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N. Cuñado · S. Callejas · M. J. García · A. Fernández
J. L. Santos

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

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Abstract Allotetraploid *Aegilops* species sharing the U genome, *Ae. columnaris* (UUMM), *Ae. ovata* (UUMM), *Ae. triaristata* (UUMM), *Ae. triuncialis* (UUCC) and *Ae. variabilis* (UUSS), regularly form bivalents at metaphase I of meiosis. The pattern of zygotene and pachytene pairing was analyzed by whole-mount surface-spreading of synaptonemal complexes under the electron microscope. The data indicated that at the zygotene stage the chromosomes were almost exclusively associated as bivalents; only a few multivalents (7%) were observed. These observations are discussed in relation to mechanisms of diploidization of polyploid meiosis.

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

Introduction

Genus *Aegilops* is distributed throughout the Mediterranean basin and in southwest and central Asia. It includes 11 diploid (2n=2x=14), 10 tetraploid (2n=4x=28), and 4 hexaploid (2n=6x=42) species (Kimber and Feldman 1987). All of the allopolyploid species mostly form bivalents at metaphase I of meiosis, which implies the existence of control mechanisms that ensure stability and fertility and confer disomic inheritance (AbuBakar and Kimber 1982; McGuire and Dvorák 1982; Cuñado 1992). In this paper we used a surface-spreading technique to analyze chromosome pairing at zygotene and pachytene in five allotetraploid *Aegilops* species sharing the U genome. The results are discussed in relation to previous studies on *Aegilops biuncialis* (UUMM, Cuñado et al. 1996) and hexaploid wheat (AABBDD, Holm 1986; Holm and Wang 1988) with respect to mechanisms of diploidization of polyploid meiosis.

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N. Cuñado (✉) · S. Callejas · M. J. García · J. L. Santos
Departamento de Genética, Facultad de Biología,
Universidad Complutense, 28040-Madrid, Spain

A. Fernández
Centro de Microscopía Electrónica, Universidad Complutense,
28040-Madrid, Spain

Materials and methods

The allotetraploids (2n=4x=28) *Ae. columnaris* (UUMM), *Ae. triaristata* (UUMM), *Ae. ovata* (UUMM, accession 9–3), *Ae. triuncialis* (UUCC, accession 4801) and *Ae. variabilis* (UUSS, accession 13-1), three plants of each, were analyzed in this study. The source of the first two species was the Estación Experimental de Aula Dei, Zaragoza (Spain) and the remainder were from the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University (Japan).

All of the 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. Two remaining anthers at zygotene or pachytene were then prepared for synaptonemal complex isolation (Holm 1986), with minor modifications: 0.03% "Triton X-100" detergent in the swelling medium, and the fixative solution contained 4% paraformaldehyde and 1.7% sucrose in distilled water, adjusted to pH 8.9 with borate buffer.

Results

Table 1 summarizes the analysis of synaptonemal complex (SC) formation and the number of zygotene nuclei studied in each species. Data from plants of the same species were pooled because they showed a similar SC behaviour. Zygotene nuclei of *Ae. columnaris* and *Ae. ovata* were not fully traced although gross chromosomal associations could be established.

Extensive bivalent formation is the main feature displayed by zygotene nuclei of all the species (see Fig. 1). In fact, only one quadrivalent in *Ae. biuncialis* (Cuñado et al. 1996) and one hexavalent in *Ae. variabilis* were observed. All bivalents showed lateral elements of equal length.

Table 1 A summary of the analysis of synaptonemal complex (SC) formation at zygotene

Species	Number of nuclei[a]	Haploid element length (μm) Mean ± SE [Range]	Range of pairing percentage	Chromosomal associations		
				14II[a]	12II+1IV[a]	Others
biuncialis[b] (UUMM)	10 (4)	1126.60 ± 40.39 [1013 – 1437]	24.0–81.3	9 (3)	– (1)	1[c]
columnaris (UUMM)	– (5)	–	–	– (5)	–	–
ovata (UUMM)	– (7)	–	–	– (7)	–	–
triaristata (UUMM)	10	1323.80 ± 30.95 [1208 – 1491]	45.5–79.8	10	–	–
triuncialis (UUCC)	10	1365.20 ± 56.15 [1101 – 1709]	18.1–88.7	10	–	–
variabilis (UUSS)	10	1311.00 ± 92.06 [855 – 1699]	45.6–92.7	9	–	1[d]

[a] Data from non-fully traced nuclei are shown in parenthesis
[b] Data taken from Cuñado et al. (1996)
[c] 13II+2I
[d] 11II+1VI

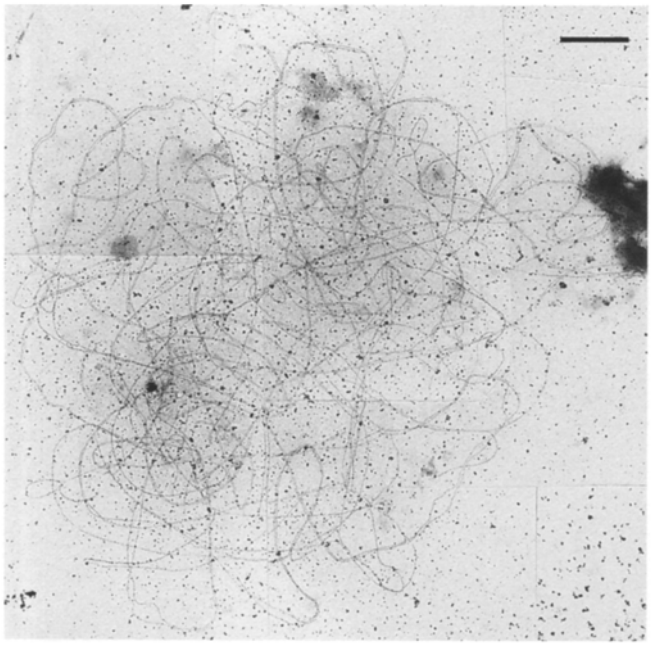

Fig. 1 Electron micrograph of a silver-stained zygotene nucleus of *Ae. triuncialis*. *Bar*: 5 μm

The frequencies of the different pairing patterns displayed by bivalents are shown in Table 2. These patterns are similar in all of the species studied. Synapsis starts at the terminal or subterminal regions. Interstitial SCs occur at numerous sites per bivalent in all but earliest stages. The maximum number observed was seven in *Ae. variabilis*. Bivalents within the same nucleus differ in the number of SC stretches and time duration of full synapsis achievement. Of the 14 nuclei analyzed, the maximum number of SC segments observed per species was 38 in *Ae. biuncialis* (67% pairing), 48 in *Ae. triuncialis* (64% pairing), 49 in *Ae. variabilis* (75% pairing) and 55 in *Ae. triaristata* (45% pairing). It seems that SC number increases in most species until more than half of the genome is paired, and then declines. The numbers of SC segments observed here are lower than those observed in rye and other plant species with larger genomes (Gillies 1985 and references therein).

The number and type of chromosomal associations observed in pachytene nuclei is given in Table 3. A total of ten quadrivalents and one hexavalent were distributed among 139 nuclei in five of the six species. However, the presence of 14 bivalents per nucleus was the most frequent chromosomal association (see Figs. 2 and 3). This latter configuration was the only one in *Ae. variabilis*.

The comparison between zygotene and pachytene results reveals that there is no correction of multivalents throughout these prophase I stages. The excess of pachytene multivalents is only due to sampling.

Discussion

In a previous paper, we reported extensive bivalent formation at zygotene in *Aegilops biuncialis* (UUMM, Cuñado et al. 1996). This pairing pattern can be extended to the other allotetraploid *Aegilops* species sharing the U genome. Therefore, the control mechanism responsible for the diploid-like meiosis of these species acts in a similar way in all of them, independent of the degree of divergence displayed by the genomes involved. The mean number of associations between chromosome arms in F_1 hybrids of diploids *Ae. longissima* (SS), *Ae. comosa* (MM) and *Ae. caudata* (CC) with *Ae. umbellulata* (UU) indicated that the C genome is closer to the U genome than to the M and S genomes, this latter one being the most divergent (Lucas and Jahier 1988). In addition, the U genome has not

Table 2 Frequencies of the different pairing patterns displayed by bivalents in the zygotene nuclei of the 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-o-	Other[b]	Total
biuncialis (UUMM)	0– 20		7	10	2	1			1				21
	20– 40		1	15	2	7	1	6	2	1			35
	40– 60		2	9	8	5	1	1	3		3		32
	60– 80		2	7	2	11		1	1				24
	80– 90			8		2							10
	90–100	10		7									17
triaristata (UUMM)	0– 20												0
	20– 40			8	1	3			2		1	2	17
	40– 60		2	21	2	10	1	3	9	3	4		55
	60– 80		3	14	3	9		1	4		2	1	37
	80– 90		1	6		2			2				11
	90–100	13	2	5									20
triuncialis (UUCC)	0– 20		7	2									9
	20– 40			4	1			1	3				9
	40– 60		12	6	2	8		2				1	31
	60– 80		6	21	3	8	5				1	2	46
	80– 90			7		3		3	6		1		20
	90–100	17	2	3	1	2							25
variabilis (UUSS)	0– 20												0
	20– 40				3	4							7
	40– 60			3	2	8		2	2		2	1	20
	60– 80		1	12	3	17		1	6	2	1	1	44
	80– 90		7	13		2		1				1	24
	90–100	25	4	9	1	1			1				42

[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	Number of nuclei[a]	Haploid element length (μm) Mean ± SE [Range]	Chromosomal associations		
			14II[a]	12II+1IV[a]	Others
biuncialis[b] (UUMM)	13 (33)	1042.92 ± 54.64 [805 – 1518]	12 (31)	1 (2)	–
columnaris (UUMM)	10 (12)	774.90 ± 26.25 [709 – 981]	8 (11)	1 (1)	1[c]
ovata (UUMM)	16 (15)	1134.62 ± 61.57 [725 – 1469]	16 (12)	– (3)	–
triaristata (UUMM)	10	905.90 ± 42.28 [693 – 1109]	9	1	–
triuncialis (UUCC)	10 (5)	1135.80 ± 32.01 [1019 – 1310]	10 (4)	– (1)	–
variabilis (UUSS)	10 (5)	1269.60 ± 83.13 [920 – 1787]	10 (5)	–	–

[a] Data from non-fully traced nuclei are shown in parenthesis
[b] Data taken from Cuñado et al. (1996)
[c] 1II+1VI

suffered important modifications during the evolution of UM and UC tetraploids (Kimber and Yen 1989; Cuñado 1993 and references therein), and the differential nature of the M and C genomes may be the result of variability within the diploid progenitors (Talbert et al. 1993; Chee et al. 1995).

In allohexaploid wheat, multivalent SCs are formed between homoeologues at zygotene, but almost homologous bivalents are almost exclusively observed by late pachytene stage (Holm and Wang 1988). A similar chromosomal behaviour is also shown by the allotetraploid *T. timophevii* (AAGG) (M. Martínez, personal communi-

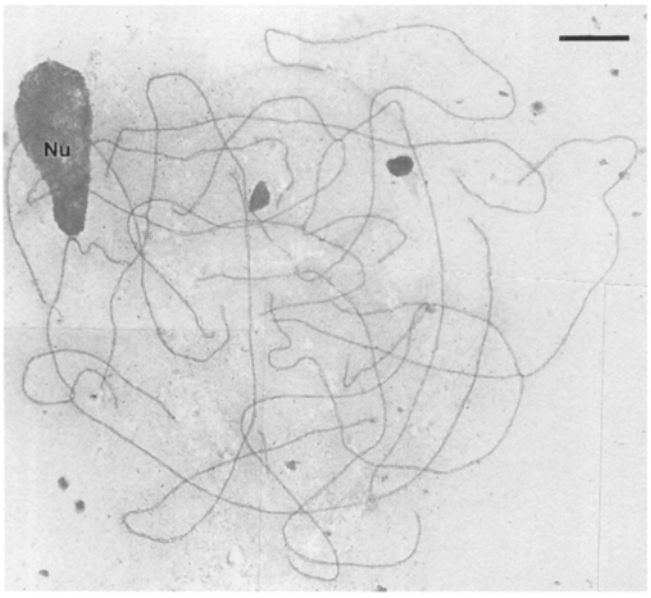

Fig. 2 *Electron micrograph* of a silver-stained pachytene nucleus (*Nu*, nucleolus) of *Ae. columnaris*. *Bar*: 5 μm.

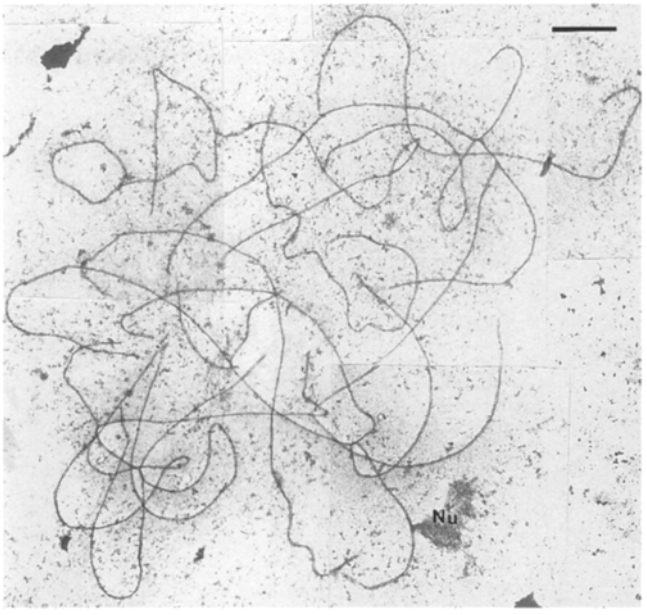

Fig. 3 Electron micrograph of a silver-stained pachytene nucleus (*Nu*, nucleolus) of *Ae. triaristata*. *Bar*: 5 μm

cation). In pentaploid *Triticum aestivum*×*T. (Aegilops) kotschyi* hybrids carrying the *Ph* or *ph* allele (Gillies 1987), the *Ph* gene effect was manifested mainly on the stringency of crossing-over while the pairing behaviour was similar in both genotypes.

The control mechanism of chromosome pairing in allotetraploid *Aegilops* species sharing the U genome may be different. We hypothesize that it mainly acts on initial synapsis in such a way that bivalent formation is achieved almost exclusively, even at zygotene. Also, it is ineffective in hemizygous condition (Cuñado et al. 1996). This control seems to be similar to that displayed by *Avena maroccana* and *A. sativa* (Jones et al. 1989) and *Festuca gigantea* (Thomas and Thomas 1993). Our data caution against the general assumption that more allopolyploids with exclusive autosyndetic pairing (at zygotene) are the exception to a more widespread behavior in which both autosyndesis and allosyndesis occur (see Gillies 1989 for references).

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