triticeae is also implied from morphology, in its net-like withering of basal leaf sheats and an abscission layer between the leaf sheath and the leaf blade, and from C-banding patterns in that its conspicuous C-bands are confined to the telomeres of long arms and it has a low amount of constitutive heterochromatin (Seberg et al. 1991). So far, the relationships of *P. sanctum* has not been studied by genome analysis. The genus *Peridictyon* has just recently been recognized as being distinct from *Festucopsis* (Seberg et al. 1991), a genus of which no material was available for this study.

The occurrence of bands in addition to the ladder-pattern in *Hordeum* species carrying the H-genome (see Bothmer et al. 1991 and references therein) facilitates distinction of these species from those with the I-, X-, and Y-genome. The same additional fragments are present in those species investigated that carry the H-genome, i.e., *Elymus sibiricus* (Dewey 1984), but they are absent in *Elymus semicostatus* which, instead of the H-genome, possesses the Y-genome (Salomon 1993). The H-genome-specific DNA fragments are also present in the hexaploid *Elytrigia repens*. Dewey (1984) concluded that *E. repens* has two homoeologous sets of the S-genome and a third genome of unknown origin, and speculated that the third genome could possibly be either the J-genome of *Thinopyrum* or the H-genome of *Hordeum*. The presence of an H-genome was postulated from meiotic pairing data of intergeneric hybrids (Cauderon and Saigne 1961; Dewey 1965; M. Assadi, personal communication), and our data further indicate that the unidentified genome in *E. repens* is presumably an H-genome.

The genomic makeup of *Leymus* was given as JN by Dewey (1984), where J originated from *Thinopyrum* and N is derived from *Psathyrostachys*. However, recently, serious doubts about the presence of a J-genome in *Leymus* have been inferred from both variation in repetitive DNA sequences (Zhang and Dvorák 1991) and meiotic pairing data (Wang and Jensen 1994). Our data may also be interpreted as indicating the absence of the J-genome in *Leymus* or, at least, that the genome of *T. junceum* is not present in *L. arenarius*.

The high copy number of dpTa1 in the genome of *Aegilops tauschii*, when compared to other of the Triticeae species examined, implies amplification in the D-genome. This observation is further supported by the high level of dpTa1 in *Triticum aestivum* (ABD) and the low copy number in *T. durum* (AB) and *T. monococcum* (A). The hybridization signal with DNA from the latter species is very weak and only present under gentle hybridization conditions. Rayburn and Gill (1986, 1987) isolated a D-genome-specific repeated DNA sequence from *Aegilops tauschii*, pAS1. However, a lack of information concerning the primary structure of this sequence prevents any conclusions about homology between dpTa1 and pAS1 from being drawn.

To understand the molecular mechanisms responsible for the heterogeneity of dpTa1, it is important to compare the primary structure of cloned representatives from different species. Recently, the monomeric unit of a tandemly repeated sequence from *H. chilense* (pHcKB6) was described by Anamthwat-Jónsson and Heslop-Harrison (1993). Sequence comparison revealed high homology (77%) to dpTa1, but also the accumulation of point mutations such as nucleotide substitutions and deletions/insertions (Fig. 4). Similarity was also found between the 3′-end of dpTa1 and a family of dispersed repetitive sequences, also from *H. chilense* (Hueros et al. 1993). This could explain the presence of bands in addition to the ladder-pattern in some species.

The dpTa1 family is characterized by a conserved genomic organization and by heterogeneity of the primary structure. The conserved organization and the presumed old age of dpTa1 support the hypothesis of Vogt (1990), which postulates that there is an inherent potential for tandemly repeated sequences to develop a locus-specific, repetitive super-structure. On the other hand, the high level of sequence divergence could make it possible to find genome-specific markers within the dpTa1 family.

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