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On the diploidization mechanism of the genus *Aegilops*: meiotic behaviour of interspecific hybrids

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Abstract Chromosomal pairing of the three diploid hybrids *Aegilops uniaristata* × *Ae. tauschii* (ND), *Ae. umbellulata* × *Ae. tauschii* (UD) and *Ae. comosa* × *Ae. uniaristata* (MN), and a triploid hybrid *Ae. cylindrica* × *Ae. caudata* (DCC), was analyzed by electron microscopy in surface-spread-prophase-I nuclei and compared with light-microscopic observations of metaphase-I cells after C-banding and fluorescence in situ hybridization. All hybrids showed extensive synapsis and complex multivalents in which up to 14 chromosomes were involved. In the diploid hybrids most metaphase-I chromosomal associations were between homoeologs, their frequencies being dependent on the relationship between the donor genomes. Despite the different overall bound-arm frequencies displayed by ND and MN hybrids at metaphase-I, chromosomes bearing rDNA sequences showed similar mean cell chromosomal association frequencies. In the triploid hybrid preferential associations involving C genomes were predominant. These observations are discussed in relation to the mechanism of diploidization showed by allotetraploid *Aegilops* species.

Key words *Aegilops* · Diploidization · Interspecific hybrid · Meiosis · Synaptonemal complex

Introduction

Allopolyploids usually arise by chromosome doubling following hybridization; therefore, they contain several structurally and genetically similar chromosome sets with the capacity for pairing and crossing-over. Despite this, they behave as diploids, owing to the almost exclusive presence of homologous bivalents at first metaphase. Synaptonemal-complex (SC) analysis has revealed that bivalent formation in allopolyploids is achieved by two major strategies. (1) The existence of conspicuous multivalent synapsis at zygotene that may be corrected by pachytene, and the suppression of crossing-over between synapsed segments of homoeologous chromosomes as occurs in *Triticum aestivum* (Holm 1986; Holm and Wang 1988), *Lotus corniculatus* (Davies et al. 1990) and *Triticum timopheevii* (Martínez et al. 1996). (2) The restriction of synapsis mostly to homologous chromosomes at zygotene, and the suppression of crossing-over at pachytene as occurs in *Festuca arundinaceae* and *Festuca gigantea* (Thomas and Thomas 1993) as well as in allotetraploid *Aegilops* species, wild wheats, (Cuñado et al. 1996 a, b, c).

Some studies on *Aegilops*-wheat hybrids have indicated the existence of diploidizing genetic systems in polyploid *Aegilops*, though a meiotic regulator comparable to the wheat *Ph* gene has not been found (Riley 1966; Sears 1976; AbuBakar and Kimber 1982; McGuire and Dvorák 1982). This system would not be equally effective in all species (Cuñado 1992 a). On the other hand, it has also been pointed out that the virtual absence of homoeologous associations at metaphase-I could be due to structural and/or molecular genome modifications that have occurred in the evolution of diploid and tetraploid species (Dvorák and McGuire 1981).

In the present paper we analyze the meiotic behaviour of interspecific *Aegilops* hybrids, by means of a surface-spreading technique for making whole-mount preparations of SCs and by C-banding and fluorescence in situ hybridization (FISH), in order to gain new insights on the diploidizing mechanism displayed by polyploid *Aegilops* (*Ae*) species. We have studied three diploid hybrids, *Ae. uniaristata* × *Ae. tauschii* (ND), *Ae. umbellulata* × *Ae. tauschii* (UD) and *Ae. comosa* × *Ae. uniaristata* (MN), and a triploid hybrid *Ae. cylindrica* × *Ae. caudata* (DCC). *Ae. uniaristata* (NN) and *Ae. tauschii* (DD) are the diploid donors of the tetraploid *Ae. ventricosa* (DDNN).The N and D genomes have not undergone significant modifications during the evolution of the poly-

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ploid and diploid species and their relationship seems to be remote (Lucas and Jahier 1988; Badaeva et al. 1996 a, b). On the other hand, the M and N genomes are very close related, whereas the relationship between the U and D genomes can be considered as an intermediate situation between those described above (Lucas and Jahier 1988; Cuñado 1992 b). The triploid hybrid represents a competitive pairing situation in which two genomes come from an established allotetraploid.

Materials and methods

The hybrids *Ae. uniaristata* (2n = 14, NN, accession J19-1) × *Ae. tauschii* (2n = 14, DD, J20-1), *Ae. umbellulata* (2n = 14, UU, J8-5) x *Ae. tauschii*, *Ae. comosa* (2n = 14, MM, J17-1) x *Ae. uniaristata*, two plants of each, and *Ae. cylindrica* (2n = 28, DDCC, J4653) x *Ae. caudata* (2n = 14, CC, J6-2), three plants, were analyzed in this study. The source of the parental species was the Plant Germ-plasm Institute (Faculty of Agriculture, Kyoto University, Japan).

All of plants were grown in a conditioned greenhouse under identical conditions with a 16-h light: 8-h dark cycle. One of the three anthers in the florets of the emerging spikes was squashed in 2% acetic orcein to locate the stages of meiosis. The two remaining anthers at zygotene or pachytene were then prepared for synaptonemal-complex isolation and silver stained as described by Cuñado et al. (1996 a, b,c).

For metaphase-I observations, the anthers were fixed in 1:3 acetic acid:ethanol and stored at 4ºC until required. C-banding was performed according to the method of Giráldez et al. (1979). A rDNA probe, pTa71 from wheat, containing the 18S, 5.8S, and 25S genes and the intergenic spacer (Gerlach and Bedbrook 1979), kindly provided by A. Cuadrado, was used. It was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by nick translation according to the manufacturer's instructions. Fluorescence in situ hybridization (FISH) was as indicated by Cuñado and Santos (1998) with minor modifications. Chromosome preparations were pre-treated with DNase-free RNase (100 µg/ml) and pepsin (500 µg/ml), dehydrated in an ethanol series, and air-dried. The hybridization mixture, consisting of 50 ng of DNA probe and 500 ng of sheared salmon sperm DNA in 50% (v/v) formamide, 10% (w/v) dextran sulphate and 2 x SSC, was denatured on a heating block at 90ºC for 10 min and placed on ice for 5 min. The probe was applied to the slides (15 µl/slide), covered with a coverslip and sealed. The slides were incubated at 75ºC for 4 min and then at 37ºC overnight in a modified thermocycler. Labelled probe was detected with 5 µg/ml of monoclonal antidigoxigenin (Sigma) and 10 µg/ml of anti-mouse Ig fluorescein (Boehringer Mannheim). Preparations were counterstained with propidium iodide (1 µg/ml) and mounted with Vectashield (Vector Laboratories).

Results

Prophase-I observations

Prophase-I nuclei from four interspecific *Aegilops* hybrids with genome constitutions ND, UD, MN, and DCC were examined in the electron microscope. Data from plants of the same type of hybrid were pooled because they showed a similar SC behaviour. Table 1 summarizes the general features of SC formation in the fully traced nuclei analyzed; namely, the total axial element length, the amount of synapsis, and the number of bivalent and multivalent configurations. In the diploid hybrids the total axial element length ranged from 797 to 1211 µm (ND), from 783 to 1203 µm (UD) and from 737 to 1301 µm (MN), while the amount of synapsis ranged from 42.3% to 100% (ND), from 43.6% to 99.1% (UD) and from 28.7% to 98.3% (MN). One of these nuclei, MN10, is shown in Fig. 1. In all cases there are significant negative correlations between the axial-element length of each nucleus and the percentage of synapsis transformed to angles ($r = -0.68$, $df = 10$, ND; $r = -0.76$, $df = 8$, UD; $r = -0.67$, $df = 13$, MN). In the triploid hybrid (DCC) the total axial-element length ranged from 1144 to 1734 µm and the amount of synapsis ranged from 67.1% to 93.1%. One of these nuclei, DCC17, is shown in Fig. 2. A significant negative correlation between axial-element length and the percentage synapsis was also observed ($r = -0.52$, $df = 16$). Although the results obtained are consistent with the view that the axial-element length decreases progressively through prophase-I as the extent of synapsis increases, no attempt to substage this period was made owing to the controversy in the interpretation of correlations of this type in hybrids (Albini and Jones 1990). Furthermore, in *Aegilops* there are no independent indicators of the prophase-I substage, such as centromeric or nucleolar morphology. Nevertheless, the presence of foldback loops and complex multivalents in the nuclei with less synapsis suggest that non-homoeologous synapsis occurs at an early stage of synapsis development in these *Aegilops* hybrids.

The widespread occurrence of multivalents, and the impossibility of the undoubted identification of homoeologous pairing situations, have focused our attention on bivalent formation. Synapsis starts at terminal or subterminal regions but interstitial SCs also occur. SC foldbacks at different positions were also observed. Within individual bivalents, extensive regions of SC formation often existed at the same time as other extensive regions that were unsynapsed. This type of bivalent inequality between the axial elements were only observed in ND and UD hybrids. However, when full synapsis is achieved the bivalents resembled those that appear in normal diploid situations; thus, some kind of adjustment of axial/lateral element lengths must take place. The bivalent-means per nucleus in the different hybrids were, according to their genome constitution, rather similar: 2.92 (ND), 2.8 (UD), 3.07 (MN) and 4.28 (DCC).

Metaphase-I observations

Although in *Aegilops* species C-heterochromatin usually appears in centromeric, pericentromeric and interstitial regions, the distribution (presence or absence) and size of C-bands (Teoh and Hutchinson 1983; Cuñado et al. 1986; Cuñado 1992 a), as well as the size and morphology of the chromosomes (Chennaveeraiah 1960), allowed us to identify some genomes in interspecific hybrids and, consequently, to analyze the different types of associations between them at metaphase-I (Table 2). In the hybrids *Ae. uniaristata* x *Ae. tauschii* (ND) and *Ae. comosa*

Table 1 A summary of the analysis of synaptonemal complex (SC) formation at prophase-I in the four interspecific *Aegilops* hybrids analyzed

Hybrid	Nucleus	Axial element length (μm)	Synapsis percentage	Chromosomal associations
Ae. uniaristata x *Ae. tauschii* (ND)	1	1211	42.3	1II+2III+1VI
	2	1014	53.6	7II
	3	1191	55.2	1I+5II+1III
	4	932	72.8	1II+2VI
	5	1003	81.7	2II+2V
	6	982	86.2	2II+2V
	7	1071	88.5	1XIV
	8	1218	92.7	1XIV
	9	909	98.5	1I+2II+1III+1IV
	10	938	98.5	7II
	11	797	100	1II+3IV
	12	867	100	7II
Ae. umbellulata x *Ae.tauschii* (UD)	1	1203	43.6	1II+1V+1VII
	2	1109	51.3	3II+2IV
	3	1032	57.9	1I+5II+1III
	4	961	69.8	2II+1X
	5	1009	72.2	1I+4II+1V
	6	983	76.8	2II+1X
	7	948	83.9	5II+1IV
	8	1102	90.8	3II+1VIII
	9	783	91.3	1II+1III+1IX
	10	815	99.1	2II+2V
Ae. comosa x *Ae. uniaristata* (MN)	1	1301	28.7	2I+2II+1III+1V
	2	1144	47.4	5II+1IV
	3	985	60.3	2I+2II+1III+1V
	4	907	66.4	2II+1X
	5	1110	74.4	7II
	6	1128	74.4	5II+1IV
	7	805	76.5	1II+1XII
	8	932	76.9	1II+2III+1VI
	9	1017	81.6	5II+1IV
	10	871	87.6	2II+1X
	11	737	88.1	4II+2III
	12	869	89.6	3II+1VIII
	13	910	89.9	3II+2IV
	14	898	90.6	2II+1IV+1VI
	15	982	98.3	2II+1X
Ae. cylindrica x *Ae. caudata* (DCC)	1	1734	67.1	5II+1XI
	2	1655	74.4	4II+1XIII
	3	1534	78.9	6II+1IX
	4	1411	79.8	3II++1III+1XII
	5	1531	82.7	4II+1XIII
	6	1451	83.0	5II+1III+1VIII
	7	1341	83.2	4II+1XIII
	8	2135	84.4	2II+1XVII
	9	1144	85.3	4II+1III+1IV+1VI
	10	1261	86.0	4II+1III+1X
	11	1652	86.2	5II+1XI
	12	1184	87.1	3II+1XV
	13	1381	87.9	6II+1IX
	14	1246	88.4	4II+1XIII
	15	1423	88.6	4II+IV+1VIII
	16	1220	89.6	5II+1IV+1VII
	17	1297	90.6	5II+1XI
	18	1258	93.1	4II+1XIII

x *Ae. uniaristata* (MN), the N genome could be differentiated from both D and M genomes by its high content of C-heterochromatin (Figs. 3 a, e). However, we were unable to distinguish the U and D genomes in the hybrid *Ae. umbellulata* x *Ae. tauschii* (UD) (Fig. 3 c). In the triploid hybrid *Ae. cylindrica* x *Ae. caudata* (DCC), because the chromosomes of the D genome are longer than those of the C genomes, homomorphic bivalents were considered to be formed by almost-homologous C chromosomes (Fig. 3 f). In the diploid hybrids, the higher the relationship between the genomes involved (MN > UD > ND) the higher is the frequency of chromosomal associations observed. The mean cell-bivalent frequencies in ND (3.16) and UD (3.36) hybrids were not very different from those obtained at prophase-I (2.92 and 2.82, respectively). This correspondence indicates that metaphase-I

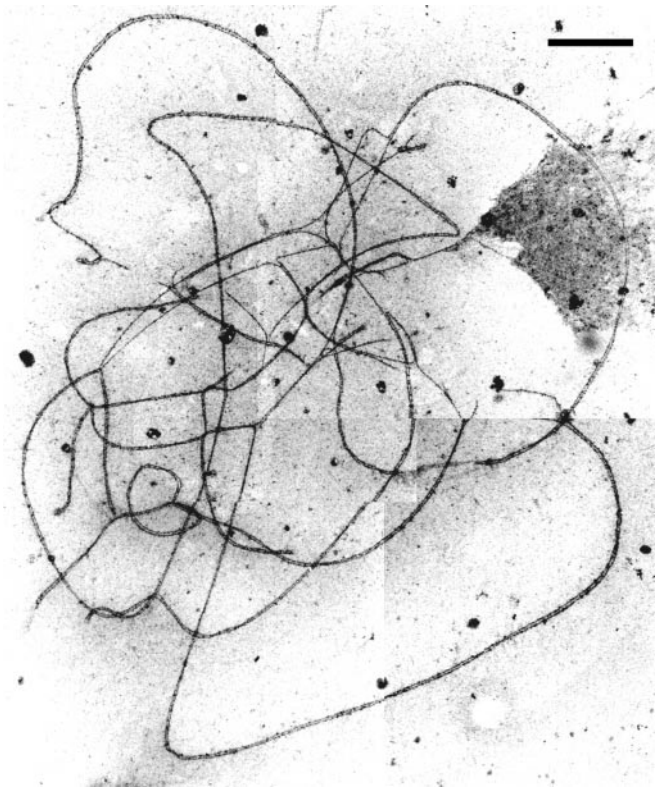

Fig. 1 Electron micrograph of a silver-stained prophase-I nucleus of the *Ae. comosa* x *Ae. uniaristata* hybrid (MN). The bar represents 5 μm.

Fig. 2. Electron micrograph of a silver stained prophase-I nucleus of the *Ae. cylindrica* x *Ae. caudata* hybrid (DCC). The bar represents 5 μm

Table 2 Mean values of the different chromosomal associations observed at metaphase-I in the *Aegilops* interspecific hybrids. Numbers into parentheses indicate the range of variation

Hybrid	No. cells	Rod bivalents		Ring bivalents	Trivalents	
		N-D	D-D	N-D	N-D-N	D-D-N
Ae. uniaristata x *Ae. tauschii* (ND)	60	3.06 (1–6)	0.02 (0–1)	0.08 (0–1)	0.13 (0–1)	0.01 (0–1)
Ae. umbellulata x *Ae. tauschii* (UD)	60	3.36 (1–6)			0.66 (0–3)	
		M-N	M-M	M-N	M-N-M	M-M-N
Ae. comosa x *Ae. uniaristata* (MN)	60	5.08 (3–7)	0.01 (0–1)	0.33 (0–2)	0.08 (0–1)	0.03 (0–1)
		C-C	C-D	C-C		
Ae. cylindrica × *Ae. caudata* (DCC)	150	3.11 (1–5)	0.03 (0–1)	3.34 (1–5)	0.21 (0–2)	

bivalents mainly derive from the SC bivalents observed at prophase-I, and supports the homoeologous nature of such bivalents. On the other hand, in the MN hybrid the mean bivalent frequency at prophase-I (3.07) is lower than that observed at metaphase-I (5.35). Therefore, a considerable number of bivalents formed by homoeologous chromosomes must have been involved in the complex multivalent associations observed at prophase-I. This is also the case in the triploid hybrid in which associations between chromosomes of the C genomes would be preferentially formed (Table 2). Many of these associations probably originated by exclusive SC bivalent formation at prophase-I.

In situ hybridization with the pTa71 probe allows the identification of the rDNA loci in *Aegilops* chromosomes (Badaeva et al. 1996 b). Regarding the hybrids analyzed here, the chromosomes carrying these loci are: 5N, 5D, 5U, 5C, 1U, 1C, 1M and 6M. All of them carry the major rDNA locus on the short arm but it is distally located in the chromosomes of the homoeology group 5 and subdistally in the 1C, 1U, 1M and 6M chromosomes. In addition, the rDNA locus is larger in the 5D chromosome than in 5C (Figs. 3 b, d, f, h). The behaviour of the chromosomes bearing rDNA sequences in the different hybrids is summarized in Table 3. The 5N and 5D chromosomes in the ND hybrid, and the 1M, 6M and 5N

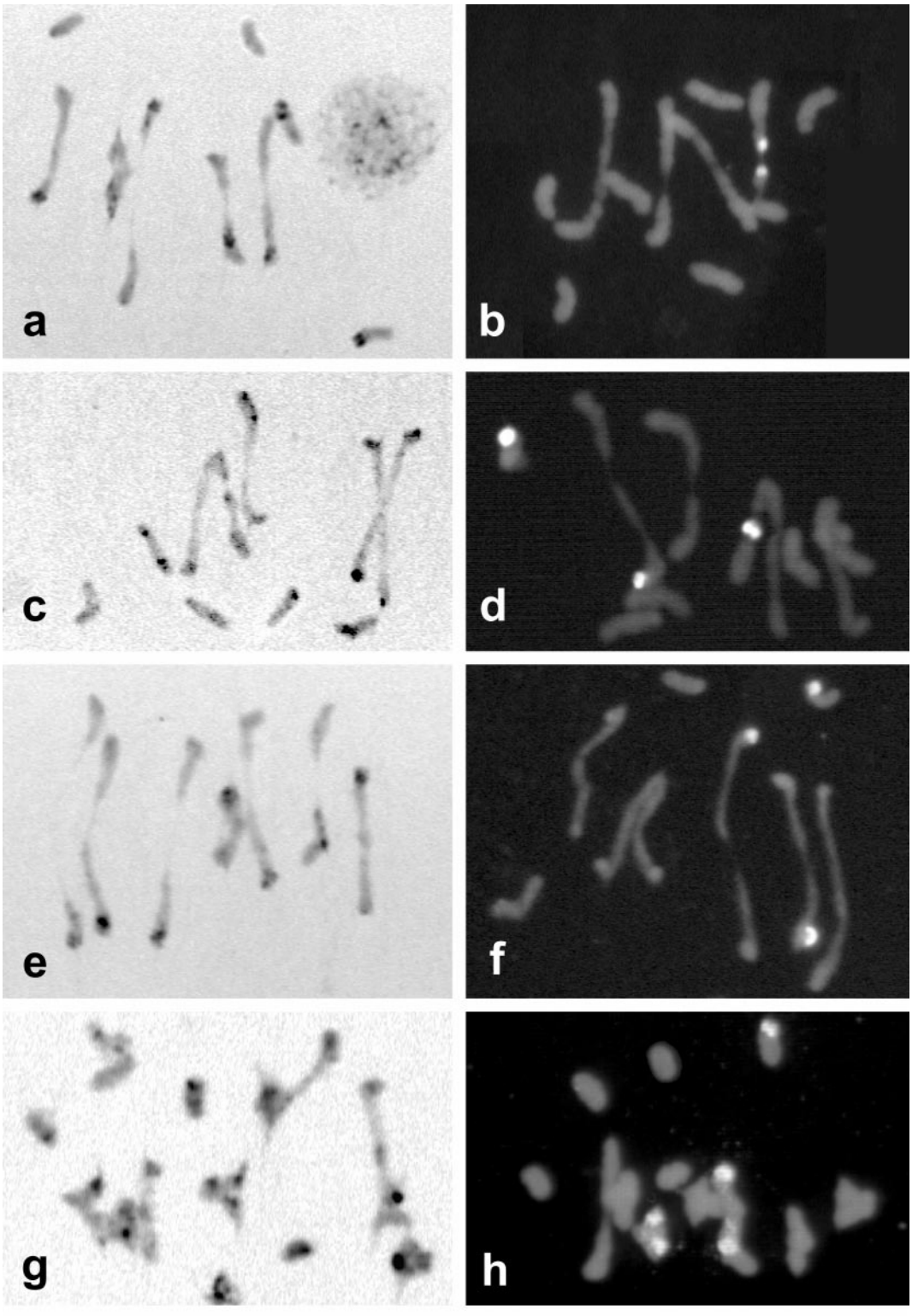

Fig. 3 Metaphase-I cells of interspecific *Aegilops* hybrids after C-banding (**a, c, e, g**) and fluorescence in situ hybridization with a labeled rDNA probe (**b, d, f, h**). *Ae. uniaristata* x *Ae. tauschii*, ND, (**a, b**). *Ae. umbellulata* x *Ae. tauschii*, UD, (**c, d**). *Ae. comosa* x *Ae. uniaristata*, MN, (**e, f**). *Ae. cylindrica* x *Ae. caudata*, DCC, (**g, h**)

chromosomes in the MN hybrid, showed similar mean-cell chromosomal association frequencies. On the other hand, the 1U, 5U and 5D chromosomes in the UD hybrid formed less bivalents but more trivalents than the chromosomes carrying rDNA sequences in the former two hybrids. Furthermore, most of these chromosomal associations seem to be heterologous (non-homoeologous),

Table 3 Mean values of the different chromosomal associations formed by chromosomes carrying rDNA sequences at metaphase-I in the different *Aegilops* hybrids analyzed

Hybrid	No. cells	Chromosomes	Bivalents		Trivalents
			Ring	Rod	
Ae.uniaristata x *Ae.tauschii* (ND)	50	5D-5N	0.72	0.06	–
		Others (12)	2.26	0.04	0.14
Ae.umbellulata x *Ae.tauschii* (UD)	50	5U- + 5D-	0.72	–	0.24
		5U-5D	0.20	–	–
		1U-	0.12	–	0.08
		Others (10)	2.20	–	0.40
Ae.comosa x *Ae. uniaristata* (MN)	50	1M- + 6M-	1.46	0.17	0.03
		5N-	0.60	0.06	–
		Others (8)	2.96	0.10	0.06
Ae. cylindrica x Ae. caudata (DCC)	90	1C-1C	0.53	0.37	0.03
		1C-	0.03	–	–
		5C-5C	0.27	0.63	–
		5C-5C-5D	–	-	0.09
		Others (10C+6D)	2.23	2.37	0.11

probably reflecting the existence of structural rearrangements between the U and D genomes. The almost exclusive observation of bivalents in which either two 1C or two 5C chromosomes were involved in the triploid DCC hybrid indicates the preference for C-C chromosomal associations over D-C (Table 3). Also, the existence of more than 50% of ring bivalents confirms the major involvement of C chromosomes in the SC bivalents observed at prophase-I.

Discussion

The meiotic behaviour displayed by the *Aegilops* hybrids, the first evolutionary stage of natural allopolyploidy, reveals that chromosomal differentiations in the donor genomes do not affect the pairing process. Indeed, all hybrids showed extensive synapsis, similar bivalent frequencies and complex multivalent configurations (Table 1). Multiple chromosomal associations, but different degrees of synaptic extension, have also been described in hybrids of the *Lolium-Festuca* complex (Jenkins and Scanlon 1987; Thomas and Morgan 1990).

Although the number of homoeologous metaphase-I bivalents involved in multivalents at prophase-I is unknown, the slight increase in the mean number of bivalents between prophase-I and metaphase-I observed in the ND and UD hybrids indicates that chiasma formation must be very effective in the maintenance of homoeologous associations until metaphase-I (Tables 1 and 2). By contrast in MN and DCC hybrids the mean number of bivalents at pachytene was much lower than at metaphase-I, probably as consequence of the maintenance of prophase bivalents and the restriction of chiasma formation to the homoeologous chromosomes involved in multivalents. If chromosomal associations at metaphase-I were at random, in the diploid 14-chromosome hybrids the ratio of intergenomic to intragenomic associations is expected to be 7:6 (Gupta and Fedak 1987). For instance, in the ND hybrid this ratio would be 3N-N:7N-D:3D-D. The results shown in Table 2 were found to be highly significant, suggesting that there were preferential homoeologous intergenomic associations. Preferential associations involving C genomes were also predominant in the DCC hybrid. All these observations suggest that SC is initiated at sites where short homologous, or partially homologous, sequence tracts are interacting but extend far into heterologous regions in order to satisfy pairing requirements (Santos et al. 1994). The early SC sites would be more effective for crossing-over, which would be responsible for the M-I chromosomal associations observed. The different frequencies of these associations in the hybrids analyzed reflect the chromosomal differentiation between the genomes involved. Chromosomal differentiation among *Aegilops* species would mainly be due to divergence in nucleotide sequence (Badaeva et al. 1996 a, b) although the existence of structural changes such as translocations, e.g. between U and D genomes (Table 3), cannot be ruled out. The differentiation appeared to be unevenly distributed among chromosomes because rDNA-bearing chromosomes show similar mean bivalent frequencies at metaphase-I in hybrids with quite different overall bound-arm frequencies (Tables 2 and 3).

McGuire and Dvorák (1982) pointed out that the diploid-like meiosis displayed by most polyploid species is probably the result of the selection of mutations in the loci involved on chromosome pairing and chiasma formation contributed by the parental genotypes. If so, mutations in genes involved in homologous recognition would have been selected in tetraploid *Aegilops* species since they show almost exclusive bivalent formation at zygotene, independently of the degree of divergence displayed by the genomes involved (Cuñado et al. 1996 a, b, c). The existence of such type of genes has been confirmed at least in *Aegilops* D genomes (Cuñado et al. 1996 b and references therein). On these grounds, it is possible to explain, for instance, the presence of multivalents at metaphase-I in the synthetic *Ae. triuncialis* and its absence in the natural allotetraploid (Kihara 1965).

The diploidizing system of *Aegilops* also works in interspecific hybrids when two almost homologous sets

are present (e.g. the DCC hybrid) because preferential prophase-I bivalent formation between chromosomes from C genomes was evident (Tables 1 and 2). However, when no homologs are present, this does not prevent synapsis between homoeologous and heterologous chromosomes, though metaphase-I chromosomal association do depend on the phylogenetic relationship between donor genomes (e.g. in the *Ae. biuncialis* x *Secale cereale* hybrid, UMR, Cuñado et al. 1996 a). Nevertheless, a certain role of the diploidizing system on the stringency of crossing-over in homologous segments, even in the hemizygous condition, cannot be ruled out because the haploid of *Ae. ovata* (UUMM) has considerably less metaphase-I chromosomal associations than the hybrid between its parents (Kihara 1937; Matsumura 1940).

Recently, Feldman et al. (1997) have proposed that two independent systems that complement each other are responsible for the diploid-like meiosis displayed by cultivated wheats. The first system is based on the non-random elimination of DNA sequences which would result in accentuating the differentiation between homoeologous chromosomes. The second system is a genic one consisting of the genes *Ph*1 and *Ph*2. It will be interesting to test whether there is also a non-random elimination of sequences from one of the two pairs of homoeologous chromosomes in tetraploid *Aegilops*; an elimination that, perhaps, should be more drastic than in wheats in order to explain the majority presence of bivalents at prophase-I in *Aegilops* species in contrast to the presence of multivalents in wheat.

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