Chromosome Research 1998, **6**, 629–637

Molecular cytogenetic analysis of tetraploid and hexaploid *Aegilops crassa*

E. D. Badaeva, B. Friebe, S. A. Zoshchuk, A. V. Zelenin & B. S. Gill

Received 2 July 1998; received in revised form and accepted for publication by J. S. Heslop-Harrison 2 September 1998

The distribution of highly repetitive DNA sequences on chromosomes of tetraploid and hexaploid cytotypes of *Aegilops crassa* ($D^{cr1}X^{cr}$ and $D^{cr1}X^{cr}D^{cr2}$ genomes) was studied using C-banding and *in situ* hybridization analyses with the pSc119, pAs1 and pTa794 DNA clones. In total, 14 tetraploid and five hexaploid accessions were examined. All chromosomes can be identified by their C-banding and ISH pattern with the pAs1 DNA clone. Only a few pSc119 hybridization sites were observed in the telomeric regions of several chromosomes. We found a high level of C-banding polymorphism and only minor variations in labeling patterns. The position of C-bands generally coincided with the location of the pAs1 sequence. Three 5S rDNA loci were detected in tetraploid *Ae. crassa*, whereas five pTa794 ISH sites were observed in *6x Ae. crassa*. All the hexaploid accessions differed from the tetraploids by a reciprocal non-centromeric translocation involving chromosomes A and N. Three additional translocations were detected in the accessions analyzed. The D^{cr1} genome of 4x *Ae. crassa* is highly modified compared with the D genome of the progenitor species *Ae. tauschii*. Because of the large amount of chromosomal rearrangements, the origin of the X^{cr} genome remains unknown. The second D^{cr2} genome of 6x Ae. crassa is different from the D^{cr1} genome but is similar to the D-genome chromosomes of *Ae. tauschii*, indicating that no additional large rearrangements occurred at the hexaploid level.

Key words: Aegilops crassa, C-banding, genome differentiation, *in situ* hybridization

Introduction

A group of related species, including *Aegilops crassa* Boiss and *Ae. vavilovii* (Zhuk.) Chennav., belong to the section Vertebrata of the genus *Aegilops*. Tetraploid ($2n = 4x = 28$, $D^{cr1}X^{cr}$) and hexaploid ($2n = 6x = 42$, $D^{cr1}X^{cr}D^{cr2}$) *Ae. crassa* constitute morphologically indistinguishable cytotypes (Zhukovsky 1928, Eig 1929, Witcombe 1983, Kimber & Feldman 1987, Van Slageren 1994). Analysis of meiotic chromosome pairing in hybrids between 4x and 6x *Ae. crassa* revealed that the hexaploid forms were derived from a hybridization between 4x *Ae. crassa* and *Ae. tauschii* Coss. (Kihara *et al*. 1959, Kimber & Zhao 1983, Zhao & Kimber 1984). *Aegilops crassa* is characterized by a large degree of morphological variation and a wide area of distribution, including Turkey, Israel, Lebanon, Syria, Iraq, Iran, Afghanistan, Transcaucasia, southern Turkmenistan, and the Pamirs and Altai mountains. The species is a common weed, growing on stony slopes, steppes, disturbed habitats and roadsides (Witcombe 1983, Kimber & Feldman 1987, Van Slageren 1994).

Meiotic pairing analysis in interspecific hybrids suggested that one of the genomes of tetraploid *Ae. crassa* was derived from the D genome of *Ae. tauschii*, and the second genome is hypothesized to have originated from the M genome of *Ae. comosa* Sm. in Sibth. and Sm. (Kihara 1940, 1957, 1963, Lilienfeld & Kihara 1951, Kihara *et al*. 1959, Kihara & Tanaka 1970, Kimber & Feldman 1987). The analysis of chloroplast and mitochondrial DNA showed that both 4x and 6x *Ae. crassa* have the same D2 cytoplasm type, which is different from the plasmons of all known diploid species. However, it was suggested that D2 might be derived from the *Ae. tauschii* cytoplasmic genome (Ogihara & Tsunewaki 1988, Tsunewaki 1993). The two genomes present in tetraploid *Ae. crassa* were shown to be substantially modified (Kihara 1940, 1957, Lilienfeld & Kihara 1951, Siddique & Jones 1967, Kihara & Tanaka 1970, Chapman & Miller 1978, Nakai 1982, Kimber & Zhao 1983, Zhao & Kimber 1984, Kimber & Feldman 1987, Zhang & Dvorak 1992, Tsunewaki 1993, Kimber 1995). *In situ* hybridization with the D-genome-specific DNA clone pAs1 confirmed that the D^{cr1} genome of tetraploid *Ae. crassa* is significantly different from the ancestral D genome of *Ae. tauschii* (Rayburn & Gill 1987).

The origin of the second genome of tetraploid *Ae. crassa* is still under discussion. The analysis of variation in nuclear repeated nucleotide sequences suggests that it may have been derived from an extinct member of the section Sitopsis (Dubcovsky & Dvorak 1995, Dvorak & Dubcovsky 1996, Dvorak 1998). However, until further evidence on the origin of this genome is obtained, the designation of X^{cr} is suggested (Dvorak 1998). In the present study, C-banding and *in situ*

E. D. Badaeva, S. A. Zoshchuk and A. V. Zelenin are at the Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Str.32, Moscow 117984, Russia. B. Friebe (corresponding author) and B. S. Gill are at the Wheat Genetics Resource Center, Department of Plant Pathology Kansas State University, Throckmorton Hall, Manhattan, KS 66506-5502, USA. Tel: (+1) 785 5326176; Fax: (+1) 785 5325692; Email: friebe@ksu.edu.

hybridization (ISH) analyses were used to establish a standard karyotype of tetraploid and hexaploid *Ae. crassa*. The results are discussed with respect to the evolutionary relationships of these species.

Materials and methods

Nineteen *Ae. crassa* accessions from the wheat germ plasm collections of the Wheat Genetics Resource Center, Kansas State University, USA; the Vavilov Institute of Plant Industry, St Petersburg, Russia; and the Gatersleben Gene Bank, Germany, were used (Table 1).

C-banding analysis

Two C-banding methods (Badaev *et al.* 1985, Gill *et al.* 1991) were used. Chromosomes of the D^{cr1} and X^{cr} genomes were designated with the letters A to N. The D^{cr2}-genome chromosomes were designated according to their homoeologous relationships (Friebe *et al.* 1992). Chromosome measurements were performed on 10–14 chromosomes of each type to determine their relative lengths (RL, length of the individual chromosome in relation to the total length of the haploid genome) and arm ratios (L/S).

DNA probes

The clone pSc119 is a 120-bp DNA sequence derived from rye (*Secale cereale*) and inserted into the plasmid pBR322 (Bedbrook *et al.* 1980). The pAs1 clone (1 kb) was isolated from *Ae. tauschii* (Rayburn & Gill 1986) and inserted into the plasmid pUC8. The pTa794 clone (410 bp) contains the 5S rRNA gene unit (120 bp) separated by a 290-bp spacer (Gerlach & Dyer 1980). All probes were labeled with biotin by nick-translation according to the manufacturer's protocol (Enzo-Diagnostic, New York, NY). In situ hybridization and signal detection were according to the previously published protocol (Badaeva *et al.* 1996a). Fifteen *Ae. crassa* accessions were C-banded and 12 accessions were analyzed by ISH. The number and location of 5S rDNA loci was determined in one tetraploid and in one hexaploid accession. The preparations were analyzed with an Olympus BH-2 microscope and microphotographs were taken using Fujicolor Super G Plus 400 and Kodak Imagelink HQ microfilm 1461.

Results

All chromosomes of 4x *Ae. crassa* hybridized with the probe pAs1. The 14 chromosome pairs of the complement can be placed into two groups based on their pAs1 ISH pattern. Five chromosome pairs were labeled heavily with large pAS1 sites (A, C, D, E and G); whereas the remaining chromosomes had fewer and smaller ISH sites. Only a few chromosomes hybridized with the probe pSc119 (data not shown).

The C-banding and pAs1 ISH patterns of all *Ae. crassa* chromosomes were diagnostic for identification of the

Table 1. Origin of the *Aegilops crassa* accessions analyzed

Accession number	Ploidy level	Analyzed by C-banding	ISH	Origin
TA1873	4x	+	+	Iran, Fars Bagdka; 12.8 km NE of Shiraz, alt. 1525 m
TA1874	4x	+	−	Iran, Fars Takht-e Jamshid historical site near Marv Dasht. alt. 1678 m
TA1875	4x	+	−	Iran, Chahar Mahall va Bakhtiari Dastana; S of Shahr-e-Kord. alt. 2074 m
TA1876	4x	−	+	Iran, about 0.8 km E of Bakhtaran (Kermanshah)
TA1877	4x	+	+	Iraq, Irbil Forest nursery, alt. 458 m
TA1880	4x	+	+	Turkey, Urfa, Ceyalpinar
TA1881	4x	+	+	Afghanistan, Kandaghar Upper Tarnak Valley Collections
TA2318	4x	−	+	Turkey, Urfa roadside. 14.6 km W of Ceyalpinar, alt. 600 m
TA2319	4x	+	+	Turkey, Urfa roadside next to field of wheat–barley–rye; 45.8 km W of Ceyalpinar, alt. 525 m
TA2320	4x	−	+	Turkey, Urfa roadside next to field of wheat–barley–rye; 45.8 km W of Ceyalpinar, alt. 525 m
TA2337	4x	+	+	Iraq, Ninawa Tall-Afar Collections
TA2214 (AE332)	4x	+	−	Iraq
TA2213 (AE290)	4x	+	−	Turkmienastan, Berge von Kopet-Dag
K-2424	4x	+	−	Armenia
K-2485	6x	+	+	Turkmienastan
TA2217 (AE847)	6x	+	−	Uzbekistan
TA1878	6x	−	+	Turkey, Izmir Agean Agric. Res. Inst. gene bank, Menemen, alt. 30 m
TA1883	6x	+	+	Italy, Instituto Orto Botanico; Rome collections
TA1884	6x	+	+	University of Manitoba Winnipeg Collections

TA, accessions from the Wheat Genetics Resource Center; K, accessions from the Vavilov Institute of Plant Industry; AE, accessions from the Gene bank Gatersleben.

complement (Figures 1 & 2). We observed significant intraspecific C-banding polymorphism. However, lines from the same geographical region were more similar than those from distant areas (Figures 3 & 4). A generalized karyotype was constructed (Figure 5). The D^{cr1}- and X^{cr}-genome chromosomes of tetraploid *Ae. crassa* were modified significantly compared with the genomes of all diploid Aegilops species and were designated with letters from A to N. The following is a description of the individual chromosomes.

Chromosome A (RL 5.97%, arm ratio 1.6) has a prominent marker C-band in the proximal region of the short arm and smaller proximal, distal and telomeric marker C-bands in the long arm. Chromosome A has a large pAs1 site at the telomere of the long arm and smaller pAs1 sites are present in proximal regions of both arms.

Chromosome B (RL 7.17%, arm ratio 1.3) has a proximal marker C-band in the long arm, and a prominent proximal and smaller distal marker C-bands are present in the short arm. Proximal, distal and telomeric pAs1 sites are present in the long arm. Chromosome C (RL 6.90%, arm ratio 2.01) has distal and telomeric marker C-bands in the short arm, and a telomeric marker C-band is present in the long arm. Additional polymorphic interstitial C-bands are present in both arms. It has large double pAs1 sites in telomeric regions of both arms and a medium-sized pAs1 site in the middle of the long arm.

Chromosome D (RL 8.64%, arm ratio 1.06) has a distally located marker C-band and a telomeric C-band in the long arm and polymorphic interstitial, distal and telomeric C-bands are present in the short arm. Chromosome D was characterized by prominent pAs1 sites in proximal and telomeric regions of the long arm.

Chromosome E is highly asymmetric (RL 6.62%, arm ratio 2.43) with a proximally located marker C-band in the short arm and a distal marker C-band in the long arm. Polymorphic telomeric C-bands are present in both arms. Chromosome E has a telomeric pAs1 site at the telomere of the short arm, and four evenly distributed pAs1 sites were observed in the long arm.

Chromosome F (RL 5.64%, arm ratio 1.03) has proximal and distal marker C-bands in the short arm, and polymorphic C-bands were observed in the proximal, interstitial and telomeric regions of the long arm. Chromosome F has two pAs1 sites in the distal region of the short arm and in proximal, distal and telomeric regions of the long arm.

Chromosome G (RL 7.22%, arm ratio 1.05) has a prominent marker C-band in the proximal region of the short arm, and distal and telomeric marker C-bands are present in the long arm. Polymorphic C-bands are present in interstitial and distal regions of the short arm. Chromosome G is similar to chromosome C with respect to its pAs1 hybridization pattern but differs from C in arm ratio and the presence of an interstitial pAs1 site in the long arm.

Chromosome H is a SAT chromosome (RL 7.36%, arm ratio 0.84) with a large satellite in the short arm. A prominent marker C-band is adjacent to the nucleolus organizer region (NOR) in the short arm, and distal and telomeric marker C-bands are present in the long arm. Chromosome H has one medium-sized pAs1 site in the satellite, and a telomeric ISH site is present in the long arm.

Chromosome I (RL 8.29%, arm ratio 1.54) has a prominent interstitial marker C-band in the short arm and a smaller distally located marker C-band in the long arm. Polymorphic C-bands were observed at the telomeres of both arms. Chromosome I has two small pAs1 sites in the distal region of the long arm.

Chromosome J (RL 8.32%, arm ratio 1.56) has proximal and distal marker C-bands in the short arm and polymorphic distal C-bands are present in the long arm. Chromosome J has a medium pAs1 site in the distal region of the short arm and a small hybridization site at the telomere of the long arm.

Chromosome K (RL 5.64%, arm ratio 1.27) has distal marker C-bands in both arms in addition to polymorphic proximal and telomeric C-bands in the long arm. Chromosomes K and L have similar pAs1 hybridization patterns. Both chromosomes have medium-sized ISH sites in distal regions of both arms. However, an additional pAs1 site was observed in the proximal region of the long arm in chromosome L.

Chromosome L (RL 6.05%, arm ratio 1.75) has a proximal and telomeric marker C-band in the short arm and an interstitial marker C-band of varying size in the long arm. The telomeric C-band in the short arm was polymorphic.

Chromosome M (RL 7.80%, arm ratio 1.78) is a SAT chromosome with a small satellite in the short arm. A prominent marker C-band is present at the NOR, and a smaller distally located marker C-band is present in the short arm. Several polymorphic interstitial and a telomeric marker C-band are present in the long arm. Small pAs1 sites are present at the telomeres of both arms.

Chromosome N (RL 8.37%, arm ratio 1.0) has a proximal marker C-band in the short and an interstitial marker C-band in the long arm. Additional polymorphic C-bands are present at interstitial and telomeric regions of both arms. Chromosome N has a prominent pAs1 site in the middle of the short arm and a smaller telomeric pAs1 sites was observed in the long arm.

Two reciprocal translocations were identified in 4x *Ae. crassa* (Figure 6). The first translocation, designated T1, involves chromosomes B and the SAT chromosome H. Overall chromosome morphology and C-banding pattern of chromosome H in the accessions TA1873, TA1874, TA1857 and TA1881 is similar to those of SAT chromosomes in diploid Aegilops species (Badaeva *et al*. 1996a,b), suggesting that the corresponding chromosomes in the remaining accessions are rearranged. The resulting Robertsonian whole-arm translocation chromosomes can be described as THL·BL and THS·BS. Whereas the untranslocated cytotype was observed in

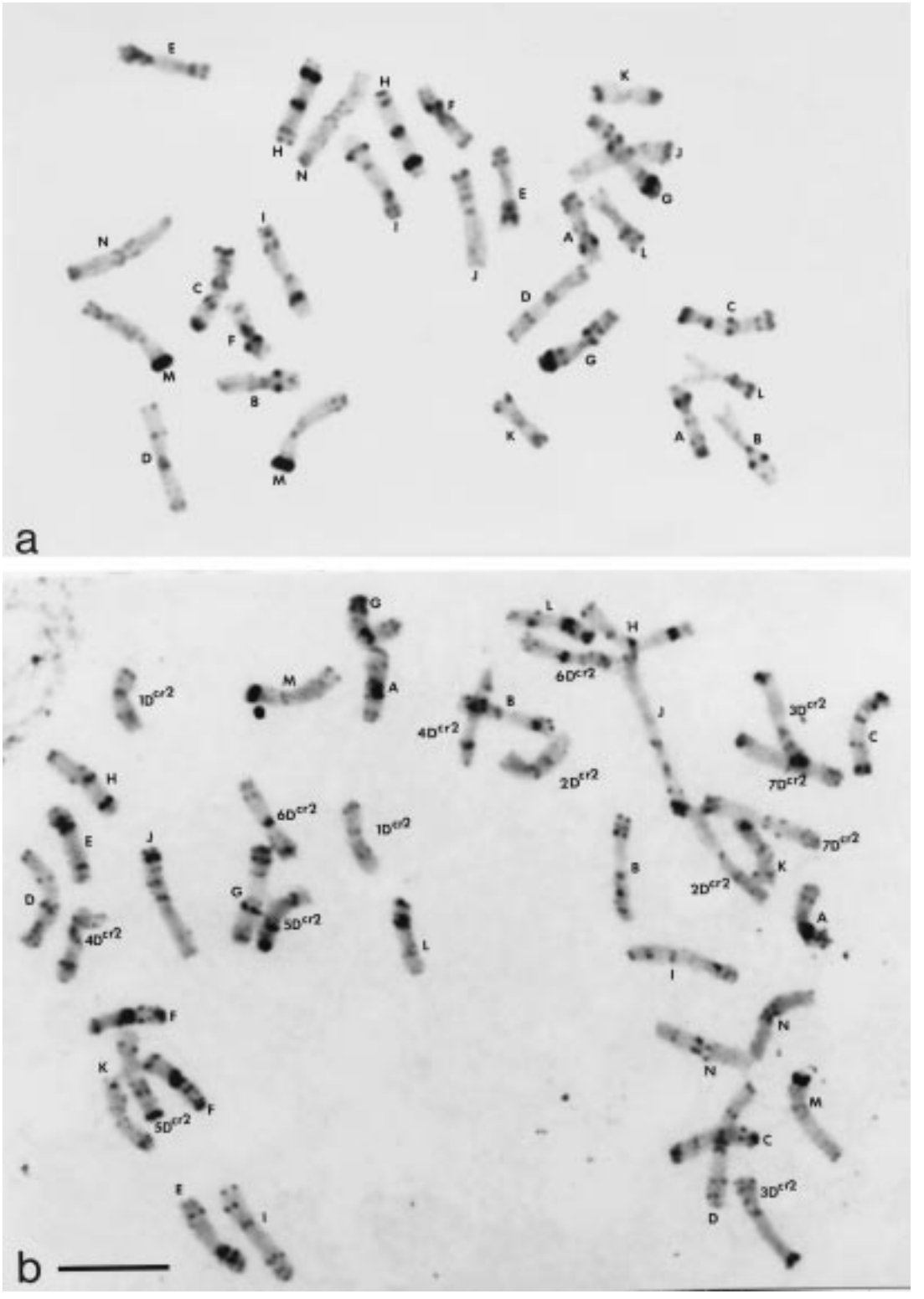

Figure 1. C-banded mitotic metaphase cells of TA1873 4x (**a**) and TA2217 6x (**b**) *Ae. crassa*. Scale bar = 10 μm.

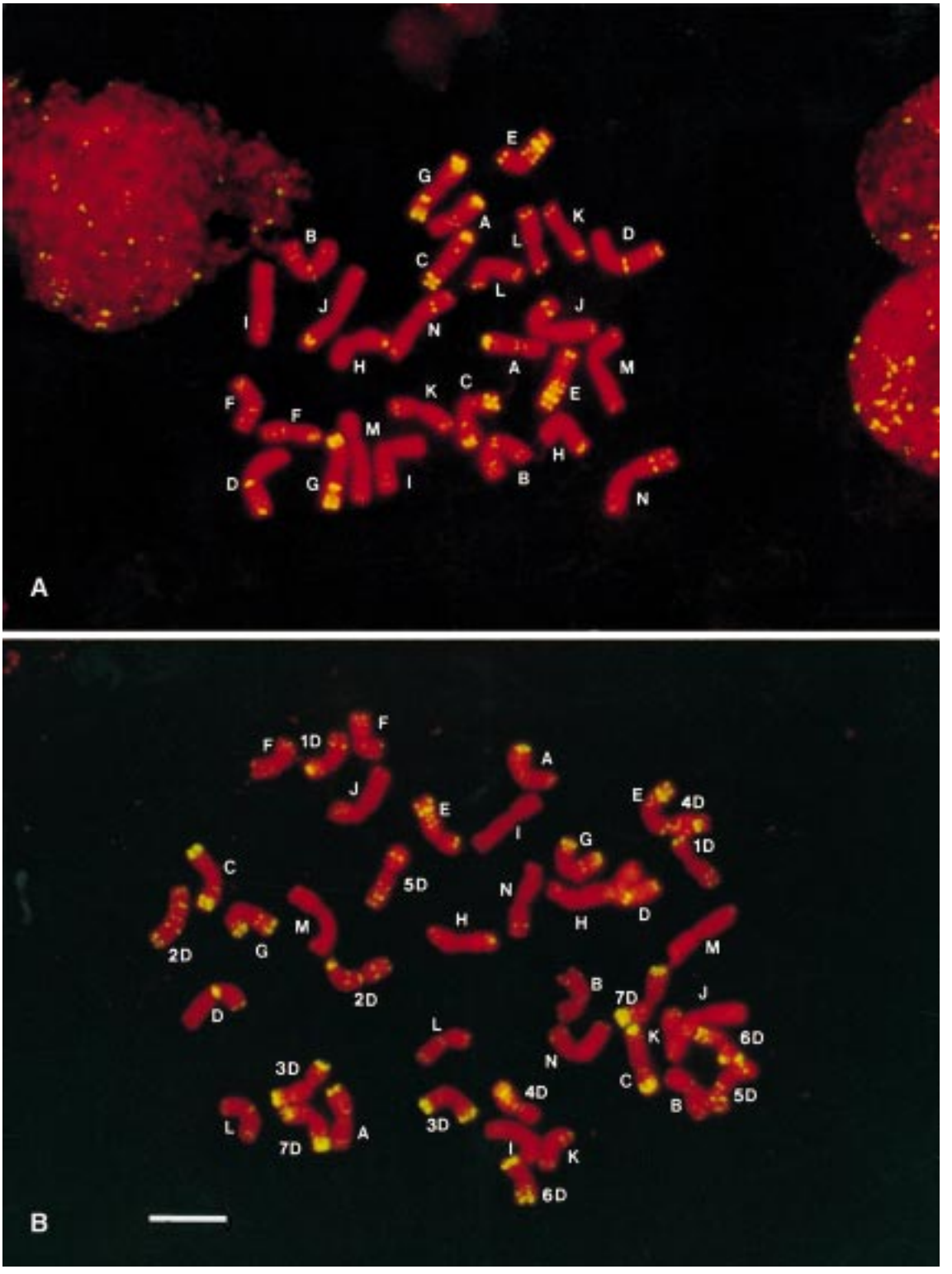

Figure 2. FISH patterns of mitotic metaphase chromosomes of TA2337 4x (**a**) and TA1883 6x (**b**) *Ae. crassa* using pAs1 as a probe. Scale bar = 10 μm.

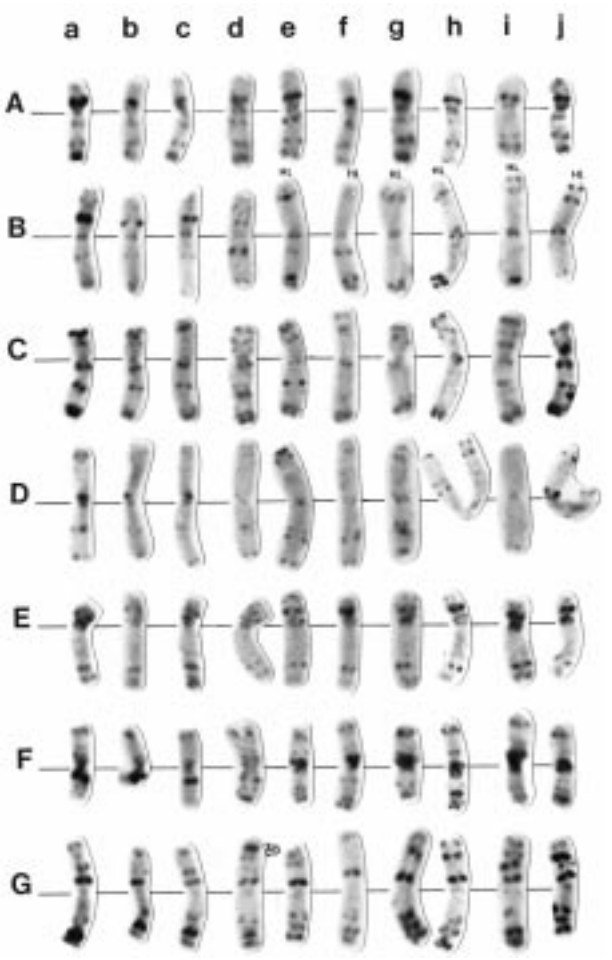

Figure 3. C-banding polymorphisms and chromosomal rearrangements detected in chromosomes A to G in different accessions of 4x *Ae. crassa*. **a** TA1873, **b** TA1874, **c** TA1875, **d** TA1881, **e** TA1877, **f** TA2337, **g** TA2214, **h** TA2319, **i** TA2213, **j** K-2424.

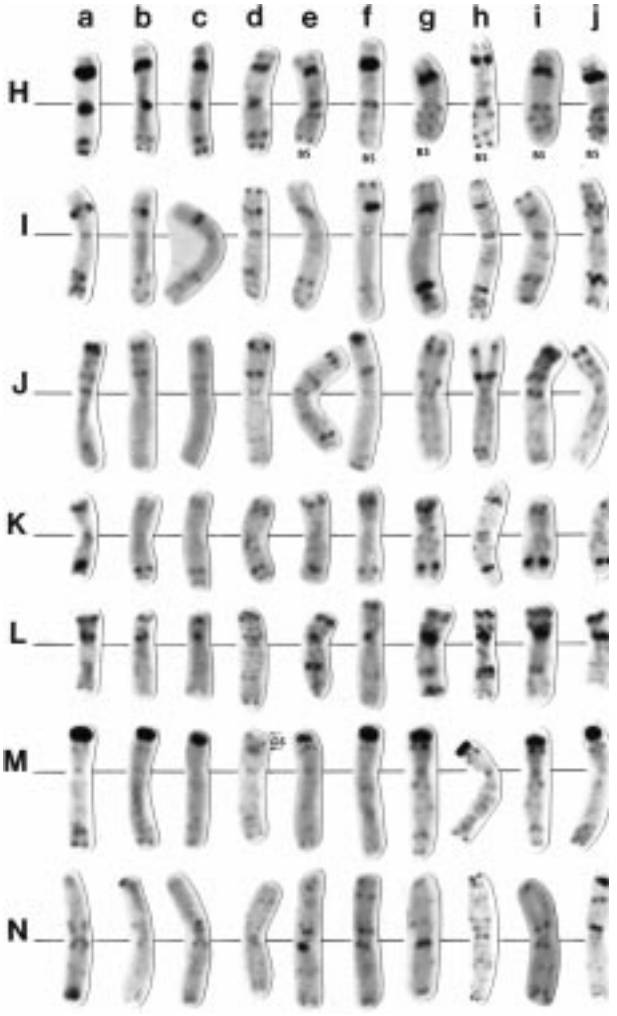

Figure 4. C-banding polymorphisms and chromosomal rearrangements detected in chromosomes H to N in different accessions of 4x *Ae. crassa*. **a** TA1873, **b** TA1874, **c** TA1875, **d** TA1881, **e** TA1877, **f** TA2337, **g** TA2214, **h** TA2319, **i** TA2213, **j** K-2424.

only some accessions from Iran, T1 was present in accessions over the entire distribution area of *Ae. crassa*. This translocation type was present in 6 of the 10 4x *Ae. crassa* accessions analyzed. The second translocation was observed in only one of the 4x *Ae. crassa* accessions from Afghanistan and was a reciprocal exchange of distal segments of the short arms of chromosomes G and M resulting in the translocation chromosomes TMS-GS·GL and TGS-MS·ML (Figure 6).

Chromosome morphologies and C-banding and pAs1 ISH patterns of the D^{cr2} chromosomes of hexaploid *Ae. crassa* were very similar to those of the D-genome chromosomes of *Ae. tauschii* and *Triticum aestivum* reported earlier (Rayburn & Gill 1987, Mukai *et al*. 1993, Badaeva *et al*. 1996a). Thus, these chromosomes were designated according to the homoeologous groups of the triticeae (Figure 7).

Two reciprocal translocations were detected in 6x *Ae. crassa* (Figure 6). T3 was present in all hexaploid accessions analyzed and identified a reciprocal exchange of small distal segments from the short arms of chromosomes A and N, resulting in the translocation chromosomes TNS-AS·AL and TAS-NS·NL. T4 was present in two of the four hexaploid accessions analyzed and identified as a Robertsonian whole-arm translocation involving chromosome B and the SAT chromosome H, resulting in the translocation chromosomes TBS·HL and THS·BL

ISH analysis with the DNA clone pTa794 revealed three 5S rDNA loci in tetraploid *Ae. crassa*. Two 5S rDNA sites were observed in very distal locations on the short arms of non-SAT chromosomes, whereas a third 5S locus was located in a short SAT-chromosome arm proximal to the secondary constriction. Five 5S rDNA loci were identified in hexaploid *Ae. crassa*. Three

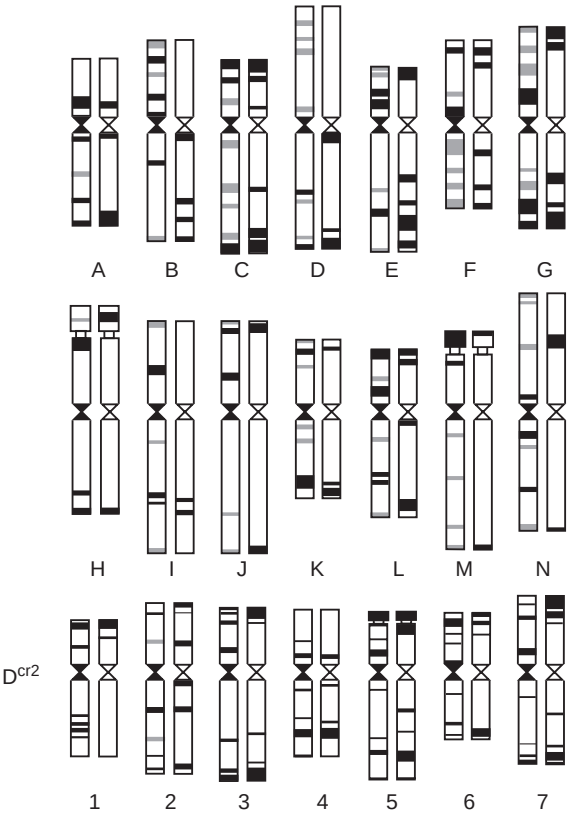

Figure 5. Generalized ideogram of the D^{cr1}–, X^{cr}- and D^{cr2}–genome chromosomes of 4x and 6x *Ae. crassa* (C-banding patterns are shown on the left and the pAs1 ISH patterns shown on the right. Marker C-bands are shown in black whereas polymorphic C-bands are shown in grey.

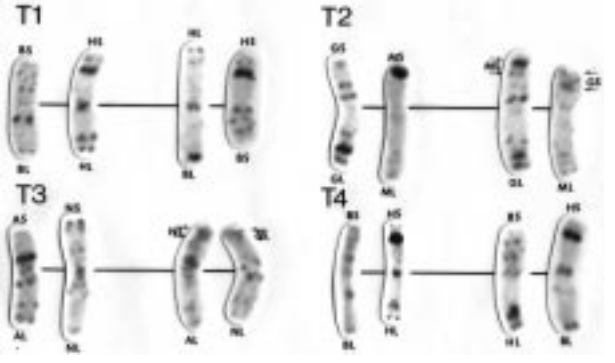

Figure 6. Chromosomal rearrangements detected in 4x and 6x *Ae. crassa*.

of these loci were derived from 4x *Ae. crassa*, and one distal and one SAT chromosome arm locus were contributed by *Ae. tauschii*.

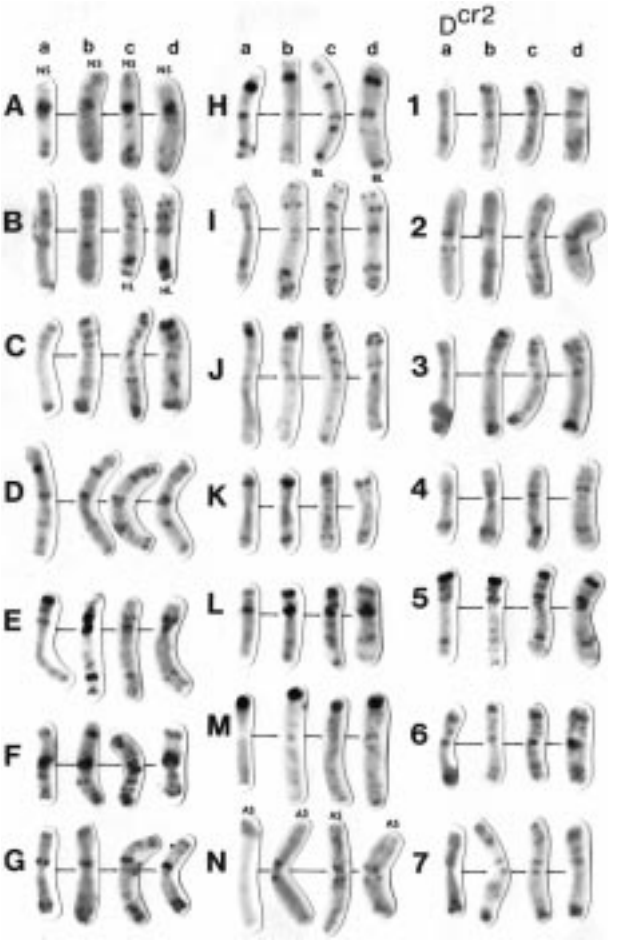

Figure 7. C-banding polymorphism and chromosomal rearrangements detected in 6x *Ae. crassa*; **a** TA1883, **b** 1884), **c** K-2485, **d** (TA2217).

Discussion

The C-banding and ISH patterns of the D^{cr1}- and X^{cr}-genome chromosomes of 4x *Ae. crassa* are different from those of all the diploid *Aegilops* species (Badaeva *et al*. 1996a). This difference is the result of many chromosomal rearrangements that occurred during the speciation process. The presence of intra- and intergenomic rearrangements is supported further by the pAs1 and pSc119 ISH pattern of 4x *Ae. crassa*. The *Ae. tauschii*-derived repeated DNA clone pAs1 detected five pairs of heavily labeled chromosomes in 4x *Ae. crassa*. Clone pAs1 is known to strongly label D-genome chromosomes, whereas smaller and fewer pAs1 ISH sites are characteristic for the M and S genomes of the putative progenitor species of the X^{cr} genome *Ae. comosa* and *Aegilops* species belonging to the section Sitopsis (Badaeva *et al*. 1996a). The pAs1 ISH patterns of the heavily labeled chromosomes of 4x *Ae. crassa* are different from those of the D genome of *Ae. tauschii*, which is in agreement with previous reports (Rayburn & Gill 1987). These data suggest that the heavily pAs1-labeled

chromosome pairs of 4x *Ae. crassa* were derived from *Ae. tauschii*. However, because of the large amount of chromosomal rearrangements that occurred during speciation of 4x *Ae. crassa*, an unambiguous genome allocation is not possible. Clone pSc119 is known to hybridize to all S-genome chromosomes of Sitopsis species (Badaeva *et al*. 1996a). However, only a few minor pSc119 ISH sites were detected in 4x *Ae. crassa*. Thus, the pSc119 ISH pattern of 4x *Ae. crassa* provides no further evidence that the X^{cr} genome was derived from an S-genome species of the section Sitopsis.

The C-banding and ISH patterns of the D^{cr2} genome of 6x *Ae. crassa* are very similar to the D-genome chromosomes of *Ae. tauschii* (Friebe *et al*. 1992, Mukai *et al*. 1993, Badaeva *et al*. 1996a). All the 6x *Ae. crassa* cytotypes had a reciprocal translocation involving chromosomes A and N. No further major chromosomal rearrangements occurred at the hexaploid level.

Tetraploid *Ae. crassa* has two SAT chromosomes (H and M), whereas three were observed in 6x *Ae. crassa*, confirming earlier reports (Chennaveeraiah 1960). Ag-NOR banding further showed that only two NORs are active in organizing nucleoli (Cermeño *et al*. 1984). ISH analysis using the 18S, 5.8S, 26S rDNA clone pTa71 detected a third NOR locus in 4x *Ae. crassa* (Yamamoto & Mukai 1995), but this locus is not actively transcribed. In diploid Aegilops species, secondary constrictions were observed on group 1, 5, and 6 chromosomes (Badaeva *et al*. 1996b). *Ae. tauschii* has only one SAT chromosome identified as 5D (Teoh *et al*. 1983, Mukai *et al*. 1991, Badaeva *et al*. 1996b). The morphology and C-banding pattern of chromosome M suggest that it was derived from a group 5 chromosome, whereas chromosome H is more metacentric and probably was derived from a group 6 chromosome. Hexaploid *Ae. crassa* has three pairs of SAT chromosomes, the group 5 and 6 chromosomes designated as M and H derived from 4x *Ae. crassa* and a third contributed by the D^{cr2} genome located on chromosome $5D^{cr2}$.

Three 5S rDNA loci were detected in 4x *Ae. crassa*: two of them are located in very distal regions of non-SAT chromosome arms, whereas the third locus was more proximal and present in a SAT chromosome arm. The 5S rDNA loci are known to be located in the short arms of group 1 and 5 chromosomes in the genus *Aegilops* (Mukai *et al*. 1990, Badaeva *et al*. 1996b). The group 1 locus is usually more distal than the group 5 locus, suggesting that the two distally located 5S rDNA loci were contributed by group 1 chromosomes, whereas the locus in the SAT chromosome arm was derived from a group 5 chromosome. Five 5S rDNA loci were detected in 6x *Ae. crassa*. Three of them were contributed by 4x *Ae crassa* and two were contributed by D^{cr2}-genome located on chromosomes $1D^{cr2}$ and $5D^{cr2}$.

The C-banding and ISH results suggest that one of the genomes of 4x *Ae crassa* was derived from the D genome of *Ae. tauschii*. Because of the large amount of inter- and intragenomic rearrangements the origin of the X^{cr} genome remains unknown. During the speciation process of 4x *Ae. crassa*, both ancestral genomes were modified substantially, resulting in chromosomal rearrangements and the redistribution of highly repetitive DNA sequences. Hexaploid *Ae. crassa* was derived from the hybridization between the tetraploid cytotype of *Ae. crassa* and *Ae. tauschii*. The speciation of 6x *Ae. crassa* was accompanied by a reciprocal translocation between the short arms of chromosomes A and N, which was present in all hexaploid accessions analyzed and can be considered specific for the 6x cytotype. The C-banding and ISH data show that the genetically related D^{cr1} and D^{cr2} genomes significantly diverged from each other. The distinct genetic differences between the tetraploid and hexaploid cytotypes of *Ae. crassa* would justify classifying them at least as different subspecies.

Acknowledgements

The authors thank to Dr A. A. Filatenko (St Petersburg, Russia) for providing the accessions. This work was supported in part by a grant no. 96-04-50731 from the Russian State Foundation for Basic Research, grant no. 96-15-97640 from the Russian State Program for the Support of Scientific Schools, a grant from the Russian State Program 'Frontiers in Genetics', and by a United States Department of Agriculture, Cooperative State Research Service special grant to the Wheat Genetics Research Center at Kansas State University, Manhattan, KS, USA. Contribution No. 98-468-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506-5502, USA.

References

Badaev NS, Badaeva ED, Bolsheva NL, Maximov NG, Zelenin AV (1985) Cytogenetic analysis of forms produced by crossing hexaploid triticale with common wheat. *Theor Appl Genet* **70**: 536–541.

Badaeva ED, Friebe B, Gill BS (1996a) Genome differentiation in Aegilops. 1. Distribution of highly repetitive DNA sequences on chromosomes of diploid species. *Genome* **39**: 293–306.

Badaeva ED, Friebe B, Gill BS (1996b) Genome differentiation in *Aegilops*. 1. Physical mapping of 5S and 18S-26S ribosomal RNA gene families in diploid species. *Genome* **39**: 1150–1158.

Bedbrook RJ, Jones J, O'Dell M, Thompson RJ, Flavell RB (1980) A molecular description of telomeric heterochromatin in *Secale* species. *Cell* **19**: 545–560.

Chapman V, Miller TE (1978) The relationship of the D genomes of hexaploid *Ae. crassa*, *Ae. vavilovii* and hexaploid wheat. *Wheat Inf Serv* **47**: 17–20.

Chennaveeraiah MS (1960) Karyomorphologic and cytotaxonomic studies in *Aegilops*. *Acta Hort Gotob* **23**: 349–360.

Cermeño MC, Orellana J, Santos JL, Lacadena JR (1984) Nucleolar activity and competition (amphiplasty) in the genus *Aegilops*. *Heredity* **52**: 603–611.

Dubcovsky J, Dvorak J (1995) Genome identification of the *Triticum crassum* complex (Poaceae) with the restriction patterns of repeated nucleotide sequences. *Am J Bot* **82**: 131–140.

Dvorak J (1998) Genome analysis in the *Triticum-Aegilops* alliance. In: Slinkard AE, eds. *Proceedings 9th International Wheat Genet Symp Saskatoon, Canada, 2–7 August 1998*. University Extension Press: University of Saskatchewan, Canada, pp 8–11.

Dvorak J, Dubcovsky J (1996) Genome analysis of polyploid species employing variation in repeated nucleotide sequences. In: Jauhar PP, ed. *Methods of Genome Analysis in Plants*. New York: CRC Press Boca Raton, pp 133–145.

Eig A (1929) Monographisch-kritische Übersicht der Gattung *Aegilops. Feddes Rep* **55**: 1–228.

Friebe B, Mukai Y, Gill BS (1992) C-banding polymorphisms in several accessions of *Triticum tauschii (Aegilops squarrosa). Genome* **35**: 192–199.

Gerlach WL, Dyer TA (1980) Sequence organization of the repeated units in the nucleus of wheat which contains 5S-rRNA genes. *Nucleic Acids Res* **8**: 4851–4865.

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberration in wheat (*Triticum aestivum). Genome* **34**: 830–839.

Kihara H (1940) Verwandschaft der *Aegilops*-Arten im Lichte der Genomanalyse. *Der Züchter* **12**: 49–62.

Kihara H (1957) Completion of genome-analysis of three 6x species of *Aegilops. Seiken Ziho* **8**: 3.

Kihara H (1963) Interspecific relationships in *Triticum* and *Aegilops. Zeiken Ziho* **15**: 1–12.

Kihara H, Tanaka M (1970) Addendum to the classification of the genus Aegilops by means of genome analysis. *Wheat Inf Serv* **30**: 1.

Kihara H, Yamashita K, Tanaka M (1959) Genomes of 6x species of *Aegilops. Wheat Inf Serv* **8**: 3–5.

Kimber G (1995) Genomic analysis and the derivation of evolutionary relationships in the wheat group – a summary. In: Raupp WJ, Gill BS, eds. *Proceedings US–Japan Symposium, Classical and Molecular Cytogenetic Analysis. 21–23 March 1994, Manhattan, Kansas, Kansas*, Agricultural Experimental Station Report 95–352D, pp 2–10.

Kimber G, Feldman M (1987) *Wild Wheat, an Introduction*. College of Agriculture University Missouri: Columbia.

Kimber G, Zhao YH (1983) The D genome of the *Triticeae. Can J Genet Cytol* **25**: 581–589.

Lilienfeld FA, Kihara H (1951) Genome-analysis in *Triticum* and *Aegilops*. X. Concluding Review. *Cytologia* **16**: 101–123.

Mukai Y, Endo TR, Gill BS (1990) Physical mapping of the 5S rRNA multigene family in common wheat. *J Hered* **81**: 290–295.

Mukai Y, Endo TR, Gill BS (1991) Physical mapping of the 18S-26S rRNA multigene family in common wheat: identification of a new locus. *Chromosoma* **100**: 71–78.

Mukai Y, Nakahara Y, Yamamoto M (1993) Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence *in situ* hybridization using total genomic and highly repeated DNA probes. *Genome* **36**: 489–494.

Nakai Y (1982) D genome donors for *Aegilops crassa* (DDMM, DDDDMM) and *Ae. vavilovii* (DDMMSS) deduce from esterase analysis by isoelectric focusing. *Jpn J Genet* **57**: 349–360.

Ogihara Y, Tsunewaki K (1988) Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. *Theor Appl Genet* **76**: 321–332.

Rayburn AL, Gill BS (1986) Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa. Plant Molecular Biol Rep* **4**: 104–109.

Rayburn AL, Gill BS (1987) Molecular analysis of the D-genome of the *Triticeae. Theor Appl Genet* **73**: 385–388.

Siddique AK, Jones JK (1967) The D genomes of *Aegilops crassa* Boiss. *Bol Genet Inst Fitotec Castelar* **4**: 29–31.

Teoh SB, Miller TE, Reader SM (1983) Intraspecific variation in C-banded chromosomes of *Aegilops comosa* and *Ae. speltoides. Theor Appl Genet* **65**: 343–348

Tsunewaki K (1993) Genome-plasmon interactions in wheat. *Jpn J Genet* **68**: 1–34.

Van Slageren MW (1994) *Wild Wheats; a Monograph of* Aegilops *L. and* Amblyopyrum *(Jaub. & Spach) Eig (Poaceae)*. Agricultural University Wageningen: the Netherlands; ICARDA: Aleppo, Syria. pp 512.

Witcombe JR (1983) *A Guide to the Species of Aegilops L. Their Taxonomy, Morphology and Distribution*. Rome: IBPGR Secretariat.

Yamamoto M, Mukai Y (1995) Physical mapping of ribosomal RNA genes in *Aegilops* and *Triticum*. In: Li S, Xin ZY, eds. *Proceedings 8th International Wheat Genetic Symposium Bejing, China, 20–25 July 1993*. pp 807–811.

Zhang HB, Dvorak J (1992) The genome origin and evolution of hexaploid *Triticum crassum* and *Triticum syriacum* determined from variation in repeated nucleotide sequences. *Genome* **35**: 806–814.

Zhao YH, Kimber G (1984) New Hybrids with D-genome wheat relatives. *Genetics* **106**: 509–515.

Zhukovsky PM (1928) A critical-systematical Survey of the species of the genus *Aegilops* L. *Bull Appl Bot Genet Plant Breed* **18**: 417–609.