The analysis of meiosis of the B genome in common wheat

N. JOUVE, J. M. GONZALEZ, A. FOMINAYA, AND E. FERRER
Department of Genetics, Faculty of Sciences, University oí' Alcalá de Henares (Madrid), Spain

Received April 9, 1984
Revised manuscript received Septembcr 17, 1984

JOUVE, N., J. M. GONZALEZ, A. FOMINAYA, and E. FERRER. 1985. The analysis of meiosis of the B genome in common wheat. Can. J. Genet. Cytol. 27: 17-22.

Two intervarictal hybrids of common wheat, *Triticum æstivum* L., are meiotically analyzed using the C-banding staining method. The C-banding pattern of nine meiotic chromosomes (4A, 7A, and the seven of the B genome) permitted their unequivocal rccognition at first metaphase plates. The pairing frequency of each B-genome chromosome arm was scored. Data en the pairing frequency of the arms, separately considered, are applied to calculate expected pairing of whole chromosomes and whole genomes. The application of mathematical models to predict the genome pairing using either equal or different frequencies per chromosome arm is discussed.

Key words: meiotic analysis, *Triticum aestivum* L., C-banding.

JOUVE, N., J. M. GONZALEZ, A. FOMINAYA et E. FERRER. 1985. The analysis of meiosis of the B genome in common wheat. Can. J. Gcnet. Cytol. 27: 17-22.

Deux hybrides intervariétales de blé commun, *Triticum aestivum* L., ont été analysées par la méthode de coloration des bandes C des chromosomes, lors de la méiose. Le pattern des bandes C de neuf chromosomes (4A, 7A et les sept du génome B) a permis de les reconnaitre sans équivoque dés les premiéres plagues métaphasiques. La fréquence d'appariement de chaque bras des chromosomes du génome B a été notéc. Ces données sur la fréquence d'appariement des bras de chromosomes, considérées séparément, ont été utilisées pour le calcul d'estimes d'appariement de chromosomes entiers et de génomes entiers. Le recours á des modeles mathématiques pour prédirc l'appariement de génomes, soit par des fréquences égales ou différentes par bras chromosomique, est discuté.

Mon c./és: analyse méiotique, *Triticum aestivum* L., bandes C.

1Traduit par le journall

Introduction

A clear understanding of individual chromosome behaviour in *Triticum* is essential to any comprehension of the causes or factors interfering with chiasma occurrence, and could be of interest for the planning of practica] experiments to allow the introduction of alien variation.

Mather (1936) showed genetically that the first chiasma is generally independent of the length of the chromosome arm. Sallee and Kimber (1978) demonstrated cytologically in wheat that there is a selection pressure for a minimum number of chiasmata per chromosome, irrespective of the length of the chromosome. Further, they demonstrated that the pairing of telocentrics is a reliable measure of the pairing of bibrachial chromosomes, thus providing a firm basis for the quantification of chromosome pairing in hybrids (Kimber and Hulse 1978).

Driscoll et al. (1979) assumed the occurrence of one chiasma per pair of synapsed arms and that the frequency of this event is equal for all arms, and developed a model enabling the prediction of chromosome pairing in hybrids (up to pentaploid) and in both euploid and aneuploid species. Further, this theory was extended to hybrids involving six genomes and to more complicated situations where both homologous and homoeologous pairing occurs in the absence of chromosome 5B of wheat (Driscoll et al. 1980).

This paper deals with the extension of the method described by Kimber and Hulse (1978) in calculating the expected pairing frequencies in wheat. It differs from that method in that the arms of the B genome are considered separately; pairing frequencies at first metaphase are scored from pollen mother cells (PMCs) stained by Giemsa in two intervarietal hybrids.

Materials and methods

The chromosome pairing at first metaphase of pollen mother cells of different arms of the B genome was investigated in two hybrids between common wheat `Chinese Spring' and the Spanish cultivars `Pané 247' and 'Alonso Peña 114'.

Anthers for meiotic analyses of PMCs were fixed without previous treatment in acetic acid — alcohol 1:3. Squash preparations of the fixed material were stained following the Giemsa C-banding technique described previously (Jouve et al. 1980).

Results and discussion

The C-banding pattern of nine meiotic chromosomes of common wheat (4A, 7A and the seyen of the B genome) was reported previously (Ferrer et al. 1984a). The individual chromosomes were analyzed as uni-

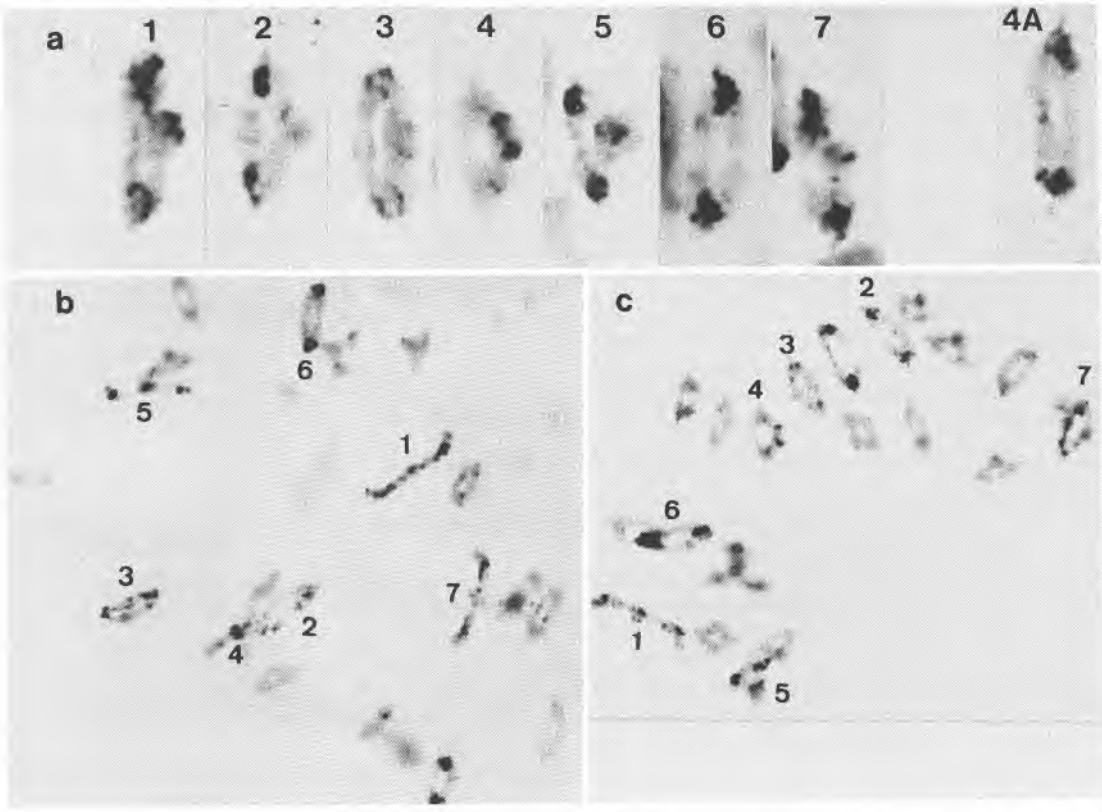

FIG. 1. *(a)* C-banded meiotic chromosomes (B genome and 4A) at first metaphase of meiosis: 1B-7B are 1-f, respectively, from left to right. Short and long arms are at the left and right, respectively, for ah l bivalents. *(b)* First metaphase plate of the hybrid `Chinese Spring' x 'Fané 247' showing three ring bivalents + four open bivalents (I B, 4B, 5B, and 7B all bound by their long arm). *(c)* First metaphase plate of the hybrid 'Chinese Spring' x 'Alonso Peña 114' showing six ring bivalents + one open bivalent (I B bound by its long arm). Scale bar = 10 hm.

valents and bivalents in F1 crosses between monosomics of `Chinese Spring' and two Spanish cultivars to aid their unequivocal recognition and characterization.

Using the C-banding technique, it is possible to distinguish among seven B-genome chromosomes which showed minor intervarietal variation and can be described as follows (Fig. 1). *(i)* Chromosome 1B (submetacentric): it presents a secondary constriction in its short arm and exhibits an obvious band near the telomere in its long arm: it presents more heterochromatin and more dispersed C-bands than any other in the B-genome karyotype. There is no intervarietal variation. *(ji)* Chromosome 2B (submetacentric): it shows a dark band close to the centromere in its short arm and a fine subterminal band in the same arm. A faint terminal band in the large arm not exhibited in `Chinese Spring' was rarely visible in some bivalents in chromosome 2B of `Pané 247' and 'Alonso Peña 114'. *(iii)* Chromosome 3B (submetacentric): it has one centromeric dark band and one intercalary band in each arm in Tané 247' and 'Alonso Peña 114'. A short band in the telomeric region of the short arm is also observed. The intercalary band of the short arm is absent in `Chinese Spring', thus allowing for the recognition and identification of the bivalent and for unequivocal distinction from 2B in both hybrids. (iv) Chromosome 4B (submetacentric): it shows one intensely stained subterminal band in its long arm in both hybrids and one faint intercalary hand in the centromeric region. There is no intervarietal variation. (y) Chromosome 5B (subtelocentric): it exhibits an intensely stained region around the centromere and one intercalary band in its long arm which was variable in size among the 5B chromosome of different karyotypes. It was middlesized in Pané 247' and very patent in `Chinese Spring' and 'Alonso Peña 114'. *(vi)* Chromosome 6B (submetacentric): it has large pericentromeric dark bands of heterochromatin, one small intercalary band in its long arm and a secondary constriction in the short arm. The satellite region is clearly distinguished from both arms in the bivalents because of its strongly condensed chromatin. This feature permits a clear distinction of the 6B bivalent from 4A and 7B.

TABLE 1. The observed and expected chromosome-to-chromosome pairing in the B genome of two intervarietal hybrids of common wheat. The expected meiotic figures are calculated under the assumptions of a mean arm pairing frequency either equal or different for each pair of chromosome arms

Chromosome		`Chinese Spring' x 'Pané 247' RB	OB,	OB,	U	X'	'Chinese Spring' x 'Alonso Peña 114' RB	OB,	OB,	U	)(2
1B	OBS	132	58	7	3		247	37	14	2	
	EXP,	152	22.4	22.4	3.2	68.71***	234.4	30.8	30.8	4	12.08***
	EXP2	132.05	57.95	6.95	3.05	0.00006	247.2	36.9	13.8	2.1	0.01
2B	OBS	166	9	24	1		257	24	15	4	
	EXP,	152	22.4	22.4	3.2	9.31***	234.4	30.9	30.9	4	10.85***
	EXP,	166.25	8.75	23.75	1.25	0.047	254.6	26.3	17.3	1.8	0.22
3B	OBS	174	21	4	1		244	31	18	7	
	EXP,	152	22.4	22.4	3.2	19.84***	234.4	30.9	30.9	4	3.15
	EXP2	173.55	21.45	4.45	0.55	0.010	240.5	34.6	21.7	3.2	0.438
4B	OBS	177	13	8	2		248	22	24	6	
	EXP,	152	22.4	22.4	3.2	17.56***	234.4	30.9	30.9	4	3.96*
	EXP2	175.75	14.25	9.25	0.75	0.409	244.6	25.4	25.4	2.8	0.50
5B	OBS	147	48	2	3		241	30	25	4	
	EXP,	152	22.4	22.4	3.2	45•99***	234.4	30.9	30.9	4	2.32
	EXP2	145.27	49.73	3.72	1.27	0.080	240.3	30.6	25.8	3.3	0.014
6B	OBS	129	47	18	6		209	51	29	II	
	EXP,	152	22.4	22.4	3.2	61.09***	234.4	30.9	30.9	4	16.77***
	EXP2	129.36	46.64	17.64	6.36	0.030	206.3	53.7	31.7	8.3	1.28
7B	OBS	138	42	14	6		203	83	12	2	
	EXP,	152	22.4	22.4	3.2	19.66***	234.4	30.9	30.9	4	102.83***
	EXP2	136.8	43.2	15.2	4.8	0.040	205	80.9	10.1	4	0.07

NOTE: RB, ring bivalent, OB,, open bivalent bound by large arrn; OB,, open bivalent bound by short arin; U. univalent; OBS, observed; EXP, expected.

*Significant at the 0.05 level.

***Significant at the 0.001 level.

This chromosome does not exhiba intervarietal variation. (*vii)* Chromosome 7B (submetacentric): it shows large bands dispersed around the centromeric regions and a fine intercalary band in the long arm. There is no intervarietal variation.

The results on individual behaviour of the seven B-genome pairs in relation to the meiotic configuration (ring or rod bivalents, and univalents) are given in Table I (OBS). Two hundred and three hundred PMCs were investigated from the hybrids `Chinese Spring' x Tané 247' and `Chinese Spring' x 'Alonso Peña 114', respectively.

Results on)(2 of a contingency test comparing the intensity of pairing distribution (ring bivalents, rod bivalents open by their long or short arm, and univalents) of each among the seven B-genome pairs in both hybrids seem to demonstrate their different behaviour: $x2 = 108.35$, df = 12, and $P < 0.001$ for `Chinese Spring' x Tané 247'; and $x2 = 100.33$, df -= 12, and $P < 0.001$ for `Chinese Spring' x 'Alonso Peña 114'.

The differences in pairing intensity among B-genome pairs can be attributed to differences in chiasma frequencies of each chromosome arm. Moreover, the different pairing frequencies of each chromosome arm can be used to predict the meiotic behaviour of whole chromosomes and whole genomes by the method of Kimber and Hulse (1978).

Chromosome length and presence of heterochromatin have been considered as the main factors affecting the intensity of pairing until now. Early in the analysis of pairing it was assumed that the chiasma frequency tended to be proportional to the length of the chromosome. However, Mather (1936, 1937, 1940) in an analysis in which the position and frequency of crossing-over was converted mathematically to chiasma frequencies, demonstrated that shorter chromosomes exhibited disproportionately high chiasma frequencies. Sallee and Kimber (1978), carried out an analysis of meiotic behaviour of telocentrics of `Chinese Spring'. A correlation comparing data of chiasma frequency per chromosome arm and the relative arm length of the same chromosome arm showed that regression of chiasma frequency on length was not significant. They concluded that there is some selection pressure for a minimum number of chiasmata per arm or per chromosome irrespective of the length of that chromosome.

TABLE 2. The observed and expected meiotic configurations of the B genome in the intervarietal common wheat hybrids analyzed. The expected patterns of pairing are estimated under either equal or different pairing frequencies for each pair of chromosome arms

Hybrid		7RB	6RB + 1OB	6RB + 1U	5RB + 2OB	5RB + 1OB + 1U	4RB + 3OB	4RB + 2OB + 1U	4RB + 1OB + 2U	3RB + 4OB	3RB + 3OB + 1U	3RB + 1OB + 3U	2RB + 5OB	Others	χ^2	df	P
'Chinese spring' × 'Alonso Peña 114'	OBS	56	113	3	63	8	27	10	1	10	4	1	2	2			
	EXP_1	53.4	104.5		77.2	10.2	38.6	7.1		9.0					21.21	5	0.001
	EXP_2	51.2	104.6		78.8	10.2	31.9	6.9		8.4	8				8.95	6	0.2–0.3
'Chinese Spring' × 'Pané 247'	OBS	25	53	8	72	5	23	5	—	5	2	—	—	2			
	EXP_1	29.2	64.7		53.2	7.8	26.1	5.9		13.1					10.25	5	0.05–0.1
	EXP_2	26.8	64.7		56.8	6.2	28.3	7.1		10.1					6.36	5	0.2–0.3

NOTE: Arrows represent aggregation of figures to application of statistical proofs.

The amount of DNA and heterochromatin present in chromosomes could also influence the variation in the pairing intensity among chromosome arms (Santos and Giraldez 1978; Loidl 1979; Naranjo and Lacadena 1982). Ferrer et al. (1984b) cytologically determined in wheat that differences in chiasmata frequencies per chromosome arm could not be explained on the basis of relative ami length only and supposed the existence of an overlapping effect of both the arm length and the amount and distribution of heterochromatin in pairing.

Whole-chromosome pairing frequency prediction

The probability of ring, rod (open by both long and short arms), and univalents can be easily calculated from the individual arm probabilities of pairing by the simple arithmetic method of Kimber and Hulse (1978). If the probability of pairing of the long arm is *piand* that of the short arm is p, then the expected probability for different meiotic figures is as follows:

RB (ring bivalent) $= p_l \cdot p_s$

OB_l (open bivalent bound by its long arm $= p_l \cdot (1 - p_s)$

OB_s (open bivalent bound by its short arm $= (1 - p_l) \cdot p_s$

U (univalent) $= (1 - p_l) \cdot (1 - p_s)$

To estimate the expected frequencies of meiotic configurations for each chromosome pair in the hybrids, two different assumptions could be applied: *(i)* ahl the pairs of chromosome arms in the B genome pair at the same frequency *(p)* that represents the mean of all observations for that genome = $\frac{\text{total number of bound arms in the cells)}}{\text{total number of arms in the cells}}$ and *(u)* each pair of chromosome arms in the B genome pair at a different frequency (p,, andp,, where *i* 1B, 2B, ..., 7B) that represents the mean of all observations for each chromosome arm separately.

The use of equivalent values to express the mean pairing per chromosome arm was discussed by Driscoll et al. (1978) and Sallee and Kimber (1978), who assumed that this does not introduce any significant perturbation into the calculation. This is correct for the type of calculations that those and other authors have made. However, the consideration of pairing frequencies of arms separately could improve the results of application of models dealing with the prediction of synapsis of complete chromosomes or the whole genome, as will be demonstrated later in this paper.

The expected meiotic figures for either equal (EXP,) or different (EXP2) frequencies of pairing together with the observed (OBS) ones are presented in Table 1. When equal probability of synapsis per arm was assumed x2 tests comparing observed and expected values gave mainly significant deviations. However, no signif-

icant differences were observed when the comparison was made between observed and expected under the assumption of different pairing probabilities for each pair of chromosome arms.

Whole-genome pairing frequency prediction

According with Kimber and Hulse (1978), once the probabilities of bivalent formation of each chromosome of a genome has been derived, the probability of the formation of the seven bivalents per cell in the material being investigated can be calculated as the product of the seven individual probabilities. Again both assumptions, equal or different pairing frequency per arm, could be considered.

In the case when all chromosome arms pair with the same frequency, one ring bivalent has a probability $r = p^2$ of being formed, and one open bivalent $o = 2p \cdot (1 - p)$. For the pair of univalents the corresponding probability is $u = (1 - p)^2$. In any case $r + o + u = 1$. Therefore, seven ring bivalents are formed with the probability of r^7. Six ring bivalents + one open bivalent can be formed in seven different ways, as the bivalent that is not formed could be the first, the second, etc. The probability of formation of this pattern is $r^6 \cdot o \cdot 7$. In a similar way, the probability of formation of six ring bivalents + one pair of univalents is $r^6 \cdot u \cdot 7$. Also in a similar way, the probability of formation of five ring bivalents + two open bivalents, five ring bivalents + two pair of univalents, five ring bivalents + one open bivalent + one pair of univalents, ...,can be calculated.

In the case when each pair of chromosome arms contributes with different frequencies to pairing, one ring bivalent r_i (i = 1B, 2B, ..., 7B) has a probability $r_i = p_{i1} \cdot p_{i2}$, one open bivalent irrespective of the arm bound, o_i, has a probability $o_i = p_{i1} \cdot (1 - p_{i2}) + (1 - p_{i1}) \cdot p_{i2}$. For the pair of univalents the corresponding probability is $u_i = (1 - p_{i1}) \cdot (1 - p_{i2})$. In any case, $r_i + o_i + u_i = 1$.

The probability distribution for the formation of cells with each pattern of pairing is the resultant of the development of the following expression:

$$(r_{1B} + o_{1B} + u_{1B}) \cdot (r_{2B} + o_{2B} + u_{2B}) \cdot \ldots \times (r_{7B} + o_{7B} + u_{7B})$$

The probability of formation of seven ring bivalents per cell is

$$r_{1B} \cdot r_{2B} \cdot r_{3B} \cdot r_{4B} \cdot r_{5B} \cdot r_{6B} \cdot r_{7B}$$

Six ring bivalents and one open bivalent can be formed in seven different ways. The probability of formation of this pattern of pairing is the sum of the following:

$$o_{1B} \cdot r_{2B} \cdot r_{3B} \cdot r_{4B} \cdot r_{5B} \cdot r_{6B} \cdot r_{7B} + r_{1B} \cdot o_{2B} \cdot r_{3B} \cdot r_{4B} \cdot r_{5B} \cdot r_{6B} \cdot r_{7B} + \ldots + r_{1B} \cdot r_{2B} \cdot r_{3B} \cdot r_{4B} \cdot r_{5B} \cdot r_{6B} \cdot o_{7B}$$

In a similar way the probability of formation of the remaining patterns of pairing could be estimated.

The frequencies of the observed and expected meiotic configurations calculated by both procedures are recorded in Table 2. It can be seen that deviations between the distribution of type of cells and the expected ones were higher when an equal frequency of pairing per pair of chromosome arms was assumed (EXP,), than in the other case (EXP2).

The evidence presented in this paper can lead to a better understanding of the meiotic behaviour of B-genome chromosomes of wheat. First, it is clear that application of C-banding to analysis of meiotic bibrachial chromosomes is a good method to score the frequencies of pairing of each pair of chromosome arms, avoiding the possible underestimation of the chiasmata frequencies obtained when telocentrics are used. The analyses of chromosome pairing made in this paper provides new evidence for the differences in pairing frequency of chromosome arms in wheat investigated by Saltee and Kimber (1978) using telocentrics. The data recorded in this contribution are in agreement with the assumption that the pairing of telocentric chromosomes is a reliable measure of the pairing of bibrachial chromosomes such as was demonstrated by those authors. Second, the prediction of rneiotic behaviour in analyses of chromosome pairing for each of the seven B-genome chromosomes is better when individual frequencies of pairing per arm are used than under the simplifying assumption that all chromosome arms pair with the same frequency. Finally, the expected frequencies of cells with different patterns of ring and rod bivalents, and univalents are more accurately calculated considering the arm-to-arm different pairing frequencies than assuming a similar behaviour for each pair of chromosome arms.

Acknowledgment

The authors thank the Comision Asesora de Investigación Científica y Técnica of Spain for the award of a grant (No. 1558-82) to support this work.

DRISCOLL, C. J., L. M. BIELIG, and N. L. DARVEY. 1979. An analysis of frequencies of chromosome configurations in wheat and wheat hybrids. Genetics, 91: 755-767.

DRISCOLL, C. J., G. H. GORDON, and G. KIMBER. 1980. Mathematics of chromosome pairing. Genetics, 95: 159-169.

FERRER, E., J. M. GONZALEZ, and N. JouvE. 1984a. Identification of C-banded chromosomes in meiosis of common wheat, *Triticum aestivum* L. Theor. Appl. Genet. 67: 257-261.

_____ 1984b. The meiotic pairing of nine wheat chromosomes. Theor. Appl. Genet. 68. In press.

JOUVE, N., M. DIEZ, and M. RODRIGUEZ. 1980. C-banding in 6x triticale x *Secale cereale* L. hybrid cytogenetics.

Theor. Appl. Genet. 57: 75-79.

KIMBER, G., and M. M. HULSE. 1978. The analysis of chromosome pairing in hybrids and the evolution of wheat. Proc. 5th Int. Wheat Genct. Symp., New Delhi. pp. 63-72.

LOIDL, J. 1979. C-band proximity of chiasmata and absence of terminalisation in *Allium flavum*. Chrornosoma, 73: 45-51.

MATHER, K. 1936. The dctermination of position in crossing over. 1. *Drosophila melanoguster.* J. Genet. 33: 207-235.

1937. The determination of position in crossing over. II. The chromosome length — chiasma frequency relation. Cytologia, Fujii Jub. vol. pp. 514-526.

_____ 1940. The determination of position in crossing over. III. The evidence of metaphase chiasmata. J. Genet. 39: 205-223.

NARANJO, T., and J. R. LACADENA. 1982. C-banding pattern and meiotic pairing in five rye chromosomes of hexaploid triticale. Theor. Appl. Genet. 61: 233-237.

SALLEE, P. J., and G. KIMBER. 1978. An analysis of the pairing of wheat telocentric chromosomes. Proc. 5th Int. Wheat Genet. Symp., New Delhi. pp. 408-419.

SANTOS, J. L., and R. GIRALDEZ. 1978. The effect of C-heterochromatin in chiasma terminalisation in *Chortippus bigutulus* L. (Acrididae, Orthoptera). Chromosoma, 70: 59-66.