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Fat element—a new marker for chromosome and genome analysis in the Triticeae

Ekaterina D. Badaeva · Svyatoslav A. Zoshchuk · Etienne Paux · Georges Gay · Natalia V. Zoshchuk · Delphine Roger · Alexander V. Zelenin · Michel Bernard · Catherine Feuillet

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Abstract Chromosomal distribution of the Fat element that was isolated from bacterial artificial chromosome (BAC) end sequences of wheat chromosome 3B was studied in 45 species representing eight genera of Poaceae (*Aegilops*, *Triticum*, *Agropyron*, *Elymus*, *Secale*, *Hordeum*, *Avena* and Triticale) using fluorescence in situ hybridisation (FISH). The Fat sequence was not present in oats and in two barley species, *Hordeum vulgare* and *Hordeum spontaneum*, that we investigated. Only very low amounts of the Fat element were detected on the chromosomes of two other barley species, *Hordeum geniculatum* and *Hordeum chilense*, with different genome compositions. The chromosomes of other cereal species exhibited distinct hybridisation patterns with the Fat probe, and labelling intensity varied significantly depending on the species or genome. The highest amount of hybridisation was detected on chromosomes of the D genome of *Aegilops* and *Triticum* and on chromosomes of the S genome of *Agropyron*. Despite the bioinformatics analysis of several BAC clones that revealed the tandem organisation of the Fat element, hybridisation with the Fat probe produces uneven, diffuse signals in the proximal regions of chromosomes. In some of the genomes we investigated, however, it also forms distinct, sharp clusters in chromosome-specific positions, and the brightest fluorescence was always observed on group 4 chromosomes. Thus, the Fat element represents a new family of Triticeae-specific, highly repeated DNA elements with a clustered–dispersed distribution pattern. These elements may have first emerged in cereal genomes at the time of divergence of the genus *Hordeum* from the last common ancestor. During subsequent evolution, the amount and chromosomal distribution of the Fat element changed due to amplification, elimination and re-distribution of this sequence. Because the labelling patterns that we detected were highly specific, the Fat element can be used as an accessory probe in FISH analysis for chromosome identification and investigation of evolutionary processes at the chromosomal level.

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E. D. Badaeva (✉) · S. A. Zoshchuk · N. V. Zoshchuk · A. V. Zelenin
Engelhardt Institute of Molecular Biology,
Russian Academy of Sciences,
Vavilov Street 32,
Moscow 119991, Russia
e-mail: katerinabadaeva@gmail.com

E. Paux · G. Gay · D. Roger · M. Bernard · C. Feuillet
INRA UBP UMR 1095, Genetics,
Diversity and Ecophysiology of Cereals,
234 Avenue du Brézet,
63100 Clermont-Ferrand, France

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Abbreviations

BAC	bacterial artificial chromosomes
dUTP	2'-dezoxyuridine-5'-triphosphate
FISH	Fluorescence in situ hybridization
LTR	long terminal repeat
PCR	polymerase chain reaction

Introduction

Repetitive DNA sequences are the major component of plant DNA. In cereals, they may comprise up to 90–95% of the nuclear genome (Flavell et al. 1974). Repetitive sequences are highly heterogenic and represent hundreds or even thousands of families that differ with respect to the length of the repeat unit, nucleotide composition, copy number and organisation in the genome (Flavell 1986; Heslop-Harrison 2000; Sharma and Raina 2005). The results of total genome sequencing of two model plant species, rice (*Oryza sativa*) and *Arabidopsis* and partial genome sequencing of some important crops such as wheat, barley, maize, sorghum, sugar beet and grapevine have revealed that the most abundant class of repetitive sequences in plants is LTR retrotransposons (The *Arabidopsis* Genome Initiative 2000; Li et al. 2004; Sequencing Project International Rice G 2005; Paux et al. 2006, 2008; Jaillon et al. 2007; Paterson et al. 2009; Schnable et al. 2009). In plant with large genomes, such as maize, wheat and barley, LTR retrotransposons constitute more than 50% of the genome. From 5% to 11% of wheat DNA is made up of DNA transposons, and the remaining classes of repeats constitute nearly 30% of the wheat genome.

Despite the abundance and outstanding diversity of repetitive DNA sequences in plants, little is known about the causes of their emergence and maintenance in plant genomes. Some authors have hypothesised that these sequences play an important role in chromosome organisation, stabilisation of chromosome structure, recognition and segregation of chromosomes in mitosis and meiosis and regulation of gene activity (Vershinin et al. 1995; Heslop-Harrison 2000; Grewal and Moazed 2003; Sharma and Raina 2005). Changes in the amount and distribution of repetitive DNA are one of the driving forces of plant evolution and speciation (Flavell et al. 1979; Dvořák and Dubkovsky 1996; Cuadrado and Jouve 2002).

It is not surprising, therefore, that repetitive sequences are increasingly attracting the attention of researchers. Over the past several decades, many new families of these sequences have been isolated and characterised (Taketa et al. 2000; Linares et al. 2001; Ananiev et al. 2002; Zhang et al. 2004; Lim et al. 2005). The development of a new vector system bacterial artificial chromosome (BAC) for DNA cloning has played a large role in promoting the discovery of new types of repetitive DNAs (Jiang et al. 1995; Li et al. 2004; Zhang et al. 2004; Charles et al. 2008). However, the number of families of repeats that have been identified and characterised is relatively low, and the number of these sequences that are currently being employed in practical plant cytogenetics is even smaller. Therefore, the search for new markers that can be used for chromosome identification and for the analysis of plant divergence and speciation is an important focus of current studies in plant biology.

Among the Triticeae species, the best studied are the repetitive sequences of rye, *Secale cereale* (Bedbrook et al. 1980; Jones and Flavell 1982a, b; Vershinin et al. 1995; Cuadrado and Jouve 2002) and *Aegilops tauschii*, the D genome donor of wheat (Rayburn and Gill 1986; Badaeva et al. 1996, 2002; Nagaki et al. 1999; Taketa et al. 2000). Recently, BAC end sequencing of the 3B chromosome of wheat resulted in the identification of a new category of tandem repeats, Fat, a highly conserved 500-bp element representing nearly 0.6% of the wheat genome (Paux et al. 2006). Fat is clustered in direct tandem repeats that are separated by a consensus 9-bp spacer sequence (CYGRTTTDB where Y is C or T, R is A or G, D is A, T or G and B is C, T or G). Each unit of the Fat element is made up of two perfect direct repeats (GAGAAGCT) at both ends and two putative autonomously replicating consensus sequences, which determine the replication origin in yeast, which suggests that amplification of the Fat element could occur through rolling circle-like replication (Paux et al. 2006). Our preliminary results revealed that this element is present in all wheat chromosomes and is the most abundant sequence in the D genome.

In the study we present here, we used fluorescence in situ hybridisation (FISH) analysis in a broad range of cereal species to trace alterations in the amount and distribution of the Fat sequence over the course of cereal evolution and to assess its possible utility for the purpose of chromosomal analysis.

Materials and methods

Materials

In total, 69 accessions from 45 species belonging to eight genera of Poaceae were studied. Twenty-four of these species belong to the genus *Aegilops* and nine belong to the genus *Triticum*. In addition, four barley species (*Hordeum*), two rye species (*Secale*), three species of the *Avena* genus and one accession for each of the Triticale, *Agropyron* and *Elymus* genera were examined. The genomic constitutions, chromosome numbers and the origin of the materials that we used are presented in Table 1.

DNA probes

A probe for Fat was synthesised using the 3B_050_N05 BAC clone, following PCR amplification, purification and labelling using a nick translation procedure. Two Fat PCR primers (GGGGAGCTTCTCACAACAAGC and TATTTACCACGGCATGTCGGG) were designed based on the sequence of 3B_050_N05_FM1 (GenBank accession number DX374230) and an approximately 460-bp fragment was amplified by PCR (30 cycles, Tm=60°C). Additional probes, *pSc119.2* (Bedbrook et al. 1980), *pAs1* (Rayburn and Gill 1986), *pTa71* (Gerlach and Bedbrook 1979) and *pTa794* (Gerlach and Dyer 1980), were used for chromosome/genome identification.

Fluorescence in situ hybridisation

Chromosomal preparations and FISH were carried out as described previously (Badaeva et al. 1996) with the following modifications. The Fat sequence was labelled with fluorescein-12-dUTP and detected with anti-fluorescein/Oregon Green rabbit IgG and Alexa Fluor® 488 conjugate (Invitrogen, Carlsbad, CA). The other probes used were labelled with biotin-16-dUTP by nick translocation according to the manufacturer's protocol (Roche, Germany) and detected with streptavidin-Cy3 (Amersham Biosciences, Piscataway, NJ). The slides were counterstained with 2 μg ml^{-1} 4′,6-diamidino-2-phenylindole in 1× phosphate-buffered saline, mounted in Vectashield medium (Vector Laboratories, Peterborough, UK) and examined on an Imager D1 microscope (Carl Zeiss, Germany). From five to 12 metaphase cells from each slide with clear, reproducible signals were selected and captured with an AxioCam HRm black-and-white camera using the AvioVision software, release 4.6.Chromosomes were identified on the basis of labelling patterns produced with the *pSc119.2*, *pAs1*, *pTa71* and *pTa794* probes and classified according to standard nomenclatures developed for each respective species (Cabrera et al. 1995; Badaeva et al. 1996, 2002, 2004; Bardsley et al. 1999; Cuadrado and Jouve 2002).

Results

Wheat

Our previous data showed that hybridisation of the Fat probe on chromosomes of common wheat produces strong, non-uniform fluorescence on 14 chromosomes of the D genome, and the remaining 28 chromosomes exhibit only pale, fuzzy hybridisation signals (Paux et al. 2006). Chromosomes of the A genome show only slightly higher fluorescence levels than B genome chromosomes (Fig. 1a; Supplementary material 1a). In addition to the diffuse signals produced, some D genome chromosomes also contain sharp, brilliantly fluorescing regions. The largest of these is detected in the short arm of chromosome 4D and several additional smaller sites are present on the short and long arms of this chromosome. Two sharp, brilliant signals are seen on the short arm of chromosome 1D. Chromosomes 6D and 7D are both marked by intense, fuzzy labelling of the proximal regions of both arms. Chromosomes 2D, 3D and 5D lacked such brilliant signals, though they were also marked by bright, non-uniform labelling. The distribution of the Fat element on chromosomes of three diploid wheat species (*T. urartu*, *T. boeoticum* and *T. monococcum*) is similar to the pattern of Fat in the A genome of polyploid wheat and consists of unevenly dispersed signals located in the proximal regions of chromosomes (Fig. 2j, k; Supplementary material 2j, k).

Table 1 The list of the materials

No.	Species	Ploidy level	Genome	Accession no.	Country of origin	Source[2]
Aegilops						
1	*Ae. speltoides* Tausch.	2*x*	S	INRA no. 37	Unknown	INRA, Rennes
2	*Ae. longissima* Schweinf. & Muschl.	2*x*	S^l	TA1912	Israel	KSU
3				k-907	Israel	VIR
4	*Ae. sharonensis* Eig.	2*x*	S^{sh}	#23	Israel, Keshon	IEHU
5	*Ae. searsii* Feldman & Kislev	2*x*	S^s	7.15	Israel	Haifa Univ.
6	*Ae. bicornis* (Forssk.) Jaub. & Spach	2*x*	S^b	k-666	Egypt	VIR
7 8				k-1328	Israel	VIR
9	*Ae. umbellulata* Zhuk.	2*x*	U	i-571748	Azerbaijan	VIR
10	*Ae. caudata* L.	2*x*	C	k-1797	Turkey	VIR
11	*Ae. comosa* Sm. in Sibth. & Sm.	2*x*	M	i-577976	Greece	VIR
12	*Ae. uniaristata* Vis.	2*x*	N	TA2688	Greece	KSU
				TA2768	Unknown	KSU
13	*Ae. tauschii* Coss.	2*x*	D	TQ-27	Israel	WIS
14				k-865	Turkey	VIR
15				i-571716	Iraq	VIR
16	*Ae. cylindrica* Host.	4*x*	CD	k-3053	Crimea	VIR
17				No. 1	Armenia	IBA
18				k-1319	Iran	VIR
19	*Ae. ventricosa* Tausch.	4*x*	D^vN^v	k-46	Unknown	VIR
20 21				No. 11 SSD32	France France	INRA, Clermont-Ferrand
22	*Ae. crassa* Boiss.	4*x*	$X^{cr}D^1$	IG-110807	Syria	ICARDA
23				IG-48477	Syria	ICARDA
24				IG-115755	Jordan	ICARDA
25				IG-48585	Jordan	ICARDA
26				IG-48581	Jordan	ICARDA
27				k-2485	Iraq	VIR
28	*Ae. triuncialis* L.	4*x*	UC	к-1757	Uzbekistan	VIR
29	*Ae. biuncialis* Vis.	4*x*	UM^b	IG47604	Cyprus	ICARDA
30				IG49059	Turkey	ICARDA
31	*Ae. geniculata* Roth.	4*x*	UM^o	IG-47761	Turkey	ICARDA
32	*Ae. peregrina* (Hack. In J.Fraser) Msire & Weller (*Ae. variabilis* Eig.)	4*x*	US	k-61	Jordan	VIR
33	*Ae. kotschyi* Boiss.	4*x*	US	TA2206	Azerbaijan	KSU
34	*Ae. columnaris* Zhuk.	4*x*	X^cU^c	TA2084	Turkey	KSU
35	*Ae. neglecta* Req. Ex Bertol. (*Ae. triaristata* Willd.)	4*x*	X^tU^t	i-573362	Palestine	VIR
36				i-570283	Turkey	VIR
37	*Ae. neglecta* Req. Ex Bertol. (*Ae. recta* (Zhuk.) Chennav.)	6*x*	X^tU^tN	k-2509	Spain	VIR
38				k-2070	Spain	VIR
39	*Ae. crassa* Boiss.	6*x*	$X^{cr}D^1D^2$	k-1005	Kazakhstan	VIR
40	*Ae. vavilovii* (Zhuk.)Chennav.	6*x*	$X^{cr}D^1S$	TA2655	Jordan	KSU
41	*Ae. juvenalis* (Thell.) Eig.	6*x*	$X^{cr}D^1U$	i-578009	Iran	VIR
Triticum						
42	*T. boeoticum* Boiss.	2*x*	A^b	INRA23733	Unknown	INRA, Clermont-Ferrand

Table 1 (continued)

No.	Species	Ploidy level	Genome	Accession no.	Country of origin	Source[2]
43	*T. monococcum* L.	2*x*	A^{m}	Du Pays du Sault	France	INRA, Clermont-Ferrand
44	*T. urartu* Thum. ex Gandil.	2*x*	A^{u}	INRA27019	Unknown	INRA, Clermont-Ferrand
45	*T. dicoccoides* Körn.	4*x*	BA	PI471750	Israel	USDA
46				TTD20	Israel	WIS
47	*T. aethiopicum* Jakubz.	4*x*	BA	k-19217	Ethiopia	VIR
48	*T. aestivum* L. em. Thell.	6*x*	BAD	Renan	France	INRA, Clermont-Ferrand
49				Tumenskaya-80	Russia	
50				Erythrospermum-59	Russia	VIGG
51					Russia	VIGG
52				L-2166	Russia	ARISER
53				k-1406	Russia	ARISER
54	*T. spelta* L.	6*x*	BAD	k-45368	Azerbaijan	VIR
55	*T. timopheevii* Zhuk.	4*x*	GA^{t}	k-6825	Georgia	VIR
56	*T. kiharae* Dorof. et Migusch.	6*x*	$GA^{t}D^{sq}$	k-47897	Russia	VIR
Triticale						
57	*Triticale*	6*x*	BAR	Central	Ukraine	PBI
Secale						
58	*S. cereale* L.	2*x*	R	Dan Nove	Poland	INRA, Clermont-Ferrand
59	*S. montanum* Guss.	2*x*	R	SM #1	Israel	Original collection
Hordeum						
60	*H. vulgare* L.	2*x*	I	INRA25160	France	INRA, Clermont-Ferrand
61	*H. spontaneum* C. Koch.	2*x*	I	IG112674	Iran	ICARDA
62	*H. chilense* Roem. et Schult.	2*x*	H^{ch}	Line 1	Spain	IAS-SCIC
63				Line 7	Spain	
64	*H. geniculatum* All.	4*x*	XX_{1}		Russia	ICIG
Agropyron						
65	*A. glaucum* Roemer & Schultes.	6*x*	$SE^{e}E^{b}$	#21	Russia	NIISHCRNZ
Elymus						
66	*E. dahuricus* Turcz. ex Grizeb.[1]	6*x*	SHY	#7	Unknown	MBG
Avena						
67	*A. prostrata* Ladiz.	2*x*	A	k-2055	Spain	VIR
68	*A. ventricosa* Bal.	2*x*	C	k-2056	Algeria	VIR
69	*A. magna* Mur. et Terr.	4*x*	DC	k-1863	Morocco	VIR

KSU Wheat Genetic and Genomics Resource Center, Kansas State University, USA, *VIR* Vavilov Institute of Plant Industry, St. Petersburg, Russia, *IEHU* Institute of Evolution, University of Haifa, Israel, *WIS* Weizmann Institute of Science, Israel, *IBA* Institute of Botany, Erevan, Armenia, *ICARDA* International Center for Agricultural Research in the Dry Areas, Aleppo, Syria, *ARISER* Agricultural Research Institute for South-East Region, Saratov, Russia, *VIGG* Vavilov Institute of General Genetics, Moscow, Russia, *PBI* Plant Breeding Institute, Odessa, Ukraine, *ICIG* Institute of Cytology and Genetics, Novosibirsk, Russia, *IAS-SCIC* Institute for Sustainable Agriculture, Cordoba, Spain, *NIISHCRNZ* Scientific-Research Agricultural Institute of the Central Zone of Non-Chernozem Region, Moscow Region, Nemchinovka, *MBG* Moscow Botanical Garden of the Russian Academy of Sciences, Russia

Diploid *Aegilops*

Ae. tauschii (D genome) chromosomes exhibit the strongest Fat element hybridisation intensities that we detected among the diploid *Aegilops* species. Distribution of the hybridisation signals in this species is similar to what we have seen in the D genome chromosomes of common wheat, though the two subspecies of *Ae. tauschii* differ in their labelling patterns on chromosomes 1D, 2D and 6D (Fig. 2g, h).

Fig. 1 Distribution of the Fat element (*green*) on chromosomes of polyploid cereal species: **a** *Triticum aestivum* (cv. Tumenskaya-80); **b** *Ae. crassa* 4*x* (k-2485); **c** *E. dahuricus*; **d** *Ae. ventricosa* (SSD32); **e** *Ae. geniculata* (IG7761); **f** *A. glaucum*; **g** *Ae. juvenalis* (i-578009); **h** *Ae. biuncialis* (IG47504); **i** *Ae. crassa* 6*x* (k-1005); **j** *Ae. columnaris* (TA2084); **k** *Ae. neglecta* (*Ae. recta*, k-2062); **l** *Ae. kotschyi* (TA2206); **m** *Ae. cylindrica* (k-1319). The chromosomes were co-hybridised with biotinylated probes *pSc119.2* (**a**, **h**, **j**, **k**, **l**) or *pAs1* (**b**–**g**, **i**, **m**) and detected with streptavidin-Cy3 (*red*). Scale bar, 10 μm. Chromosomes of polyploid wheat and *Aegilops* are designated according to (Badaeva et al. 2002, 2004; Schneider et al. 2003); chromosomes of *Ae. columnaris* with unknown homology and genome affinity are designated with letters (*A*–*J*)

In the two studied accessions of the subspecies *strangulata*, chromosome 1D contains brilliant signals in the distal part of the short arm that are absent in the respective chromosome of the accession k-865 (ssp. *tauschii*). By contrast, in the latter case, we observe a clear signal in the distal part of the chromosome 6D short arm (Fig. 2o; Supplementary material 2g, h, o). Alteration of pale and brightly fluorescing regions characterises the long and the short arms of chromosome 2D of ssp. *tauschii*, while in ssp. *strangulata*, such non-uniform labelling is observed in the long arm only.

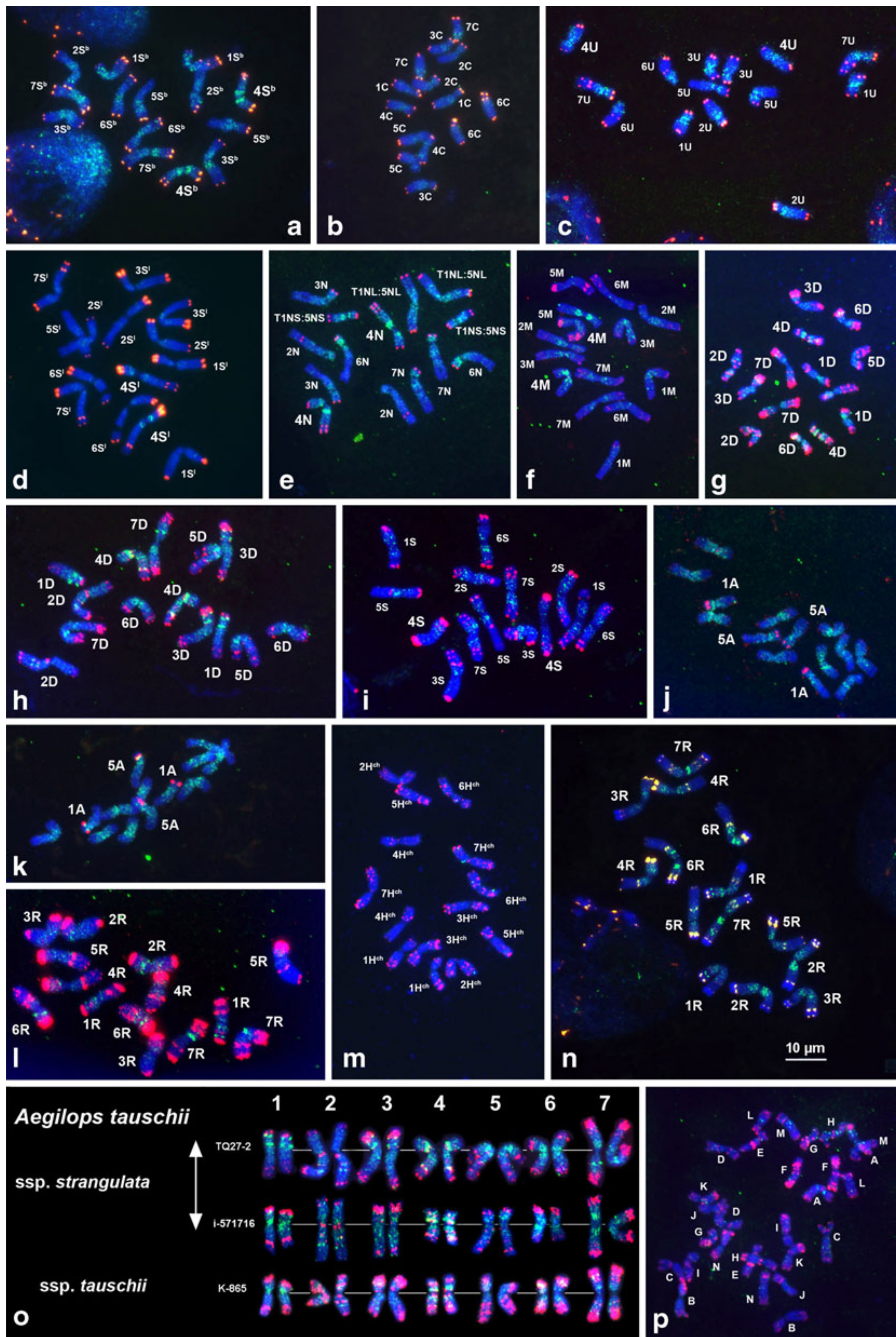
Aegilops uniaristata (N genome) and *Aegilops comosa* (M genome) show a moderate amount of hybridisation with the Fat probe (Fig. 2e, f,), while the intensity of hybridisation to *Aegilops umbellulata* (U genome) and the *Aegilops caudata* (C genome) chromosomes is noticeably weaker (Fig. 2b, c; Supplementary material 2b, c, e, f). In species of the Sitopsis (S genome) group, labelling intensity is highest for *Aegilops bicornis* (Fig. 2a), followed by *Aegilops longissima* and *Aegilops sharonensis* (Fig. 2d), and only weak hybridisation is seen in *Aegilops speltoides* (Fig. 2i; Supplementary material 2a, d, i). For all the genomes we investigated in this group, the Fat element is found in an uneven, diffuse pattern, localised to the proximal regions of all chromosomes, with the brightest fluorescence being seen on group 4 chromosomes.

Polyploid *Aegilops* of the D genome cluster

FISH analysis of *Aegilops cylindrica* reveals significant differences in fluorescence intensity of the Fat probe between the C and D genomes (Fig. 1m); hybridisation to chromosomes of the C genome is very poor, but the D genome chromosomes are heavily labelled. The distribution of signals produced by the Fat and *pAs1* probes on the D genome chromosomes of *Ae. cylindrica* is similar to the hybridisation patterns on the chromosomes of diploid *Ae. tauschii* ssp. *tauschii*. In another tetraploid species of the D genome cluster, *Aegilops ventricosa*, all chromosomes hybridise with the Fat element probe, though fluorescence intensity on the D^v genome chromosomes is slightly higher than on the N^v genome chromosomes (Fig. 1d). The hybridisation patterns seen on the D genome chromosomes of both this tetraploid species and the diploid *Ae. tauschii* ssp. *tauschii* are virtually the same; however, there are differences in the labelling patterns of some N^v genome chromosomes of *Ae. ventricosa* compared with *Ae. uniaristata* (Supplementary material 1d, m; 2e, h, o).

The tetraploid species *Aegilops crassa*, the most ancient polyploid *Aegilops* species, originated from hybridisation of *Ae. tauschii* with an extinct diploid species of the Sitopsis group (Zhang and Dvorak 1992; Badaeva et al. 2002). All *Ae. crassa* chromosomes hybridise with the Fat probe with approximately the same intensity (Fig. 1b; Supplementary material 1b). However, labelling patterns of the D^1 and X^{cr} genomes differ from their putative diploid ancestors. The overall fluorescence seen on chromosomes $1D^1$, $3D^1$, $6D^1$ and $7D^1$ is weaker, and chromosome $1D^1$ does not exhibit any distinct signals in its short arm. Chromosome $4D^1$ is missing a small distal Fat site that is present in the short arm of the respective chromosome of the ancestral species. Fluorescence intensity of the X^{cr} genome chromosomes is noticeably higher than in genomes of all species of the Sitopsis group and is comparable with what we have seen on chromosomes in the D^1 genome. Thus, large, brilliant signals are present in the short arm of chromosome $3X^{cr}$ and the long arms of chromosomes $6X^{cr}$ and $7X^{cr}$. Significant changes in the *pAs1* and Fat-labelling patterns seen on chromosomes $2D^1$ and $5D^1$ and the emergence of large clusters of Fat element on some X^{cr} genome chromosomes are probably caused by species-specific translocations with the D genome chromosomes. At the same time, intergenomic translocations cannot explain the overall increase of labelling intensity of the X^{cr} genome chromosomes. This could indicate that the Fat element expanded over the course of *Ae. crassa* evolution from the D^1 to the X^{cr} genome.

The tetraploid *Ae. crassa* is the parental form of three hexaploid species—*Ae. crassa* (genome $D^1X^{cr}D^2$), *Aegilops vavilovii* (genome $D^1X^{cr}S$) and *Aegilops juvenalis* (genome $D^1X^{cr}U$). FISH analysis reveals that labelling patterns of the hexaploid *Ae. crassa* (Fig. 1i) and *Ae. vavilovii* chromosomes are the same to what is seen in the parental species. In contrast, we found significant alterations in the distribution of the Fat probe on the chromosomes of all three genomes of *Ae. juvenalis* (Fig. 1g; Supplementary material 2b, g, i), some of which are likely to be due to chromosomal rearrangements that have occurred over the course of evolution (Badaeva et al. 2002).


10 µm
Aegilops tauschii
1 2 3 4 5 6 7
ssp. strangulata
TQ27-2
i-571716
ssp. tauschii
K-865

◀ **Fig. 2** Distribution of the Fat element (*green*) on chromosomes of the diploid *Aegilops* and rye species and diploid and polyploid barleys: **a** *Ae. bicornis* (k-1328); **b** *Ae. caudata* (k-1797); **c** *Ae. umbellulata* (i-571748); **d** *Ae. longissima* (k-907); **e** *Ae. uniaristata* (TA2768); **f** *Ae. comosa* (i-577976); **g** *Ae. tauschii* ssp. *tauschii* (k-865); **h** *Ae. tauschii* ssp. *strangulata* (TQ27); **i** *Ae. speltoides* (#37); **j** *T. urartu* (INRA27019); **k** *T. monococcum* (cv. Du Pays du Sault); **l** *S. cereale* (cv. Dan Nove); **m** *H. chilense* (#7); **n** *S. montanum* (Israel, El-Kuneitra); **p** *H. geniculatum* ($2n=4x=28$). **o** The distribution of Fat (*green*) and *pAs1* (*red*) probes on chromosomes of *Ae. tauschii* ssp. *strangulata* (TQ27 and i-571716) and ssp. *tauschii* (k-865). The chromosomes were co-hybridised with biotinylated probes *pSc119.2* (**a**–**e**, **i**, **l**, **n**), *pAs1* (**f**–**h**, **m**, **p**) and *pTa794* (**j**, **k**) and detected with streptavidin-Cy3 (*red*). Scale bar, 10 µm. Chromosomes of rye are classified according to Cuadrado and Jouve (2002), chromosomes of *H. chilense* are classified according to Cabrera et al. (1995) and chromosomes of *Aegilops* species are classified according to Badaeva et al. (1996)

Polyploid *Aegilops* of the U genome cluster

This group of polyploid *Aegilops* includes seven tetraploid and one hexaploid species, all of which possess the U genome in combination with the C (*Aegilops triuncialis*), S (*Aegilops peregrina*, *Aegilops kotschyi*), M (*Aegilops biuncialis*, *Aegilops geniculata*) or X genome (*Aegilops columnaris*, *Aegilops neglecta* 4*x* (=*Aegilops triaristata*), *Ae. neglecta* 6*x* (=*Aegilops recta*)). The chromosomes in this group all exhibit moderate, diffuse hybridisation patterns with the Fat probe. Irrespective of the genome constitution of these polyploid species, all of the chromosomes in their karyotypes have similar fluorescence intensities. However, in some species, such as *Ae. biuncialis* (Fig. 1h), *Ae. geniculata* (Fig. 1e), *Ae. columnaris* (Fig. 1j), *Ae. neglecta* 6*x* (Fig. 1k), *Ae. kotschyi* (Fig. 1l) and *Ae. peregrina*, we did find one to several clusters of Fat probe hybridisation that permitted the identification of at least a few chromosomes in their karyotypes (Supplementary material 1e, h, j, k, l).

Other cereals

We carried out our FISH analysis with the Fat probe on two rye species (*Secale cereale* and *Secale montanum*), on hexaploid Triticale, four species of barley (*Hordeum vulgare*, *Hordeum spontaneum*, *Hordeum geniculatum* and *Hordeum chilense*), three oats with different genome constitutions (*Avena prostrata*, *Avena ventricosa* and *Avena magna*), *Elymus dahuricus* and *Agropyron glaucum* (Table 1). No hybridisation with the Fat probe is detected in the genomes of any of the three oat species or in two of the barley species, *H. spontaneum* and *H. vulgare* (I genome). In two other barley species, the diploid *H. chilense* (H genome) and the tetraploid *H. geniculatum* (XX_1 genome), very weak signals are observed (Fig. 2m, p). Distinct hybridisation with the Fat probe is detected in all of the other Triticeae species that we investigated. All chromosomes of *S. cereale* and *S. montanum* and the R genome chromosomes in the karyotype of Triticale are labelled in their proximal region, and chromosomes 1R, 4R, 6R and 7R have additional chromosome-specific clusters of Fat probe hybridisation (Fig. 2l, n). In the hexaploid wheatgrass species *A. glaucum*, 14 chromosomes are heavily labelled, four other chromosomes possess small distinct clusters of Fat probe in pericentromeric regions, and the remaining 24 chromosomes hybridise poorly with this probe (Fig. 1f). In contrast, only eight of 42 chromosomes of *E. dahuricus* exhibit weak, diffuse Fat signals in their proximal regions (Fig. 1c; Supplementary material 1c, f; 2l, m, n, p).

Discussion

The results we present here demonstrate that the Fat element is a Triticeae-specific sequence. It is absent from the *Aveneae* species and appears to have arisen first in barley species (*H. chilense* and *H. geniculatum*) among the cereal genomes that we investigated in this study. In these barleys, we observe very weak, scarce signals distributed not specifically along all their chromosomes. In two other *Hordeum* species—*H. vulgare* and *H. spontaneum*, no hybridisation with the Fat probe have been detected. At the same time, in all of the other Triticeae species, we observe distinct hybridisation with the Fat probe. Based on these results, it seems likely that this sequence may have first appeared in the genomes of cereal species at the time of divergence of the genus *Hordeum*, estimated to be ~11 MYA (Huang et al. 2002). During subsequent evolution, the amount and distribution of Fat element changed in a species-specific and genome-specific manner.

The genus *Secale* is probably diverged from the common ancestor of the Triticeae next after barley (Flavell et al. 1977; Huang et al. 2002). In two rye species, *S. montanum* and *S. cereale*, we observe clear, dispersed labelling and several brilliant, clustered signals in chromosome-specific positions. These results indicate

that evolution of rye was accompanied by amplification of the Fat sequence. Noteworthy, hybridisation with the Fat probe is completely absent from the regions of telomeric heterochromarin that are known to be formed by the arrays of tandemly repeated DNA families, some of which are found only in rye (Bedbrook et al. 1980; Jones and Flavell 1982b; Vershinin et al. 1995; Cuadrado and Jouve 2002). Based on these observations, we assume that amplification of the Fat sequence occurred independently of the emergence and amplification of the rye-specific repetitive DNAs.

Two other genera of the Triticeae that are *Elymus* and *Agropyron* diverged from the common ancestor of cereals prior to wheat/*Aegilops* complex. These two genera include a broad range of diploid and polyploid, annual and perennial species with different genome compositions (Dewey 1984). In our study, we analysed two hexaploid species representing each of these genera. FISH analysis reveals sharp differences between *E. dahuricus* and *A. glaucum* in hybridisation patterns of the Fat sequence on their chromosomes. Fourteen of 42 wheatgrass chromosomes are intensely labelled with the Fat probe and four other chromosomes possess small, but clear signals in their pericentromeric regions. This pattern is similar to what was obtained earlier in *A. glaucum* using FISH with the genomic DNA of *Pseudoroegneria strigosa* as a probe (Chen et al. 1998). Based in this observation, we suggest that these are the St genome chromosomes that are labelled with the Fat sequence, and the genome of the putative diploid progenitor of *A. glaucum* may possess a high amount of the Fat element. On the other hand, a diploid species from the *Pseudoroegneria* genus is thought to be the St genome to the polyploid *Elymus* species (Sun et al. 2007). However, only little amount of hybridisation with the Fat probe is detected on a few chromosomes of *E. dahuricus*. Clear differences in the amount of Fat probe hybridisation between the related genomes in karyotypes of the two hexaploid species can be due to different mechanisms: (1) the ancestral *Pseudoroegneria* forms that were the St genome donors to *Agropyron* and *Elymus* differ substantially in the amount of Fat element or (2) the Fat element was amplified in the St genome of *A. glaucum* over the course of polyploidisation.

The Fat sequence is found in all species of *Triticum* and *Aegilops*; however, it is most abundant in the D genome. Therefore, massive amplification of the Fat element may have occurred in the ancestral form of *Ae. tauschii*. In other species of *Aegilops*, the amount of Fat element present is probably similar to the level that was present in the ancient progenitor of *Aegilops* and wheat or increased slightly or decreased. It is of interest that the fluorescence intensity of Fat probe hybridisation is always higher in those *Aegilops* species that also show stronger hybridisation with the *pAs1* probe. Thus, amplification of the Fat element over the course of the evolution of the *Aegilops* species is probably connected to amplification of the *pAs1* sequence.

Evolution of *Ae. comosa* and *Ae. uniaristata* belonging to the Comopyron section was most likely accompanied by an increase in the amount of the Fat element, as their genomes show a relatively high level of hybridisation with the Fat probe. All diploid *Aegilops* species of the Sitopsis group have similar distribution pattern of the Fat probe; however, lower fluorescence intensities on the chromosomes of more 'advanced' species, such as *Ae. longissima* and *Ae. sharonensis*, compared with *Ae. bicornis* that is considered to be more 'primitive' (Dvořák and Zhang 1992) is probably indicative of gradual elimination of the Fat sequence in the course of divergence of this group. Diploid wheat species all have the same amount and distribution of the Fat element which is probably similar to what was present in the genome of the putative progenitor of *Triticum*/*Aegilops* complex.

Formation of polyploid *Triticum* and *Aegilops* species can also lead to alterations in the amount and distribution of the Fat element in one or two parental genomes; however, it is a gradual process that can probably be affected by the genome composition of a polyploid. In this study, we have not revealed any changes in the labelling patterns of parental genomes in such species as common wheat and *T. timopheevii*, *Ae. cylindrica*, *Ae. triuncialis*, *Ae. peregrina* and *Ae. kotschyi*, *Ae. recta*, hexaploid *Ae. crassa* and *Ae. vavilovii*, all of which are considered as 'recent' polyploids (Zhang et al. 1992; Dubkovsky and Dvořák 1994, 1995; Linc et al. 1999; Feldman 2001; Huang et al. 2002). Only little modifications are found in tetraploid emmer wheat and *Ae. ventricosa*. The origin of tetraploid wheat is dated back to ~0.5 MYA (Huang et al. 2002) and, although the age of *Ae. ventricosa* has not been determined, some modifications of the parental genomes detected in this species using different approaches suggest that it is not a recent polyploid (Dubkovsky and Dvořák 1994; Bardsley et al. 1999; Badaeva et al. 2002, 2008). By contrast, the significant changes are found in the

tetraploid *Ae. crassa*, the most ancient *Aegilops* species (Dubkovsky and Dvořák 1995). Hybridisation of the Fat probe to the X^{cr} genome chromosomes is significantly higher than in any of the diploid species of the Sitopsis group; what is more, some chromosomes also possess brilliant clusters of the Fat probe hybridisation which are not found in genomes of the putative progenitor species. An increase in the amount of the Fat element in the X^{cr} genome of *Ae. crassa* can be caused by the expansion of the Fat sequence from the D to the X^{cr} genome and its local amplification in certain sites of the X^{cr} genome chromosomes over the course of long-term co-evolution of two sharply different genomes in a single nucleus.

The significant changes of the Fat probe labelling patterns are also found in some other *Aegilops* species with the UM (*Ae. geniculata* and *Ae. biuncialis*) or UX genome composition (*Ae. columnaris* and *Ae. neglecta*) and in hexaploid *Ae. juvenalis* with the D^{1}X^{cr}U type of nuclear genome. Although some of these changes are likely to be due to chromosomal rearrangements identified earlier in these species (Friebe et al. 1999; Badaeva et al. 2002, 2004; Schneider et al. 2008), the emergence of sharp, brilliant clusters of the Fat probe hybridisation suggests that the formation of these polyploids has promoted local amplification of the Fat sequence in one or several chromosomal sites. As the significant reorganisations of parental genomes in the above-mentioned *Aegilops* species at the molecular and chromosomal levels are also shown by other authors (Abubakar and Kimber 1982; Kimber et al. 1988; Yen and Kimber 1990, 1992; Dubkovsky and Dvořák 1995; Resta et al. 1996), we assume that the alterations of the Fat probe distribution that we observed in our study are probably one of the constituents of these processes. Because all these polyploids share the same genome inherited from diploid *Ae. umbellulata*, it could be the U genome that promotes the processes of genome changes in the polyploids.

The tandem organisation of the Fat element was first hypothesised on the basis of analysis of 11 Mb of random BES from wheat chromosome 3B (Paux et al. 2006). However, according to FISH analysis, the distribution of the Fat probe is predominantly dispersed within proximal regions of chromosomes. Most families of tandem repeats that have been described so far are usually located in subtelomeric, pericentromeric or interstitial chromosome regions, and in FISH experiments, these elements appear as sharp, distinct signals on chromosomes (Badaeva et al. 1996; Heslop-Harrison 2000; Sharma and Raina 2005). Dispersed labelling is usually seen for mobile elements, in particular LTR retrotransposons (Katsiotis et al. 1996; Belyayev et al. 2001, 2005). Some of these elements can also form clusters in certain chromosome regions (Schmidt et al. 1995; Staton et al. 2009; Thomas et al. 2009). However, the Fat element exhibits no homology to any known retrotransposon families (Paux et al. 2006). Moreover, the labelling intensity that is characteristic of some of the retrotransposon families that have been mapped is usually much weaker than the hybridisation pattern that we see for the Fat element.

Construction of a BAC-based integrated physical map of the largest (995 Mb) wheat chromosome 3B (Paux et al. 2008) also revealed complicated genome organisation of the Fat sequence. Thus, in the BAC clone TAACSPALLhA_1119J14 (accession number AC216572), which is 131 kb long, 264 Fat repeat copies, 465 bp each form long tandems spanning over 129,880 bp. Several other BAC clones are found to carry more than 10 tandemly organised Fat repeats and, in most BACs, two or three copies of the Fat element are interspersed within several kilobase-long stretches of other repetitive sequences. Taking together our molecular and FISH results, we can conclude that, in genomes of cereal species, the Fat element is organised into tandem arrays varying in length (from few to several hundred copies) that are interspersed with arrays of other types of repetitive sequences. Thus, the Fat element represents a new family of Triticeae-specific repeated DNA elements exhibiting a clustered–dispersed distribution and preferential localisation to proximal chromosome regions. Though no classes of repetitive DNA with similarity to Fat have been found in the Triticeae (Sharma and Raina 2005), they have been identified in other plants. In particular, two families of satellite DNA with dispersed distribution have been identified in the *Aveneae*: *pAm1*, specific to the C genome (Solano et al. 1992), and *pAs120a*, specific to the A genome (Irigoyen et al. 2001).

In our analyses, we found that chromosomes of the D genome of *Ae. tauschii* and the polyploid species that are derived from it and chromosomes of some other species of *Aegilops*, *Secale* and *Agropyron* exhibit rather conservative and specific Fat probe hybridisation. Therefore, the Fat element, like other families of repetitive sequences that have been

cloned, can be used in FISH analysis for chromosome identification and investigation of evolutionary processes at the chromosomal level.

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