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The pattern of zygotene and pachytene pairing in allotetraploid *Aegilops* species sharing the *D* genome

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Abstract Chromosome pairing behaviour of the allotetraploid *Aegilops* species sharing the D genome, *Ae. crassa* (DDMM), *Ae. cylindrica* (DDCC) and *Ae. ventricosa* (DDNN), was analyzed by electron microscopy in surface-spread prophase-I nuclei. Synaptonemal-complex analysis at zygotene and pachytene revealed that synapsis in the allotetraploids was mostly between homologous chromosomes, although a few multivalents were also formed. Only homologous bivalents were observed at metaphase-I. It is concluded that the mechanism controlling bivalent formation in these species acts mainly at zygotene by restricting pairing to homologous chromosomes, but also acts at pachytene by preventing chiasma formation in homoeologous associations. These observations are discussed in relation to mechanisms of diploidization of polyploid meiosis.

Key words *Aegilops* · Allotetraploid · Diploidization · Pairing control · Synaptonemal complex

Introduction

Although most allopolyploids form homologous bivalents at metaphase-I, the existence of homologous and homoeologous chromosome sets within the same nucleus makes it reasonable to expect variability in their pairing behaviour. This hypothesis has been confirmed after synaptonemal-complex analysis where two major strategies shared by allotetraploids and allohexaploids are involved:

(1) *Triticum aestivum* cv Chinese Spring (Hobolth 1981; Jenkins 1983; Holm 1986; Holm and Wang 1988), the pentaploid hybrid *Triticum kotschyi*×*T. aestivum* (Gillies 1987), the synthetic allotetraploids *Lolium temulentum*×*Lolium perenne* (Jenkins 1985;1986) and *Lolium multiflorum*×*Festuca drymeja* (Thomas 1990), and the segmental allotetraploid *Lotus corniculatus* (Davies et al. 1990) all show conspicuous multivalent pairing at zygotene which may be corrected by pachytene before crossing-over occurs, and where it does not crossing-over is suppressed between paired segments of homoeologous chromosomes.

(2) On the other hand, in the artificial allotetraploid *Lycopersicum esculentum*×*Solanum lycopersicoides* (Menzel 1964), the allohexaploids *Avena maroccana, Avena sativa, Festuca arundinaceae* and *Festuca gigantea* (Jones et al. 1989; Thomas and Thomas 1993), and in allotetraploid *Aegilops* species sharing the U genome (Cuñado et al. 1996 a, b), initial synapsis is mostly restricted to homologous chromosomes.

In the present paper we describe the pattern of zygotene and pachytene pairing in the allotetraploid *Aegilops* species sharing the D genome, namely *Ae. crassa*, *Ae. cylindrica* and *Ae. ventricosa*, in order to elucidate the mechanisms of pairing in the genus *Aegilops* and to compare them with those displayed by its close relatives.

Materials and methods

Three plants each of the allotetraploids (2n=4x=28) *Aegilops crassa* (DDMM, accession 21-2), *Aegilops cylindrica* (DDCC, accession 4653) and *Aegilops ventricosa* (DDNN, accession 22-6), were analyzed in this study. The source of these species was the Plant Germplasm Institute, Faculty of Agriculture, Kyoto University (Japan).

All 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 as indicated by Holm (1986), with minor modifications: namely, the presence of 0.03% "Triton X-100" detergent in the

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swelling medium, and with the fixative solution containing 4% paraformaldehyde and 1.7% sucrose in distilled water, adjusted to pH 8.9 with borate buffer. The preparations were then dried overnight on a warm plate at 37°C, rinsed, and air-dried. For silver impregnation, a few drops of 33% $AgNO_3$ solution were placed on the preparations which were then covered with a patch of nylon cloth. Nuclei were examined using a Jeol-1010 electron microscope and photographed on Kodak 4489 film.

Results

Nuclei from zygotene to pachytene from *Ae. crassa*, *Ae. cylindrica* and *Ae. ventricosa* were examined in the electron microscope. Data from plants of the same species were pooled because they showed a similar synaptonemal-complex (SC) behaviour. Table 1 summarizes the general features of SC formation in the zygotene nuclei analyzed, namely: the total haploid lateral element length, the amount of synapsis and the number of bivalent and multivalent configurations. Only three nuclei of *Ae. cylindrica* were not fully traced because they showed some SC distortions, although even here the nature of chromosomal associations could be inferred from the number of pairing partner switches observed. In *Ae. crassa* the number of SC segments ranged from 23 to 57 per nucleus, in *Ae. cylindrica* from 22 to 48, and in *Ae. ventricosa* from 15 to 58, the maximum number (57, *Ae. crassa*; 48, *Ae. cylindrica*; 58, *Ae. ventricosa*) occurring in nuclei that were 61%, 63%, and 41% synapsed, respectively. These numbers of SC segments are significantly lower than those observed in rye (2n=14), 76 in 45% synapsed nucleus with a maximum of 13 SCs per bivalents (Gillies 1985), and other plants with larger genomes, such as *Tradescantia* (Hasenkampf 1985) and *Lilium*, in which 36 SCs were observed in one bivalent (Holm 1977).

Table 1 A summary of the analysis of synaptonemal complex (SC) formation at zygotene in three species of *Aegilops* with a D genome

Species	Nucleus	Haploid element length (μm)	Synapsis percentage	No. SC segments	Bivalents	Quadrivalents
Ae. crassa	C1	1976	38.5	52	14	0
	C2	1118	49.9	45	14	0
	C3	1215	52.7	50	14	0
	C4	1735	60.1	43	14	0
	C5	1357	60.6	57	14	0
	C6	1104	67.3	50	14	0
	C7	951	78.6	37	14	0
	C8	1174	79.0	39	14	0
	C9	904	84.2	37	14	0
	C10	934	89.7	23	14	0
Mean		1246.8±111.9	–	–	14.0±0.0	0.0±0.0
Ae. cylindrica	Y1	1781	48.7	38	14	0
	Y2	1344	57.0	38	12	1
	Y3	945	60.7	23	14	0
	Y4	1464	63.0	48	14	0
	Y5	1068	64.9	38	14	0
	Y6	1371	67.7	46	14	0
	Y7	1094	69.8	26	14	0
	Y8	1049	70.3	28	14	0
	Y9	1427	75.2	22	14	0
	Y10	1225	76.5	41	14	0
	Y11	1174	79.8	23	14	0
	Y12	1389	85.6	31	14	0
	Y13	–	–	–	12	1
	Y14–Y15	–	–	–	14	0
Mean		1277.5±66.8	–	–	13.7±0.2	0.1±0.1
Ae. ventricosa	V1	1248	34.7	29	12	1
	V2	1417	36.7	40	14	0
	V3	1593	38.5	32	14	0
	V4	1400	40.4	36	14	0
	V5	1932	41.3	58	14	0
	V6	1438	60.2	36	14	0
	V7	1353	74.2	45	12	1
	V8	1061	88.2	17	14	0
	V9	935	88.4	15	10	2
	V10	1011	90.2	18	14	0
Mean		1338.8±93.8	–	–	13.2±0.4	0.4±0.2

Extensive bivalent formation is the main feature displayed by zygotene nuclei of all three species (see Fig. 1). In fact, only two quadrivalents in *Ae. cylindrica* and four quadrivalents in *Ae. ventricosa* were observed. Lateral elements of equal length were present in all bivalents.

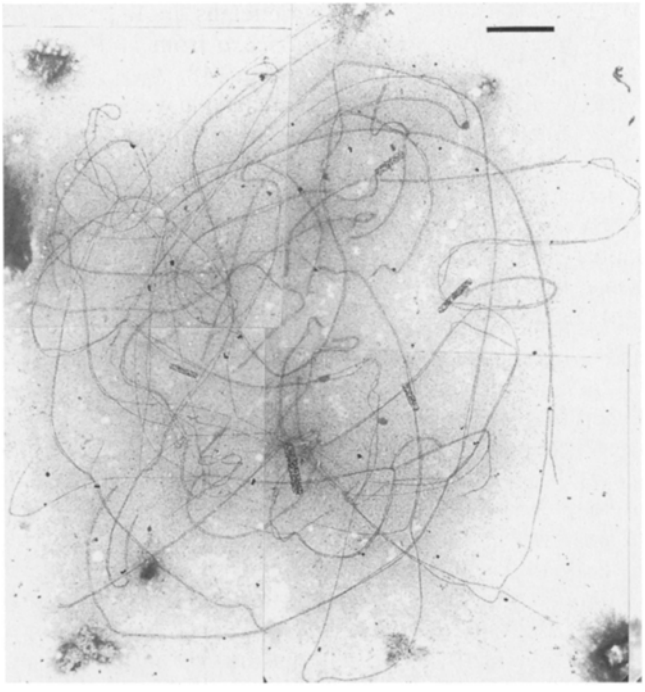

Fig. 1 Electron micrograph of a silver-stained zygotene nucleus of *Ae. cylindrica*. The bar represents 5 μm

The pairing patterns displayed by the bivalents are similar in the three species studied (Table 2) and resembled those seen in allotetraploid *Aegilops* species sharing the U genome (Cuñado et al. 1996 a,b). Synapsis starts at terminal or subterminal regions although in all but the earliest stage, and at numerous sites per bivalent, interstitial SCs also occur. Within individual bivalents, extensive regions of SC formation often existed at the same time as other extensive regions that were unpaired. Additionally, in the same nucleus one bivalent might have multiple SC segments while other bivalents had only a few.

The number and type of chromosomal associations observed in pachytene nuclei is given in Table 3. A total of 16 quadrivalents (one in *Ae. crassa*, nine in *Ae. cylindrica*, and six in *Ae. ventricosa*) and one hexavalent (*Ae. ventricosa*) were distributed among 86 nuclei. However, the presence of 14 bivalents per nucleus was the most frequent chromosomal association (see Figs. 2 and 3).

Discussion

The results of the present SC analysis show that, in the three allotetraploid *Aegilops* species sharing the D genome, pairing during prophase-I is mostly as bivalents, although a few multivalents are formed (see Tables 1 and 3). The frequency of multivalents in *Ae. crassa* is 0.00 at zygotene and 0.08 at pachytene, compared with 0.13 and 0.19 in *Ae. cylindrica*, and 0.40 and 0.27 in *Ae. ventricosa*; therefore, there does not seem to be a correction of multivalents between these prophase-I stages. Since only bivalents were

Table 2 Frequencies of the different pairing patterns displayed by bivalents in the zygotene nuclei of the three species analyzed

Species	% Bivalent pairing	Patterns of synapsis progression in bivalents[a]										
		–––	––<	–o–	–o–<	–o–o–	–o–o–<	–o–o–o–	–o–o–o–<	–o–o–o–o–	Others[b]	Total
A. crassa	0–20			1					2			3
	20–40		2	3	2	3	2	2		2	5	21
	40–60		1	1	3	3	4	4	3	8		27
	60–80		4	1	6	11	4	9	2	5	2	44
	80–90			4	1	8	2	3			1	19
	90–100	10	1	11		3		1				26
Ae. cylindrica	0–20		2		1		1					4
	20–40		2	6	3	2	2	2				17
	40–60		7	11	2	7	2	4		6	3	42
	60–80		3	10	2	15	1	5		2	2	40
	80–90		3	11		6		3				23
	90–100	24	1	9	3	2		1				40
Ae. ventricosa	0–20			2		4	1	1				8
	20–40		1	6	2	11	2	4	2		1	29
	40–60		2	11	4	10		2	1	3	2	35
	60–80		3	7	1	5				1	3	20
	80–90			6	2	3	1					12
	90–100	26		2								28

[a] –, paired region; <, >, o, unpaired region. Length differences of these regions are not considered
[b] More than three interstitial pairing initiation sites

Table 3 A summary of the analyzed nuclei at pachytene

Species	No. nuclei[a]	Haploid element length (μm), mean±SE [range]	Chromosomal associations		
			14II[a]	12II+1IV[a]	Others
Ae. crassa (DDMM)	12	910.1±60.75 (513–1311)	11	1	–
Ae. cylindrica (DDCC)	24 (24)	885.0±30.85 (578–1235)	19 (20)	5 (4)	–
Ae. ventricosa (DDNN)	19 (7)	1046.2±37.74 (712–1340)	16 (5)	1 (2)	2[b]

[a] Data from non-fully traced nuclei are shown in parenthesis
[b] 11II+1VI; 8II+3IV

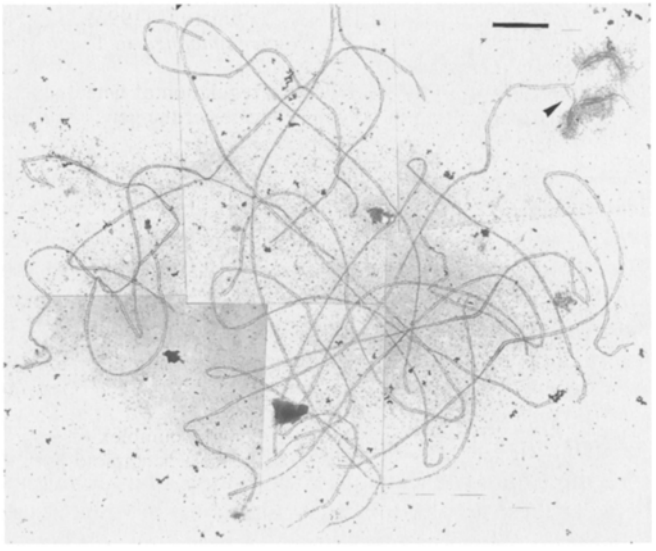

Fig. 2 Electron micrograph of a silver-stained pachytene nucleus of *Ae. crassa*. The *arrowhead* indicates axial thickenings in the unsynapsed NOR region. The bar represents 5 μm

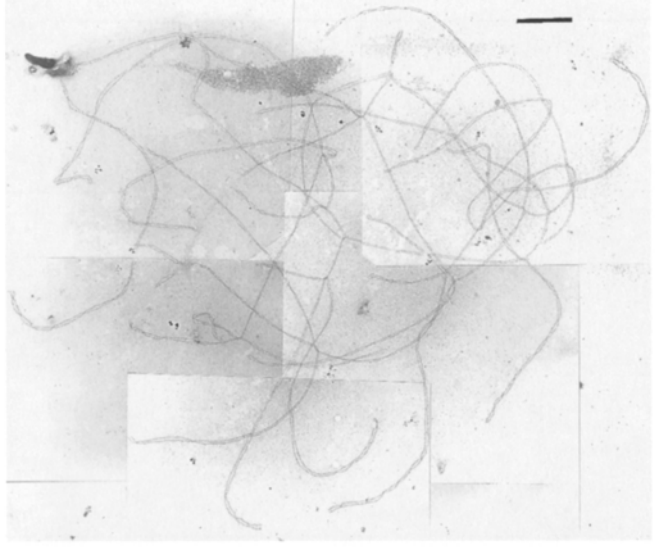

Fig. 3 Electron micrograph of a silver-stained pachytene nucleus of *Ae. ventricosa*. The bar represents 5 μm

observed at metaphase-I (Cuñado 1992), a suppression of cross-overs in homoeologous associations has to occur.

The control mechanism responsible for the diploid-like meiosis of these species acts in a similar way in all of them, and is independent of the degree of divergence displayed by the genomes involved. Although some differences between the D genomes of the species analyzed here have been detected (Kimber and Zhao 1983; Rayburn and Gill 1987), it is also known that *Aegilops* D genomes are able to compensate for the absence of chromosome 3D of *Triticum aestivum* (AbuBakar and Kimber 1982; McGuire and Dvorák 1982). This indicates that in these genomes there is a homoeologous pairing-suppressor at least as effective as the *Ph*2 gene found in *T. aestivum* (Mello-Sampayo 1971; Driscoll 1972). From the results obtained, it is suggested that these D genes act predominantly by increasing the stringency of homologous synapsis at early stages of meiotic prophase. This hypothesis is also supported by the fact that the mean number of multivalents per nucleus at mid-zygotene is lower in *T. aestivum* (AABBDD, 42 chromosomes; Holm and Wang 1988) than in *Triticum timopheevii* (AAGG, 28 chromosomes; Martínez et al. 1996), 1.09 and 2.14 respectively.

It is noteworthying that all tetraploid *Aegilops* species containing either the U genome (Cuñado et al. 1996 a, b) or the D genome (this paper) show almost exclusive bivalent formation at zygotene without any multivalent elimination mechanism. This behaviour clearly contrasts with that displayed by the allopolyploid *Triticum* species studied to-date in which the mean number of lateral elements involved in multivalents at mid-zygotene, late-zygotene, and pachytene are 5.45, 4.57, and 0.85 in *T. aestivum* (Holm and Wang 1988) and 8.57, 4.00, and 2.16 in *T. timopheevii* (Martínez et al. 1996). A more pronounced stringency of crossing-over to homologous segments would be produced in *T. aestivum* by the *Ph*1 gene located in chromosome 5B (Gillies 1987).

The reason for the striking differences observed between the synaptic behaviour displayed by wild and cultivated wheats remains unknown although it might be related to the different types of selection to which they have been subjected.

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