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Metaphase I bound arms frequency and genome analysis in wheat-*Aegilops* hybrids

1. *Ae. variabilis*-wheat and *Ae. kotschyi*-wheat hybrids with low and high homoeologous pairing

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Summary. Meiotic associations of different wheat-*Aegilops variabilis* and wheat-*Ae. kotschyi* hybrid combinations with low and high homoeologous pairing were analyzed at metaphase I. Five types of pairing involving wheat and *Aegilops* genomes were identified by using C-banding. A genotype that seems to promote homoeologous pairing has been found in *Ae. variabilis* var. *cylindrostachys*. Its effect is detectable in the low pairing hybrids but not in the high ones. Pairing affinity has been analyzed on the basis of metaphase I associations in the low and high homoeologous pairing hybrids, and in bivalents and multivalents in the high pairing hybrids. The results indicate that the amount of bound arms of each type of identifiable association relative to the total associations formed (relative contribution) was not maintained, either between the different levels of pairing (low and high) or between different meiotic configurations (bivalents and multivalents). These findings seem to indicate that quantifications of genomic relationships based on the amount of chromosome pairing at metaphase I must be carefully done in this type of hybrid combinations.

Key words: Wheat-*Aegilops* hybrids – Genome analysis – Homoeologous pairing

Introduction

The determination of genome relationships on the basis of homoeologous pairing taken as a measurement of the affinities between different species has been very useful in establishing the evolutionary relationships in many taxa (Rosenberg 1909; Kihara 1954; Rajhathy and Thomas 1974).

The most accurate procedure for genomic analysis is to produce triploid hybrids between allotetraploids and their putative diploid ancestors. The formation of a basic number of bivalents is the triploid hybrid would usually be interpreted as clear evidence for genome homology. Thus, genomic analysis is based on measurements of the total amount of chromosome pairing per cell. However, the determination of genomic homology becomes more difficult in high polyploids, when the mean of bivalents per cell in the hybrids is not the basic number or when the frequencies of multivalents are considerable. In addition, pairing frequencies could be altered either by the existence of gene(s) that regulate(s) homoeologous pairing (Sears 1976) or by environmental factors (Ress and Naylor 1960; Couzin and Fox 1974). This is essentially the current picture of most hybrids analyzed in allotetraploid series. For these reasons, theoretical models have been developed to study genome relationships in Triticineae (Driscoll 1979; Kimber and Alonso 1981; Kimber et al. 1981; Alonso and Kimber 1981; Espinasse and Kimber 1981). All of them measure the similarities of two or more genomes on the basis of the frequencies of metaphase I pairing, assuming very restrictive premises, mainly due to the impossibility of distinguishing chromosomes of different genomes with traditional staining techniques.

C-banding techniques have demonstrated their potential importance in analyzing genome affinities directly. However, there are very few cases in the literature in which such studies have been carried out (Cuñado et al. 1986; Orellana et al. 1988). In this work we use a C-banding technique to study the meiotic behavior of specific metaphase I associations involving different genomes in

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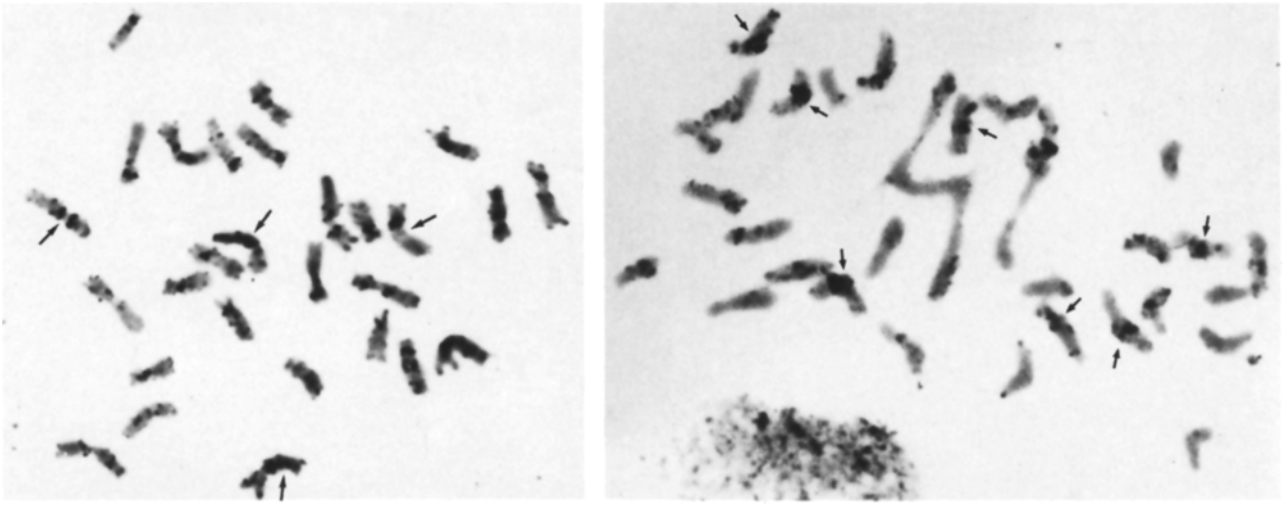

Fig. 1. Mitotic metaphase of *Aegilops variabilis* var. *cylindrostachys*. *Arrows* indicate chromosomes with a very similar C-banding pattern to those of the B genome

Fig. 2. Metaphase I of CSAevt hybrid plant. *Arrows* indicate chromosomes of the B genome

low and high homoeologous pairing wheat-*Aegilops kotschyi* and wheat-*Ae. variabilis* hybrid combinations.

Materials and methods

Chinese Spring euploid plants (CS) and high pairing mutants (*ph1b*) (Sears 1977), both with genome constitution AABBDD, were crossed as females with *Aegilops kotschyi* var. *leptostachya* (Aekl), *Ae. variabilis* var. *typica* (Aevt), and *Ae. variabilis* var. *cylindrostachys* (Aevc) (all with genome constitution UUSS), as males, in order to obtain wheat-*Aegilops* hybrid plants. For meiotic cells, anthers of wheat-*Aegilops* hybrids were fixed in acetic:ethanol (1:3) and stored 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

The differential C-banding patterns of wheat and *Aegilops* chromosomes allow the following three different chromosome groups to be distinguished at meiosis in the wheat-*Aegilops* hybrid plants: (i) A and D genomes of Chinese Spring characterized by the absence of C-heterochromatin markers, (ii) B genome of wheat formed by chromosomes with prominent and pericentrometric C-heterochromatin blocks, and (iii) chromosomes of U and S genomes with scattered and disperse C-heterochromatin.

In the hybrids (genome constitution ABDUS), ten types of association at metaphase I could be found, but only five groups are distinguishable, namely, associations between chromosomes of A and D (A-D), A or D and U or S (AD-US), U or S and B (US-B), A or D and B (AD-B), and U and S genomes (U-S).

Chromosomes of A and D genomes were pooled because they showed an undistinguishable C-banding pattern. Genomes U and S were also considered as a whole due to their very similar C-banding patterns, in spite of the fact that both could be differentiated at mitosis by chromosome size (see Fig. 1); however, these differences are not expressed at metaphase I where the degree of chromosome condensation makes identification difficult (see Figs. 2 and 3).

In all hybrids, chromosomes of the B genome were easily distinguishable, except for those in which *Ae. variabilis* var. *cylindrostachys* was used. In these cases, two chromosomes (probably S chromosomes) with a very similar C-banding pattern to those of the B genome were observed, and subsequently these were considered as chromosomes of the B genome in all data scored. This assumption leads to an increase in AD-B frequencies and to a decrease in AD-US and U-S types, the US-B type being affected when associations take place between both chromosomes and the U genome, while associations between the two B-like C-banding chromosomes of *Ae. variabilis* and those of the B genome of wheat can be easily identified (see Fig. 3a).

Table 1 shows the number of ring (R) and rod (O) bivalents, univalents, and multivalents for each type of distinguishable configuration observed in the hybrids with low (CSAekl, CSAevt, and CSAevc) and high (ph1bAekl, ph1bAevt, and ph1bAevc) homoeologous pairing. As expected, meiotic associations at metaphase

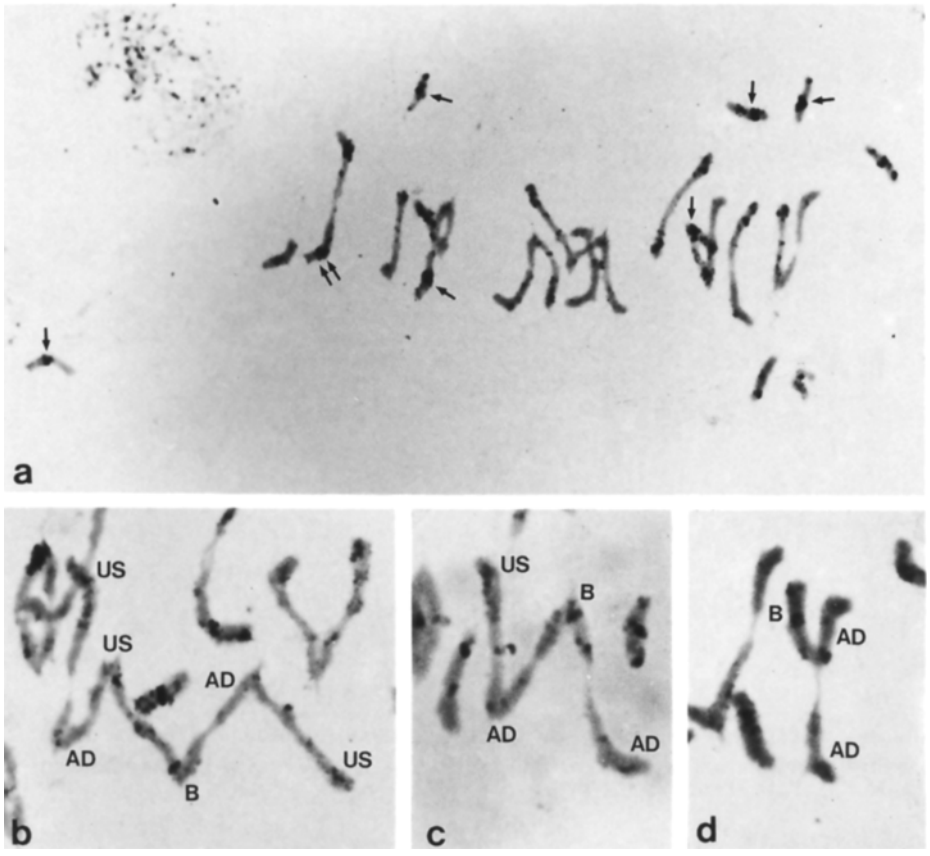

Fig. 3. **a** Metaphase I of ph1bAevc hybrid plant. *Arrows* indicate chromosomes of the B genome. *Double arrows* indicate: **a** bivalent formed by one B and one S chromosome, **b** hexavalent, **c** quadrivalent, and **d** Y-shaped trivalent. Chromosomes implicated in each configuration are indicated

I increase in hybrids in which the *Ph* locus was inactive (Fig. 3a).

In the hybrids with low homoeologous pairing, multivalents are mainly represented by V-shaped trivalents, and only one cell with one quadrivalent was observed in CSAevt (see Fig. 2). However, more complex configurations were frequently observed in the hybrids with high homoeologous pairing. In these cases, the maximum number of chromosomes one would expect to be associated in the largest meiotic configuration is five, if all chromosomes for a given homoeologous group are paired. However, a certain number of hexavalents has been found (see Fig. 3b). This type of configuration could only be explained by the existence of translocations between the different genomes in the hybrids. This assumption is in agreement with the appearance of trivalents formed only by the chromosomes of A and D genomes or only by those of U and S genomes, as observed in this work.

The existence of reciprocal translocations involving different homoeologous groups has been described in wheat (Sears 1954; Baker and McIntosh 1966; Kobrehel and Feillet 1975) and in *Ae. variabilis* and *Ae. kotschyi* (Furuta 1981; Kawahara 1988). It is also well known that this cytogenetic mechanism has accompanied the evolutionary process of the Triticineae.

From the meiotic configurations observed at metaphase I, the number of associations for the five distinguishable groups (A-D, AD-US, AD-B, US-B, and U-S) could be estimated as the minimum number of chiasmata that could explain each configuration. Obviously, configurations in which more than two chromosome arms were associated at the same region, as in Y-shaped and frying pan trivalents, were considered undetermined (Un) (Fig. 3d).

Tables 2 and 3 show the number of bonds observed for each identifiable type in the hybrid plants with low (CSAekl, CSAevt, CSAevc) and high (ph1bAekl, ph1bAevt, ph1bAevc) homoeologous pairing, respectively. In the hybrids with low homoeologous pairing, the number of bound arms in multivalents has not been included because of their low frequency.

In both CSAekl and CSAevt, the frequencies of bound arms per cell at metaphase I were very similar, since no significant deviation was detected. However, the mean number of bonds per cell seems to be different and higher in CSAevc, the differences being significant for most types when *t*-tests were performed (see Table 4).

These results indicate that the genotype of *Ae. variabilis* ssp. *cylindrostachys* (Aevc) used in this work increases meiotic associations between homoeologous chromosomes.

Table 1. Number of the different meiotic configurations observed for the five distinguishable types of pairing in the low and high homoeologous pairing hybrid crosses

Cross	No. plants	No. cells	Meiotic configurations																
			Bivalents										Univalents			Multivalents			
			A-D		AD-US		AD-B		US-B		U-S		US	AD	B	III	IV	V	VI
			R	O	R	O	R	O	R	O	R	O							
CSAekl	5	250	2	135	–	186	–	13	1	97	1	63	3,078	3,013	1,639	8	–	–	–
CSAevt	3	150	3	50	–	100	–	2	4	56	1	46	1,837	1,884	927	4	1	–	–
CSAevc	5	250	11	149	2	300	–	35	–	104	2	112	2,829	2,813	1,606	24	–	–	–
Total	13	650	16	334	2	586	–	50	5	257	4	221	7,744	7,710	4,172	36	1	–	–
ph1bAekl	3	87	84	86	13	164	6	41	31	131	4	60	408	339	319	199	30	2	2
ph1bAevt	3	86	92	84	12	161	5	66	56	110	19	50	337	224	259	189	61	9	4
ph1bAevc	1	25	30	26	8	54	1	3	7	54	2	20	84	94	92	54	7	1	–
Total	7	198	206	196	33	379	12	110	94	295	25	130	829	657	670	442	98	12	6

R: ring bivalents, O: open bivalents, III: trivalents, IV: quadrivalents, V: pentavalents, VI: hexavalents

Table 2. Number of bound arms observed for each type of distinguishable association observed at metaphase I in low homoeologous pairing hybrids plants

Plant	Associations in bivalents						Total associations					
	A-D	AD-US	AD-B	US-B	U-S	Total	A-D	AD-US	AD-B	US-B	U-S	Total
CSAekl-1	41	46	3	21	16	127	44	53	3	21	16	137
CSAekl-2	16	41	3	17	11	88	17	42	3	17	11	90
CSAekl-3	33	34	3	18	17	105	34	35	3	18	17	107
CSAekl-4	24	21	4	14	15	78	24	21	4	14	15	78
CSAekl-5	25	44	–	29	6	104	25	46	–	29	6	106
Total	139	186	13	99	65	502	144	197	13	99	65	518
CSAevt-1	22	29	–	20	13	84	22	30	–	22	13	87
CSAevt-2	17	33	1	22	25	98	18	36	1	22	25	102
CSAevt-3	17	38	1	22	10	88	18	40	1	22	11	92
Total	56	100	2	64	48	270	58	106	2	66	49	281
CSAevc-1	44	66	11	23	21	165	47	71	12	24	25	179
CSAevc-2	20	56	7	17	14	114	21	59	8	17	15	120
CSAevc-3	48	73	8	16	29	174	50	80	9	16	29	184
CSAevc-4	27	61	7	22	31	148	27	66	7	23	35	158
CSAevc-5	32	48	2	26	21	129	34	54	2	26	21	137
Total	171	304	35	104	116	730	179	330	38	106	125	778

In the high-pairing hybrids, only plants ph1bAekl and ph1bAevt could be compared, because data from only one plant could be analyzed for ph1bAevc. The comparisons between the former two hybrids seem to indicate a similar behavior at metaphase I, since the differences were never significant when *t*-tests were performed (Table 4). Although no comparison could be made with ph1bAevc, it seems to be clear that the mean number of bonds per cell for each type is no higher than that observed in ph1bAekl or ph1bAevt. These findings indicate that the increase in homoeologous associations observed in the low-pairing hybrids due to the Aevc genotype is not detectable in the high-pairing ones.

The existence of gene(s) that promote(s) homoeologous pairing in wheat hybrid combinations is known (Sears 1976). The promotion effect could be due to the activity of major genes as those described in *Ae. longissima* (Mello-Sampayo 1971), *Ae. caudata* (Upadhya 1966), *Ae. mutica* (Riley 1966; Dover and Riley 1972), and *Ae. speltoides* (Riley et al. 1961), or to a polygenic system

Table 3. Number of bound arms for each type of distinguishable association observed at metaphase I in high homoeologous hybrid plants. Undetermined associations (Un)

Plant	Associations in bivalents						Associations in multivalents						Total associations						
	A-D	AD-US	AD-B	US-B	U-S	Total	A-D	AD-US	AD-B	US-B	U-S	Total	A-D	AD-US	AD-B	US-B	U-S	Un	Total
ph1bAekl-1	76	68	18	70	28	260	26	73	9	19	30	157	102	141	27	89	58	14	417
ph1bAekl-2	84	68	20	83	19	274	30	81	17	21	24	173	114	149	37	104	43	2	447
ph1bAekl-3	94	54	15	40	21	224	43	81	3	18	21	166	137	135	18	58	42	2	390
Total	254	190	53	193	68	758	99	235	29	58	75	496	353	425	82	251	143	18	1,254
ph1bAevt-1	93	59	26	86	19	283	36	68	15	25	16	160	129	127	41	111	35	4	443
ph1bAevt-2	105	60	33	67	49	314	55	95	25	19	35	229	160	155	58	86	84	24	543
ph1bAevt-3	70	66	17	69	20	242	46	102	14	28	17	207	116	168	31	97	37	10	449
Total	268	185	76	222	88	839	137	265	54	72	68	596	405	450	130	294	156	38	1,435
ph1bAevc-1	86	70	5	68	24	253	22	72	7	14	14	129	108	142	12	82	38	4	382

such as those found in *Ae. speltoides* (Dvorák 1972; Chen and Dvorák 1984) and *Secale cereale* (Feldman 1968; Lelley 1976; Dvorák 1977; Cuadrado and Romero 1984).

The differences detected here are probably due to the existence of a polygenic system, since the levels of pairing are too low to consider them as qualitative effects, although the effect of a single gene can result in the observed differences. Moreover, this is in agreement with the variation of the mean number of bound arms per cell observed by other authors in the same type of hybrids (Driscoll and Quinn 1968; Sears 1977; McGuire and Dvorák 1982; Ceoloni et al. 1986).

The inactivation of the *Ph* locus could produce an increase in pairing in such a way that the small differences due to the promoting effect of the Aevc genotype could not be detected in the high-pairing hybrids. On the other hand, the effects of the *ph1b* allele and the promoter could not be additive (Riley and Law 1965).

Generally, the five types of specific association identified in this work showed a tendency to exhibit a certain relative order, i.e., AD-US > A-D > US-B > U-S > AD-B, that is generally maintained in low and high homoeologous pairing hybrids.

The AD-US excess can be explained by the higher number of genomes involved in this type of association, whereas in the remaining types, the number of probable chromosomes to be associated is lower and consequently the number of possible combinations to pair is also lower.

The types of association in which equal numbers of genomes are involved could provide information about the genome affinities expressed, since the same mean number of bound arms per cell is expected for all types if there is no preferential pairing (Table 5). The comparisons between AD-B and US-B types, both in the low- and high-pairing hybrids, indicate that affinities between the U or S genome and the B genome of wheat are higher than between the A or D and B genomes. It has been reported that genomes U and S of *Ae.variabilis* and *Ae. kotschyi* derived from those of *Ae. umbellulata* and *Ae. sharonensis,* respectively (Tanaka 1955). *Ae. sharonensis* and other diploid *Aegilops* species of section Sitopsis are the probable donors of the B genome to wheat (see Kerby and Kuspira 1987, 1988 for a review). Thus, genome S of *Ae. variabilis* and *Ae. kotschyi* could be very closely related to the B genome of wheat and is probably responsible for the preferential pairing found.

Likewise, associations between A and D genomes are higher than those between U and S genomes (Table 5). It is well known that A and D genomes pair more frequently than they do with the B genome. From metaphase I data is seems clear that both genomes are more closely related to each other than US-B genomes, although A and D genomes seem to be less related phylogenetically

Table 4. Mean number of bound arms per cell for each type of distinguishable association observed at metaphase I in both low and high homoeologous pairing crosses. The comparisons among crosses within the same level of pairing are also included

Cross	Mean number of associations per cell						
	Type of association						
	A-D	AD-US	AD-B	US-B	U-S	Total	
CSAekl	0.58	0.79	0.05	0.40	0.26	2.07	
CSAevt	0.39	0.71	0.01	0.44	0.33	1.87	
CSAevc	0.72	1.32	0.15	0.42	0.50	3.11	
ph1bAekl	4.05	4.90	0.94	2.90	1.66	14.45	
ph1bAevt	4.69	5.24	1.50	3.43	1.79	16.65	
ph1bAevc	4.32	5.68	0.48	3.28	1.52	15.44	
Comparisons	*t*-values						*df*
CSAekl-CSAevt	1.504	0.538	2.064	0.645	0.797	0.729	6
CSAekl-CSAevc	0.961	3.748**	2.831*	0.432	3.029*	3.307*	8
CSAevt-CSAevc	2.188	4.772**	3.169*	0.302	1.561	3.737**	6
ph1bAekl-ph1bAevt	1.490	0.720	1.850	0.910	0.230	2.130	4

* Significant at the 5% level
** Significant at the 1% level

Table 5. Comparisons by paired *t*-tests between the mean number of associations per cell observed for those types involving the same number of genomes (AD-B/US-B, A-D/U-S) in all crosses analyzed

Cross	Type of association			
	AD-B	US-B	A-D	U-S
CSAekl	0.05	0.40	0.58	0.26
t-value (*df*=4)	5.349**		4.061*	
CSAevt	0.01	0.44	0.39	0.33
t-value (*df*=2)	63.999***		0.596	
CSAevc	0.15	0.42	0.72	0.50
t-value (*df*=4)	4.518*		1.954	
Total	0.08	0.42	0.59	0.37
t-value (*df*=12)	9.345***		3.682**	
ph1bAekl	0.94	2.90	4.05	1.66
t-value (*df*=2)	6.235*		5.375*	
ph1bAevt	1.50	3.43	4.69	1.79
t-value (*df*=2)	3.884		11.938**	
Total	1.12	3.18	4.36	1.70
t-value (*df*=6)	8.144***		12.363***	

[a] Data from ph1bAevc are also included
* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

to each other than S and B genomes (Sears 1966; Dvorák 1976; McGuire and Dvorák 1982).

If the mean number of associated arms at metaphase I is a good indication of the affinities between the different genomes that are in competition to pair, one can expect that these affinities would be maintained in hybrids with low- and high-pairing, as well as in different meiotic configurations within the same level of pairing. Obviously, the mean number of associations per cell alone is not an accurate measure of their affinities. The between-plant differences with respect to the levels of pairing (low and high) make it necessary to develop a method that takes the relative contribution of each type of bond analyzed at metaphase I into account. Thus, we consider that using the mean number of bonds relative to the total associations formed (relative contribution) may be the best way to estimate genomic affinities.

The comparisons carried out between genotypes within the low-pairing hybrids were only significant for US-B and AD-B types when the mean numbers of bonds relative to the total associations of CSAevt and CSAevc formed were compared (AD-B CSAevt mean=0.01, CSAevc mean=0.05, $t=2.75$, $0.05>P>0.01$, $df=6$; US-B CSAaevt mean=0.24, CSAevc mean=0.14, $t=4.31$, $0.01>P>0.001$, $df=6$). In these hybrids no distinction between different meiotic configurations, namely, bivalents and multivalents, was made due to the low frequency of multivalents found. The existence of two B-like C-banding chromosomes in the hybrids in which *Ae. variabilis* var. *cylindrostachys* (Aevc) was used could explain the excess of AD-B bonds found. However, the influence of these two chromosomes does not seem to be too high, because we should also expect deviations for AD-US, US-B, and U-S types of association, deviations that were not observed in this work.

In the hybrids with high homoeologous pairing, only plants of the crosses ph1bAekl and ph1bAevt were compared and no significant deviation was obtained, either when bivalents or multivalents were considered. These

Table 6. Comparisons between the relative contributions in bivalents and multivalents for all types of distinguishable association in the high homoeologous pairing crosses. In all cases paired, *t*-tests were performed

Cross	Mean number of associations/Total associations									
	A-D		AD-US		AD-B		US-B		U-S	
	Biv	Mult	Biv	Mult	Biv	Mult	Biv	Mult	Biv	Mult
ph1bAekl	0.34	0.20	0.25	0.47	0.07	0.06	0.25	0.12	0.09	0.15
t-value ($df=2$)	12.12**		15.37**		0.43		4.06		5.27*	
ph1bAevt	0.32	0.23	0.22	0.44	0.09	0.09	0.27	0.13	0.10	0.11
t-value ($df=2$)	9.83*		4,414.57***		0.00		24.25**		0.55	
ph1bAevc	0.34	0.17	0.28	0.56	0.02	0.05	0.27	0.12	0.09	0.11
Total	0.33	0.21	0.24	0.47	0.07	0.07	0.26	0.12	0.10	0.13
t-value ($df=6$)	8.65 ***		23.00***		0.00		10.69***		2.59*	

[a] Data from ph1bAevc are also included
* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

results indicate that the relative contributions of the different types of association to the total bonds are maintained quite well at the same level of pairing.

In order to determine whether or not the relative contributions are maintained in different meiotic configurations within the same level of pairing, the mean number of bonds for each type of association was compared between bivalents and multivalents in the high homoeologous pairing hybrids, where the frequency of both types of configuration is high enough to use paired *t*-tests (Table 6). In most of the comparisons, the relative contributions differed in bivalents and multivalents, which leads to the conclusion that the contribution of each type of association is not maintained in the same weight in different meiotic configurations. For example, if the associations occur between A and D genomes, the relative contribution of this type is higher in bivalents than in multivalents, and the same behavior can be observed in associations formed between U or S genomes with the genome B. However, types AD-US and U-S are higher in multivalents. This discrepancy observed between bivalents and multivalents is due, in part, to the presence of A-D and US-B ring bivalents.

Driscoll et al. (1979), analyzing meiotic behavior of Chinese Spring × *Ae. variabilis*, found an excess of ring bivalents at the expense of the number of multivalents expected under their mathematical model. The discrepancy between observed and expected closed bivalents was numerically greater when chromosome 5B was lacking. This is probably due to the existence of preferential pairing, i.e., those genomes that were more closely related would produce ring bivalents more frequently than those less related. Thus, A and D, or U, S, and B chromosomes form more ring bivalents when meiotic pairing increases (see Table 1).

It is known that the U genome of *Ae. variabilis* is characterized by the presence of acrocentric chromosomes (Chennaveeraiah 1960), whereas A, B, D, and S chromosomes are metacentric. The short arms of the U genome might show low association frequencies and consequently may lead to an excess of AD-US and U-S open bivalents. This same behavior would be expected for US-B bivalents if pairing was at random, but most of the US-B associations might actually involve S-B chromosomes and therefore they might form ring bivalents.

The frequencies of ring bivalents have a great influence on determining relative contributions, since at least two chiasmata have occurred in these configurations. In fact, the order using the mean number of bound arms relative to the total bonds is different in bivalents (A-D > AD-US > US-B > U-S > AD-B) and multivalents (AD-US > A-D > U-S > US-B > AD-B) (see Table 6). These results indicate that comparisons between hybrids with different levels of pairing using meiotic configurations can lead to erroneous conclusions in estimating genomic affinities, because deviations can arise from differences in the number of each meiotic configuration (bivalents, multivalents). For this reason, the comparisons between hybrids with high and low homoeologous pairing have been done using the relative contribution calculated only from bivalents in both types of hybrids with the same *Aegilops* genotype (Table 7). In most cases the differences were significant and, again, the relative contributions of types A-D, AD-B, and US-B were greater in the high homoeologous pairing plants, and those of types AD-US and U-S were greater in the low-pairing ones.

It has been reported that the suppression of *Ph* locus activity produces two effects: an increase in synapsis and crossing-over frequency between homoeologoes, and ex-

Table 7. Comparisons of the relative contribution for all types of distinguishable association in bivalents between the low and high pairing hybrids

Aegilops parent	Mean number of associations/Total associations									
	A-D		AD-US		AD-B		US-B		U-S	
	CS	ph1b	CS	ph1b	CS	ph1b	CS	ph1b	CS	ph1b
Aekl	0.27	0.34	0.37	0.25	0.03	0.07	0.20	0.25	0.13	0.09
t-value (*df*=6)	1.46		2.49*		4.06**		1.24		1.47	
Aevt	0.21	0.32	0.37	0.22	0.01	0.09	0.24	0.27	0.17	0.10
t-value (*df*=4)	3.62*		4.02*		6.93**		0.92		1.32	
Aevc	0.23	0.34	0.42	0.28	0.05	0.02	0.20	0.27	0.16	0.09
Total	0.24	0.33	0.39	0.24	0.03	0.07	0.18	0.26	0.15	0.10
t-value (*df*=18)	3.61**		5.82***		3.51**		2.97		2.71*	

[a] Data from Aevc are also included
* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

pression of residual affinities that are not detected under normal conditions (Kimber and Alonso 1981). The differences between low- and high-pairing hybrids might be explained by the appearance of residual affinities. Such residual affinities should be expressed in the same way in bivalents and multivalents in the high homoeologous pairing hybrids and, because the differences between such types of configurations are very clear, this possibility must be ruled out. In fact, it is not very logical to ascribe any discrepancy between expected and observed data to residual genome affinities, since any deviation could be included in this class.

If the mean number of associated arms at metaphase I is taken as an estimation of genome affinities, genome relationships will depend on the pairing that is effective for chiasma formation. Therefore, actual genome affinities can be overestimated in hybrids with high pairing where a number of ring bivalents (with at least two chiasmata) have occurred. Although obviously chiasma frequency and genome affinities are related, one can assume that genome relationships depend only on the number of configurations involving chromosomes of different genomes, irrespective of the number of chiasmata formed. Thus, genome affinities would be measured on the number of bivalents formed between the genomes that compete.

In the low-pairing hybrids, the mean number of bound arms per cell is very similar to the mean number of bivalents per cell due to the low number of ring bivalents, and the relative order is the same when both types of means are used (AD-US > A-D > US-B > U-S > AD-B). This relative order is also maintained in the high-pairing hybrids when bivalent frequencies are used, but this order is different between low- and high-pairing hybrids when the mean number of bound arms relative to the total bonds for each type of pairing in bivalents is used. As already pointed out, this is probably du to an excess of ring bivalents in the high-pairing hybrids. These results seem to indicate that chiasma frequency plays an important role in the relative contribution of each type of specific association to the total amount of pairing observed at metaphase I.

It has been claimed on several occasions (Kimber 1984) that chromosome pairing is the most practical and reliable method of determining phylogenetic relationships in allopolyploid series. Data presented in this work indicate that numerical quantifications of genomic relationships based on the amount of chromosome pairing at metaphase I in hybrid combinations, even if all chromosomes are identified at meiosis, must be carefully considered in the establishment of the evolutionary process.

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