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Meiotic behaviour of Un, D and R genomes in the amphiploid *Aegilops ventricosa – Secale cereale* and the parental species

M. C. Cermeño, N. Cuñado and J. Orellana
Departamento de Genética, Facultad de Biología, Universidad Complutense, E-28040 Madrid, Spain

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Summary. Meiotic pairing frequencies of the Un and D genomes of *Ae. ventricosa* and the R of *S. cereale* could be easily established at metaphase I in *Aegilops ventricosa – Secale cereale* amphiploid plants as well as in its parental species by using the C-banding technique procedure. The results show a high diminution of chromosome pairing for all genomes in the amphiploid with respect to its parental species probably due to C-heterochromatin content and/or genotypic or cryptic interactions between the three genomes.

Key words: Meiotic pairing – C-banding – *Aegilops ventricosa – S. cereale* – Amphiploidy of *Aegilops ventricosa – S. cereale*

Introduction

Meiotic irregularities have been extensively studied in triticale in great detail by using differential staining techniques but only rarely in other amphiploids of related species (for review, see Gupta and Phiyadarshan 1982).

Telomeric C-bands of rye chromosomes and intercalary or centromeric bands of wheat chromosomes seem to influence chromosome pairing in triticales and different hypotheses have been suggested to explain the meiotic irregularities found in these plants (Thomas and Kaltsikes 1974, 1976; Merker 1976; Roupakias and Kaltsikes 1977).

Because of the very close evolutionary relationships between *Aegilops* and *Triticum* meiotic analyses of *Aegilops – Secale* amphiploid plants could provide new information for explaining pairing failures in triticales. Low chiasma frequency and univalents have already been observed at metaphase I in *Ae. ventricosa – S. cereale* amphiploid plants but the contribution of each genome in the univalent formation could not be determined due to the conventional staining techniques employed (Dosba and Jahier ,1981). For this reason, studies should be made with techniques which allow the pairing of each genome in a given amphiploid to be distinguished.

Aegilops ventricosa is an allotetraploid species which probably originated from spontaneous hybridization between *Ae. squarrosa* and *Ae. uniaristata* (Kimber et al. 1983; Kimber and Zhao 1983). C-banding techniques have revealed differences in localization of heterochromatin between the rye and *Aegilops* chromosomes (Teoh and Hutchinson 1983; Teoh et al. 1983). Consequently, in the *Ae. ventricosa – S. cereale* amphiploid it should be possible to identify the two genomes of *Aegilops* and the one of *Secale*. This would allow the analysis of its meiotic behaviour and comparison with the parental species.

Material and methods

Plants of diploid rye cv. 'La Raña' (Orellana and Giraldez 1981), *Aegilops uniaristata*, *Aegilops ventricosa* and the *Aegilops ventricosa* × *Secale cereale* amphiploid formed the material for this study.

Seeds of the *Ae. ventricosa* × *S. cereale* amphiploid were kindly supplied by Dr. F. Dosba, Plant Breeding Station, Research Center of Rennes, INRA, France.

Ae. uniaristata and *Ae. ventricosa* were obtained from The Aula Dei, Experimental Station, CSIC, Zaragoza, Spain.

To obtain mitotic metaphase cells, seeds were germinated on wet filter paper in Petri dishes at 20 °C. When primary roots were 1 cm long, they were excised and immersed in tap water at 0 °C for 48 h to shorten the chromosomes. Subsequently, the tips were fixed in acetic-ethanol 1 : 3. To analyze the meiotic cells, anthers were fixed in acetic-ethanol 1 : 3. Both root tips and anthers were maintained in the fixative liquid for 1–4 months at 3–4 °C. The fixed material was squashed and stained following the Giemsa C-banding technique described previously (Giraldez et al. 1979).

Results and discussion

Studies of genome relationships in interspecific hybrids of *Aegilops* seem to indicate that *Aegilops ventricosa* (DDUnUn) is an allotetraploid species which probably originated from the spontaneous hybridization between *Ae. squarrosa* (DD) and *Ae. uniaristata* (UnUn) (see Kimber et al. 1983; Kimber and Zhao 1983). When C-banding technique was applied to mitotic chromosomes of *Aegilops ventricosa* they could be classified into two groups according to their C-banding pattern.

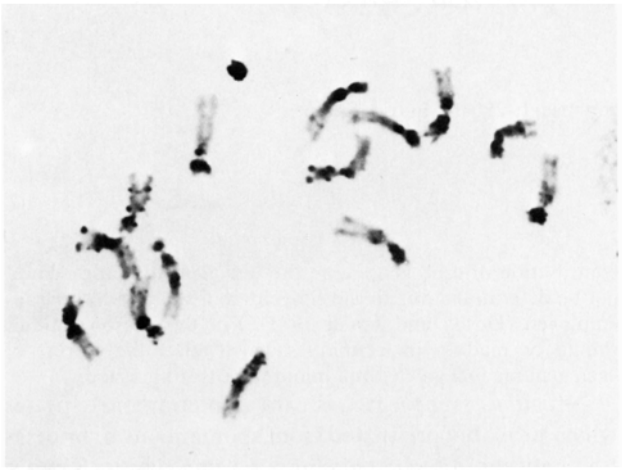

Fig. 1. C-banded somatic metaphase cell of *Aegilops uniaristata*

The first group is formed of chromosomes with prominent centromeric, pericentromeric and interstitial or dispersed thin bands; the other one, by chromosomes with clearly observable centromeric bands and smaller but clearly visible interstitial and/or telomeric bands. These two C-banding patterns are very similar to those reported by Teoh and Hutchinson (1983) for *Ae. uniaristata* and *Ae. squarrosa,* respectively. Moreover, the correspondence was very close when the mitotic metaphase cells of *Ae. uniaristata* (Fig. 1) and *Ae. squarrosa* were banded.

While on the one hand there is a good correspondence between the centromere position found in the two groups of chromosomes and that of their hypothetical ancester diploid species. There are, however, some differences which can imply significative evolutionary modifications. Perhaps the Un and D designations for both genomes of *Ae. ventricosa* in this paper, should be reformed.

All amphiploid plants showed, as expected, the same C-banding pattern either for *Ae. ventricosa* or rye chromosomes which, as usual, show C-heterochromatin blocks located preferentially in most of the telomeres (Fig. 2).

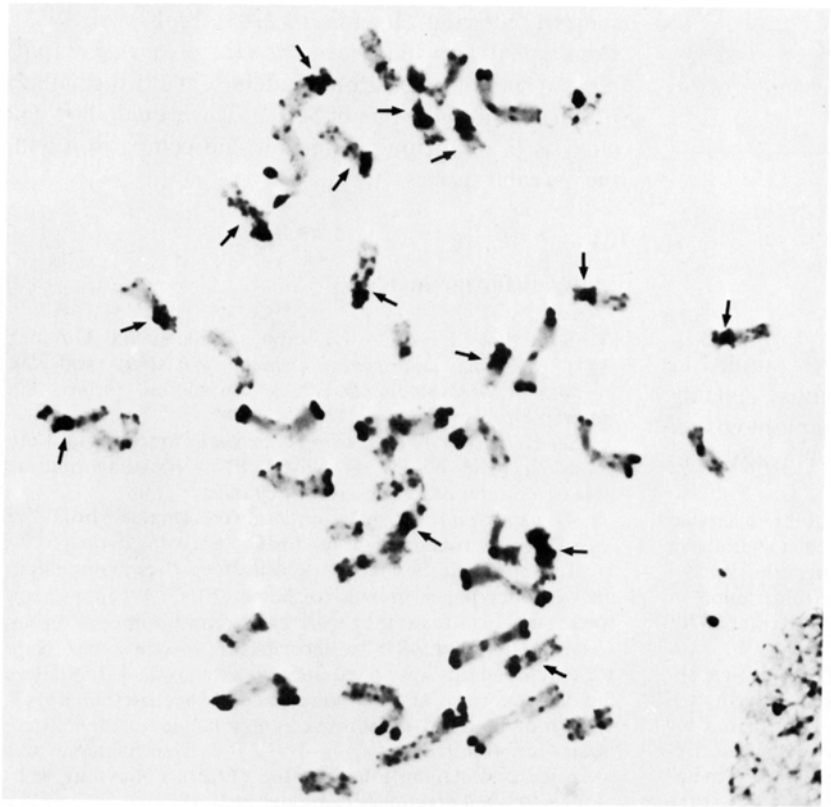

Fig. 2. C-banded somatic metaphase cell of *Ae. ventricosa* × *S. cereale* amphiploid. *Arrows* indicate the Un genome chromosomes

Table 1. Frequencies of the different meiotic configurations of the Un, D and R genomes in *Aegilops ventricosa, Secale cereale* and their amphiploid

Aegilops ventricosa									*Secale cereale*				
Plant	Un				D				Plant	R			
	R	O	U	$\bar{X}$	R	O	U	$\bar{X}$		R	O	U	$\bar{X}$
Av 1	169	40	1	12.60	203	7	–	13.77	Raña 1	676	24	–	13.76
Av 2	159	49	2	12.23	197	13	–	13.57	Raña 2	673	27	–	13.73
Av 3	183	27	–	13.10	203	7	–	13.77	Raña 3	672	28	–	13.72
Av 4	167	41	2	12.50	197	13	–	13.57	Raña 4	670	30	–	13.70
Av 5	179	30	1	12.93	183	27	–	13.10	Raña 5	649	51	–	13.49

Aegilops ventricosa × *Secale cereale* amphiploid

Plant	Un				D				R			
	R	O	U	$\bar{X}$	R	O	U	$\bar{X}$	R	O	U	$\bar{X}$
AvSe 1	200	128	22	10.48	315	33	2	13.26	125	177	48	8.54
AvSe 2	187	133	30	10.14	307	40	3	13.08	104	194	52	8.04
AvSe 3	173	147	30	9.86	290	60	–	12.80	65	247	38	7.54
AvSe 4	175	143	32	9.86	297	51	2	12.90	70	221	59	7.22
AvSe 5	183	136	31	10.04	286	56	8	12.56	74	223	53	7.42

Table 2. Comparison of the mean meiotic pairing of the D, Un and R genomes in the *Aegilops ventricosa* × *Secale cereale* amphiploid with that of its parental species

	No. plants	No. cells	$\bar{X}$ Bonds per cell		
			D	Un	R
Ae. ventricosa	5	150	13.702	12.669	–
S. cereale	5	500	–	–	13.68
Ae. ventricosa–S. cereale	5	250	12.920	10.076	7.952

t (R) = 24.3118; d.f. = 8; $P < 0.001$
t (D) = 5.9447; d.f. = 8; $P < 0.001$
t (Un) = 13.4030; d.f. = 8; $P < 0.001$;

Using C-bands as cytological markers it is possible to identify every meiotic configuration at metaphase I involving the Un, D or R chromosomes in the amphiploid as well as in its parental species (Table 1) (Fig. 3). Therefore, the amphiploid provides a suitable material for analyzing the degree in which C-heterochromatin affects meiotic pairing in each genome.

The mean number of bound arms at metaphase I was lower in the amphiploid plants than in the parental species for the three genomes analyzed, the differences being highly significant in all cases when *t*-tests were performed (Table 2).

There is a clear correlation between the C-heterochromatin content and the decrease in pairing which each genome shows in the amphiploid with respect to the parental species. That is, genomes R and Un, with more heterochromatin, have a pairing reduction of 41.88 and 20.47%, respectively, whereas the pairing of the D genome (more euchromatic) is only reduced by 5.7%.

It has been reported that in triticale and wheat-rye hybrids telomeric heterochromatin in single or double dosages reduce the meiotic pairing of chromosomes carrying it, (see Gupta and Priyadarshan 1982). This does not occur in rye itself (Merker 1976; Naranjo and Lacadena 1980). In addition, it is well-known that C-heterochromatin is late in replicating. Thomas and Kaltsikes (1974) suggested that the effect of heterochromatin could be produced by an overlap between the processes of DNA replication and meiotic prophase pairing. This interference between DNA replication and meiotic pairing could be critical in triticale because a shorter meiotic cycle has been forced upon the rye genome (Merker 1976). These facts could explain the meiotic behaviour of rye chromosomes observed in the *Ae. ventricosa* × *S. cereale* amphiploid plants. Nevertheless, another factor must be taken in account: rye chromosome partners are homozygous due to the colchicine treatment used for obtaining the amphiploid and it is well known that the homozygosity produced by inbreeding leads to reductions in chromosome pairing in diploid rye (see Lamm 1936; Rees 1955). Thus, rye genomes in the amphiploid could be considered to be very similar to those of an inbred line and, consequently, a low pairing frequency is expected with respect to an open pollinated variety. This possibility may explain, in part, the pairing diminution observed for rye chromosomes; however the values of the mean number of bound arms per cell, are much more lower than those usually found in inbred lines (Orellana et al. 1984).

Merker (1976) concluded that not only telomeric, but also the proximal or intercalary heterochromatin could influence

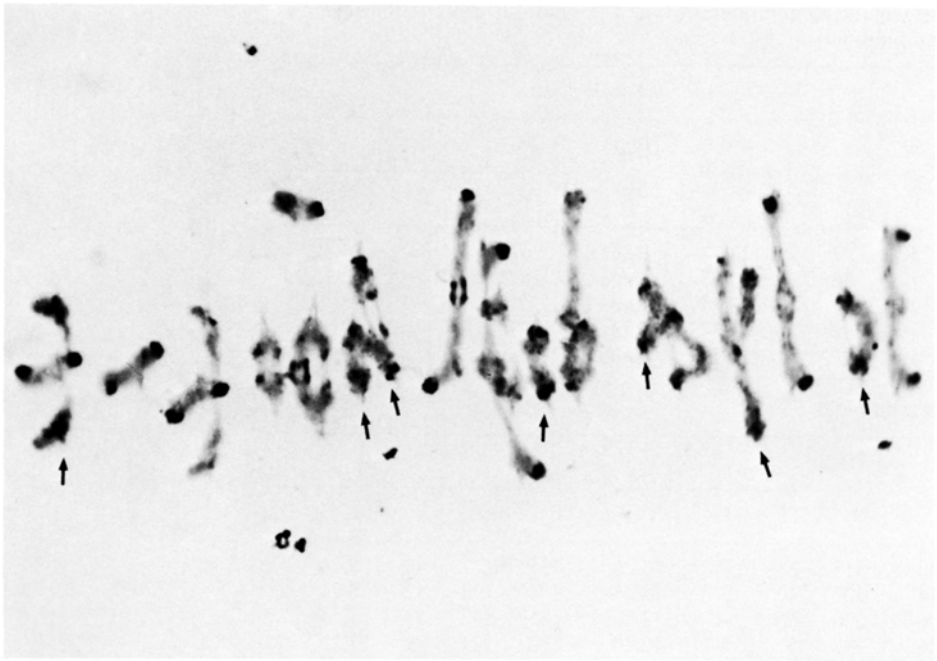

Fig. 3. C-banded meiotic metaphase cell of *Ae. ventricosa* × *S. cereale* amphiploid. *Arrows* designate the Un genome chromosomes

chromosome pairing and that effect was correlated with the size of the heterochromatin block. If this was true, one would expect the same pairing levels for Un and D genomes in *Ae. ventricosa* and in the amphiploid. From the results of Tables 1 and 2 it is clear that in the amphiploid there is a high pairing diminution for Un and low for D genome. Therefore, the single presence of heterochromatin in Un is not enough to explain the results found.

Roupakias and Kaltsikes (1977) inferred that C-heterochromatin on one chromosome can influence chromosome pairing in other chromosomes. This fact might explain all results observed because the addition of the rye genome to those of *Ae. ventricosa* would imply an increase in C-heterochromatin and, consequently, meiotic pairing would be lower, the most heterochromatic genomes being the most affected. However, it is very difficult to imagine how C-heterochromatin located in one chromosome can influence other ones.

On the other hand, cryptic interactions between the genomes of rye and *Aegilops* chromosomes could be considered to be one plausible explanation for pairing failures found as this has been observed by Orellana et al. (1984) in wheat-rye addition and substitution lines. In that case, the addition of any rye chromosome to wheat would produce a decrease in wheat-wheat chromosome pairing. A similar situation can occur in this amphiploid.

Moreover, specific interactions between rye and *Aegilops ventricosa* genotypes in regulating meiosis similar to those observed by Lelley (1979) in wheat-rye hybrid plants must be also taken into account.

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