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Genomic analysis in the genus *Aegilops*. II. Interspecific hybrids between polyploid species sharing two common genomes

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Hybrids between polyploid *Aegilops* species sharing two common genomes were analysed at metaphase I by using a C-banding technique in order to establish genome relationships. In all cases it allowed discrimination between associations of chromosomes with similar morphology and C-banding belonging to the same genomes (homomorphic associations) and associations involving different chromosomes (heteromorphic associations). In the hybrids involving *Ae. variabilis* and *Ae. kotschyi*, (UUSS), it was also possible to identify the U and S genomes, which are shared by the tetraploid species, and their analysis indicated that the genomes of both species are essentially unaltered. However, the data of the *Ae. crassa(6x)* × *Ae. vavilovii* (DDDMMN) hybrid showed that the divergences between the shared genomes are at present substantial despite their common origin. By contrast, in the case of the *Ae. triaristata(6x)* × *Ae. triaristata(4x)* (UUMMN) hybrid the data did not confirm that the hexaploid species arose from the tetraploid one.

Keywords: *Aegilops*, C-banding, chromosome associations at metaphase I, genomic analysis, interspecific hybrids.

Introduction

As indicated in the previous paper (see the Introduction in Cuñado 1993), the utilization of differential staining techniques makes possible the recognition of chromosomes and/or genomes in *Aegilops* species and, consequently, the identification of the chromosomes involved in meiotic associations of appropriate interspecific hybrids, thus permitting the analysis of their genomic relationships.

In the present work the meiotic behaviour of hybrids between tetraploid and hexaploid species of *Aegilops* sharing two common genomes is analysed by using the C-banding technique.

Materials and methods

The interspecific hybrids analysed were obtained by crossing tetraploid and/or hexaploid *Aegilops* species which shared two common genomes.

1 US, in *Ae. variabilis* (UUSS) × *Ae. variabilis var. typica* (UUSS), *Ae. variabilis* v. *typica* (UUSS) × *Ae. kostchyi* (UUSS) and reciprocal;

2 UM, in *Ae. triaristata(6x)* (UUMMNN) × *Ae. triaristata(4x)* (UUMM);

3 DM, in *Ae. crassa(6x)* (DDDDMM) × *Ae. vavilovii* (DDMMSS).

To designate the genomes of the different *Aegilops* species, the nomenclature proposed by Kimber & Tsunewaki (1988) was followed.

The handling of the hybrid seeds and the cytological techniques are described in the previous paper (Cuñado, 1993).

Results

From the comparison of the meiotic behaviour of the intraspecific hybrid *Ae. variabilis* × *Ae. variabilis v. typica* and the interspecific hybrids between *Ae. variabilis v. typica* and *Ae. kotschyi*, it was possible to analyse whether the U and S genomes, shared by the tetraploid species, have been altered during their evolution. In addition, the C-banding technique allows the associations of chromosomes belonging to the U genomes to be distinguished from those of chromosomes of the S genomes, which show a higher amount of heterochromatin (Fig. 1a) (Table 1) (see C-banding descriptions in Cuñado, 1992).

The three types of hybrid show a rather regular meiotic behaviour forming bivalents almost exclusively

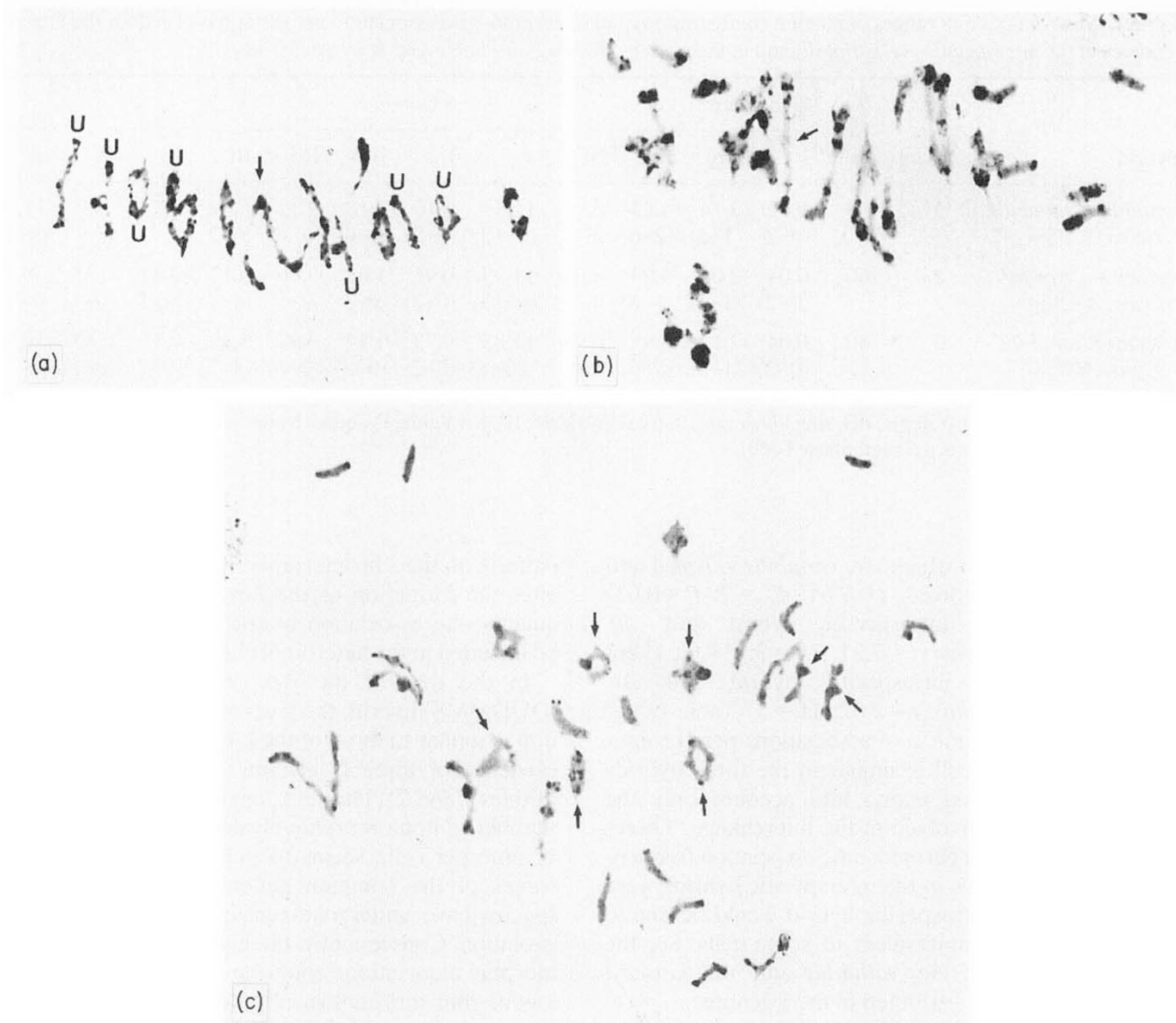

Fig. 1 C-banded metaphase I cells of interspecific *Aegilops* hybrids. (a) *Ae. variabilis* v. *typica* × *Ae. kotschyi* (UUSS); bivalents of the U genome are indicated by its symbol; arrow indicates a quadrivalent formed by S chromosomes. (b) *Ae. triaristata(6x)* × *Ae. triaristata(4x)* (UUMMN). (c) *Ae. crassa(6x)* × *Ae. vavilovii* (DDDMMS). In (b) and (c), arrows indicate homomorphic chromosome associations or bivalents.

at metaphase I even in *Ae. variabilis* × *Ae. kotschyi* hybrids where one quadrivalent (or one trivalent plus one univalent) formed by S chromosomes appears in some cells (Table 1) (Fig. 1a). So, one can conclude that the S genomes from these tetraploid species, *Ae. variabilis* and *Ae. kotschyi*, differ in a reciprocal translocation. These results are in agreement with those reported by Furuta (1981) although this author found a higher number of interchanges in some hybrids involving other varieties.

It is noticeable that there are differences in the total frequencies of chromosome association per metaphase I cell between the two reciprocal hybrids between *Ae. variabilis* and *Ae. kotschyi* ($t = 3.03$; d.f. = 2; $P < 0.05$). In contrast, the intraspecific hybrid of *Ae. variabilis* presents a level of chromosome associations similar to those of the *Ae. kotschyi* × *Ae. variabilis* hybrid ($t = 1.81$; d.f. = 3) but different to those of the reciprocal hybrid ($t = 9.02$; d.f. = 3; $P < 0.001$).

When the comparisons are made at the genomic level, it is observed that the means of associations between chromosomes of the U genomes are similar in the three hybrids. However, the frequencies of chromosome associations of the S genomes differ significantly

Table 1 Mean values and ranges of meiotic configurations and of chromosome association per metaphase I cell for the U and S genomes in the intraspecific *Ae. variabilis* and in the interspecific *Ae. variabilis* × *Ae. kostchyi* hybrids

Hybrid	No. plants	No. cells	U Genome					S Genome						
			I	IIro	IIri	IIt	$\bar{x}$	I	IIro	IIri	IIt	III	IV	$\bar{x}$
variabilis × *variabilis* v. *typica* (UUSS)	3	90	0.02 (0–2)	2.14 (1–5)	4.84 (2–6)	6.99 (6–7)	11.83 (9–13)	0.16 (0–2)	2.18 (1–4)	4.72 (3–6)	6.90 (6–7)	—	—	11.61 (9–13)
variabilis v. typica × *kotschyi* (UUSS)	2	60	0.04 (0–2)	2.05 (1–4)	4.93 (3–6)	6.98 (6–7)	11.93 (9–13)	0.95 (0–3)	1.62 (0–3)	3.50 (2–5)	5.12 (4–7)	0.47 (0–1)	0.35 (0–1)	9.78 (8–12)
kotschyi × *variabilis* v. *typica* (UUSS)	2	60	0.04 (0–2)	2.12 (1–3)	4.87 (3–6)	6.98 (6–7)	11.85 (10–13)	0.73 (0–2)	1.13 (0–3)	3.97 (3–5)	5.10 (4–7)	0.45 (0–1)	0.43 (0–1)	10.38 (9–12)

(I, univalents; IIro, rod bivalents, IIri, ring bivalents, IIt, total bivalents, III, trivalents; IV, quadrivalents; $\bar{x}$, mean number of chromosome associations per metaphase I cell).

in the two hybrids in which *Ae. variabilis* v. *typica* and *Ae. kotschyi* are involved ($t = 7.01$; d.f. = 2; $P < 0.01$) and between the intraspecific hybrid and *Ae. variabilis* × *Ae. kotschyi* ($t = 7.21$; d.f. = 3; $P < 0.01$) but not between the intraspecific hybrid and *Ae. kotschyi* × *Ae. variabilis* ($t = 2.02$; d.f. = 3) (Table 1).

Nevertheless, the mean of associations per chromosome arm and per cell is similar in the three hybrids when it is calculated taking into account only the chromosomes not involved in the interchange. Therefore, the fact that the chromosome association frequencies of the S genomes in the interspecific hybrids were lower than in the intraspecific hybrid would be due to the existence of a multivalent in some cells. So, the differences between *Ae. variabilis* and *Ae. kotschyi* could be exclusively attributed to the S genome.

On the other hand, in the *Ae. triaristata(6x)* × *Ae. triaristata(4x)* (UUMMN) and *Ae. crassa(6x)* × *Ae. vavilovii* (DDDMMS) hybrids, the staining technique used allowed the associations between chromosomes with similar morphology and C-banding pattern belonging to the common genomes (homomorphic associations) and those involving different chromosomes (heteromorphic associations) to be distinguished (Table 2) (Fig. 1, b, c).

In the *Ae. triaristata(6x)* × *Ae. triaristata(4x)* (UUMMN) hybrid, the frequencies of homomorphic and, even, the total associations per metaphase I cell are lower than those of the UUSS hybrids mentioned above (Tables 1 and 2). This result seems to indicate that the genomes U and M, present in the tetraploid and hexaploid forms of *Ae. triaristata*, have suffered substantial alterations. In addition, a maximum number of five homomorphic bivalents per metaphase I cell has been observed which can be attributed to changes that occurred in the morphology and/or C-banding pattern of the chromosomes from *Ae. triaristata(4x)* after the formation of the hexaploid species. Consequently, the association of such chromosomes would be included in the heteromorphic class.

In the case of the *Ae. crassa(6x)* × *Ae. vavilovii* (DDDMMS) hybrid, the level of chromosome association is similar to those of the UUSS hybrids despite the existence of three D genomes and two M genomes (Tables 1 and 2). This fact, together with the rather low number of homomorphic bivalents found (a maximum of nine per cell), seems to indicate that the chromosomes of the common genomes of both hexaploid species have undergone certain changes during their evolution. Consequently, the high frequency of heteromorphic associations could be partially due to homologous (but morphologically different) chromosomes, whereas the association frequency between homoeologous chromosomes from the genomes D, M and S should be rather low.

It is worth mentioning the high frequency of trivalents formed by two homomorphic chromosomes and a third heteromorphic but not very different one (Fig. 1c) (Table 2). These trivalents could probably be formed by chromosomes of the three D genomes, in which case one can assume that one of the D genomes has changed in relation to the other two but their meiotic affinity is maintained. However, some of the trivalents as well as the heptavalents observed could be attributed to the existence of interchanges (Table 2).

Discussion

Hybrids involving Ae. variabilis *and* Ae. kostchyi

The chromosome association frequency observed in the intervarietal *Ae. variabilis* × *Ae. variabilis* v.

Table 2 Mean values and ranges of meiotic configurations and of homomorphic and heteromorphic chromosome associations per metaphase I cell in the *Ae. triaristata(6x)* × *Ae. triaristata(4x)* and *Ae. crassa(6x)* × *Ae. vavilovii* hybrids

Hybrid	No. plants	No. cells	I	Homomorphic associations				Heteromorphic associations						
				IIro	IIri	IIt	$\bar{x}$	IIro	IIri	III	IV	V	VI+VII	$\bar{x}$
triaristata(6x) × *triaristata(4x)* (UUMMN)	8	250	12.61 (7–21)	1.37 (0–4)	1.38 (0–3)	2.78 (0–5)	4.28 (0–4)	4.85 (1–9)	0.38 (0–2)	1.46 (0–4)	0.41 (0–2)	0.07 (0–1)	—	9.98 (5–15)
crassa(6x) × *vavilovii* (DDDMMS)	2	100	13.09 (9–17)	0.64 (0–3)	5.38 (3–8)	6.02 (4–9)	14.86 (11–19)	1.43 (0–4)	0.96 (0–2)	2.33 (0–5)	0.50 (0–2)	0.29 (0–2)	0.27 (0–1)	8.99 (5–13)

(I, univalents; IIro, rod bivalents; IIri, ring bivalents; IIt, total bivalents; III, trivalents; IV, quadrivalents; V, pentavalents; VI + VII, hexavalents plus heptavalents; $\bar{x}$, mean number of chromosome associations per cell).

typica(UUSS) hybrid is lower than that reported in the parental *Ae. variabilis* v. *typica*, 25.30 (Cuñado, 1992). This behaviour is similar to that of intervarietal hybrids of common wheat, *Triticum aestivum* (Waranabe, 1962; Dvorak & McGuire, 1981; Vega *et al.*, 1987). This decrease in association frequency (hybrid desynapsis) cannot be attributed to structural changes since multivalents are not observed (Table 1). However, some authors accept the possibility of the existence of cytologically undetectable chromosomal differences (Dvorak & McGuire, 1981; Dvorak & Appels, 1982).

On the other hand, polyploid species of *Aegilops* are autogamous and, consequently, homozygous. The heterozygosity produced in the intervarietal hybrid of *Ae. variabilis* might be responsible for the decrease in association frequency as suggested by Riley & Law (1965) in intervarietal hybrids of common wheat. Both factors could lead to a decrease in the frequency of chromosome association not only in the intervarietal hybrid but also in the interspecific hybrids between *Ae. kotschyi* (UUSS) and *Ae. variabilis* (UUSS) (Table 1).

When the mean of chromosome associations of the U and S genomes in the three UUSS hybrids is compared to the parental *Ae. variabilis* v. *typica*, 12.50 in the U genome and 12.80 in the S genome, (Cuñado, 1992), it was observed that the S genome shows a higher decrease than the U genome. In addition, this reduction in the U genome is similar in the three hybrids, while in the S genome it is higher in the two interspecific hybrids (Table 1). This behaviour can be attributed to the presence of a multivalent in many of the cells due to a translocation difference between the two species (Fig. 1a), since the association frequency between the remaining five pairs of chromosomes of the S genome is similar to that of the intraspecific hybrid.

Several authors (Dvorak & McGuire, 1981; Ferrer *et al.*, 1984; Vega *et al.*, 1987) reported a differential behaviour of the chromosomes of intervarietal wheat hybrids, suggesting that the chromosomes with a greater content of C-heterochromatin showed a greater decrease in their association frequencies. In *Ae. variabilis* and *Ae. kostchyi.*, the S genome has more heterochromatin content than the U genome (Fig. 1a), however this would not explain the different levels of chromosome associations between the two reciprocal interspecific hybrids (Table 1).

Lucas & Jahier (1988) found differences in the meiotic behaviour between reciprocal crosses of some diploid *Aegilops* species attributable, at least partially, to nuclear–cytoplasmic interactions. Although a similar reasoning could be applied to the reciprocal hybrids between *Ae. variabilis* and *Ae. kostchyi*, it seems difficult to explain that the cytoplasms of the two species influence differentially the meiotic behaviour of the S genomes but not the U genomes.

Kimber & Feldman (1987) consider that *Ae. variabilis* and *Ae. kotschyi* are two species closely related although some data seem to suggest that they are actually the same species. For instance, the intraspecific variability found in the acid phosphatase electrophoretic pattern of several lines of *Ae. variabilis* is large and Nakai & Tsuji (1984) observed that the pattern of one such line was similar to that of *Ae. kotschyi.* In addition, large variability of chromosomal interchanges (Kawahara, 1986) and association frequencies at metaphase I (Furuta, 1981) in different lines of both species has been found. The results from the three types of UUSS hybrids analysed in this work indicate that the differences in the metaphase I associations between both species are not greater than between the two varieties of the same species and, in this case, *Ae. kotschyi* and *Ae. variabilis* v. *typica* seem to differ in one reciprocal translocation between chromosomes of the S genomes (Table 1) (Fig. 1a).

Ae. triaristata(6x) ×Ae. triaristata(4x) *hybrid*

According to Kihara (1963), *Ae. triaristata(6x)* (UUMMNN) arose from a cross between *Ae. triaristata(4x)* (UUMM) and a diploid species related to *Ae. uniaristata* (NN); in consequence, the hybrid between the hexaploid and the tetraploid forms of *Ae. triaristata* should have repeated the genomes U and M. However, the frequencies of homomorphic chromosome associations and bivalents are lower than those of the UUSS hybrids mentioned above (Tables 1 and 2). Likewise, these frequencies and, even, the total frequencies are lower than those observed in *Ae. triuncialis* × *Ae. variabilis* (UUCS) and *Ae. biuncialis* × *Ae. triuncialis* (UUMC) hybrids in which only the U genome was in double dose (Cuñado, 1993). These results do not seem to confirm the fact that the tetraploid and hexaploid forms of *Ae. triaristata* share two genomes. Even considering only one genome in common (U genome), there seems to be a greater differentiation between tetraploid and hexaploid *Ae. triaristata* than between the tetraploid species mentioned above.

One possible explanation for these results could be that the tetraploid form which gave rise to the hexaploid form was very different to the present time tetraploid species. However, this seems not be right because Kimber & Yen (1989), analysing the meiotic behaviour of *Ae. triaristata(4x)* × *Ae. umbellulata*, demonstrated that the U genome of *Ae. triaristata(4x)* is practically unchanged in relation to its diploid donor, *Ae. umbellulata* (UU). Summarizing, one can conclude that after the origin of *Ae. triaristata(6x)*, the chromosomes arising from the tetraploid parental species suffered many structural (morphological and C-banding patterns) changes or else that *Ae. triaristata(6x)* did not come from *Ae. triaristata(4x)*. In any case, it does not seem reasonable to maintain either the present genomic symbols or the names of the two species; in fact, Kimber & Feldman (1987) proposed renaming the tetraploid and hexaploid species *Ae. neglecta* and *Ae. recta*, respectively.

Ae. crassa(6x) × Ae. vavilovii hybrid

It is accepted that *Ae. crassa(6x)* (DDDDMM) and *Ae. vavilovii* (DDMMSS) arose from *Ae. crassa(4x)* (DDMM) and, consequently, they share the genomes D and M. According to Kihara (1963) the third genome of *Ae. crassa(6x)* arose from *Ae. squarrosa* (DD) but differed from the other D genome. However, from isozymatic analyses Nakai (1982) suggested that they could come from a duplication of the D genome of *Ae. crassa(4x)*. In the case of *Ae. vavilovii* (DDMMSS), its third genome arose from a diploid species having the S genome, probably *Ae. longissima* (Kimber & Feldman, 1987).

The analysis of the meiotic behaviour of the hybrids between both hexaploid species with *Ae. squarrosa* and with the tetraploid species carrying the D genome suggests that the two genomes of *Ae. crassa(6x)* are very similar to each other but rather different from those of *Ae. squarrosa* (DD), *Ae. cylindrica* (DDCC) and *Ae. ventricosa* (DDNN) the difference being even greater in relation to the D genome of *Ae. vavilovii* (Chapman & Miller, 1978; Espinasse & Kimber, 1981; Kimber & Zhao, 1983; Zhao & Kimber, 1984).

The analysis of the interspecific *Ae. crassa(6x)* × *Ae. vavilovii* (DDDMMS) hybrid seems to confirm that both species arose from the same tetraploid species, *Ae. crassa* (DDMM), although there are some morphological and/or C-banding pattern differences between several chromosomes belonging to the D and M genomes (Table 2) (Fig. 1c). The existence of chromosomal rearrangements is evident both in *Ae. vavilovii* and *Ae. crassa(6x)* (Kimber & Zhao, 1983), thus explaining the high frequency of multivalents observed in the hybrid (Table 2). Although the occurrence of preferential associations between the chromosomes of the two D genomes from *Ae. crassa(6x)* is probable, they seem to be very similar to the D genome of *Ae. vavilovii* since some metaphase I cells showing five trivalents have been found (Table 2). These trivalents were formed by two homomorphic chromosomes, with the third one being not very different from the morphological point of view.

In summary, from the results obtained in this work it can be concluded that the utilization of the C-banding technique to the analysis of chromosome association at metaphase I in hybrid combinations allows one to draw conclusions on the affinity and evolutionary relationships of the genomes shared by the parental species. These results suggest the necessity for revision of cytotaxonomy data of the genus *Aegilops* based on conventional staining techniques as well as the obtention and analysis of new hybrid combinations.

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