Molecular cytogenetic analysis of *Aegilops cylindrica* Host

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Abstract: The genomic constitution of *Aegilops cylindrica* Host (2*n* = 4*x* = 28, $D^cD^cC^cC^c$) was analyzed by C-banding, genomic in situ hybridization (GISH), and fluorescence in situ hybridization (FISH) using the DNA clones pSc119, pAs1, pTa71, and pTA794. The C-banding patterns of the D^c- and C^c-genome chromosomes of *Ae. cylindrica* are similar to those of D-and C-genome chromosomes of the diploid progenitor species *Ae. tauschii* Coss. and *Ae. caudata* L., respectively. These similarities permitted the genome allocation and identification of the homoeologous relationships of the *Ae. cylindrica* chromosomes. FISH analysis detected one major 18S-5.8S-25S rDNA locus in the short arm of chromosome $1C^c$. Minor 18S-5.8S-25S rDNA loci were mapped in the short arms of $5D^c$ and $5C^c$. 5S rDNA loci were identified in the short arm of chromosomes $1C^c$, $5D^c$, $5C^c$, and $1D^c$. GISH analysis detected intergenomic translocation in three of the five *Ae. cylindrica* accessions. The breakpoints in all translocations were non-centromeric with similar-sized segment exchanges.

Key words: *Aegilops cylindrica*, C-banding, GISH, FISH, genome evolution.

Résumé : La constitution génomique de l'*Aegilops cylindrica* Host (2*n* = 4*x* = 28, $D^cD^cC^cC^c$) a été analysée par révélation des bandes C, par hybridation génomique in situ (GISH) et par hybridation in situ en fluorescence (FISH) à l'aide des clones d'ADN pSc119, pAs1, pTa71 et pTA794. Les motifs de bandes C des chromosomes des génomes D^c et C^c de l'*Ae. cylindrica* sont semblables à ceux des chromosomes des génomes D et C chez les espèces donatrices *Ae. tauschii* Coss. et *Ae. caudata* L., respectivement. Ces similitudes ont permis d'attribuer les génomes et d'identifier les relations homéologues entre les chromosomes de l'*Ae. cylindrica*. L'analyse FISH a détecté un locus majeur d'ADNr 18S-5,8S-25S sur la bras court du chromosome $1C^c$. Des loci mineurs d'ADNr 18S-5,8S-25S ont été localisés sur les bras courts des chromosomes $5D^c$ et $5C^c$. Des loci d'ADNr 5S ont été situés sur les bras courts des chromosomes $1C^c$, $5D^c$, $5C^c$ et $1D^c$. Une analyse GISH a détecté des translocations intergénomiques chez trois des cinq accessions de l'*Ae. cylindrica*. Les points d'échange pour toutes ces translocations n'étaient pas centromériques et les échanges impliquaient des segments de taille semblable.

Mots clés : *Aegilops cylindrica*, révélation des bandes C, GISH, FISH, évolution des génomes.

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Introduction

Aegilops cylindrica Host (2*n* = 4*x* = 28, $D^cD^cC^cC^c$), an autogamous, tetraploid wild relative of bread wheat *Triticum aestivum* L. (2*n* = 6*x* = 42, AABBDD), is native to the Mediterranean, Middle East, Asia, and was also introduced to the Great Plains and the Pacific northwest of the United States (Kimber and Feldman 1987; van Slageren 1994). The genomic constitution of *Ae. cylindrica* was determined by analyses of chromosome pairing (Sax and Sax 1924; Kihara 1931; Kihara and Matsumura 1941; Sears 1944; McFadden and Sears 1946), storage proteins (Johnson 1967; Masci et al. 1992), isozymes (Jaaska 1981; Nakai 1981), and differences in restriction length patterns of repeated nucleotide sequences (Dubcovsky and Dvorak 1994). These studies identified the diploid species *Ae. caudata* L. (2*n* = 2*x* = 14, CC) as the donor of the C^c genome and *Ae. tauschii* Coss. (2*n* = 2*x* = 14, DD) as the donor of the D^c genome of *Ae. cylindrica*. The cytoplasm of *Ae. cylindrica* was contributed by *Ae. tauschii* (Maan 1976; Tsunewaki 1989, 1996).

Detailed karyotypic data including C-banding patterns and the distribution of four repetitive DNA sequences have been reported recently for all the diploid *Aegilops* species (Badaeva et al. 1996*a*, 1996*b*). This has set the stage for the analysis of genome differentiation of the polyploid *Aegilops* species. Here we report on the genome structure of *Ae. cylindrica* based on the results of C-banding, fluorescence in situ hybridization (FISH), and genomic in situ hybridization (GISH) analyses.

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Materials and methods

Five accessions of *Ae. cylindrica* were analyzed. TA2201 (= AE663) (var. *pauciaristat* Eig) collected in France, TA2202 (= AE889) (var. *aristulata* (Zhuk.) Tzvel.) collected in Romania, TA2203 (= AE746) collected in Iran, and TA2204 (= AE719) collected in Armenia and kindly provided by Dr. K. Hammer, IPK Gatersleben, Germany. The accession TA2205 (= BGRC1461) of unknown origin was obtained from the germplasm bank at Braunschweig, Germany. The *Aegilops* nomenclature follows that proposed by van Slageren (1994).

For chromosome identification, the C-banding protocol described by Gill et al. (1991) was used.

FISH analysis was used to map four repetitive DNA sequences on metaphase chromosomes of *Ae. cylindrica* accessions TA2201, TA2202, TA2203, and TA2204. The probe pTa71 contains an insert of the clone pTA71 (Gerlach and Bedbrook 1979) recloned in the *Eco*RI site of the vector pUC19 (O'Dell, personal communication). The 9.05 kbp fragment is a part of rDNA repetitive unit consisting of one copy each of 18S rDNA, 5.8S rDNA, 25S rDNA, and an intergenic spacer from wheat cv. Chinese Spring. The probe pTA794 contains a 410 bp *Bam*HI fragment including an intergenic spacer and is part of 5S rDNA gene family isolated from wheat cv. Chinese Spring and inserted into the plasmid pBR322 (Gerlach and Dyer 1980). The probe pAs1 contains a 1 kb long DNA fragment isolated from *Ae. tauschii* in the plasmid pUC8 (Rayburn and Gill 1986*a*). The clone pSc119 contains a 120 bp repeat derived from an *Eco*RI relic DNA of rye cv. King II inserted into the plasmid pBR322 (Bedbrook et al. 1980).

Root tip meristem preparation and FISH were carried out according to Pickering et al. (1997).

Probes were labeled by nick translation in the presence of FluoroGreen and FluoroRed (Amersham) as described in Table 1. The hybridization solution per slide (30 μL) contained 50% formamide, 2 × SSC, 10% dextran sulfate, 0.1% sodium dodecyl sulfate, 50 ng/μL carrier DNA, and a combination of two 400 pg/μL fluorochrome-labeled probes pTa71 and pTA79. The slides were treated for 10 min at 80°C followed by hybridization for 6 h at 37°C and subsequent washing to a stringency of 82–84%. Chromosomes were counterstained with 1 μg/mL 4′,6-diamidino-2-phenylindole·2HCl and mounted with antifade solution. After documentation of the FISH sites, the probes were stripped from the slides in 50% formamide and 0.5 × SSC. The same slides were then rehybridized using a probe combination of pSc119 and pAs1 with the same conditions as described for the first round of hybridization. Chromosome preparations of the accessions TA2201, TA2202, and TA2203 also were hybridized with the probe combinations of pTa71 with pAs1, and pTa71 with pSc119. Signals were visualized using an Olympus BH-2 microscope equipped for phase contrast and epifluorescence. Images were captured with a SPOT CCD camera using the appropriate SPOT software (Diagnostics Instruments, Inc.) and processed using the PhotoShop 4.0 software (Adobe). Images were printed on a Kodak ds 8650 PS color printer.

GISH analyses followed the protocol of Reader et al. (1994) with minor modifications. Total genomic DNA was isolated from freeze-dried leaf tissue of 3-week-old seedlings of *Ae. caudata* and *Ae. tauschii* according to Sharp et al. (1988). The *Ae. caudata* DNA was digested with *Sau*3A1 and labeled with FluoroGreen by nick translation and used as hybridization probe for detecting C-genome specific chromatin. Unlabeled *Ae. tauschii* DNA was added as a competitor at 70 times excess to the probe amount in order to block D-genome specific and common sequences in the hybridization step. Fifty microlitres of denatured hybridization solution containing 2 × SSC, 10% dextran sulfate, 0.2% sodium dodecyl sulfate, and 1 ng/μL labeled probe DNA together with the competitor DNA, were loaded per slide and incubated for 2 h at 65°C. Following appropriate washes, the slides were mounted with antifade solution and fluorescence signals recorded on Fujicolor 400 ASA film, scanned, digitized, and processed by using PhotoShop 4.0 software. Images were printed as described for FISH prints.

Table 1. Probe and fluorochrome combinations used for FISH mapping.

Probe	Fluorochrome	$Excitation_{max}$ (nm)	$Emission_{max}$ (nm)
pAs1	FluoroGreen Fluorescein-11-dUTP	490	520
Sc119	FluoroGreen Fluorescein-11-dUTP	490	520
Sc119	FluoroRed Rhodamine-4-dUTP	545	575
Ta71	FluoroRed Rhodamine-4-dUTP	545	575

The D^c-genome chromosomes of *Ae. cylindrica* were assigned according to their homoeologous groups by comparison with the standard karyotype of the D-genome progenitor species *Ae. tauschii* (Friebe et al. 1992*a*). The assignment of the C^c-genome chromosomes of *Ae. cylindrica* was based on the comparison with the standard karyotype of the C-genome progenitor species *Ae. caudata* (Friebe et al. 1992*b*). The C^c-genome chromosomes were designated according to the nomenclature of Friebe et al. (1992*b*) with letters from A to G because the homoeology of most of these chromosomes remains to be established.

Results

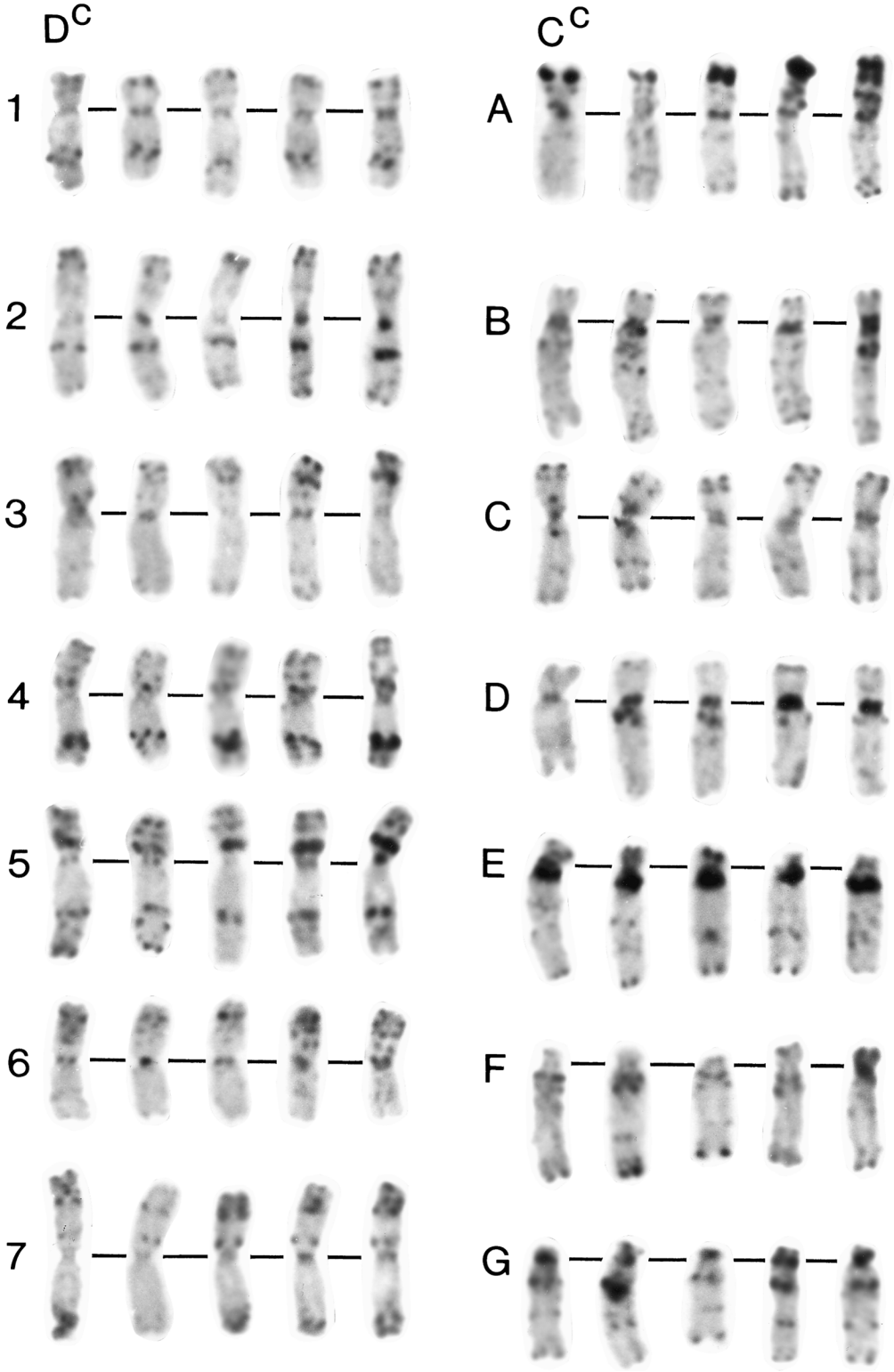
C-banding analysis

The C-banding patterns of the D^c- and C^c-genome chromosomes of *Ae. cylindrica* are similar to those of the D- and C-genome chromosomes of the diploid progenitor species *Ae. tauschii* and *Ae. caudata*, respectively (Fig. 1). Only minor variation in C-banding patterns were observed for chromosomes in both the D^c and C^c genomes of *Ae. cylindrica*. The range of C-band polymorphism was much smaller compared to the variation in C-banding patterns observed in *Ae. tauschii* and *Ae. caudata* (Fig. 1). Chromosome $5D^c$ in accession TA2202 had an unbanded segment at the telomeric region of the short arm, which suggests that this chromosome might be structurally rearranged.

Mapping of rDNA loci

The rDNA loci (NOR and 5S) were mapped by FISH using the probe combination pTa71 and pTa794 (Fig. 2*b* and 2*c*) and sequential FISH with the probe combination pSc119 and pAs1 to identify individual chromosomes (Rayburn and Gill 1986*b*, Badaeva et al. 1996*a*) on the same metaphase plate (see Fig.2*a*–2*e*). The tetramethylrhodamine-labeled pTa71 probe generated 6 red hybridization sites with 3 different levels of fluorescence intensity (Fig. 2*b*). Two major signal sites detect the 18S-5.8S-25S rDNA loci at the nucleolar organizer regions (NORs) in the short arms of chromosome $1C^c$ (open arrows). The 4 smaller FISH sites belong to 18S-5.8S-25S rDNA loci at NORs in the short arms of chromosomes $5D^c$ (solid arrow heads) and $5C^c$ (open arrow heads). The level of signal intensity at the NORs of chromosome $1C^c$ is higher than that of the $5C^c$ chromosome. Chromosome $1C^c$ (solid arrows) can be distinguished from $5C^c$ by the presence of a secondary constriction at metaphase under phase contrast followed by C-banding analysis (Fig. 2*a*).

Fig. 1. C-banding polymorphism detected in *Aegilops cylindrica* (left to right): TA2205, TA2201, TA2204, TA2203, and TA2202. The horizontal black line indicates the centromere.



Simultaneous FISH using a fluorescein-labeled pTA794 probe identifies 8 green 5S rDNA sites (4 loci) (Fig. 2*c*). Three loci are located proximally to the NORs on chromosome pairs $1C^c$, $5D^c$, and $5C^c$. An additional locus is present at a very distal position in the short arm of chromosome pair $1D^c$ (solid arrows in Fig. 2*c*).

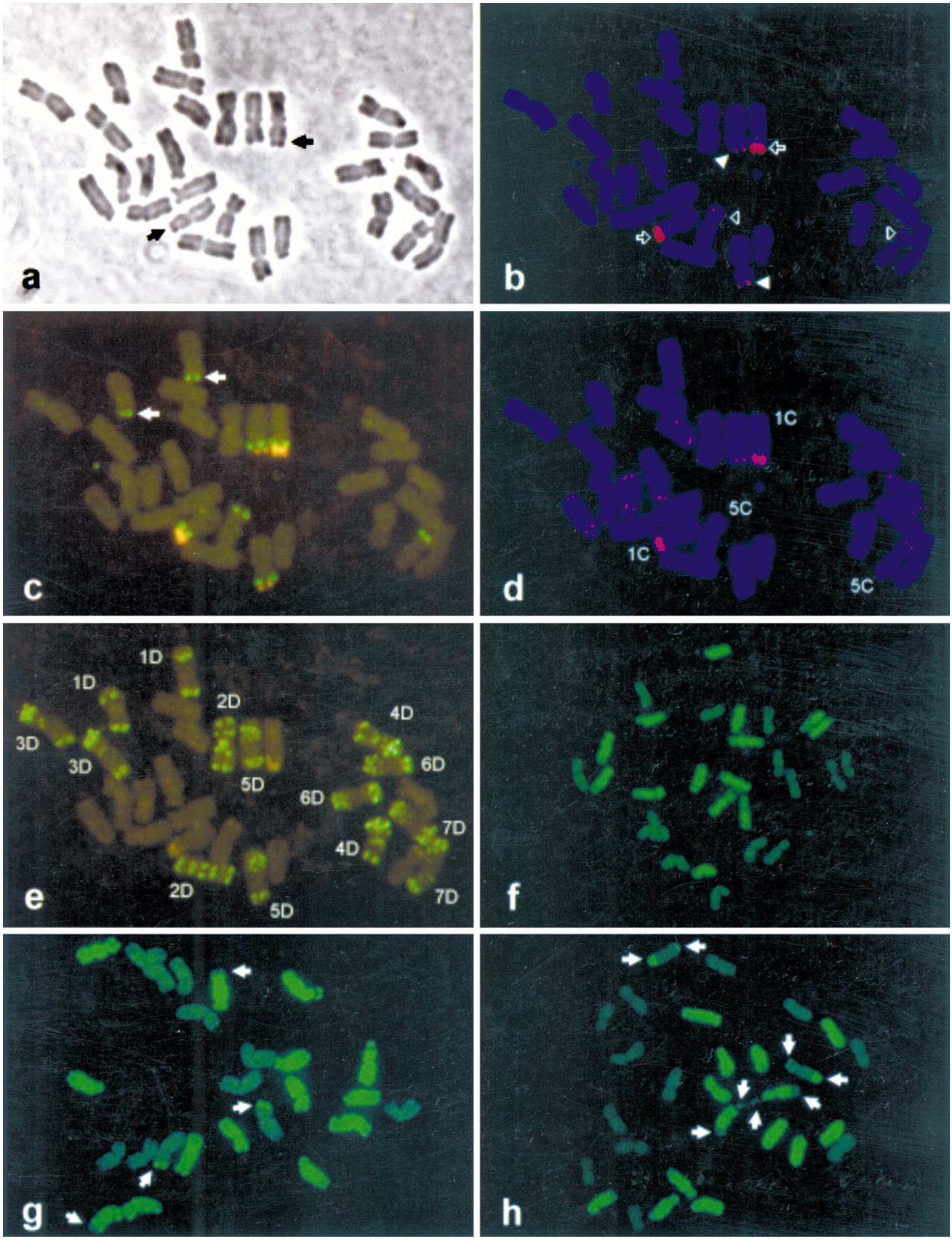


Fig. 2. (*a*) Mitotic metaphase chromosomes of *Aegilops cylindrica* accession TA2204 in phase contrast, arrows mark the secondary constrictions on chromosomes $1C^c$. (*b*) Same cell after FISH with pTa71 and DAPI counterstaining. Six red signals mark NORs, open arrows point to the NORs on chromosome $1C^c$, solid arrowheads point to the NORs on chromosome $5D^c$, and open arrowheads point to the NORs on chromosome $5C^c$. (*c*) Same cell after FISH with pTa71 and pTA794, green signals mark eight 5S-rDNA clusters and orange signals mark NORs. (*d*) Same cell after FISH with pSc119, red signal patterns enable the identification of chromosomes $1C^c$ and $5C^c$. (*e*) Same cell after FISH with pAs1, green FISH sites allow the identification of all D^c genome chromosomes. (*f*) GISH patterns of metaphase in *Ae. cylindrica* accession TA2204, bright green signals mark C^c-genome chromosomes, arrowheads point to the interstitial GISH site on chromosome $7D^c$. (*g*) GISH patterns of metaphase in *Ae. cylindrica* accession TA2202 with one reciprocal intergenomic translocation marked by arrows. (*h*) GISH pattern of metaphase of *Ae. cylindrica* accession TA2201 with two reciprocal intergenomic translocations, arrows and arrowhead point to the corresponding interchanged segments.

The accessions TA2201, TA2202, and TA2203 had the same number of hybridization sites for NOR and 5S rDNA loci per genome with similar signal intensities.

Intergenomic translocations

GISH analysis detected intergenomic translocations in 3 of 5 accessions analyzed (Fig. 2). The accessions TA2204 and TA2205 showed no obvious translocations (Fig. 2*f*). The accession TA2203 was homozygous for a reciprocal translocation involving a long acrocentric C^c-genome and a submetacentric D^c-genome chromosome (Fig. 2*g*). The translocation breakpoint in the C^c-genome chromosome is located in the long arm at a fraction length of about 0.85 with the distal 15% of the arm derived from a D^c-genome chromosome. The D^c-genome chromosome involved in this interchange is submetacentric with the breakpoint located in the short arm at a fraction length of approximately 0.70 with the distal 30% of the arm derived from a C^c-genome chromosome. Sizes and arm ratios of the translocation chromosomes suggest that either chromosome B or D from the C^c genome and chromosome 3D or 5D from the D^c genome are involved in this segment interchange.

The accession TA2202 segregated for two different genotypes. Three plants were homozygous for an intergenomic translocation involving a satellite chromosome (SAT) from the C^c genome with the breakpoint at a fraction length of about 0.85 in the long arm, and the long arm of a submetacentric D^c-genome chromosome with the breakpoint at a fraction length of about 0.85 (not shown). The C^c-genome chromosome involved in this translocation was identified by the presence of a secondary constriction as chromosome A. Size and arm ratio of the D^c-genome translocation chromosome identify it as either chromosome 3D or 5D. Five plants of accession TA2202 did not have any intergenomic translocation.

Six out of 8 plants in accession TA2201 were homozygous for a double intergenomic reciprocal translocation involving the four arms of SAT chromosomes $1C^c$ (chromosome A) and $5D^c$ (Fig. 2*h*). FISH using either the probes pTa71 and pAs1 or the probes pTa71 and pSc119 revealed that the SAT chromosomes $1C^c$ and $5D^c$ were involved in the multiple translocation event (not shown). The distal regions of both arms of the translocation chromosome pair $1C^c$ were unlabeled, indicating that they were derived from the D^c genome. The breakpoint in the short arm is located in the satellite and in the long arm at a fraction length of about 0.70. The distal regions of both arms of chromosome pair $5D^c$ were brightly labeled indicating their origin from the C^c genome. The breakpoints were at fraction lengths of about 0.85 in the short arm and about 0.70 in the long arm (Fig. 2*h*). Two plants lacked the translocation.

Chromosome $7D^c$ had an interstitial GISH site in the middle of the genetically short arm in all accessions analyzed (Fig. 2*f*), most likely caused by cross-hybridization of genomic *Ae. caudata* DNA to this region.

Discussion

Wild relatives of wheat are a valuable source of genetic variation that can be utilized in wheat improvement. However, the directed exploitation of this variability requires detailed knowledge of the genetic and cytogenetic structure of these species. This study is part of a long-term project aimed at producing standard karyotypes of all the species in the *Triticum–Aegilops* complex (discussed in Gill and Friebe 1998). Cytogenetic systems can be established using the classical approach as illustrated for the polyploid species *Ae. geniculata* (Friebe et al. 1999) or the molecular cytogenetic approach as demonstrated here. In the present study C-banding analysis and molecular probes coupled with FISH and GISH analyses were successfully used to determine cytogenetic and phylogenetic affinities for most of the *Ae. cylindrica* chromosomes. At present, the only missing link is the paucity of FISH genetic markers (Lapitan et al. 1997).

The present analysis showed that both the D^c and C^c genomes of *Ae. cylindrica* are very similar to the D and C genomes of the diploid progenitor species *Ae. tauschii* and *Ae. caudata* (Friebe et al. 1992*a*, 1992*b*). The amount of C-band polymorphism was much higher in the diploid species compared to that observed in *Ae. cylindrica*. Close similarity between the D-genome chromosomes of *Ae. tauschii* and the D^c genome of *Ae. cylindrica* also was indicated by the distribution pattern of a D-genome specific DNA probe pAs1 (Rayburn and Gill 1986*b*; Badaeva et al. 1996*a*).

The homoeologous relationships of only some of the C-genome chromosomes of *Ae. caudata* was established unambiguously. Compensating chromosome substitution lines were obtained for the combinations A(1D), C(5D) (Friebe et al. 1992*b*), and B(2D) (Endo 1996) indicating that chromosomes A, C, and B are homoeologous to groups 1, 5, and 2 of wheat, respectively. The remaining *Ae. caudata* chromosomes D, E, F, and G show homoeology to more than one group, based on isozyme (Schmidt et al. 1993) and restriction fragment length polymorphism (RFLP) analyses (Peil et al. 1998; Schubert et al. 1998; K.S. Gill, personal communication). These chromosomes are structurally rearranged. Structural rearrangements in C-genome chromosomes of *Ae. caudata* and U- and N-genome chromosomes of *Ae. umbellulata* Zhuk., and *Ae. uniaristata* Vis. also are indicated by

highly asymmetrical karyotypes, compared to the more symmetrical karyotypes of the remaining diploid species of this genus (Badaeva et al. 1996*a*, 1996*b*). Recently, RFLP analysis provided direct evidence for the presence of chromosomal rearrangements in the U genome of *Ae. umbellulata* (Zhang et al. 1998). All 7 chromosomes had at least one rearrangement compared to wheat.

Chennaveeraiah (1960) detected only one pair of SAT chromosomes, whereas Pathak (1940) reported two pairs of chromosomes with secondary constrictions in *Ae. cylindrica*. Ag-NOR banding later confirmed that *Ae. cylindrica* has two pairs of transcriptional active NORs that belong to the C^c genome, whereas the *Ae. tauschii*-derived NOR on chromosome 5D is suppressed (amphiplasty) (Cermeno et al. 1984).

FISH analysis using the 18S-5.8S-25S rDNA probe pTa71 detected two major loci in the short arms of chromosomes $1C^c$ and $5D^c$ and a minor 18S-5.8S-25S-rDNA cluster in the short arm of chromosome $5C^c$ of *Ae. cylindrica*. The diploid species *Ae. caudata* has two pairs of SAT chromosomes that were identified as chromosomes A = 1C and C = 5C, whereas *Ae. tauschii* has only one SAT chromosome pair that was identified as chromosome 5D (Chennaveeraiah 1960; Cermeno et al. 1984; Friebe et al. 1992*a*, 1992*b*). FISH analysis detected two major 18S-5.8S-25S rDNA clusters in the short arms of chromosomes 1C and 5C of *Ae. caudata*, whereas only one 18S-5.8S-25S rDNA FISH site was detected in the short arm of chromosome 5D of *Ae. tauschii* (Badaeva et al. 1996*b*). Some of the *Ae. tauschii* accessions analyzed were polymorphic for an additional minor 18S-5.8S-25S rDNA site located in the genetically short arm of chromosome 7D (Mukai et al. 1991; Badaeva et al. 1996*b*). However, no rDNA cluster was detected on chromosomes $7D^c$ in any of the *Ae. cylindrica* accessions analyzed (Fig. 2*b*).

Ag-NOR banding showed that in *Ae. cylindrica* only two pairs of NORs, belonging to the C^c genome, are transcriptionally active. The nucleolar activity of the NOR on chromosome $5D^c$ is speculated to be suppressed (amphiplasty) (Cermeno et al. 1984). The present analysis suggests that the lack of nucleolar activity of the NOR on chromosome $5D^c$ is not only the result of suppression, but might also be caused by the loss of most of the 18S-5.8S-25S rDNA genes at this site. A similar loss in copy number of 18S-5.8S-25S rDNA genes occurred in the A/A^t genome of *Triticum turgidum* and *T. timopheevii* (Jiang and Gill 1994).

FISH analysis using the 5S rDNA probe pTA 794 detected two pairs of FISH sites in both the D^c and C^c genome of *Ae. cylindrica*, corresponding to the two 5S rDNA loci observed in the short arms of the homoeologous group 1 and 5 chromosomes of *Ae. caudata* and *Ae. tauschii* (Badaeva et al. 1996*b*).

GISH analysis revealed the presence of reciprocal intergenomic translocations in 3 of the 6 different *Ae. cylindrica* accessions analyzed. All translocations had the breakpoints in noncentromeric regions. C-banding analysis failed to detect these translocations because of similarities in C-banding patterns of the interchanged segments. Only one structural rearrangement involving the short arm of chromosome $5D^c$ in the accession TA2202 was detected by C-banding analysis. This rearrangement remained undetected by GISH analysis suggesting that it occurred within the D^c genome.

C-banding analysis detected only a small number of chromosomal translocations among the different accessions of diploid *Aegilops* species analyzed (Friebe and Gill 1996; Friebe et al. 1992*a*, 1992*b*, 1993, 1995*a*, 1995*b*, 1996). Chromosome morphology and C-banding patterns suggest that most translocations probably were derived from the centric breakage–fusion mechanism of univalents at meiotic metaphase I. In *Ae. tauschii*, 2 of 16 different accessions analyzed were homozygous for reciprocal whole arm translocations involving chromosomes 1D and 7D in TA2462 and 1D and 3D in KU20-7 (Friebe et al. 1992*a*; Friebe and Gill 1996), although no structural rearrangements were detected among the 19 accessions of *Ae. caudata* analyzed (Friebe et al. 1992*b*).

Gametocidal chromosomes, which are preferentially transmitted and known to induce chromosomal rearrangements, were identified in the C genome of *Ae. caudata* and in the derived C^c genome of *Ae. cylindrica* and C^t genome of *Ae. triuncialis* (Endo 1990; Köszegi et al. 1998). In *Ae. caudata* and in *Ae. triuncialis*, the gametocidal chromosome belongs to the homoeologous group 3, whereas in *Ae. cylindrica* it is a group 2 chromosome (Endo 1996). The large amount of chromosomal rearrangements detected in the present study might be the result of *Gc* gene activity following the hybridization event. However, one must note that the exchanged segments were of similar length and that the origin of these translocatons through homoeologous recombination remains an intriguing prospect that needs to be pursued in the future.

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