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Genome differentiation in *Aegilops*. 4. Evolution of the U-genome cluster

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Abstract. Phylogenetic relationships of polyploid *Aegilops* species sharing the U-genome were investigated by analyzing heterochromatin banding patterns of their somatic metaphase chromosomes as revealed by C-banding and fluorescence in situ hybridization (FISH) with the heterochromatin-limited repetitive DNA probes pSc119, pAs1, as well as the distribution of NOR and 5S DNA loci revealed by pTa71 (18S-26S rDNA), and pTa794 (5S rDNA) probes. Seven tetraploid (*Ae. triuncialis*, *Ae. peregrina*, *Ae. kotschyi*, *Ae. geniculata*, *Ae. biuncialis*, *Ae. columnaris*, and 4x *Ae. neglecta*) and one hexaploid (6x *Ae. neglecta*) *Aegilops* species of the U-genome cluster were studied. The U^t and C^t chromosomes of 4x *Ae. triuncialis* (U^tC^t) were similar to the diploid donors *Ae. umbellulata* (U) and *Ae. caudata* (C). However, the size of the NOR locus on chromosome $5U^t$ was reduced. Karyotypic analyses confirmed that 4x *Ae. peregrina* (S^pU^p) was derived from a hybridization of the diploid species *Ae. umbellulata* with *Ae. longissima*, whereas *Ae. umbellulata* and *Ae. sharonensis* (or an immediate precursor) were the diploid progenitor species of *Ae. kotschyi* (S^kU^k). In both 4x species, the NORs on S-genome chromosomes were inactivated and were accompanied with a decrease or loss of rDNA sequences. Karyotypes of the tetraploid species, *Ae. geniculata* (U^gM^g) and *Ae. biuncialis* (U^bM^b) differed from each other and from the putative diploid progenitors *Ae. umbellulata* and *Ae. comosa* indicating that various types of chromosomal alterations occurred during speciation. Inactivation of major NORs on the M-genome chromosomes, redistribution of 5S rDNA sites, and loss of some minor 18S-26S rDNA loci were observed in *Ae. geniculata* and *Ae. biuncialis*. Significant differences in the total amount and distribution of heterochromatin, the number and location of 5S and 18S-26S rDNA loci observed between *Ae. columnaris* (U^cX^c)/4x *Ae. neglecta* (U^nX^n) and *Ae. geniculata*/*Ae. biuncialis* indicate that these species have different origins. Similarities in C-banding and FISH patterns of most *Ae. columnaris* and 4x *Ae. neglecta* chromosomes suggest that they were probably derived from a common ancestor, whereas distinct differences of three chromosome pairs may indicate that the divergence of these species was probably associated with chromosomal rearrangements and/or introgressive hybridization. *Ae. umbellulata* contributed the U genome, however, the source of their second genomes remains unknown. The formation of 6x

Ae. neglecta ($U^nX^nN^n$) was not associated with large modifications of the parental genomes.

Key words: *Triticum*, *Aegilops*, wild wheats, karyotype evolution, C-banding, fluorescence in situ hybridization.

Introduction

The genus *Aegilops* L. includes 11 diploid and 12 polyploid species (Zhukovsky 1928, Eig 1929, Hammer 1980, Witcombe 1983, Löwe 1984, van Slageren 1994). The present study follows the classification of van Slageren except that *Ae. mutica* Boiss. is considered as belonging to the genus *Aegilops*. All diploid species possess distinct genomes and they can be easily discriminated on the basis of plant morphology. In contrast, polyploid *Aegilops* showed no clear morphological borders between species and numerous intermediate forms have been discovered (Zohary and Feldman 1962, Feldman 1965a–c, Zohary 1966).

Investigations of the origin of polyploid *Aegilops* showed that the genomes of some species are very similar to those of the diploid progenitors, whereas they are modified in other species (Kihara 1954). Kihara suggested that either presently extinct species were the donors of the modified genomes or they were significantly rearranged during evolution. According to an alternative hypothesis (Zohary and Feldman 1962, Kimber and Feldman 1987), the rate of parental genome modification in polyploid species is different. As a rule, one genome is very close or even identical to the parental (pivotal genome), whereas the second (differential genome) is modified.

This hypothesis suggested that most polyploid *Aegilops* species were derived from the hybridization of a limited number of 'primordial' tetraploids sharing a common genome. Further speciation was accompanied by complete or segmental chromosome substitutions in their differential genomes. Based on investigations of plant morphology and meiotic chromosome pairing in interspecific hybrids, Zohary and Feldman (1962) proposed that introgressive hybridization has played a significant role in the process of intraspecific divergence of polyploid *Aegilops*. However, some facts contradict this suggestion (Chennaveeraiah 1960, Talbert et al. 1993, Dubcovsky and Dvorák 1994, Resta et al. 1996).

Three pivotal genomes, A, D, and U were identified in the *Triticum*/*Aegilops* complex, and all polyploid species were subdivided into three clusters. All diploid and polyploid wheats were included in the A-genome cluster. The D-genome cluster contained diploid *Ae. tauschii* Coss. and five polyploid species of sections *Vertebrata* and *Cylindropyron*. The U-genome cluster includes diploid *Ae. umbellulata* Zhuk. and seven polyploid species of section *Pleionathera* (Morris and Sears 1967, Kimber and Feldman 1987).

In tetraploid species, the U-genome can be combined with the C (*Ae. triuncialis* L.), S (*Ae. peregrina* Hack. in J. Fraser Maire & Weiller and *Ae. kotschyi* Boiss.), or M genomes (*Ae. geniculata* Roth., *Ae. biuncialis* Vis., *Ae. columnaris* Zhuk., 4x *Ae. neglecta* Req. ex Bertol., and 6x *Ae. neglecta* has the genome constitution $U^nM^nN^n$) (Lilienfeld 1951; Kihara 1954, 1963; Morris and Sears 1967; Kimber and Feldman 1987; Kimber and Tsunewaki 1989; van Slageren 1994). However, the origin of *Ae. columnaris* and *Ae. neglecta* is still under discussion. Thus, Dvorák (1998) assigned them the genome formula UX.

The U genomes in all species of this cluster are similar to each other and to that of the parental diploid species *Ae. umbellulata*, whereas the second genomes are modified compared to the original (Kihara 1954, 1963; Chennaveeraiah 1960; Kimber and Abu-Bakar 1981; Kimber and Zhao 1983; Kimber and Feldman 1987; Kimber and Yen 1989). The extent of genome modification varies between species. Both genomes of *Ae. triuncialis* were found to be nearly identical to those of the parental species (Kihara 1954, Chennaveeraiah 1960, Kimber and Yen 1989, Zhang and Dvorák 1992, Dubcovsky and Dvorák 1994, Dvorák and Dubcovsky 1996). Significant changes of the parental genomes in *Ae.*

columnaris and *Ae. neglecta* prevented the identification of the origin of these species (Resta et al. 1996).

In the present work, we analyzed the heterochromatin structure in karyotypes of eight polyploid species of the U-genome cluster using C-banding and fluorescence in situ hybridization (FISH) with the heterochromatin-specific DNA probes pSc119, and pAs1 (non-coding highly repeated DNA sequence), as well as rDNA loci using pTa71 (18S-26S rDNA), and pTa794 (5S rDNA) probes. The karyotypes and NOR loci were compared between diploid and derived polyploids to analyze phylogenetic relationships.

Materials and methods

Twenty-one accessions of *Ae. triuncialis*; 20 of *Ae. peregrina*, 10 of each *Ae. kotschyi*, *Ae. geniculata*, and 4x *Ae. neglecta*; 27 accessions of *Ae. biuncialis*; 23 accessions of *Ae. columnaris*; and 11 accessions of 6x *Ae. neglecta* of diverse origin were examined (Table 1). One accession of *Ae. triuncialis* (k-2981) was a mixture of *Ae. triuncialis* and *Ae. biuncialis*, and the respective forms were designated as k-2981a (*Ae. triuncialis*) and k-2981b (*Ae. biuncialis*). Three original samples of *Ae. triuncialis*, two of *Ae. columnaris*, and one of *Ae. neglecta* were collected by Drs. A.G. Gukasyan and E.A. Nazarova in different regions of Armenia. One sample of *Ae. triuncialis* was collected by Dr. O.V. Muravenko in Turkmenistan. The other accessions are maintained in the germplasm collections of the Vavilov Institute of Plant Industry (WIR, Russia), Wheat Genetics Resource Center, Kansas State University (U.S.A.), USDA-ARS Small Grains Collection, Aberdeen, Idaho (U.S.A.), Kyoto University (Japan), and the Weizmann Institute of Science (Israel).

DNA probes: The pAs1 clone (1 kb) was isolated from *Ae. tauschii* (Rayburn and Gill 1986) and inserted into the pUC8 plasmid. The pSc119 clone is a 120-bp DNA sequence derived from rye (*Secale cereale* L.) and inserted into the plasmid pBR322 (Bedbrook et al. 1980). Both pAs1 and pSc119 map to heterochromatic regions of cereal chromosomes. The pTa794 clone is a 410-bp *Bam*HI fragment of 5S rDNA isolated from *T. aestivum* and cloned in the pBR322 plasmid (Gerlach and Dyer 1980). The pTa71 clone is a 9-kb *Eco*RI fragment of 18S-26S rDNA isolated from bread wheat (Gerlach and Bedbrook 1979) and recloned in the pUC19 plasmid. All probes were labeled with either digoxigenin-11-dUTP or biotin-16-dUTP (Boehringer-Mannheim, Germany) by nick-translation according to manufacturer's protocol.

In situ hybridization and signal detection were as described by Badaeva et al. (1996a). After FISH, the images of metaphase chromosomes were taken with a CCD camera (Cool Snap, U.S.A.) on a Leitz Wetzlar microscope and processed in Adobe Photoshop software (version 7.0).

C-banding was performed according to the previously published protocol (Badaeva et al. 1994) except that a 1.5 to 2 h treatment with 0.2% colchicine of root-tip meristematic tissues was used to block and collect cells at metaphase stage of mitosis. Chromosome preparations were analyzed using an Olympus BH-2 microscope. Microphotographs were taken using Mikrat-N (Russia) or Imagelink HQ (Canada) microfilm and printed on Kodak RC-II F-4 (U.S.A.) or Bromexpress-1 N (Russia) photographic paper.

Chromosomes of *Ae. peregrina* and *Ae. geniculata* were classified according to standard genetic nomenclature (Friebe et al. 1996a, 1999). Chromosomes of other species were classified on the basis of similarity with *Ae. umbellulata* U-genome chromosomes and with other diploid and polyploid species of the genus *Aegilops* (Friebe and Gill 1996; Friebe et al. 1992, 1993, 1995, 1996b; Badaeva et al. 1996a, 1999, 2002a, b; Vishnyakova et al. 1997).

Results

Aegilops triuncialis. ($2n = 4x = 28$, U^tC^t) is a tetraploid species consisting of two varieties *triuncialis* and *persica*. *Ae. triuncialis* originated from hybridization of *Ae. umbellulata* with *Ae. caudata* L. (Kihara 1949, 1954; Kimber and Yen 1989; Kimber and Tsunewaki 1989). Meiotic pairing and karyotype analyses (Senyaninova-Korchagina 1932; Kihara 1949, 1954; Lilienfeld 1951; Chennaveeraiah 1960; Tsunewaki 1980; Kimber and Yen 1989) and variation of repeated nucleotide sequences (Dubcovsky and Dvorák 1994) showed that the genomes of *Ae. triuncialis* are not altered relative to the parental genomes.

Table 1. The list of *Aegilops* accessions analyzed and their sources; i, k – St Petersburg, Russia; TA – the Wheat Genetics Resource Center, Kansas State University, U.S.A.; PI – USDA-ARS, Aberdeen, Idaho, U.S.A.; KU – Kyoto University, Japan; TX, TN – Weizmann Institute of Science, Rehovot, Israel; and G – accessions from the original collection sites. An * indicated those accessions analyzed by FISH

Accession #	Country of origin	Province	Collection site	Altitude (m)
Ae. triuncialis var. *triuncialis*				
TA1748	Afghanistan	Faryab	20 mi W of Maimana.	915
G-10	Armenia		Kafan region, between villages of Arzvanyk and Kafan.	
G-14	Armenia		Near Erevan, dry slope in gorge of the Razdan River.	
G-15*	Armenia		Ekhegnadzor region, shore of rivulet near village of Getan.	
i-571284	Cyprus		Klepini, E Kyrenia.	
k-1965	Iran	Fars	26 km from Shiraz.	1,540
k-2472*	Italy		Unknown.	
i-108908	Kyrgyzstan		Unknown.	
i-571164*	Lebanon		Unknown.	
i-571217	Morocco		Dar Chaoui, 20 km from Tarle to Rabat.	
i-571017	Pakistan	Balochistan	Attok Zai, 16 km NE of Quetta.	
k-3419	Turkey	Van	43 km N of Vaka.	
G-11	Turkmenistan	Achal	SW Kopet Dag, dry river bed Makhtum-Kala.	
Ae. triuncialis var. *persica*				
k-146	Azerbaijan		Stepanakert region, Nagorny Karabakh, village of Dagadan.	1,030
k-1174	Azerbaijan		Nakhichevan region, Aznavurt.	1,500
k-940	Afghanistan	Faryab	Road from Darzob–Maimana–Kaisor.	540
k-2981a	Syria		NE of Sen Simon.	
k-1558	Turkmenistan	Achal	Ashkabad region, Firuza.	
k-1559*	Turkmenistan		Kelata.	
k-1714	Turkmenistan	Balkan	Kara-Kala, shore of Sumbar River, 100 m upstream of the Aiderinka River.	
k-1757	Uzbekistan	Tashkent	Bostanlyk, Tashkent region, Khadjikent station.	
Ae. peregrina				
i-571289	Cyprus		Kabo Greco.	
KU13-2	Israel	Tel Aviv	Suburbs of Tel Aviv.	
TA1885	Israel	Central	Costal plain, 10 km E of Rehovot.	
TA1887	Israel	Southern	4 km E of Ashqelon.	
TA1888	Israel	Jerusalem	Campus of Jerusalem University.	
TA1889	Israel	Central	3 km SE of Rehovot.	
TA1891*	Israel	Central	3 km SE of Rehovot.	

Table 1 (continued)

TA1919	Israel	Central	3 km SE of Rehovot.	
TA1986	Israel	Jerusalem	In the garden of the Shepherd Hotel.	
k-61	Jordan		Unknown.	
i-570636	Jordan		10 km W of Salt toward Jordan Valley.	750
KU6664	Jordan	West Bank	9 km NW of Fari'ah al Jiftliq (Dead Sea–Fari'ah al Jiftlik).	
KU6668	Jordan	Al Balqa'	Basin of the Jordan River (A), Amman–Salt–Dead Sea.	
KU6701	Jordan	Al Balqa'	Basin of the Jordan River (B), Amman–Salt–Dead Sea.	
KU13-1	Lebanon	Ash Shamal	Suburbs of Tripoli (Tarabulus).	
TA1893*	Turkey	Konya	Opposite Eregli.	1,250
k-3281	Syria	Al Ladhiqiyah	Lattakia.	
i-570515	Syria		Between Sakhra and Samtha.	
k-644	Unknown			
TA1896*	Unknown		Collection of the University of Reading, United Kingdom.	
Ae. kotschyi				
KU14-1	Egypt	Shamal Sina	El' Arish, Sinai Peninsula.	
KU14-3	Egypt	Shamal Sina	El' Arish, Sinai Penninsula.	
KU6609	Egypt		69 km from rest house between Cairo–Alexandria.	
k-201*	Israel		Unknown.	
TA1979*	Israel	Southern	25 km S of Be'er Sheva'.	
TA1980	Israel	HaNegev	14 km NW of Be'er Sheva'.	
KU6626	Israel	Jerusalem	In the garden of the Shepherd Hotel.	
i-570604	Jordan	Shamakh	S of Shoubak.	1,215
i-573482	Jordan	Haiyan	Ruweibid, near Bal'amo.	
TA1974	Unknown		Collection of the University of Manitoba, Winnipeg, Canada.	
Ae. biuncialis				
k-738	Azerbaijan		Unknown.	
PI428557	Azerbaijan		Unknown.	
PI554176	Bosnia & Herzegovinia	Herzegovinia	2 km E of Mostar on Highway 17.	70
i-571306	Cyprus		Anarita, near Timi.	
PI483025*	Cyprus		Paphos, 3 km NNW from Baths of Aphrodite.	20
PI378189	Greece	Macedonia	Kumanovo.	
PI550956	Greece	Macedonia	2 km from Kallithea, Chalkidiki.	10
k-3280	Greece		Unknown.	
TN08	Israel	Hagalil	Along Zefat-Dalton Road, upper Galilee.	

Table 1 (continued)

Accession #	Country of origin	Province	Collection site	Altitude (m)
TN07	Israel	Hagalil	3 km S of Manara, upper Galilee.	
TN03	Israel		Along Mas"ada-Majdal Sharms Road, upper Golan Heights.	
PI487282	Jordan	Irbid		900
i-570579	Jordan		Unknown.	
k-2682*	Lebanon		Unknown.	
k-3293	Russian Federation		Krasnodar region.	
k-2981b	Syria		NE of Sen Simon.	
k-2651	Syria		Unknown.	
PI487215	Syria	Idlib		400
PI542148	Turkey	Urfa	On the State Farm at Ceylanpinar.	350
PI573358	Turkey	Bilecik	14 km N of Bilicik.	120
TA2000	Turkey	Bursa	11 km NW of Karacabey.	20
TA2078	Turkey	Elazig	1 km E of Elazig, Arapkir–Elazig–Malatya junction.	
TA2079	Turkey	Gaziantep	19 km N of Gaziantep.	800
TA2080	Turkey	Turkey	9 km N of Araban at province border.	1,000
TA2081	Turkey	Antalya	4 km SW of Korkuteli.	1,100
k-3007*	Ukraine	Crimea	Coast of Arabat Gulf in stone quarry.	
PI374367	Yugoslavia	Serbia	Kosovo, Srbvovac kod Zvecana.	600
Ae. geniculata				
i-571296	Cyprus		Near the Moni Power Station.	
k-2807*	Israel		Mai'anit.	
TA1701	Turkey	Hatay	1 km N of Iskenderum.	50
TA1799	Turkey	Canakkale	70 km E of Can.	
TA1800	Turkey	Kirkareli	Babaeski.	
TA1702*	Unknown		Collection of the Gradina Botanica, Cluj, Romania.	
TA1813	Unknown		Collection of the Institute Orto Botanico, Rome, Italy.	
TA1815	Unknown		Collection of the University of Reading, United Kingdom.	
TA1816	Unknown		Collection of the Botanical Garden of the University Zürich, Switzerland.	
TA1817	Unknown			
Ae. columnaris				
G-13	Armenia	Ararat	Near the village of Urznadzor.	
k-564*	Armenia		Azizbek, near village of Khandzorut.	
k-1193	Armenia		Abovyan, near village of Shor-Bulakh.	

Table 1 (continued)

k-1512	Armenia	Yerevan	Near Yerevan at gorge of Razdan River.	
PI428558	Azerbaijan		Unknown.	
i-571163	Lebanon		Unknown.	
i-571185	Lebanon		Unknown.	
i-571172	Lebanon		Unknown.	
i-571173*	Syria		Unknown.	
i-577972*	Syria		Unknown.	
i-570236	Turkey	Ankara	14 km SW of Keskin.	
TA2084	Turkey	Hatay	1 km N of Iskenderum.	50
TA2106	Turkey	Konya	Unknown.	
PI276968	Turkey	Konya	Unknown.	
PI554180	Turkey	Kars	35 km W of Tuzluca.	1,010
PI554184	Turkey	Malatya	5 km S of Sivas–Malatya province border.	1,500
PI554185	Turkey	Van	2 km SE of Van on road to Gurpinar.	1,810
PI554190	Turkey	Van	29 km SE of Van.	1,790
PI560506	Turkey	Van	3 km W of Ermisler, roadside along Van Lake.	1,630
PI564180	Turkey	Icel	58 km SW of Silifke, 4 km W of Ovacik, Mersin.	250
PI564181	Turkey	Icel	58 km SW of Silifke, 4 km W of Ovacik, Mersin.	250
PI542191	Unknown		Collection of the Gene Bank at Izmir, Turkey.	
TX01	Unknown			
4x *Ae. neglecta*				
k-2711	Algeria		Unknown.	
G-12*	Armenia	Kafan	Near Kafan Mount.	
k-2387	Italy		Unknown.	
i-570283*	Turkey	Elazig	3 km NW of Keban.	1,100
k-3475	Turkey	Dyarbakyr	10 km NE of Dyarbakyr, Agricultural Institute.	
k-3529	Turkey	Ankara	Kizicahaman.	
TA2156	Turkey	Konya	32 km W of Konya.	1,450
TA2157	Turkey	Menemen	South slope of hill.	45
TA2162	Turkey	Dyarbakyr	Junction of Dyarbakyr–Bismil road on Highway 380.	900
k-1932	Unknown			
6x *Ae. neglecta*				
k-1469	Azerbaijan	Zangelan	By road from Zangelan–Kafan.	
k-1654	Bulgaria	Burgas	Village of Primorsko.	
k-2059	Greece		30 km W of Baza.	

Table 1 (continued)

Accession #	Country of origin	Province	Collection site	Altitude (m)
k-2410*	Greece	Macedonia	Road from Larisa–Antalia.	1,100
k-1638	Greece		Unknown.	
k-3264	Italy		Unknown.	
i-571245	Morocco		10 km of Aguelmous.	
k-2712	Portugal		Unknown.	
k-2416	Spain		Unknown.	
k-2063*	Spain		Road from Trudjilo–Merida.	
k-2062*	Spain		Unknown	

Three types of plasmons are recognized in *Ae. triuncialis*. The first corresponds to that of *Ae. umbellulata*, the second to *Ae. caudata*, and the third to *Ae. mutica* (Endo and Tsunewaki 1975, Tsunewaki 1996). Based on the analysis of interspecific hybrids, Waines and Barnhart (1992) suggested that *Ae. umbellulata* was a female parent of *Ae. triuncialis* var. *triuncialis*, and *Ae. caudata* was the female parent of var. *persica*. Differences in karyotype structure between the two varieties have been reported previously (Senyaninova-Korchagina 1932, Chennaveeraiah 1960).

We examined karyotypes of 21 accessions of *Ae. triuncialis* (13 of var. *triuncialis* and 8 of var. *persica*) by C-banding and of four accessions (three of var. *triuncialis* and one of var. *persica*) were analyzed by FISH. The karyotype structure of the accessions studied (Fig. 1) was similar to that described by Senyaninova-Korchagina (1932) and later by Chennaveeraiah (1960) for *Ae. triuncialis* var. *persica*.

All *Ae. triuncialis* chromosomes had distinct C-banding patterns similar to those of the parental species *Ae. umbellulata* and *Ae. caudata* (Friebe et al. 1992, 1995; Badaeva et al. 1996a; Vishnyakova et al. 1997), which allowed identification and classification of *Ae. triuncialis* chromosomes according to standard genetic nomenclature. Only limited intraspecific polymorphism was observed, manifested in the presence or absence of certain bands and variation in C-band size (Fig. 1). Chromosomal rearrangements found in three of the 21 accessions were represented by single or multiple translocations with centromeric (Robertsonian translocations) (**T1:** $T3U^tS{\cdot}D^tS + T3U^tL{\cdot}D^tL + T7U^tS{\cdot}G^tS + T7U^tL{\cdot}B^tL + TB^tS{\cdot}G^tL$), G14-2 (Fig. 1d) or inter-stitial (**T2**: $T1U^tS{\cdot}1U^tL\text{-}4U^tS + T4U^tL{\cdot}\ 4U^tS\text{-}1U^tL$), i-571217 (Fig. 1a); **T3**: $TB^tS{\cdot}B^tL\text{-}7U^tL + T7U^tS{\cdot}7U^tL\text{-}B^tL$, k-1965 (Fig. 1d) breakpoints and by a paracentric inversion **invA**^{t} ($1C^t$), k-1559-1 (Fig. 1e). The origin of additional modified chromosomes (chromosome $1U^t$ in k-940, Fig. 1g; chromosome $5U^t$ in k-146 and k-1965, Fig. 1h, i) remains unknown.

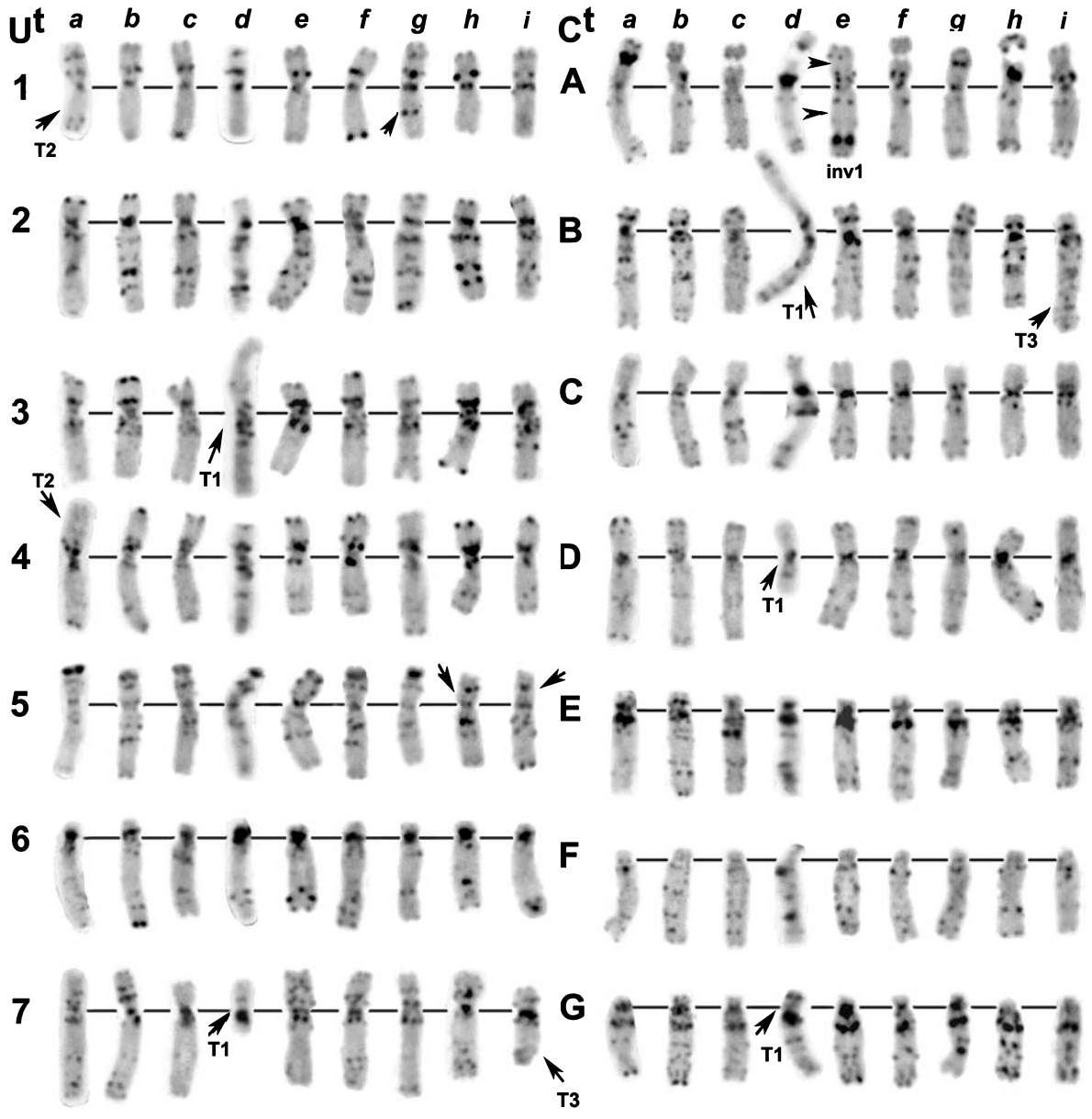

Fig. 1. C-banding polymorphism of *Ae. triuncialis*: (**a**) i-571217, (**b**) k-1714, (**c**) k-2472, (**d**) G14-2, (**e**) k-1559-1, (**f**) k-1559-2, (**g**) k-940, (**h**) k-146, (**i**) k-1965. **T1**: $T3U^tS{\cdot}D^tS + T3U^tL{\cdot}D^tL + T7U^tS{\cdot}G^tS + T7U^tL{\cdot}B^tL + TB^tS{\cdot}G^tL$; **T2**: $T1U^tS{\cdot}1U^tL\text{-}4U^tS + T4U^tL{\cdot}4U^tS\text{-}1U^tL$; **T3**: $TB^tS{\cdot}B^tL\text{-}7U^tL + T7U^tS{\cdot}7U^tL\text{-}B^tL$; **inv1**: paracentric inversion of chromosome A^t

FISH with pSc119 revealed distinct hybridization sites in the telomeric regions of one or both arms of 12 chromosome pairs of *Ae. triuncialis*. Interstitial FISH sites were observed in the long arms of chromosomes $7U^t$ and one C^t-genome chromosome (F^t) (Fig. 2d). Comparison of the pSc119-labeling patterns with the parental species shows that both the number and intensity of hybridization signals on *Ae. triuncialis* chromosomes decreased (Badaeva et al. 1996a).

An extended secondary constriction always was observed on chromosome A^t ($1C^t$), whereas the satellites on the U^t-genome chromosomes usually were not visible. Two active nucleolar organizing regions (NOR) were observed in both varieties of *Ae. triuncialis* using Ag-NOR banding (Cermeño et al. 1984), and four major NOR loci were revealed using FISH (Figs. 3a, 4), confirming previous observations (Yamamoto 1992). The signal size decreased in the order $A(1C^t) > 5U^t > 1U^t >> C(5C^t)$. A faint signal was occasionally observed in the middle of the long arm of chromosome $6U^t$. Four approximately equal 5S rDNA sites were detected in all chromosomes of homoeologous groups 1 and 5 (Fig. 3a). Thus, the distribution of ribosomal RNA gene families on chromosomes of *Ae. triuncialis* is similar to those of the parental species (Badaeva et al. 1996b) (Fig. 4), suggesting that speciation in *Ae. triuncialis* was not associated with large modifications of the parental genomes. The low amount of C-banding polymorphism and the low frequency of chromosomal rearrangements may reflect a comparatively recent origin of *Ae. triuncialis*.

Aegilops peregrina and *Ae. kotschyi* are two closely related tetraploid species ($2n = 4x = 28$) with the genome constitution S^pU^p and S^kU^k, respectively (Kihara 1937, 1940, 1949, 1954; Tanaka 1955; Kimber and Feldman 1987; Waines and Barnhart 1992; van Slageren 1994). *Aegilops umbellulata* contributed the U genome (Kimber and Yen 1988, 1989; Zhang and Dvorák 1992), and several other species have been suggested as possible sources of the second genome. Kihara (1949) proposed that this genome was contributed by a species of the Sitopsis group. Other authors considered an unknown species of the M-genome group (Chennaveeraiah 1960), *Ae. searsii* Feldman & Kislev ex Hammer (Ogihara and Tsunewaki 1988), *Ae. longissima* Schweinf. & Muschl. (Kihara 1946, Talbert et al. 1991, Friebe et al. 1996a) or the immediate precursor of *Ae. longissima* and *Ae. sharonensis* Eig. (Zhang et al. 1992). The degree of S-genome modification in these species also was estimated differently; some authors reported that it is modified (Kihara 1940, 1946, 1949, 1954; Zohary and Feldman 1962; Chennaveeraiah 1960; Kimber and Yen 1989), and others observed no differences compared with the parental species (Zhang et al. 1992; Friebe et al. 1996). The cytoplasms of *Ae. peregrina* and *Ae. kotschyi* are similar to the plasmon of *Ae. searsii* (Ogihara and Tsunewaki 1988, Siregar et al. 1988, Tsunewaki 1996).

Twenty accessions of *Ae. peregrina* and 10 accessions of *Ae. kotschyi* were examined by C-banding and one accessions of *Ae. kotschyi* and three accessions of *Ae. peregrina* were analyzed by FISH (Table 1). All *Ae. peregrina* chromosomes are highly heterochromatic and show distinct C-banding patterns (Fig. 5) allowing their identification and classification according to the standard genetic nomenclature (Friebe et al. 1996a). The U^p genome of *Ae. peregrina* is similar to that of *Ae. umbellulata* (Friebe et al. 1995, 1996a; Badaeva et al. 1996a; Vishnyakova et al. 1997). However, some differences observed between the corresponding chromosomes in the size and position of C-bands may reflect the fact that the U^p genome of *Ae. peregrina* is modified. At the same time, the S^p genome is nearly identical to the S^l genome of *Ae. longissima* (Friebe et al. 1993, Badaeva et al. 1996a, Friebe and Gill 1996) indicating little modification, which contradicts earlier findings (Chennaveeraiah 1960, Zohary and Feldman 1962).

Significant C-banding polymorphism was detected in *Ae. peregrina* (Fig. 5). The frequency of chromosomal aberrations was comparatively low, which agrees with meiotic pairing data (Furuta 1981a; Kawahara 1986, 1988). Only four accessions had rearranged chromosomes (chromosomes $5S^p$, $6S^p$ in k-644 in Fig. 5b; chromosomes $3U^p$, $1S^p$, $3S^p$ and $7S^p$ in k-61 in Fig. 5i; chromosomes $3U^p$, $4U^p$, $5U^p$, $1S^p$ in KU6627 in Fig. 5c; and chromosome $1U^p$ in KU6664 in Fig. 5g) whose origin is unknown because of differences in morphology and C-band position and number. All other chromosomes in these lines are normal.

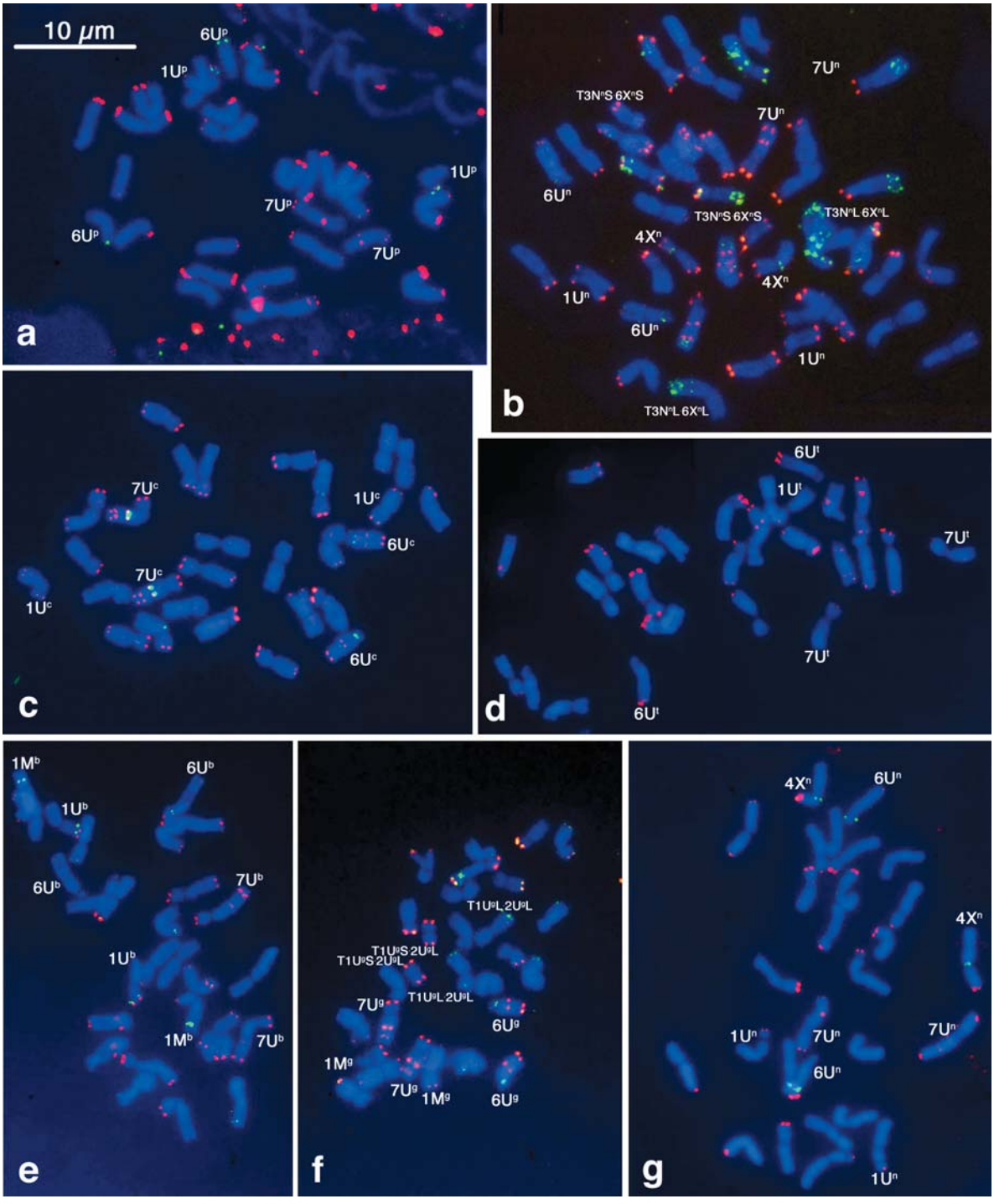

Fig. 2. Multicolor FISH with probes pSc119 and pAs1 on chromosomes of: (**a**) *Ae. peregrina* (KU14-1), (**b**) 6x *Ae. neglecta* (k-2062), (**c**) *Ae. columnaris* (k-564), (**d**) *Ae. triuncialis* (i-570164), (**e**) *Ae. biuncialis* (k-3007), (**f**) *Ae. geniculata* (k-2807), (**g**) 4x *Ae. neglecta* (k-570283). The probe pSc119 was labeled with biotin and detected with avidin-Cy3 and the probe pAs1 was labeled with digoxigenin and detected with anti-digoxigenin-FITC, Fab fragment. Scale bar =10 μm

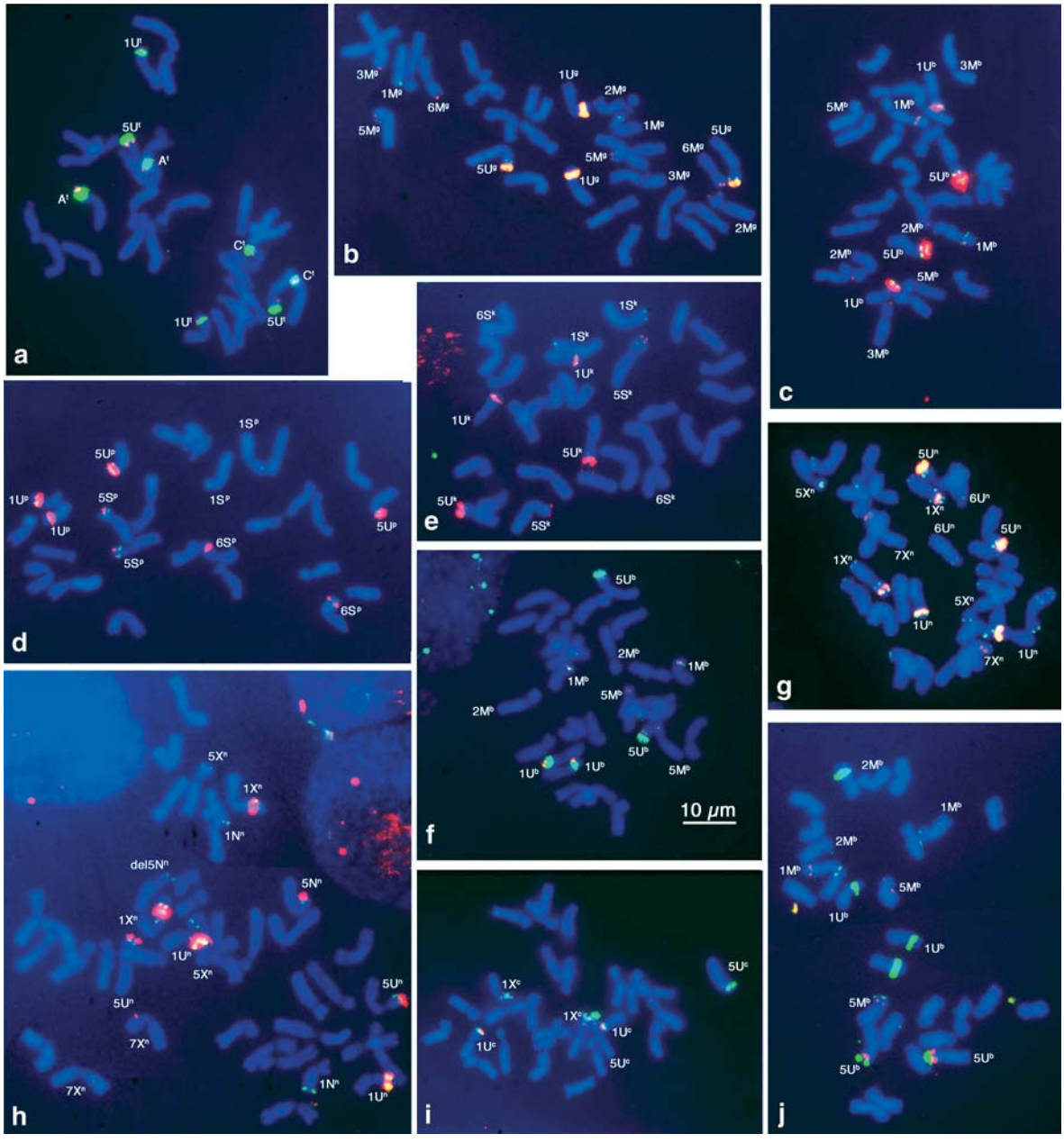

Fig. 3. Multicolor FISH with probes pTa794 (5S rDNA) and pTa71 (18S-26S rDNA) on chromosomes of: (**a**) *Ae. triuncialis* (k-2472), (**b**) *Ae. geniculata* (TA1702), (**c**) *Ae. biuncialis* (k-3007), (**d**) *Ae. peregrina* (KU14-1), (**e**) *Ae. kotschyi* (k-201), (**f**) *Ae. biuncialis* (k-2682), (**g**) 4x *Ae. neglecta* (G-12), (**h**) 6x *Ae. neglecta* (k-2062), (**i**) *Ae. columnaris* (i-571173), (**j**) *Ae. biuncialis* (PI429025). Combinations bio-pTa71 + dig-pTa794 (b, c, d, e, g, h) or dig-pTa71 + bio-pTa794 (a, f, i, j) were used. Biotinylated probes were detected with avidin-Cy3 and digoxigenin-labeled probes were detected with anti-digoxigenin-FITC, Fab fragment. Scale bar =10 μm

Therefore, these chromosomes did not likely originate as a result of simple translocations. These modified chromosomes might probably have arisen as a result of introgression of genetic material from a related species followed by meiotic recombination. Previous studies based on isozyme (Hart and Tuleen 1983) and C-banding analyses (Friebe et al.

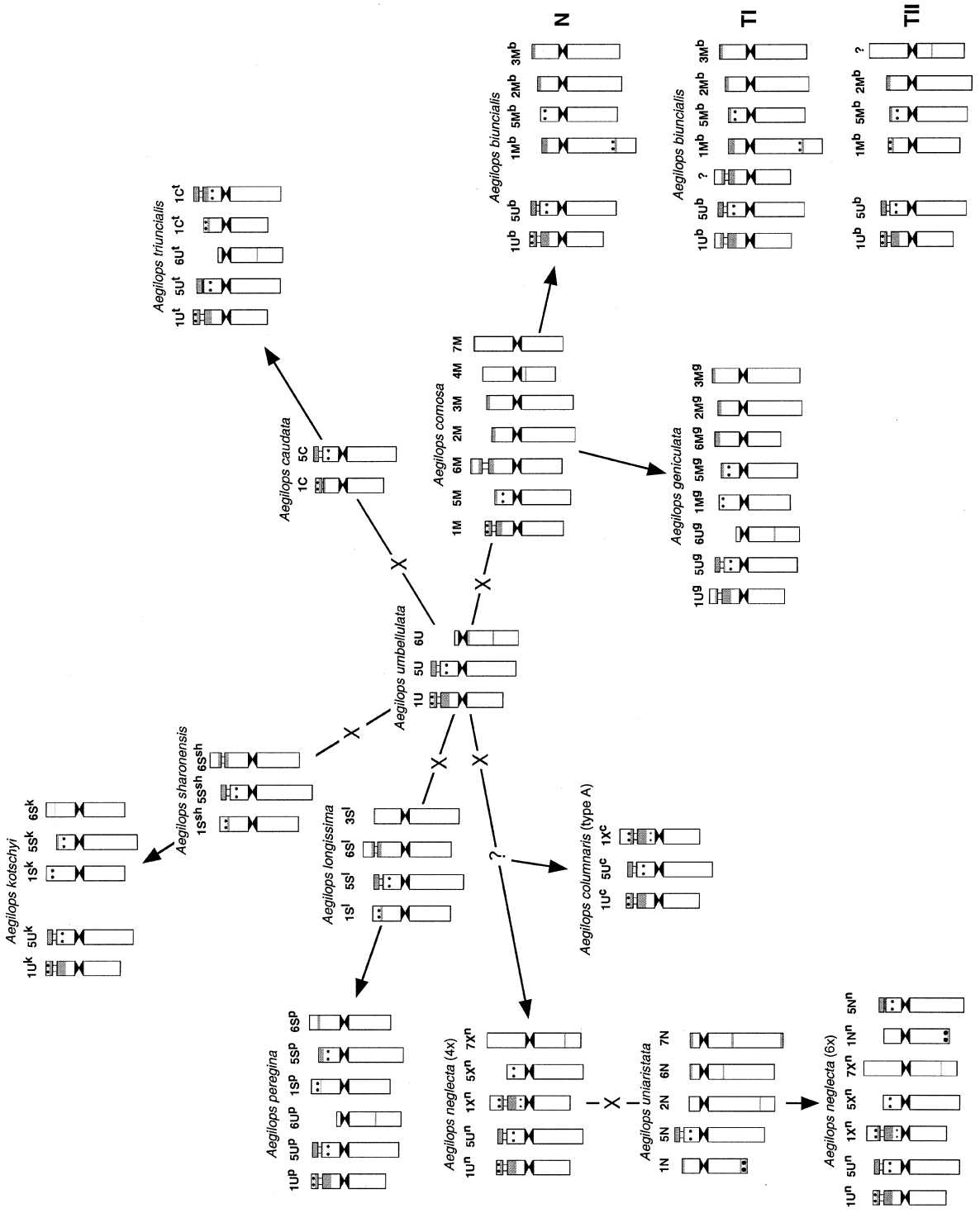

Fig. 4. Distribution of 18S-26S (squares) and 5S (circles) rRNA gene loci on chromosomes on polyploid *Aegilops* species and their supposed diploid ancestors

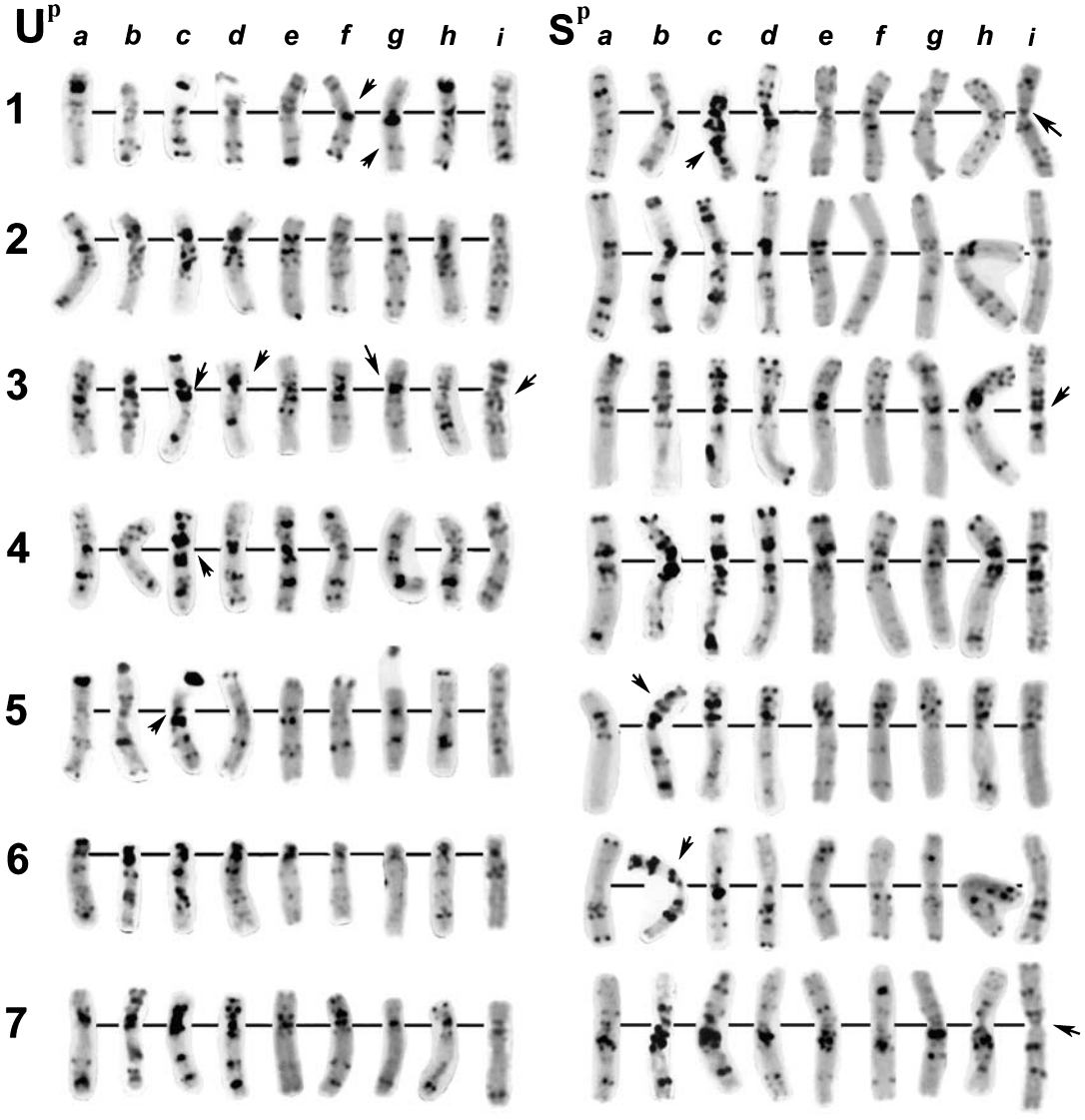

Fig. 5. C-banding polymorphism of *Ae. peregrina*: (**a**) TA1888, (**b**) k-644, (**c**) KU6627, (**d**) TA1896, (**e**) KU6668, (**f**) KU13-2, (**g**) KU6664, (**h**) KU6701, (**i**) k-61. Modified chromosomes are marked with arrows

1993, 1996a; Friebe and Gill 1996) revealed that chromosomes $4S^l$ and $7S^l$ of *Ae. longissima* are involved in a species-specific reciprocal translocation that is absent in *Ae. sharonensis*. The presence of this translocation in *Ae. peregrina* and its absence in *Ae. kotschyi* suggests that *Ae. peregrina* originated from the hybridization of *Ae. umbellulata* and *Ae. longissima* and that *Ae. kotschyi* originated from the hybridization of *Ae. umbellulata* and *Ae. sharonensis*.

Although *Ae. kotschyi* has the same genome constitution as *Ae. peregrina*, the C-banding patterns were different (Figs. 5, 6) Comparison of *Ae. kotschyi* with the diploid ancestors *Ae. umbellulata* and *Ae. sharonensis* revealed a higher degree of genome modification in *Ae. kotschyi* compared to *Ae. peregrina*. Chromosomes $4S^p$ and $7S^p$ of *Ae. peregrina* are nearly identical to $4S^l$ and $7S^l$ of *Ae. longissima*, whereas chromosomes $4S^k$ and $7S^k$ of *Ae. kotschyi* are similar to $4S^{sh}$ and $7S^{sh}$ of *Ae. sharonensis*.

Most of the rearrangements observed are Robertsonian translocations and involved U-U genome chromosomes (**T1**: T4U^{k}-S·6U^{k}L + T6U^{k}S·4U^{k}L, in KU14-1, TA1974, TA1975, Fig. 6c–e), U-S genome chromosomes (**T4**: T7U^{k}S·3S^{k}S + T7U^{k}L·3S^{k}L, KU6626, Fig. 6g), or S-S genome chromosomes (**T2**: T3S^{k}S·7S^{k}S + T3S^{k}L·7S^{k}L, KU6609, Fig. 6a). In translocation type **T3** (KU14-3, Fig. 6b) the breakpoints were located in interstitial regions of the long arm of chromosome 3S^{k} and the short arm of chromosome 5S^{k}. In addition, evidence suggests that this accession also has several chromosome substitutions. The C-banding patterns of chromosomes 5U^{k}, 6U^{k}, 4S^{k}, 6S^{k}, and 7S^{k} of this accession are similar to those of the corresponding chromosomes of *Ae. peregrina* rather than those of *Ae. kotschyi*.

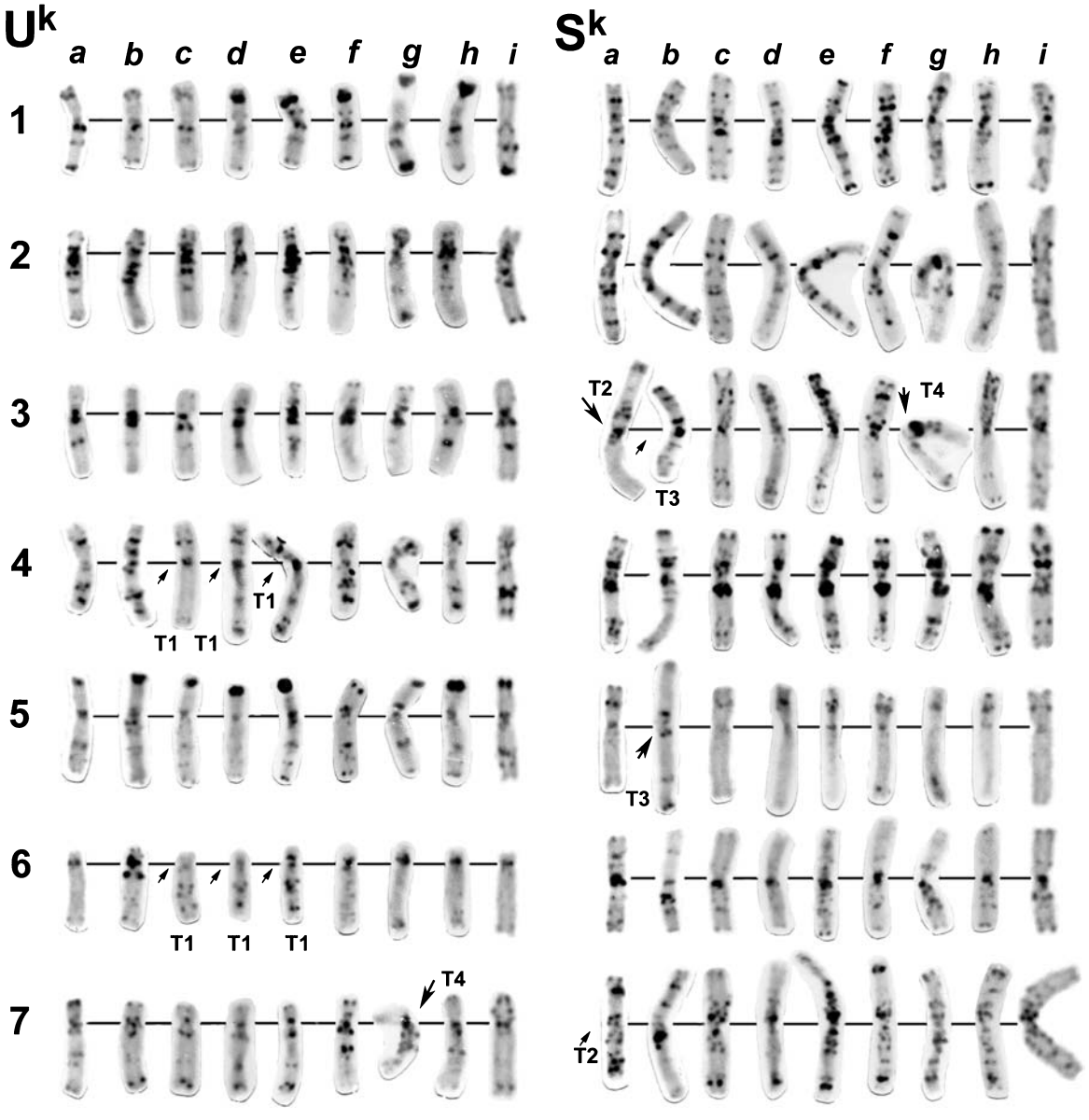

Fig. 6. C-banding polymorphism of *Ae. kotschyi*: (**a**) KU6609, (**b**) KU14-3, (**c**) KU14-1, (**d**) TA1974, (**e**) TA1975, (**f**) TA1980, (**g**) KU6626, (**h**) TA1979, (**i**) i-457292. **T1**: T4U^{k}S·6U^{k}L + T6U^{k}S·4U^{k}L; **T2**: T3S^{k}S·7S^{k}S + T3S^{k}L·7S^{k}L; **T3**: T3S^{k}S·3S^{k}L-5S^{k}S + T3S^{k}L-5S^{k}S·5S^{k}L; **T4**: T7U^{k}S·3S^{k}S + T7U^{k}L·3S^{k}

FISH with clone pSc119 revealed signals of various sizes in telomeric regions of either the short or both arms of 13 chromosome pairs in *Ae. peregrina* and in 12 pairs of *Ae. kotschyi* (Fig. 2a). Interstitial pSc119 FISH sites were detected in the long arm of chromosomes $7U^p$ and $7U^k$. In both species, chromosome 6U had no pSc119 FISH site although distinct telomeric and interstitial FISH sites are present in *Ae. umbellulata* (Badaeva et al. 1996a). However, in the tetraploid species this chromosome has a telomeric and interstitial pAs1 FISH site that is absent in *Ae. umbellulata*. Another pAs1 FISH site was detected in the middle of the satellite of chromosome $1U^p$ of *Ae. peregrina*.

In contrast to Chennaveeraiah (1960), we found only two pairs of satellite chromosomes in *Ae. peregrina* and *Ae. kotschyi*, which coincides with the number of active NORs detected by Ag-NOR staining (Cermeño et al. 1984) and *in situ* hybridization (Yamamoto 1992). In both species, major NORs were observed on group 1 and 5 U-genome chromosomes. *Ae. peregrina* and *Ae. kotschyi* also have several minor 18S-26S rDNA sites. Two consistent minor loci detected on group 5 and 6 S-genome chromosomes of both species were smaller in *Ae. kotschyi* than in *Ae. peregrina*. Thus, the suppression of nucleolar activity of the S-genome chromosomes is associated with a significant reduction in copy number of 18S-26S rRNA genes. The hybridization patterns with pTa794 were similar in both species; four similar-sized 5S rDNA loci were located on chromosomes of homoeologous groups 1 and 5 (Fig. 3d, e; Fig. 4).

The group of UM-genome species. Previously, four tetraploid *Aegilops* species of the Section *Pleionathera* generally were accepted to have the genome constitution UM (Kihara 1937, 1949, 1954; Lilienfeld 1951; Kimber and Feldman 1987; Kimber and Tsunewaki 1989). The plasmon of *Ae. geniculata* is similar to the plasmon of *Ae. mutica*. The plasmons of *Ae. biuncialis*, *Ae. columnaris*, and *Ae. neglecta* are nearly identical, but modified compared to that of *Ae. umbellulata* (Tsunewaki 1993, 1996).

Meiotic pairing analysis of *Ae. biuncialis* and *Ae. neglecta* indicated only little differentiation of their U genomes (Kihara 1954, Feldman 1965a–c, Kimber and Abu-Bakar 1981, Kimber and Feldman 1987, Kimber and Yen 1989), whereas the data on *Ae. geniculata* and *Ae. columnaris* are conflicting. The U^g genome in two accessions of *Ae. geniculata* was similar to that of *Ae. umbellulata*, but two other accessions were different (Kimber et al. 1988, Kimber and Yen 1989). No intraspecific variability was observed by molecular marker analysis (Talbert et al. 1993), indicating that the modifications may be caused by chromosomal rearrangements. The meiotic pairing data in *Ae. columnaris*/*Ae. umbellulata* hybrids also are conflicting (Kimber and Yen 1988, 1989). However, the large amount of variability observed using molecular markers indicates that the intraspecific heterogeneity in *Ae. columnaris* is caused by alien introgression rather than by chromosomal aberrations.

The presence of modified M genomes in *Ae. geniculata* and *Ae. biuncialis* was further confirmed by meiotic, cytogenetic, and molecular analyses (Kihara 1954, Kimber and Abu-Bakar 1981, Kimber et al. 1988, Talbert et al. 1993, Resta et al. 1996, Friebe et al. 1999), whereas the origin of the X genomes of *Ae. columnaris* and *Ae. neglecta* is still under discussion (Yen et al. 1992, Resta et al. 1996, Dvorák 1998). Kihara (1937, 1949) suggested that these species are related to *Ae. biuncialis*. Because 5 to 10 bivalents were observed in *Ae. biuncialis*/*Ae. columnaris* hybrids and more than 12 bivalents were observed in *Ae. columnaris*/*Ae. neglecta* hybrids, the genome formula UM was assigned to *Ae. columnaris* and *Ae. neglecta*. Chennaveeraiah (1960) observed karyotypic similarities in *Ae. columnaris* and *Ae. biuncialis*. However, evidence indicates that *Ae. columnaris* and *Ae. neglecta* are not related to the M-genome progenitor species *Ae. comosa* Sm. in Sibth. & Sm. ($2n = 2x = 14$, M) (Talbert et al. 1993, Resta et al. 1996, Badaeva 2002a).

Hexaploid *Ae. neglecta* originated from hybridization of tetraploid *Ae. neglecta* with *Ae. uniaristata* Vis. (2n = 2x = 14, N). Fourteen bivalents are formed at meiosis in 6x *Ae. neglecta*/4x *Ae. neglecta* hybrids, whereas 10 to 11 bivalents were observed in 6x *Ae. neglecta*/*Ae. ventricosa* Tausch. (2n = 4x = 28, D^vN^v) hybrids, indicating the presence of a common genome (Kihara 1937). The presence of an N genome in 6x *Ae. neglecta* was confirmed by karyotype analysis (Chennaveeraiah 1960). Meiotic pairing analysis did not provide evidence that the third genome in 6x *Ae. neglecta* is related to the N genome of *Ae. uniaristata* (Yen and Kimber 1992), suggesting that the N^n genome of 6x *Ae. neglecta* is significantly modified.

Aegilops geniculata. Ten accessions of *Ae. geniculata* (2n = 4x = 28, U^gM^g) were analyzed using C-banding and two accessions were analyzed by FISH. Chromosome morphology and C-banding patterns in most accessions were similar to those reported previously (Pathak 1940, Chennaveeraiah 1960, Friebe et al. 1999) allowing chromosome designations according to the standard nomenclature (Friebe et al. 1999). Comparing *Ae. geniculata* with its diploid ancestors *Ae. umbellulata* and *Ae. comosa* revealed differences in morphology and C-banding patterns of many chromosomes belonging both to the M^g and U^g genomes, indicating that both genomes are modified, which agrees with previous data (Kimber and Abu-Bakar 1981, Kimber et al. 1988, Kimber and Yen 1989, Yen and Kimber 1990, Talbert et al. 1993).

Ae. geniculata is characterized by significant C-banding and translocation polymorphism. As a rule, accessions from the same geographic area (i.e. TA1813, TA1702 and TA1816, Europe, Fig. 7a–c; TA1815 and k-2807, Israel, Fig. 7e, i; and TA1799, TA1701, TA1800, Turkey, Fig. 7f–h) are more similar than accessions from distant regions. Six lines had a normal karyotype, whereas various types of modified chromosomes were observed in four lines. Some modifications were the result of either Robertsonian translocations (**T2**: $T1U^gS{\cdot}2U^gS + T1U^gL{\cdot}2U^gL$, k-2807 in Fig. 7h) or translocations with interstitial breakpoints (**T1**: $T4U^gS{\cdot}4U^gL\text{-}6U^gL + T6U^gS{\cdot}6U^gL\text{-}4U^gL$, TA1701 in Fig. 7f). The origin of other modified chromosomes is unknown. Some chromosomes differed from the normal chromosome complement in morphology, whereas others had a normal morphology but differed in the C-banding pattern. These modified chromosomes may have occurred as a result of alien introgression followed by meiotic recombination.

Chromosome modifications were found mostly in Turkish accessions of *Ae. geniculata*. Previously, Furuta (1981b) also reported a high frequency of chromosomal rearrangements in accessions of *Ae. geniculata* from this geographic area. On the other hand, Yen and Kimber (1990) did not observe modifications of the U^g-genome chromosomes in five Turkish accessions of *Ae. geniculata*.

FISH with pSc119 revealed signals on 10 chromosome pairs and five chromosome pairs had pAs1 FISH sites. Large or medium-sized pSc119 FISH sites were observed in telomeric regions of either one or both chromosome arms (Fig. 3f). Interstitial pSc119 FISH sites were located in the long arms of $6U^g$ and $7U^g$, which were identified on the basis of their similarity with the corresponding chromosomes of *Ae. umbellulata* (Badaeva et al. 1996a, Castilho and Heslop-Harrison 1995). The pAs1 FISH sites were faint and located in interstitial chromosome regions and, in general, resembled the pAs1-labeling pattern of *Ae. comosa* chromosomes (Badaeva et al. 1996a, 1999).

Two pairs of SAT chromosomes were observed in *Ae. geniculata* confirming previous results (Pathak 1940, Chennaveeraiah 1960, Cermeño et al. 1984, Yamamoto 1992, Yamamoto and Mukai 1995). Multicolor FISH with the probes pTa71 and pTa794 detected two major and four minor 18S-26S rDNA and three 5S rDNA sites (Fig. 3b), whose location was different from those in the parental species (Fig. 4). Chromosome $1U^g$ had a major NOR but lacked the 5S rDNA site and $1M^g$ only had the 5S rDNA locus. Small 5S rDNA loci were

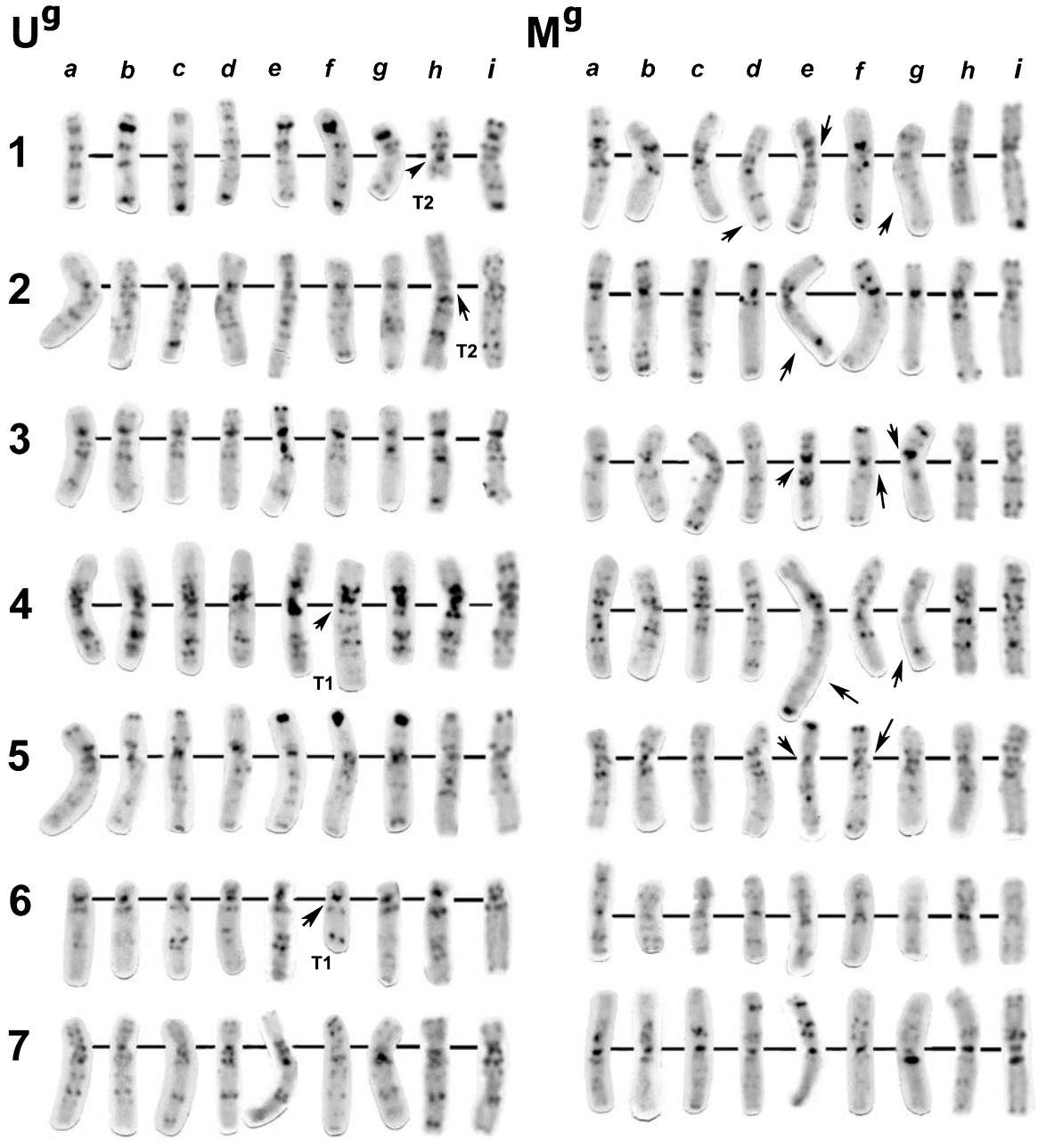

Fig. 7. C-banding polymorphism of *Ae. geniculata*: (**a**) TA1813, (**b**) TA1702, (**c**) TA1817, (**d**) TA1815, (**e**) TA1799, (**f**) TA1701, (**g**) TA1800, (**h**) k-2807 (**i**) k-571296. Modified chromosomes are indicated with arrows. **T1**: $T4U^gS{\cdot}4U^gL\text{-}6U^gL + T6U^gS{\cdot}6U^gL\text{-}4U^gL$; **T2**: $T1U^gS{\cdot}2U^gS + T1U^gL{\cdot}2U^gL$

detected in the short arms of all group-5 chromosomes, which also had either major ($5U^g$) or minor ($5M^g$) 18S-26S rDNA loci. Constant minor pTa71 FISH sites of various intensities were detected in telomeric regions of two large submetacentric chromosomes pairs, presumably $2M^g$ and $3M^g$, and in a small metacentric chromosome pair. The latter probably is the derivative of chromosome 6M of *Ae. comosa*, which lost the NOR during the speciation of *Ae. geniculata*. A weak hybridization signal was occasionally observed in an

interstitial regions of the long arm of chromosome $6U^g$, which also is present in chromosome 6U of *Ae. umbellulata* (Badaeva et al. 1996b).

Our data confirm that *Ae. geniculata* speciation was accompanied by modification of both parental genomes as a result of amplification, deletion, and re-distribution of various classes of repetitive DNA sequences and chromosomal rearrangements.

Aegilops biuncialis. We analyzed 27 accessions of *Ae. biuncialis* of diverse origins by C-banding, and three with different chromosomal types were chosen for FISH. All chromosomes had characteristic C-banding patterns that allowed their identification (Fig. 8). *Ae. biuncialis* chromosomes were classified on the basis of their similarity with chromosomes of the related tetraploid species *Ae. geniculata* and the putative ancestors *Ae. umbellulata* and *Ae. comosa*. Morphologies and C-banding patterns of chromosomes $1U^b$, $5U^b$, and $5M^b$ are similar to those of the corresponding chromosomes of the parental species. Thus, the remaining SAT chromosome was designated as $1M^b$ although its arm ratio is different compared to 1M of *Ae. comosa*, indicating that chromosome $1M^b$ is highly rearranged.

We observed karyotype variation in *Ae. biuncialis* and, thus, this species was subdivided into three major groups. The first group (chromosome type *N*) is the most widespread and includes accessions from all geographic regions except Cyprus (Fig. 8a–e, h, i). This chromosomal type is considered as typical. Examination of 22 lines of chromosomal type *N* revealed variation of C-banding patterns with accessions from the same area being more similar. Characteristic C-banding patterns of chromosomes $4U^b$, $2M^b$, $6M^b$, and $7M^b$ divided the accessions of chromosomal type *N* into two subgroups: European (Balkan countries, Southern Russia, and Ukraine) and Near East (Turkey, Israel, Jordan, Syria, and Azerbaijan). However, there were many intermediate forms between the two subgroups. The frequency of chromosomal aberrations was low. We identified a translocation between chromosomes $1U^b$ and $5U^b$ with interstitial breakpoints (**T1**: $T1U^bL{\cdot}1U^bS\text{-}5U^bS$ + $T5U\text{-}^{bL\cdot5Ub}S\text{-}1U^bS$) in two lines from Turkey (TA2079 and TA2080) (Fig. 8a), and an interstitial translocation between chromosomes $1U^b$ and $3U^b$ (**T2**: $T1U^bL{\cdot}1U^bS\text{-}3U^bS$ + $T3U^bL{\cdot}3U^bS\text{-}1U^bS$) in another Turkish accession (TA2078) (Fig. 8b).

Telomeric pSc119 FISH sites of various intensities and few interstitial sites were observed in 11 chromosome pairs with the labeling pattern similar to that of *Ae. geniculata*. However, differences between these species were observed after FISH with the probe pAs1 (Fig. 2e, f), i.e., a prominent pAs1 FISH site detected in the distal part of the long arms of chromosomes $1M^b$ and $2M^b$ and in the short arm of $1U^b$ present in *Ae. biuncialis* were absent in *Ae. geniculata*.

Two major NORs were detected in chromosome-type **N** using FISH with pTa71 and pTa794 (Fig. 3c), and they were located on chromosomes $1U^b$ and $5U^b$. Minor pTa71 sites were detected in telomeric regions of the short arms of $2M^b$ and $3M^b$, whereas $1M^b$ had two loci, one at the telomere of short arm and one in the distal third of the long arm. Chromosome $1M^b$ also had a 5S rDNA locus in the long arm close to the minor NOR. Two additional 5S rDNA loci, associated with major NORs, were located on $1U^b$ and $5U^b$, and another 5S rDNA locus was detected on chromosome $5M^b$. Thus, although *Ae. biuncialis* has two major and several minor NORs similar to *Ae. geniculata* their relative location is different (Fig. 4). These species also differ in the number and location of 5S rDNA sites.

The second and third group of *Ae. biuncialis*, designated as chromosome-type **I** and **II** differed from the normal and from each other in karyotype structure, the amount and distribution of C-bands, and pTa71 and pTa794 FISH patterns. Four accessions from Israel and Cyprus (TN03, TN08, PI483025, and i-571306), had chromosome-type **I**. These forms were relative uniform and differed in morphology and C-banding patterns of chromosomes $1U^b$,

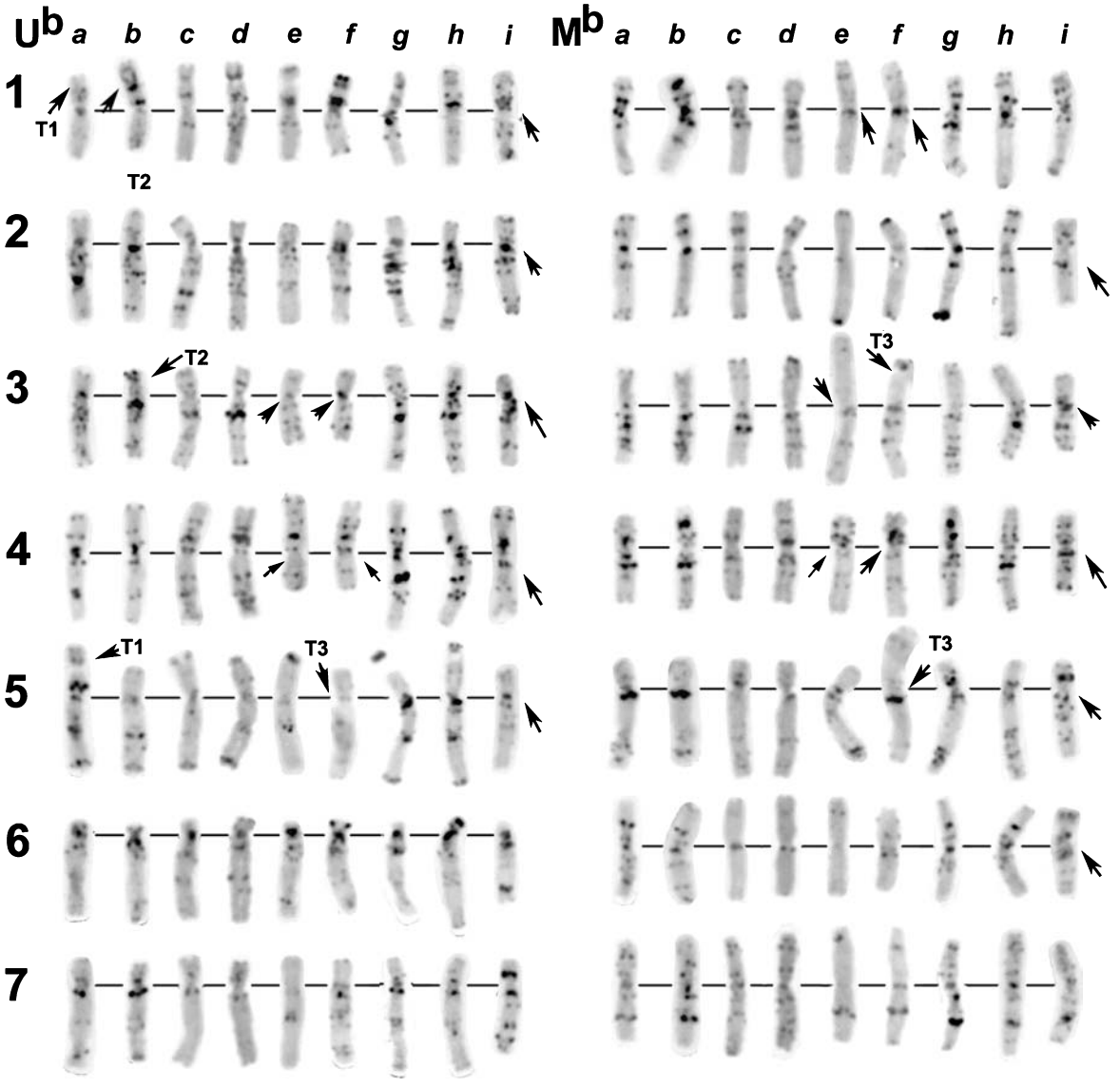

Fig. 8. C-banding polymorphism of *Ae. biuncialis*: (**a**) TA2079, (**b**) TA2078, (**c**) PI374367, (**d**) k-3093, (**e**) PI483025, (**f**) TN03, (**g**) TN07, (**h**) PI487282, (**i**) k-2682. Modified chromosomes are indicated with arrows. **T1**: T1U^{b}L·1U^{b}S-5U^{b}S + T5U^{b}L·5U^{b}S-1U^{b}S; **T2**: T1U^{b}L·1U^{b}S-3U^{b}S + T3U^{b}L·3U^{b}S-1U^{b}S; **T3**: cyclic translocation T5U^{b}S·3M^{b}L + T5U^{b}L·5M^{b}L + T3M^{b}S·5M^{b}S

3U^b, 4U^b, 1M^b, 4M^b, 5M^b, and 7M^b (Fig. 8e, f). In contrast to chromosomal type **N** these lines had three pairs of SAT chromosomes, two were identified as chromosomes 1U^b and 5U^b, whereas the identity of the third SAT chromosome remains unknown. Both Israeli accessions (TN03, TN08) had cyclic Robertsonian translocations between chromosomes 5U^b, 3M^b, and 5M^b (**T3**: T5U^{b}S·3M^{b}L + T5U^{b}L·5M^{b}L + T3M^{b}S·5M^{b}S) (Fig. 8f). As the result of this translocation a segment of the short arm of chromosome 5U^b including the satellite was transferred to the short arm of chromosome 3M^b.

Chromosome-type I is similar to the normal type in pSc119 and pAs1 FISH patterns, however, they differ in the location of pTa71 and pTa794 FISH sites (Fig. 3j). Three major

pTa71 FISH sites were observed on chromosomes $1U^b$, $5U^b$, and a pair of modified chromosomes of unknown origin and three major pTa794 FISH sites are present on chromosome arms $1M^bL$, $5U^bS$, and $5M^bS$.

One accession from Lebanon (k-2682) was placed into a separate chromosome type **II** (Fig. 8i, Fig. 3f). This line differs significantly from other accessions in C-banding patterns of most chromosomes. Two major NORs located on chromosomes $1U^b$ and $5U^b$ were associated with 5S rDNA, loci and small pTa71 and pTa794 FISH sites were detected in the short arm of $1M^b$. Minor pTa71 and pTa794 FISH sites were observed in the short arm of chromosomes $5M^b$ and additional minor 18S-26S rDNA loci were detected in a telomeric region of $2M^bS$ and in the middle of the arm of an unidentified large metacentric chromosome. Thus, the number and distribution of 18S-26S and 5S rDNA loci in accession k-2682 differed significantly from those of the other lines.

C-banding and FISH analyses revealed significant intraspecific differentiation in *Ae. biuncialis*. The relationships between the different chromosome types are unknown. Similarity in the FISH patterns of the normal and chromosome-type **I** support a common origin. However, chromosome type **II** probably is derived either from a separate hybridization event or could be of hybrid origin. Distinct differences in the karyotype structure suggest that the divergence of chromosome-types **N** and **I** was accompanied by large chromosomal rearrangements and, probably, also by introgression of genetic material from other species.

Ae. columnaris. Although some chromosomes of *Ae. columnaris* resembled those of *Ae. geniculata* and *Ae. biuncialis*, these species differed significantly in their karyotype structure, the amount and distribution of C-bands, and hybridization patterns of 5S and 18S-26S rDNA probes and highly repetitive DNA sequences. The genetic nomenclature of *Ae. columnaris* chromosomes have not been established, and we tentatively assigned chromosomes to homoeologous groups and genomes on the basis of their similarity with chromosomes of other *Aegilops* species (Fig. 9). However, this classification should be considered preliminary.

Examination of 23 accessions of *Ae. columnaris* revealed broad intraspecific polymorphism. On the basis of karyotype structure, this species can be subdivided into two major chromosomal types, **A** and **B**. Eighteen accessions were assigned to chromosomal type **A** (Fig. 9a–g). They showed significant polymorphism due to variability in both C-banding patterns and chromosomal rearrangements. Chromosomal aberrations represented by single or complex translocations with centromeric and non-centromeric breakpoints and paracentric inversions were identified in 10 of 18 accessions of chromosomal type **A**.

A paracentric inversion in chromosome $7U^c$ (**inv1**) was the most common type of chromosomal aberrations found in six accessions from various locations in Turkey and Azerbaijan (Fig. 9e). A Robertsonian translocation between chromosomes $1U^c$ and $5U^c$ (**T1**: $T1U^cS{\cdot}5U^cS + T1U^cL{\cdot}5U^cL$) with inactivation of the NOR on the $5U^cS$ arm was identified in two accessions (k-1512 and k-1193) from Armenia (Fig. 9a). A second Robertsonian translocation between chromosomes $1X^c$ and $3U^c$ (**T2**: $T3U^cS{\cdot}1X^cS + T3U^cL{\cdot}1X^cL$) associated with the loss of nucleolar activity of the $1X^cS$ arm was observed in the Turkish accession PI560506 (Fig. 9c). Both **T1** and **T2** translocations were identified in another Turkish accession (PI554180, Fig. 9b). A translocation with interstitial breakpoints between chromosomes $4U^c$ and $7U^c$ (**T3**: $T7U^cS{\cdot}7U^cL\text{-}4U^cL + T4U^cS{\cdot}4U^cL\text{-}7U^cL$) was identified in two accessions (i-571172 and i-571163) from Lebanon (Fig. 9f).

FISH with probes pSc119 and pAs1 detected polymorphic hybridization patterns in *Ae. columnaris* (Fig. 2c). Large to medium pSc119 sites were observed in telomeric regions of one or both arms of 11 of 14 chromosome pairs, and interstitial sites were observed in long arms of chromosomes $6U^c$ and $7U^c$. FISH with pAs1 revealed signals of different intensities on five pairs of chromosomes.

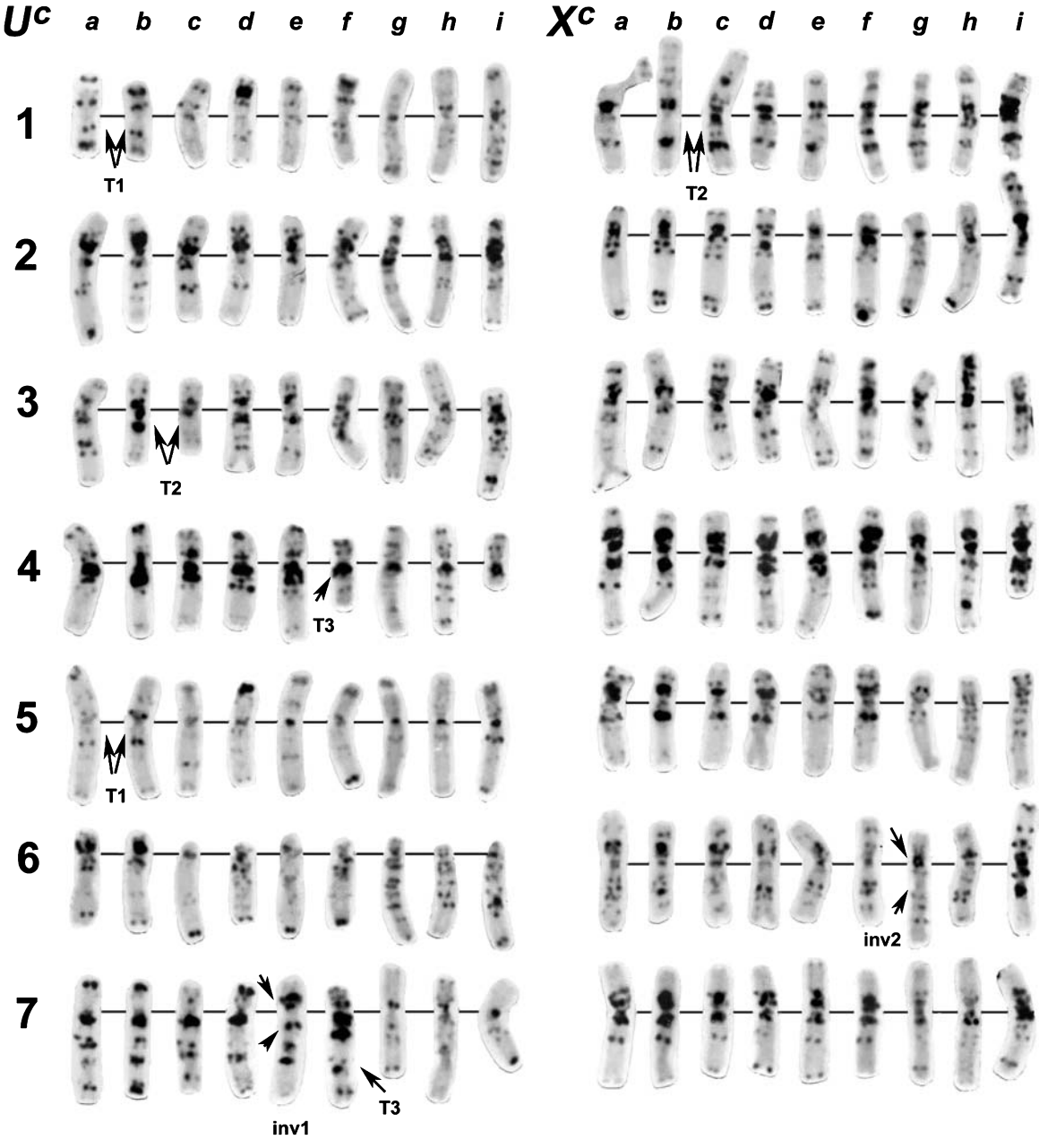

Fig. 9. C-banding polymorphism of *Ae. columnaris* chromosomes: (**a**) k-1193, (**b**) PI554180, (**c**) PI560506, (**d**) G-1, (**e**) TA2106, (**f**) i-571172, (**g**) i-577972, (**h**) PI564180, (**i**) PI564181. **T1**: T1U^{c}S·5U^{c}S + 1U^{c}L·5U^{c}L; **T2**: T3U^{c}S·1X^{c}S + T3U^{c}L·1X^{c}L; **T3**: T7U^{c}S·7U^{c}L-4U^{c}L + T4U^{c}S·4U^{c}L-7U^{c}L; **inv1**: paracentric inversion of chromosome 7U^c; **inv2**: paracentric inversion of chromosome 6X^c

A large polymorphic site present in the long arm of chromosome 7U^c, proximal and interstitial sites in the long arm of chromosome 6U^c, and a small pAs1 FISH site in the satellite of chromosome 1U^c were also observed in the corresponding chromosomes of *Ae. peregrina*, *Ae. kotschyi*, *Ae. geniculata*, and *Ae. biuncialis*.

Ae. columnaris is characterized by a unique distribution of two ribosomal probes (Fig. 3i, Fig. 4). Two major NOR were detected on

chromosomes $1U^c$ and $5U^c$. These chromosomes also had 5S rDNA sites distal and proximal to major NORs. The third major 18S-26S rDNA locus was observed in the short arm of the chromosome $1X^c$, which also had two 5S rDNA loci; one in the distal part of satellite and another in the short arm proximal to the NOR. Thus, *Ae. columnaris* (chromosomal type **A**) has three major NORs and four 5S rDNA loci, two of which are located in one chromosome arm. No minor NOR were observed. Such a distribution of 18S-26S and 5S rDNA loci has no analogs in diploid (Badaeva et al. 1996b) or polyploid *Aegilops*/*Triticum* species except *Ae. neglecta* as will be discussed below.

Four Turkish accessions of *Ae. columnaris* belong to chromosome-type **B** (Fig. 9h, i) and one accession from Syria (i-577972, Fig. 9g) occupied an intermediate position between chromosome-types **A** and **B**. A paracentric inversion was detected in chromosome $6X^c$ in the accession i-577972 (Fig. 9g). The structure and C-banding patterns of most chromosomes of chromosome-type **B** were different from those of chromosome-type **A**. Only chromosomes $5U^c$, $6U^c$, $1X^c$, $6X^c$, and $7X^x$ had similar morphology and/or C-banding patterns. Chromosome-type **B** consists of two groups. The first includes two accessions from southern Turkey (PI564181 from Icel and TA2084 from Iskenderum), and the second group consists of two accessions from two distant sites in Turkey (Izmir and Kars). A large amount of chromosome modification in these forms suggests that their divergence may have been associated with chromosomal rearrangements and alien introgressions.

4x Ae. neglecta (2n = 4x = 28, U^nX^n) and 6x Ae. neglecta (2n = 6x = 42, $U^nX^nN^n$). All chromosomes of these species can be identified on the basis of morphology and C-banding patterns. The U^n- and X^n-genome chromosomes of 4x *Ae. neglecta* and 6x *Ae. neglecta* are nearly identical (Fig. 10, Fig. 11). They also were highly similar to those of the chromosome-type **A** of *Ae. columnaris*. For this reason, we classified the 4x *Ae. neglecta* chromosomes according to the nomenclature used for *Ae. columnaris* (Fig. 9). However, irrespective of the high similarities between these species, they can be easily discriminated by the morphology and C-banding patterns of three X-genome chromosomes (2X, 3X, and 5X) (Figs. 9, 10). The N^n genome of 6x *Ae. neglecta* is similar to that of *Ae. uniaristata*, and, thus, they were classified according to the standard genetic nomenclature developed for this species (Friebe et al. 1996b) with further modifications (Iqbal et al. 2000).

Comparing 10 accessions of 4x *Ae. neglecta* and 11 accessions of 6x *Ae. neglecta* from diverse geographical regions revealed comparatively low variation in C-banding patterns and translocation polymorphism (Figs. 10, 11). Three types of Robertsonian translocations were found in 4x *Ae. neglecta*. The first translocation involving chromosomes $6U^n$ and $7X^n$ (**T1**: $T6U^nS{\cdot}7X^nL + T6U^nL{\cdot}7X^nS$) was identified in accession TA2157 (Fig. 10e). The second translocation (k-3475) involved chromosomes $1U^n$ and $2X^n$ ($T1U^nS{\cdot}2X^nL + T1U^nL{\cdot}2X^nS$) (Fig. 10d). The third translocation present in the accession k-3529 involved chromosomes $5U^n$ and $4X^n$ ($T5U^nS{\cdot}4X^nS + T5U^nL{\cdot}4X^nL$) (Fig. 10h).

Translocations were rather common in 6x *Ae. neglecta*. Four accessions from Spain, Portugal, and Morocco (k-2062, k-2063, k-2711, and i-571245) had a Robertsonian translocation between chromosomes $6X^n$ and $3N^n$ (**T4**: $T6X^nS{\cdot}3N^nS + T6X^nL{\cdot}3N^nL$) (Fig. 11a, b). Another Robertsonian translocation (**T5**: $T2X^nS{\cdot}3N^nL + T2X^nL{\cdot}3N^nS$) was identified in accession k-1654 from Bulgaria (Fig. 11d).

FISH with probe pSc119 detected signals of various sizes in telomeric regions of either one or both arms of 12 chromosome pairs of 4x *Ae. neglecta*, whereas an interstitial pSc119 FISH site was detected only in the long arm of chromosome $7U^n$ (Fig. 2g). Two distinct pAs1 FISH sites were observed in proximal regions of chromosomes $6U^n$ and $4X^n$. The pSc119 and pAs1 FISH patterns on U^n- and X^n-genome chromosomes of 6x *Ae. neglecta* were

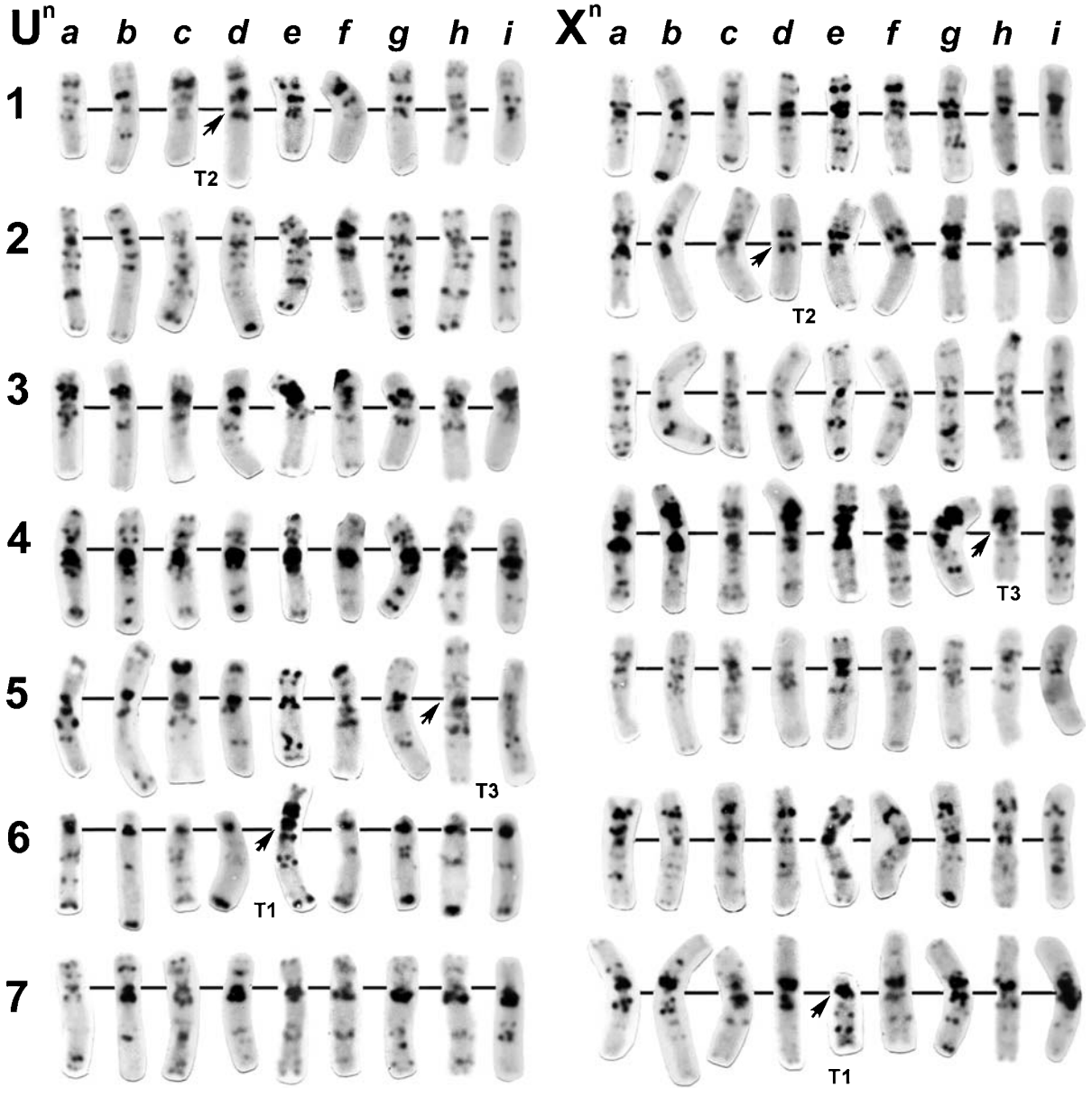

Fig. 10. C-banding polymorphism of 4x *Ae. neglecta*: (**a**) k-2711, (**b**) i-570283, (**c**) G- (original accession from Armenia), (**d**) k-3475, (**e**) TA2157, (**f**) TA2156, (**g**) TA2162, (**h**) k-3529, (**i**) k-1932. **T1**: $T6U^nS{\cdot}7X^nL$ + $T6U^nL{\cdot}7X^nS$; **T2**: $T1U^nS{\cdot}2X^nL$ + $T1U^nL{\cdot}2X^nS$; **T3**: $T5U^nS{\cdot}4X^nS$ + $T5U^nL{\cdot}4X^nL$

nearly identical to those of 4x *Ae. neglecta* (Fig. 2b). No differences in the labeling pattern of the N-genome chromosomes were observed between 6x *Ae. neglecta*, *Ae. ventricosa*, and *Ae. uniaristata* (Badaeva et al. 1996a, Bardsley et al. 1999, Iqbal et al. 2000). Therefore, the N^n genome of 6x *Ae. neglecta* does not appear to be structurally modified as was suggested by Yen and Kimber (1992).

Previously, one pair of SAT chromosomes was reported in 4x *Ae. neglecta* and two were observed in 6x *Ae. neglecta* (Senyaninova-Korchagina 1932, Chennaveeraiah 1960). We observed three pairs of SAT chromosomes in

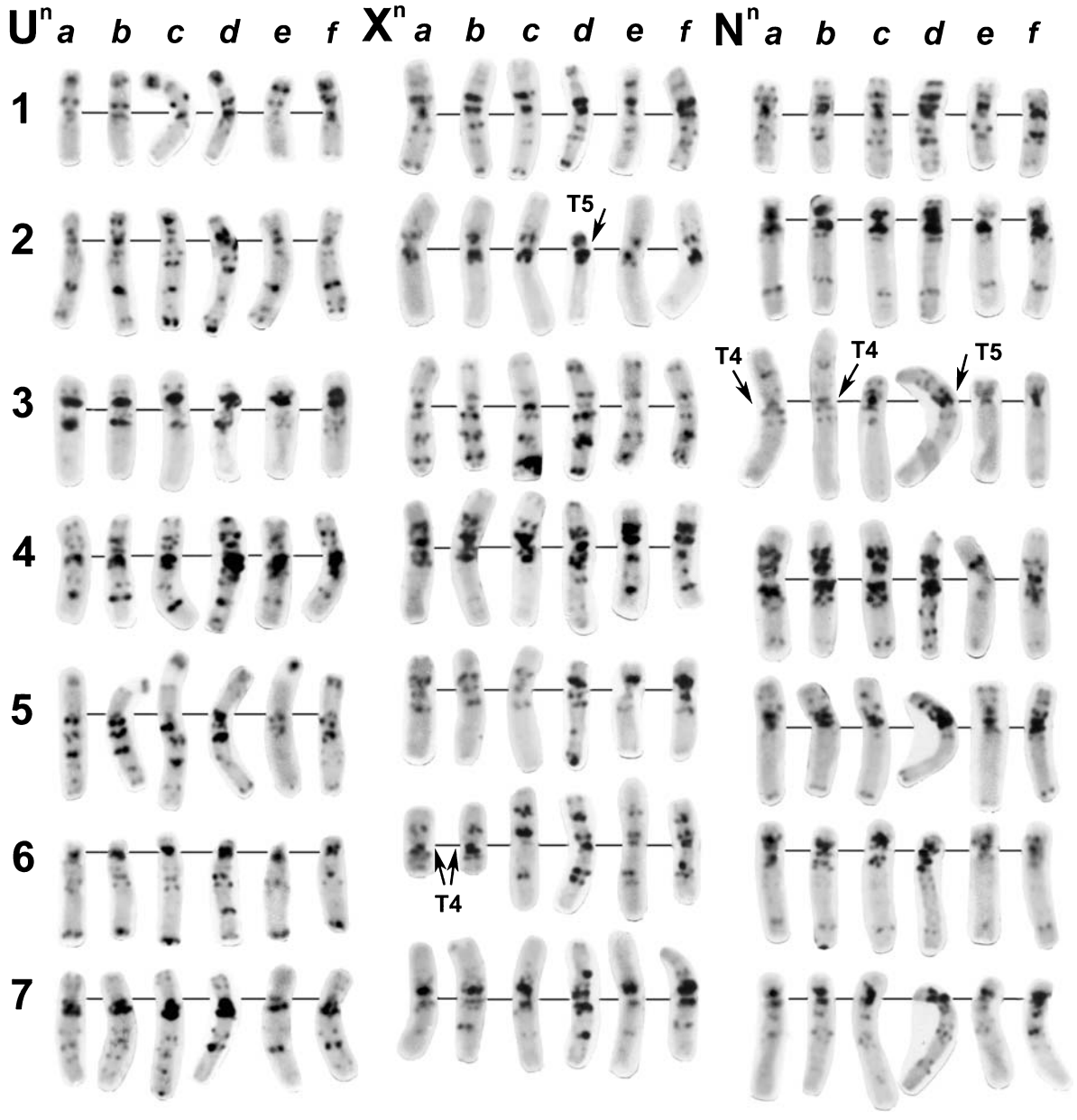

Fig. 11. C-banding polymorphism of 6x *Ae. neglecta*: (**a**) i-571245, (**b**) k-2712, (**c**) k-3462, (**d**) k-1654, (**e**) k-1469, (**f**) k-1638. **T4**: $T6X^nS{\cdot}3N^nS + T6XS^nL{\cdot}3N^nL$; **T5**: $T2X^nS{\cdot}3N^nL + T2X^nL{\cdot}3N^nS$

both species, two on U^n- and one on X^n-genome chromosomes, which is in agreement with Ag-NOR banding (Cermeño et al. 1984) and in situ hybridization with DNA probe pTa71 (Yamamoto 1992). Active NORs probably are located on chromosomes $1U^n$, $5U^n$, and $1X^n$. The pTa71 and pTa794 FISH patterns of 4x *Ae. neglecta* overall are similar to those of *Ae. columnaris* (chromosome-type **A**) (Fig. 3g) with 18S-26S rDNA and 5S rDNA loci on chromosomes $1U^n$, $5U^n$, and $1X^n$. However, 4x *Ae. neglecta* had an additional minor 18S-26S rDNA locus in the distal region of the long arm of chromosome $7X^n$ and an additional 5S rDNA locus in the short arm of chromosome $5X^n$ (Fig. 3g) that are not present in *Ae. columnaris* (Fig. 4).

Four major ($1U^n$, $5U^n$, $1X^n$, $5N^n$) and two minor ($7X^n$ and $1N^n$) NORs and seven 5S rDNA loci on all group-1 and group-5 chromosomes were detected in 6x *Ae. neglecta* (in one plant of accession k-2062 the major NOR locus on chromosome $5N^n$ is deleted (Fig. 3h). Thus, the number of major NORs and 5S rDNA loci in 6x *Ae. neglecta* is similar to the total number of these loci in the parental species. However, the number of minor 18S-26S rDNA sites was smaller than expected. Nine minor NORs were detected in *Ae. uniaristata* (Badaeva et al. 1996a, Iqbal et al. 2000), but only one of them, located in the $1N^nL$ arms is present in 6x *Ae. neglecta*. The 18S-26S rRNA locus on chromosome $7X^n$ of 6x *Ae. neglecta* originated from 4x *Ae. neglecta*.

Discussion

Examination of seven polyploid *Aegilops* species of the Section *Pleionathera* using C-banding and FISH analyses with four DNA probes confirmed that they all shared a U genome similar to the U genome of the diploid species *Ae. umbellulata*. However, some changes in the corresponding chromosomes in the polyploids were observed as is discussed below.

Chromosome 1U is one of the two SAT chromosomes of diploid *Ae. umbellulata* and is one of the most conserved chromosomes among the different polyploid species with only small variations in C-banding and FISH patterns. The loss of the 5S rDNA locus on 1U observed in *Ae. geniculata* is probably caused by a species-specific translocation.

Chromosome 6U of *Ae. umbellulata* is an acrocentric chromosome and can be identified unambiguously. Chromosome 6U of *Ae. umbellulata* has large telomeric and interstitial pSc119 FISH sites in the long arm and small pAs1 FISH sites in pericentric regions of both arms. Polymorphic minor NOR loci were detected in the middle and pericentric regions of the long arm (Badaeva et al. 1996a, Castilho and Heslop-Harrison 1995). The FISH patterns of the corresponding chromosomes in the polyploid species were diverse. In *Ae. peregrina*, $6U^p$ lacked a pSc119 site but a telomeric pAs1 FISH site was observed in the long arm. *Ae. kotschyi* $6U^k$ had a pericentric and an interstitial pAs1 FISH site. Similar pericentric and interstitial pAs1 sites of different intensities are present in chromosomes $6U^b$, $6U^g$, and $6U^c$.

Chromosome 7U of *Ae. umbellulata* has only pSc119-specific FISH sites: two in telomeric regions of both arms and two located interstitially in the long arm. No hybridization with clone pAs1 was detected (Castilho and Heslop-Harrison 1995, Badaeva et al. 1996a). Chromosome 7U of polyploid *Aegilops* species always had pSc119 sites at the telomere of the short arm and in the middle of long arm; subtelomeric and/or telomeric pSc119 sites were polymorphic. In addition, some species had distinct pAs1 hybridization sites. Large and small FISH sites were detected in the middle of the long arm of chromosomes $7U^c$ and $7U^g$, respectively.

The similar location of new pAs1 hybridization sites in chromosomes 6U and 7U of different polyploid species suggests that they probably originated by the amplification of a similar preexisting sequence in 6U and 7U of *Ae. umbellulata* although in lower copy number. Liu et al. (1998a, b) showed that rapid changes in copy number of certain DNA sequences occur in newly synthesized amphiploids of the *Triticum*/*Aegilops* complex.

Our study confirmed the origin of the second genomes in most polyploids of the Section *Pleionathera*. However, we also detected certain level of modification of the second genome in some species. The S^p genome of *Ae. peregrina* is thought to be considerably modified (Kihara 1940, 1949, 1954; Zohary and Feldman 1962; Chennaveeraiah 1960; Kimber and Yen 1989). However, C-banding and FISH analyses strongly suggest that the S^p genome was derived from *Ae. longissima*, and that it is not structurally altered relative to the parental species, in agreement with data of Zhang et al. (1992) and Friebe et al. (1996a). C-banding analysis showed that the S^k genome of *Ae. kotschyi* was

derived from either the S^{sh} genome of *Ae. sharonensis* or its immediate precursor. The high frequency of chromosomal aberrations and reduction in the number and size of 18S-26S rDNA loci observed in the S^k genome compared to the S^{sh} and S^p genomes suggests that *Ae. kotschyi* is an older species than *Ae. peregrina* (Fig. 4).

The present study confirms that the second genomes of *Ae. geniculata* and *Ae. biuncialis* are derivatives of the M genome of *Ae. comosa*. The M genomes in both species are modified as a result of amplification, elimination, and redistribution of highly repetitive DNA sequences and chromosomal rearrangements. Significant intraspecific heterogeneity was observed in both species, indicating that these types either were derived from independent hybridization events or their divergence was associated with major chromosomal rearrangements and introgressive hybridization. These results may indicate that both of these species are still undergoing an intensive speciation process.

The origin of second genomes of *Ae. columnaris* and 4x *Ae. neglecta* remains unknown. Both species are closely related, as indicated by similarities in C-banding and hybridization patterns with pSc119 and pAs1 probes and the distribution of 18S-26S and 5S rDNA loci. However, the X genomes of 4x *Ae. neglecta* and *Ae. columnaris* (chromosome-type **A**) differ in morphology and C-banding patterns, the number and location of 5S rDNA loci, and the number of minor NORs. Two hypotheses may explain the origin of these species: (1) they occurred as a result of hybridization of *Ae. umbellulata* with a species not belonging to the *Triticum*/*Aegilops* complex (Chennaveeraiah 1960, Resta et al. 1996), or (2) that a species of the Section Comopyron was the donor of the second genome of *Ae. neglecta* and *Ae. columnaris*, which was significantly rearranged during speciation (Kihara 1937, 1963). Our data do not support a close relationship of the X and M genome, because they differ significantly in chromosome morphology, the amount and distribution of C-heterochromatin, the hybridization patterns of probes pSc119 and pAs1, the number and location of 5S rDNA loci, and the number and location of major and minor NORs. However, neither is there support in favor of the first hypothesis, because no species with a genome similar to the X genome has been discovered among the *Aegilops*, *Triticum*, *Agropyron*, *Hordeum*, and *Avena* species studied to date. Introgressive hybridization is considered to be one of the possible mechanisms of speciation in the Triticeae (Zohary and Feldman 1962). Assuming that 4x *Ae. neglecta* and *Ae. columnaris* arose via such a mechanism, their X genomes may be of hybrid origin.

Previously, we have shown that formation of polyploid species of the D-genome cluster always led to inactivation or even deletion of NORs of the D-genome chromosome (Badaeva et al. 2002b). In contrast, in polyploid species of the U-genome cluster, generally the NOR on chromosomes 1U and 5U remain active, while those of the differential genomes are suppressed.

The pSc119 and pAs1 FISH patterns on chromosomes of polyploid species of the D-genome cluster were highly conserved (Badaeva et al. 1998, 2002b). The distribution of two ribosomal RNA gene families in all species of this group was not changed compared to those of the parental species except for the loss of the major NOR on chromosome 5D in *Ae. ventricosa* and in 4x and 6x Ae. crassa Boiss. (Badaeva et al. 1998, 2002b; Bardsley et al. 1999). The present study detected polymorphism in pSc119 and pAs1 hybridization patterns in several accessions of *Ae. columnaris* and *Ae. biuncialis*. Significant variation in the distribution of 18S-26S and 5S rDNA loci was observed in *Ae. geniculata*, *Ae. biuncialis*, *Ae. columnaris*, and 4x *Ae. neglecta*, which suggests that the formation of U-genome polyploid species was associated with major chromosomal rearrangements and probably introgressive hybridization.

C-banding analysis of polyploid species of the D-genome cluster revealed a comparatively

low level of chromosomal aberrations (Linc et al. 1999; Badaeva et al. 1998, 2002b). Chromosomal rearrangements were represented by single or double translocations with predominantly centromeric breakpoints. The number of translocations in these species included 1 to 3 different types and their frequencies never exceeded 50%. The U-genome polyploids displayed a wide spectrum of chromosomal aberrations including single and complex translocations with centromeric and interstitial breakpoints and paracentric inversions. The frequencies of these chromosomal rearrangements varied significantly between species from 20% in *Ae. triuncialis* to more than 60% in *Ae. columnaris*. Many species of this group have significantly modified chromosomes whose origin are unknown and probably were derived from alien introgression with subsequent chromosome recombination.

The above results suggest that the species of the U-genome cluster generally are characterized by a comparatively high genome instability, which probably was induced by their pivotal genomes. An "outburst" of genetic variability in response to hybridization, termed hybrid disgenesis has been discovered in *Drosophila* (Pyatkov et al. 2002) and in plants (McClintock 1984). Molecular-genetic analysis revealed that this phenomenon is caused by mobilization of certain types of transposable elements (Lonnig and Saedler 1997, Kidwell and Lisch 2000, Pyatkov et al. 2002). Therefore, we can speculate that the genome instability of polyploid *Aegilops* species of the U-genome cluster may be caused by a similar mechanisms and that the U-genome progenitor species *Ae. umbellulata* may have certain classes of genetic elements that are capable of inducing chromosomal rearrangements hybridization in other genomes.

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