METAPHASE I BOUND ARMS AND CROSSING OVER FREQUENCY IN RYE. IV. TRITICALE–RYE HYBRIDS

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SUMMARY

The correspondence between the number of metaphase I associations (bonds) and the chiasma frequency of specific chromosome arms of rye has been analysed in the meiosis of Triticale–rye hybrid plants. These plants were heterozygous for prominent telomeric C-bands in the two arms of chromosome 1R. From the frequency of bound arms at metaphase I and the frequency of recombined chromosome at anaphase I in the arms considered, estimates of chiasma frequency can be made under the assumption that at least one chiasma per bond was formed. However, these estimates lead to the absurdity of negative chiasma frequencies. The discrepancy between metaphase I and anaphase I results, due to an excess of anaphase I parental chromosomes, can be explained on the assumption that some of the bonds appearing at metaphase I are achiasmata and a remnant of prophase pairing.

1. INTRODUCTION

In Triticinae, the number of chiasmata cannot be determined at diplotene. At metaphase I, often only associations (bonds) between bivalent arms can be observed, these bonds being interpreted traditionally as an indication of the presence of at least one chiasma.

In previous studies (Giraldez and Orellana, 1979; Orellana and Giraldez, 1981, 1983) a procedure was used for estimating chiasma frequency in specific chromosome arms of rye marked with Giemsa banding in meiosis. Plants differing in respect to the C-banding pattern of specific chromosome arms were used. From the frequency of being bound at metaphase I and the frequency of recombinant chromosomes at anaphase I or metaphase II in the arms considered, estimates of chiasma frequencies were derived.

The materials employed in those instances were an open pollinated variety of rye (Orellana and Giraldez, 1981), the F₁s between rye inbred lines (Giraldez and Orellana, 1979; Orellana and Giraldez, 1981) and partially desynaptic plants obtained from the backcrosses between those F₁s and their parental inbred lines (Orellana and Giraldez, 1983).

The results indicated that more than one chiasma per arm could be formed in some chromosomes of the cultivar and the F₁s. However, in desynaptic plants an excess of anaphase I parental type chromosomes was found, being interpreted as produced by the existence of non-chiasmate bonds, i.e., some of the bonds appearing at metaphase I were actually a remnant of prophase pairing, no chiasmata being formed between the arms involved.

In the present work, the analysis has been extended to chromosome 1R in Triticale–rye hybrids. The existence of metaphase I 1R bivalents having no chiasmata is demonstrated.

2. MATERIAL AND METHODS

Four Triticale-rye hybrid plants (genome constitution ABRR) obtained from the cross between a hexaploid Triticale (cv. Cachirulo) and a rye inbred line (Line E) formed the material for this study.

In order to obtain mitotic metaphase cells, seeds were germinated on wetted filter paper in Petri dishes at room temperature. When primary roots were 1 cm long they were excised and immersed in tap water at 0°C for 24 h to shorten the chromosomes. Subsequently, the tips were fixed in acetic alcohol 1 : 3. For meiotic cells, anthers were fixed in acetic alcohol 1 : 3.

Both root tips and anthers were maintained in the fixative during one to four months at 3–4°C. The fixed material was squashed and stained following the Giensa C-banding technique described previously (Giraldez *et al.*, 1979).

3. RESULTS

Plates 1(a)–(b) show C-banded mitotic metaphases of the Triticale (cv. Cachirulo) and rye inbred line plants used in the cross from which the Triticale-rye hybrids were obtained. There are differences in the C-banding pattern of most rye chromosomes, those of Triticale having a higher amount of telomeric C-heterochromatin than the ones of inbred line E.

Using the C-banding technique it was possible to identify chromosome pair 1R in the meiosis of the Triticale-rye hybrids analyzed. As expected, these plants were heterozygous for the C-banding pattern of this chromosome pair. Chromosome 1R proceeding from Triticale had C-heterochromatin blocks in both telomeres whereas the one proceeding from inbred line E had almost no telomeric C-heterochromatin.

Plates 2(a)–(b) show some examples of metaphase I C-banded cells of the Triticale-rye hybrid plants studied.

Table 1 shows the three types of middle metaphase I chromosome pairing observed (rye-rye, wheat-wheat and wheat-rye) indicating the frequencies of the different configurations for chromosome pair 1R (R, ring bivalents; O1, open bivalents with the long arm bound; Os, open bivalents with the short arm bound; U, univalents) and for the rest of rye and wheat chromosomes (R, ring bivalents; O, open bivalents; U, univalents).

It is worth mentioning that, at metaphase I, univalents and not bound arms of chromosomes forming open bivalents showed no evidence of recombination, *i.e.*, the two chromatids of these chromosome arms showed the same banding pattern.

On the other hand, all wheat-rye bivalents were open, the two 1R chromosomes being involved with almost the same frequency. Wheat-rye trivalents were formed by two wheat and one rye chromosome. In all wheat-rye associations of chromosome 1R only long arm was involved.

A similar conclusion to that obtained by Naranjo (1982) can be deduced from table 1. There is a preferential occurrence of wheat-rye meiotic pairing between chromosomes of homoeologous group 1, in spite of the fact that in rye-rye associations the average number of bound arms was lower in pair 1R than in the remaining rye chromosome pairs. This suggests a greater affinity or a more efficient pairing between wheat and rye chromosomes for homoeologous group 1.

PLATE 1. C-banded somatic metaphase cells. (a) Plant of the hexaploid Triticale cv. Cachirulo.
(b) Plant of inbred line E of rye.

PLATE 2. C-banded metaphase I cells of the Triticale $\times$ rye hybrids. (a) The $1R^L$ chromosome arm is bound. (b) Both arms of chromosome $1R$ are unbound.

PLATE 3. C-banded anaphase I cells of the Triticale $\times$ rye hybrid plants. (a) Parental type for both 1R chromosome arms. (b) Recombinant type for 1R^L chromosome arm and parental type for 1R^S chromosome arm.

TABLE 1

The three types of middle metaphase I chromosome pairing observed (rye-rye, wheat-wheat and wheat-rye) indicating the frequencies of the different configurations for chromosome 1R (R, ring bivalent; Ol, open bivalent with the long arm bound; Os, open bivalent with the short arm bound; U, univalents) and for the remaining rye and wheat chromosomes (R, ring bivalents; O, open bivalents; U, univalents). 50 metaphase I cells per plant were observed

Plant	Rye-rye associations						Wheat-rye associations					
	Chromosome 1R			Remaining rye chromosomes			Wheat-wheat associations			Chromosome 1R involved		
	R	Ol	Os	U	R	O	U	R	O	U	Bivalents*	Trivalentst
CHE-1	1	25	2	41	226	62	22	21	115	424	3	-
CHE-2	4	23	1	40	197	90	22	21	104	444	3	-
CHE-3	6	21	-	44	197	91	20	14	105	458	1	1
CHE-4	1	24	3	39	212	77	18	26	110	421	5	-
Total	12	93	6	164	832	320	82	82	434	1747	12	2
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* All wheat-rye bivalents were open with the long arm bound. Chromosome 1R proceeding from triticales was involved in 5 of them, chromosome 1R proceeding from the inbred line was involved in the remaining 7 wheat-rye bivalents.

† Wheat-rye trivalents were formed by two wheat and one rye chromosome. In the two wheat-rye trivalents in which it was involved, chromosome 1R was the one proceeding from the triticales and had the long arm bound.

		Anaphase I cell types						
		I	II	III	IV	V	VI	VII
Minimum number of chiasmata formed
	short arm	0	0	1	1	1	1	2 or 0
	long arm	0	1	0	1	1	1	0 or 2

FIG. 1. The different metaphase I cell types expected in respect to the constitution of their chromosome 1R (showing or not evidence of recombination in the two arms) and the minimum number of chiasmata necessary for their appearance.

As chromosome pair 1R was heterozygous for the presence of telomeric C-bands in both chromosome arms, seven different types of anaphase I in respect to the constitution of their chromosome 1R (proceeding from cells in which wheat-rye pairing for this chromosome did not occur) can be expected. Fig. 1 shows diagrammatically the different anaphase I types expected as well as the minimum number of chiasmata necessary for their appearance.

Now, if a bound arm at metaphase I corresponds to only one chiasma, the expected frequencies of the different anaphase I types can be estimated from metaphase I results (table 1): Type I would proceed from cells having a univalent pair; type II would proceed from cells with open bivalents with the long arm bound; type III would proceed from cells with open bivalents with the short arm bound and cells having ring bivalents would produce types IV, V and VI with the relative frequencies 1:1:2, assuming that there is no chromatid chiasma interference across centromere.

50 anaphase I cells per plant were observed, examples are shown in plate 3. No evidence of recombination between wheat and rye chromosomes could be observed at this phase.

Table 2 shows the comparison between the observed and the expected frequencies of the different anaphase I types for chromosomes 1R under the assumption of a 1:1 correspondence between chiasmata and metaphase I bonds. The difference between observed and expected values is highly significant, an excess of anaphase I chromosomes showing no evidence of recombination being found.

The possibility of more than one chiasma per bond can also be considered: for a bivalent arm, if there is no chromatid chiasma interference, the frequency of anaphase I chromosomes showing evidence of recombination would be (Mather, 1935):

$$Fr = f_1 + \frac{1}{2}f_2 + \frac{3}{4}f_3 + \dots + \frac{2}{3}[1 - (-\frac{1}{2})^n]fn$$

and the frequency of anaphase I chromosome arms showing no evidence of recombination:

$$Fp = f_0 + \frac{1}{2}f_2 + \frac{1}{4}f_3 + \dots + \frac{1}{3}[1 - (-\frac{1}{2})^{n-1}]fn$$

in which $f_0, f_1, f_2, \dots, f_n$ are the frequencies of 0, 1, 2, $\dots, n$ chiasmata respectively.

TABLE 2

The comparison between the observed and the expected frequencies of the different anaphase I types for chromosomes 1R (see fig. 3) under the assumption of a 1:1 correspondence between chiasmata and metaphase I bonds. 50 anaphase I cells per plant were observed

Plant	Anaphase I types							
	I	II	III	IV	V	VI	VII	
Che-1	Obs.	34	16	—	—	—	—	—
	Exp.	22	25	2	0.25	0.25	0.5	0
CHE-2	Obs.	35	15	—	—	—	—	—
	Exp.	22	23	1	1	1	2	0
CHE-3	Obs.	32	18	—	—	—	—	—
	Exp.	23	21	—	1.5	1.5	3	0
CHE-4	Obs.	37	13	—	—	—	—	—
	Exp.	22	24	3	0.25	0.25	0.5	0
Total	Obs.	138	62	—	—	—	—	—
	Exp.	89	93	6	3	3	6	0

Contingency χ^2 between observed and expected values in totals (anaphase I types III, IV, V and VI have been added): $\chi^2 = 34.777$; $0.001 > p$.

If

$$R = \frac{1}{2}f_2 + \frac{3}{4}f_3 + \dots + \frac{2}{3}[1 - (-\frac{1}{2})^n]fn$$

and

$$P = \frac{1}{2}f_2 + \frac{1}{4}f_3 + \dots + \frac{1}{3}[1 - (-\frac{1}{2})^{n-1}]fn$$

we can rewrite Mather's equations as:

$$Fr = f_1 + R; \quad Fp = f_0 + P.$$

f_0 can be estimated from the metaphase I results since it equals the frequency with which the arm considered is not bound. Fr and Fp can be estimated from the anaphase I observations.

Now, if a maximum of two chiasmata per arm are formed, $P = R = \frac{1}{2}f_2$, and in this case f_1 and f_2 can be deduced.

Table 3 shows the estimated values of f_0 , f_1 and f_2 for each 1R chromosome arm in the plants studied under the assumption of a maximum of two chiasmata per arm (in these estimates, 1R chromosome arms involved in wheat-rye associations have been considered as not bound arms). The estimates lead to the absurdities of a negative value of f_1 in the short arm and to a lower value of f_1 than f_0 or f_2 in the long arm.

If more than two chiasmata per arm were formed no estimates of $f_1, f_2, \dots, f_n$ can be made, however, in this case $R > P$, and the value of f_1 would be lower than the one estimated under the assumption of a maximum of two chiasmata per arm (table 3), the absurdity being increased.

4. DISCUSSION

The excess of anaphase I parental chromosomes found in the meiosis of the Triticale-rye hybrids analysed in this work do not fit the expectation

TABLE 3

The estimated (*) frequencies of 0 (f_0), 1 (f_1) and 2 (f_2) chiasmata in the two arms of chromosome 1R, under the assumption of a maximum of two chiasmata per arm

Plant	Short arm			Long arm		
	f_0	f_1	f_2	f_0	f_1	f_2
CHE-1	0.94	-0.06	0.12	0.48	0.12	0.40
CHE-2	0.90	-0.10	0.20	0.46	0.06	0.48
CHE-3	0.88	-0.12	0.24	0.46	0.18	0.36
CHE-4	0.92	-0.08	0.16	0.50	0.02	0.48
Total	0.91	-0.09	0.18	0.475	0.095	0.43

(*) f_0 = frequency of not bound bivalent arms at metaphase I (table 1). (1R chromosome arms involved in wheat-rye associations have been considered as not bound arms). $f_2 = 2(Fp - f_0)$; Fp = frequency of anaphase I cells showing no evidence of recombination in the chromosome arm considered (table 2). $f_1 = 1 - f_0 - f_2$.

neither under the hypothesis of one bond—one chiasma (table 2) nor under the possibility of more than one chiasma per bond being formed (table 3).

On the other hand, although the data could be explained in part with the existence of chromatid chiasma interference (*i.e.*, all bonds in the short arm and most bonds in the long arm being the result of two chiasmata in which the same two chromatids are involved), a too complicated picture would result and therefore this possibility can certainly be excluded.

The existence of non-chiasmate bonds proposed earlier (Orellana and Giraldez, 1983) in desynaptic rye would easily explain an excess of anaphase I parental chromosomes when compared to the frequency with which a given chromosome arm is not bound at metaphase I. These non-chiasmate bonds would represent a remnant of prophase pairing that could be similar to the process found in achiasmate organisms in which chromosomes remain paired till anaphase I onset (see Baker *et al.*, 1976). The existence of non-chiasmate associations at metaphase I in organisms that do form chiasmata has also been claimed by John (1976) and King and John (1980) as an explanation for the terminal chromosome associations found in grasshoppers.

Under this point of view, a bivalent arm can have three different possibilities at metaphase I: it can be bound having at least one chiasma (with a probability $CB = f_1 + f_2 + \dots + f_n$), it can be bound having no chiasmata (with a probability NCB) or it can be not bound (with a probability NB). In this case, the relationships between anaphase I and metaphase I results would be:

$$Fr = f_1 + \frac{1}{2}f_2 + \frac{3}{4}f_3 + \dots + \frac{2}{3}[1 - (-\frac{1}{2})^n]f_n$$

$$Fp = NB + NCB + \frac{1}{2}f_2 + \frac{1}{4}f_3 + \dots + \frac{1}{3}[1 - (-\frac{1}{2})^{n-1}]f_n.$$

Cytologically, only two possibilities can be distinguished at metaphase I, bound arms ($B = CB + NCB$) and not bound arms (NB). So, only if a maximum of one chiasma is formed in the bivalent arm considered the frequency with which non chiasmate bonds (NCB) are formed can be deduced.

In the hybrids analysed here this is probably the expected situation, given the low number of bonds formed when compared to normal rye. In this case all bonds formed in the short arm of bivalent 1R would be non-chiasmate while in the long arm non-chiasmate bonds would appear with a probability $NCB = 0.215$.

This value can be compared with the one obtained in an earlier work (Orellana and Giraldez, 1983) for the same chromosome arm in desynaptic plants of rye (B1PEE). In that instance the frequency with which the long arm of bivalent 1R was not bound at metaphase I was $NB = 0.0225$ and the frequency of anaphase I parental chromosomes was $Fp = 0.2072$. The probability of non-chiasmate bonds assuming that a maximum of one chiasma was formed was $NCB = Fp - NB = 0.1847$.

In the present work a higher frequency of unbound arms at metaphase I ($NB = 0.475$) is accompanied by a higher frequency of non-chiasmate bonds. This can be explained if we consider that in these hybrids chiasma formation is more severely affected than bound arm formation, i.e., the proportion of metaphase I bonds that are the result of one chiasma is lower in these hybrids than in the desynaptic plants mentioned above, the frequency of non-chiasmate bonds being increased.

The results obtained in the Triticinae concerning interspecific gene transference by means of meiotic recombination support the existence of chiasmata between chromosomes of the different species involved. However, in this work no evidence of recombination between chromosomes of wheat and rye has been observed. If the existence of non-chiasmate bonds is extended to homoeologous metaphase I bonds, the frequency of interspecific recombination in Triticinae can be smaller than the one estimated from cytological observations at metaphase I if a minimum of one chiasma per bond is considered.

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