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CHROMOSOMA

Metaphase I Bonds, Crossing-over Frequency, and Genetic Length of Specific Chromosome Arms of Rye

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Abstract. Using a Giemsa C-banding procedure three chromosome pairs (3, 6 and 7) have been identified in meiosis of the F1 of a cross between two rye inbred lines. Two of these chromosome pairs (3 and 7) were heterozygous for a prominent telomeric heterochromatic band. The comparison between the frequencies of the different meiotic configurations at metaphase I, anaphase I and metaphase II presented by these two chromosome pairs has allowed the estimation of the chiasma frequency and the genetic length of the chromosome arms 3 short and 7 long.

Introduction

In rye, the number of chiasmata cannot be determined at diplotene. Occasionally, depending on the material, reasonable estimates can be made at metaphase I. Often, only an association between bivalent arms can be observed, and then chiasma frequency is simply considered equivalent to the frequency of associated (bound) arms at metaphase I. A bound arm, however, merely indicates the presence of at least one chiasma, the actual number of chiasmata remaining undertermined. Methods to transform bound arm frequencies to chiasma frequencies and genetic length, using functions related to mapping functions (Sybenga, 1975), are still far from perfect.

In addition, it is impossible to recognise specific bivalents of rye when traditional staining procedures are used. Without chromosomal markers, which may affect the results, chiasma frequencies or even frequencies of being bound, cannot be estimated for specific chromosome arms.

In the present study, a procedure in some aspects similar to that employed in the demonstration of crossing-over inhibition in rye anthers cultured with colchicine (de la Peña et al., 1979), has been used. Chiasma frequencies have been estimated in specific chromosome arms of rye, using Giemsa C-banding in meiosis of a hybrid between inbred lines which differ in respect to terminal C-heterochromatin in two chromosome arms. From the frequency of being

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bound at metaphase I and the frequency of recombination at anaphase I and metaphase II in the arms considered, estimates of actual chiasma frequencies have been derived.

Material and Methods

Two inbred lines of rye *Secale cereale* (P and E) and eight plants of the F1 of the cross between two individual plants of these two lines (PE) formed the material for this study. The inbred lines were obtained by self pollination during 14 (P) and 17 (E) generations at the Experimental Station of Aula Dei (C.S.I.C., Zaragoza, Spain).

In order to obtain mitotic metaphase cells, seeds were germinated on wetted 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 24 h to shorten the chromosomes. After that 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 two to four months at 3–4° C.

Slides were prepared by squashing the fixed material with a drop of 45% acetic acid. After removal of the coverslip by the dry ice method, slides were rapidly immersed in absolute alcohol for 1 to 24 h. This variation in time of dehydration was seen not to affect subsequent C-banding definition. Slides were dried with a hair dryer just before the beginning of C-banding.

Air dried slides were immersed in 0.2 N HCl at 60° C for 3 min, washed vigorously in tap water and placed in a saturated solution of $Ba(OH)_2$ at room temperature for 10 min, washed again in tap water and immersed in $2 \times$ SSC at 60° C for 1 h. Slides were then stained with a Giemsa solution prepared with 3 ml of Giemsa (Gurr's R66) and 100 ml phosphate buffer pH 7.

Staining was checked until appropriate contrast was obtained. Then, slides were washed in tap water and rapidly air dried, immersed in xylene for 5 min and mounted in DPX.

Results

In the two inbred lines studied all seven chromosome pairs could be distinguished in C-banded mitotic metaphases. Within each inbred line the banding pattern of the two chromosomes of each pair of homologues was practically identical and no variation between plants was observed. Chromosomes have been classified previously according to their average arm length ratio, except the nucleolar organizer chromosome which was classified as number 7, and the classification compared with that of others (Giraldez et al., 1979).

Figure 1 shows mitotic metaphase plates of the two inbred lines and their F1. The short arm of chromosome 3 and the long arm of chromosome 7 show apparent differences in the amount of telomeric C-heterochromatin in the two inbred lines. These differences are better appreciated in their F1 in which chromosomes derived from the two lines have been stained under the same conditions (Fig. 1c). In the plants of this F1 there are three chromosome pairs (3, 6 and 7) in which at least one chromosome bears a prominent telomeric band. Chromosome pair 3 is submetacentric and heterozygous for a prominent telomeric band in its short arm; chromosome pair 6 is homozygous for a telomeric band in the short arm and its two arms are very different in length; and chromosome pair 7 is submetacentric, heterozygous for a prominent telomeric band in its long arm and having a heterochromatic band near the nucleolar organizer region. Thus, these three chromosome pairs can be unequivocally identified at metaphase I, anaphase I and metaphase II in pollen mother cells.

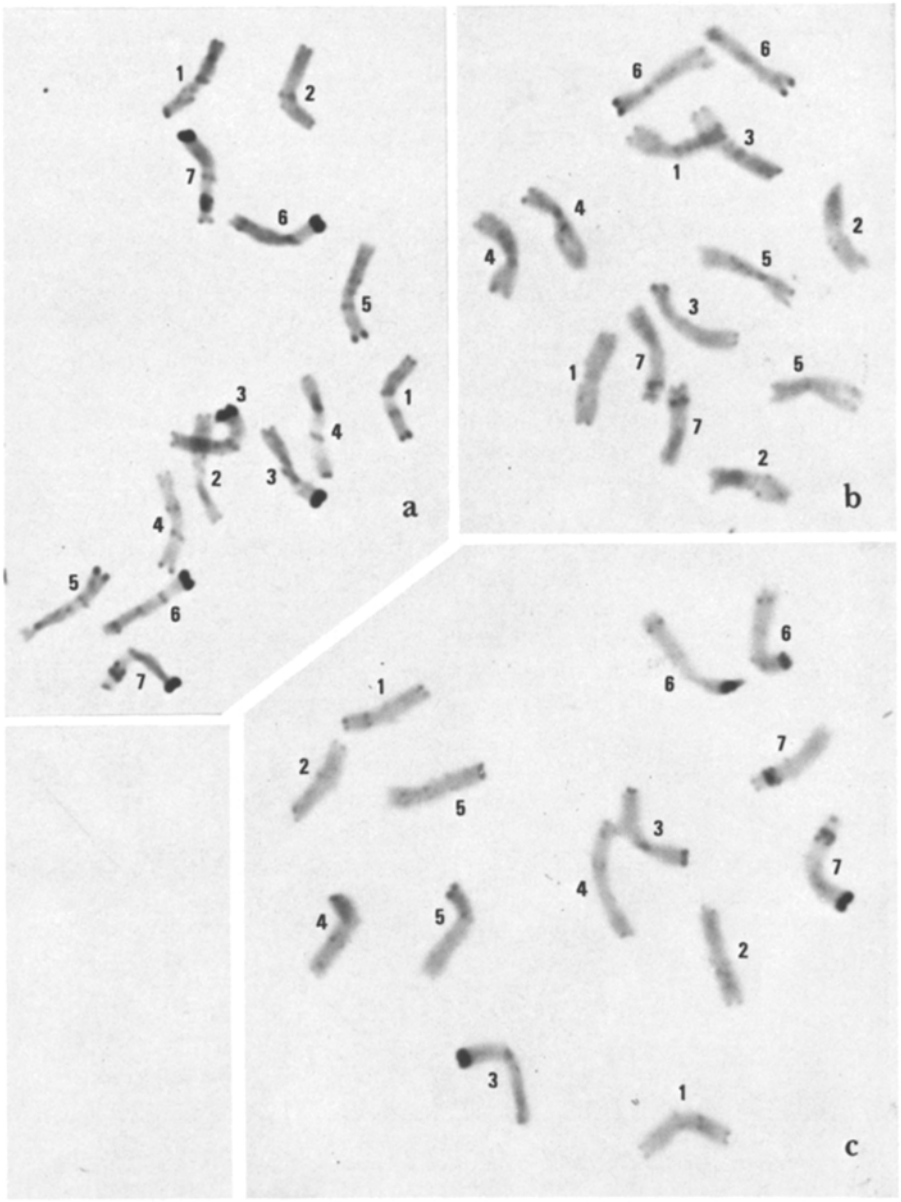

Fig. 1 a–c. Mitotic metaphase plates of inbred line P (**a**), inbred line E (**b**) and their F1 (**c**). In the F1 differences in banding pattern between the two homologues of chromosome pairs 3 and 7 are apparent

Figure 2a–c shows C-banded metaphase I cells of these F1 plants in which bivalents 3, 6 and 7 can be distinguished.

The four possible configurations in these three bivalents at metaphase I, i.e., ring bivalents (R), open bivalents in which the long arm was bound (Ol), open bivalents in which the short arm was bound (Os), and univalent pairs (U) could be identified. Table 1 shows the frequency of these configurations for the three bivalents in 500 metaphase I cells (10 anthers, 50 cells each).

Two types of both chromosomes 3 and 7 can appear at anaphase I and at metaphase II:

(i) Parental type (3p, 7p): both chromatids have the same telomeric heterochromatin constitution, showing no evidence of recombination.

(ii) Recombinant type (3r, 7r): each chromatid has a different telomeric heterochromatin constitution, showing evidence of recombination.

Figure 2e–f shows C-banded anaphase I cells in which the two types of chromosomes (p and r) can be distinguished. In agreement with the results of Jones (1978) recombination inside the heterochromatic C-bands in heterozygotes was never observed.

Table 2 gives the frequencies of the different anaphase I and metaphase II cells in respect to the two types of each of chromosomes 3 and 7.

Let f0, f1, f2 ... fn be the frequencies with which 0, 1, 2 ... n crossing-overs (chiasmata) take place in a bivalent arm heterozygous for telomeric heterochromatin. In absence of chromatid interference the frequencies of recombinant and parental chromosomes arising from the first meiotic division can be deduced as follows:

Number of chiasmata	Frequency	Expected frequency of the chromosome types arising from the first meiotic division	
		Recombinant (r)	Parental (p)
0	f0	–	f0
1	f1	f1	–
2	f2	1/2 f2	1/2 f2
3	f3	3/4 f3	1/4 f3
4	f4	5/8 f4	3/8 f4
...	...	...	...
n	fn	$\frac{2-(-1)^n(1/2)^{n-1}}{3}$ fn	$\frac{(-1)^n(1/2)^{n-1}+1}{3}$ fn

Then, the expected frequency of recombinant type chromosomes would be:

$$Fr = f1 + 1/2\, f2 + 3/4\, f3 + 5/8\, f4 + \ldots \quad (1)$$

and the expected frequency of parental type:

$$Fp = f0 + 1/2\, f2 + 1/4\, f3 + 3/8\, f4 + \ldots \quad (2)$$

Now, when a bivalent arm heterozygous for telomeric heterochromatin is not bound at metaphase I the two chromatids of each chromosome have the

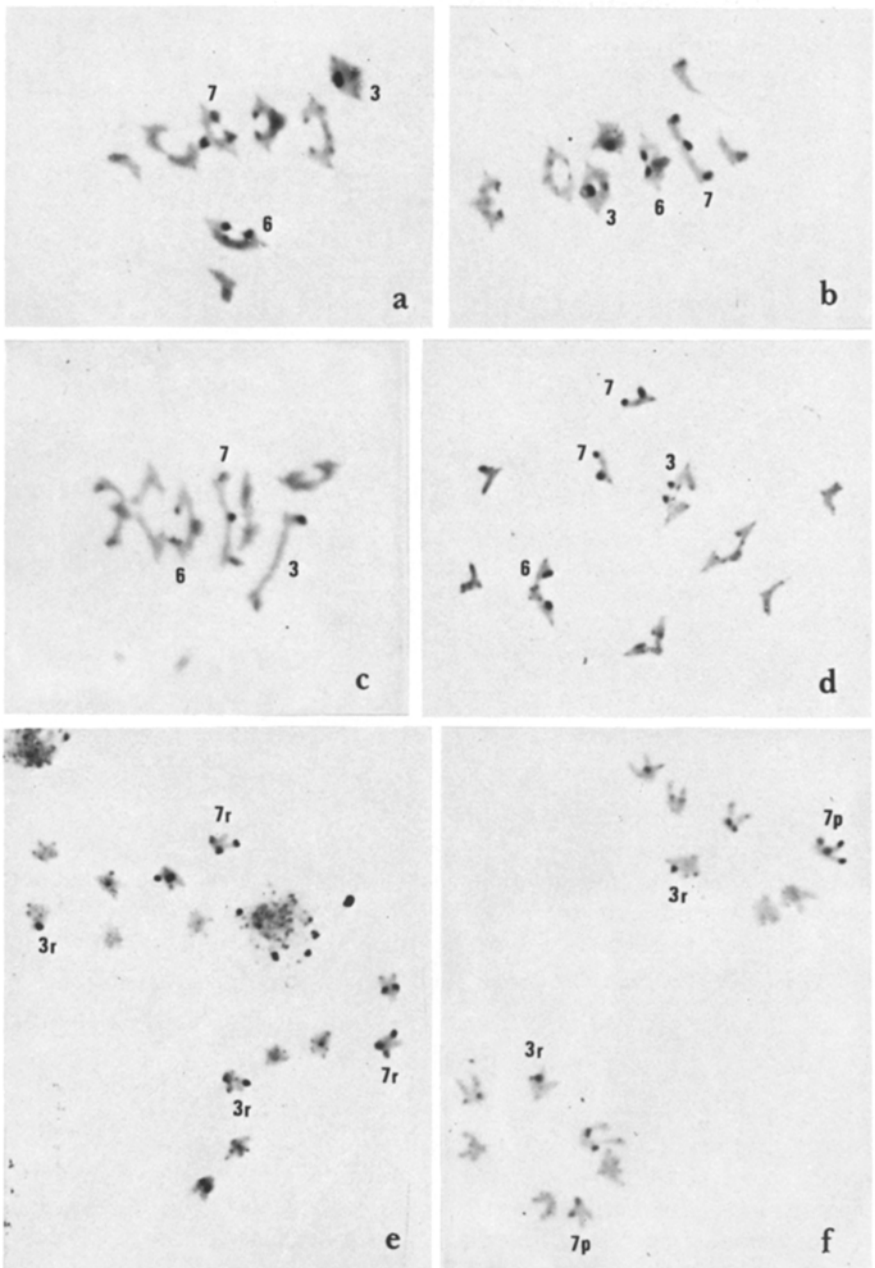

Fig. 2a–f. Meiotic cells of the F1 of P × E. **a–c** Metaphase I. In **c** the short arm of bivalent 3 is not bound, both chromatids having the same telomeric heterochromatin constitution in each arm. **d** Anaphase I, beginning. Both chromosome pairs 3 and 7 show evidence of recombination. **e–f** Anaphase I cells in which parental (*p*) and recombinant (*r*) type chromosomes can be distinguished

Table 1. Numbers of ring bivalents (R), open bivalents with long arm bound (Ol), open bivalents with short arm bound (Os) and univalent pairs (U) for chromosomes 3, 6 and 7 in 500 metaphase I complete cells of rye. Mean number of bound arms per cell 12.71 ±0.049

Configuration	Chromosome			
	3	6	7	1+2+4+5 (pooled)
R	468	475	292	1,640
Ol	26	24	199	342[a]
Os	6	1	8	
U	–	–	1	18

[a] In chromosomes 1, 2, 4 and 5 the two types of open bivalents could not be distinguished

Table 2. Frequencies of anaphase I and metaphase II cells with parental (p) and recombinant (r) chromosomes 3 and 7

Chromosome		Anaphase I cells	Metaphase II cells	Total (Anaphase I poles + Metaphase II cells)
3	7			
r	r	141	618	900
r	p	9	27	45
p	r	7	37	51
p	p	–	4	4

same heterochromatin constitution (Fig. 2c) indicating that no chiasmata have been lost in this material. Then, the frequency of not bound arms at metaphase I can be taken as a good estimate of f0.

If the chiasma frequencies follow a Poisson distribution (assuming no chiasma interference), the value of the distribution mean (λ) can be derived from:

$$f0 = e^{-\lambda}$$

and subsequently the expected values f1, f2, f3, ..., fn.

From (1) and (2) Fr and Fp can be calculated.

Table 3 shows the comparison between the observed frequencies of recombinant and parental type chromosomes arising from the first meiotic division and the corresponding expected frequencies. Both chromosomes 3 and 7 show a significant excess of recombinant types.

To explain these discrepancies one can assume that a bivalent arm being bound at metaphase I is the result of only one chiasma. Then, the probabilities for the short arm of bivalent 3 and the long arm of bivalent 7 to be bound at metaphase I would equal the frequencies of recombinant type chromosomes 3r and 7r respectively. Table 4 shows the comparison between the frequencies of the two possible configurations (bound and not bound) for the heterozygous

Table 3. Comparison between the observed frequencies of recombinant (r) and parental (p) type chromosomes arising from the first meiotic division and the corresponding expected frequencies under the assumption of a Poisson distribution of chiasmata

Chromosome	Anaphase I				Metaphase II				Total (anaphase I poles + metaphase II cells)			
	Obs.		Exp.		Obs.		Exp.		Obs.		Exp.	
	r	p	r	p	r	p	r	p	r	p	r	p
3	150	7	103.4	53.6	645	41	451.9	234.1	945	55	658.8	341.2
7	148	9	104.6	52.4	655	31	456.9	229.1	951	49	666.1	333.9
χ^2 tests												
Chromosome 3	61.516 ($p \ll 0.001$)				84.768 ($p \ll 0.001$)				128.809 ($p \ll 0.001$)			
Chromosome 7	53.953 ($p \ll 0.001$)				93.087 ($p \ll 0.001$)				131.088 ($p \ll 0.001$)			

Only half of the total Metaphase II observations have been considered independent observations

Table 4. Comparison between the frequencies of the two possible metaphase I configurations (bound and not bound) in the heterozygous arm of bivalents 3 and 7, and the corresponding frequencies of recombinant (r) and parental (p) type chromosomes in anaphase I and Metaphase II cells

Chromosome arm	Metaphase I		Anaphase I cells		Metaphase II cells		Total (anaphase I poles + metaphase II cells)	
	Bound	Not bound	r	p	r	p	r	p
3 (short)	474	26	150	7	645	41	945	55
7 (long)	491	9	148	9	655	31	951	49
	Contingency χ^2 tests							
	Metaphase I – Anaphase I		Metaphase I – Metaphase II		Anaphase I – Metaphase II		Metaphase I – Total (AI + M II)	
Chromosome 3	0.1377 ($0.8 > p > 0.7$)		0.2355 ($0.7 > p > 0.5$)		0.4775 ($0.5 > p > 0.3$)		0.0444 ($0.9 > p > 0.8$)	
Chromosome 7	6.9342 ($0.01 > p > 0.001$)		5.3298 ($0.05 > p > 0.01$)		0.3404 ($0.7 > p > 0.5$)		7.4204 ($0.01 > p > 0.001$)	

Only half of the total metaphase II observations have been considered independent observations

arm of each bivalent at metaphase I and the frequencies of recombinant and parental type chromosomes at anaphase I and metaphase II. There is a good fit for chromosome 3, whereas for chromosome 7 there is a significant excess of AI–MII parental chromosomes.

Discussion

In the chromosome arms analysed a highly significant deviation of the distribution of chiasmata from a Poisson series has been demonstrated (Table 3). This can be taken as an evidence of within arm chiasma interference (Haldane, 1931). The fit between the frequencies of bound arms at metaphase I and recombinant type chromosomes of pair 3 (Table 4) indicates that in the short arm of this chromosome a maximum of one chiasma is produced. In the plants of the F1 analysed the frequencies of 0 and 1 chiasma in the short arm of chromosome 3 would be respectively $f0=0.052$ and $f1=0.948$.

The excess of parental type chromosomes of pair 7 when compared with bound arm frequency at metaphase I (Table 4) suggests that some of the metaphase I bonds in the long arm of this chromosome are the result of more than one chiasma.

Table 5 shows the length (expressed as the percent of the total haploid complement) and arm length ratio of chromosomes 3 and 7 of inbred line P (taken from Giraldez et al., 1979). As can be seen, the long arm of chromosome 7 is longer than the short arm of chromosome 3. The difference in length, together with the fact that in the short arm of chromosome 3 only one chiasma is formed as a maximum, makes it reasonable to assume that in the long arm of chromosome 7 a maximum of two chiasmata are formed.

If this is true, the frequencies f0, f1 and f2 of 0, 1 and 2 chiasmata respectively, in the long arm of chromosome 7 can be deduced as follows:

$f0=$frequency of not bound arms at metaphase $I=0.018$

$Fp=f0+1/2\,f2=0.049 \quad f2=0.062$

$f0+f1+f2=1 \quad f1=0.92$

Now, if the chiasma frequency is known, the genetic length (L) for these two chromosome arms (3 short and 7 long) can be estimated from: $L=50(f1+2\,f2+3\,f3+\ldots+n\,fn)$.

$L(3\text{ short})=47.4$

$L(7\text{ long})=52.2$

Moreover, since we have a theoretically expected chiasma distribution based on class 0 (Poisson) and a derived "true" distribution, we can attempt an estimate of interference (I) in the two chromosome arms under study, following two different ways:

(i) Coincidence (C) can be estimated from the ratio between "observed" variance (derived from distribution of 0, 1 and 2 chiasmata deduced previously)

Table 5. Relative length (expressed as the percent of the total haploid complement) and arm length ratio at mitotic metaphase of the chromosomes 3 and 7 of inbred line P (taken from Giraldez et al., 1979)

	Chromosome	
	3	7
Length	14.43 ± 0.21	13.77 ± 0.19
Arm ratio	1.24 ± 0.02	1.45 ± 0.02

and theoretical variance in case of random chiasma distribution (=mean). Interference equals 1-C (Sybenga, 1975).

I(3 short) = 0.9247
I(7 long) = 0.9301

(ii) Coincidence (C) can be also estimated from the ratio between "observed" double crossovers (frequency of two chiasmata) and expected doubles (and triples etc.). Again, interference equals 1-C. Of course, as the "observed" frequency of two chiasmata in short arm of chromosome 3 equals 0, the interference value for this chromosome arm estimated by this method is 1.

I(3 short) = 1
I(7 long) = 0.9317

Then, comparison between these two approaches is only possible for chromosome 7. In the long arm of this chromosome, the interference values obtained by these two methods are practically the same.

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