Z. Pflanzenzüchtg. **93**, 199—211 (1984)

ISSN 0044-3298 / InterCode: ZEPZAD

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Metaphase I Bound Arms and Crossing Over Frequency in Rye

II. Inbred Lines

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With 4 figures and 4 tables

Received August 3, 1983 / Accepted August 8, 1983

Abstract

Using a Giemsa C-banding procedure it has been possible to identify at meiosis several chromosome pairs of three inbred lines of rye showing desynapsis. The probability of bonds in specific bivalent arms has been analysed. On the other hand, the comparison of the numbers of bound arms per cell in different moments of metaphase I has shown the existence of bound arm loss during this stage. It is concluded that: (1) there are significant differences in the frequency of bonds between bivalent arms within lines and within bivalent arms between lines that cannot be explained either by the different arm length or by the C-heterochromatin content, (2) there is a good fit between the observed distributions of bound arms per cell and the corresponding binomial ones, in spite of the fact that two of the basic assumptions under which a binomial series can be expected are not true (the probability of being bound is not the same in all bivalent arms and the probability for a bivalent arm to be bound is not the same in the different metaphase I cells of a line, due to bound arm loss). The reason for the fit is that these two factors have opposite effects in the variance of the bound arms per cell distribution.

Key words: *Secale cereale* — inbred rye — metaphase I — chiasma frequency

In rye, it is impossible to recognize specific bivalents at meiosis when traditional staining procedures are used. Also, the similarities in C-banding pattern between the different chromosome pairs of normal rye makes it difficult to distinguish between the different rye bivalents, even when C-banding

technique is employed. This difficulty has been solved in part by the use of chromosome markers involving structural or numerical changes (SYBENGA 1975). However, the results concerning chromosome behaviour can be affected in these cases by the modifications in the karyotype.

In normal diploid rye, most studies concerning bound arm frequency and distribution at metaphase I have been described in statistical terms. These have assumed either that the probability of being bound is the same for all chromosome arms of the complement and that the probability of the different meiotic configurations is also the same for the different bivalents, or that each bivalent arm has a different probability according to its length. Under these assumptions, theoretical distributions of bound arms per cell can be deduced and their fit with the observed distributions tested. However, a good fit with these theoretical distributions does not necessarily mean that these basic assumptions are true.

The analysis of this problem is the main aim of this work. Using a Giemsa C-banding procedure it has been possible to identify some specific chromosomes at meiosis of three inbred lines of rye (*Secale cereale*) differing in the degree of desynapsis. Differences in bound arm frequency between chromosomes within lines as well as within chromosomes between lines are reported. The possible reasons for these differences, as well as the good agreement between predicted and observed distributions of bound arms per cell are discussed.

Materials and Methods

Plants of the three inbred lines of rye (*Secale cereale*) E, P and M, earlier described by GIRALDEZ et al. (1979) were studied.

To obtain mitotic metaphase cells, seeds were germinated on wet filter paper in Petri dishes at room temperature. When primary roots were 1—2 cm long they were excised and immersed in tap water at 0 °C for 48 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. The fixed material was squashed and stained following the Giemsa C-banding technique described previously (GIRALDEZ and ORELLANA 1979).

The chromosome nomenclature used was the one proposed at the Workshop on Rye Chromosome Nomenclature and Homoeology Relationships, held at Wageningen, The Netherlands (SYBENGA 1983). The tentative correspondence between this nomenclature and the one used in previous works (GIRALDEZ et al. 1979) is:

Present nomenclature:	1R	2R	3R	4R	5R	6R	7R
GIRALDEZ et al. (1979)	7	3	2	4	6	5	1

Results

1. The distribution of bound arms per cell at metaphase I

On the assumption that (1) the probability of being bound at metaphase I is the same for all chromosome arms of the complement, (2) there is no interchromosomal interference and (3) the probability for a bivalent arm to be bound is the same in all cells, then the distribution of bound arms per

Tab. 1 Distributions of numbers of bound arms per cell at metaphase I in the three inbred lines, compared to the corresponding binomial distributions

Number of bound arms per cell	Inbred line P		M		E	
	observed	expected	observed	expected	observed	expected
14	31	23.9	17	14.4	20	16.5
13	90	98.9	69	71.4	72	73.9
12	171	190.3	154	164.2	152	153.4
11	241	225.3	247	232.2	215	195.9
10	204	183.4	229	225.7	157	172.0
9	95	108.6	149	159.6	96	109.8
8	48	48.2	81	84.6	51	52.6
7	16	16.3	42	34.2	24	19.2
6 or less	4	5.1	12	13.5	13	6.7
χ^2	10.222		4.978		12.881	
	$0.3 > p > 0.2$		$0.8 > p > 0.7$		$0.2 > p > 0.1$	
d.f.	8		8		8	

cell must fit a binomial series. *Table 1* shows the distributions of bound arms per cell at metaphase I in the three lines analysed compared with the corresponding binomial distributions. Only anthers having all PMCs at metaphase I were scored (8 to 10 plants per line, 100 cells per plant). In all cases there is a good fit between observed and expected values.

2. Bound arm frequency in specific bivalents

The comparison between C-banded chromosomes at mitosis and meiosis in the three lines (*Figs. 1, 2* and *3*) indicated that four chromosome pairs of inbred line P (1R, 2R, 5R and 6R) and three chromosome pairs of inbred lines M and E (1R, 2R and 6R) could be identified at metaphase I. The homologous relationships between these chromosomes have been ascertained by studying the pairing in hybrids between these inbred lines (P×E, GIRALDEZ and ORELLANA 1979, P×M, ORELLANA and GIRALDEZ 1981). *Table 2* shows the number of metaphase I 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) for each of the identifiable chromosomes in the three lines. *Table 3* shows the number of bonds formed by each bivalent arm.

From these results three main conclusions can be drawn:

(1) Within each chromosome pair, both arms form bonds independently, i.e., there is no interference across the centromere. Only chromosome 6R of line P shows a significant excess of ring bivalents and univalents (negative interference).

(2) Within each line, there are differences between bivalent arms in the probability of being bound. Although short arms always show a lower fre-

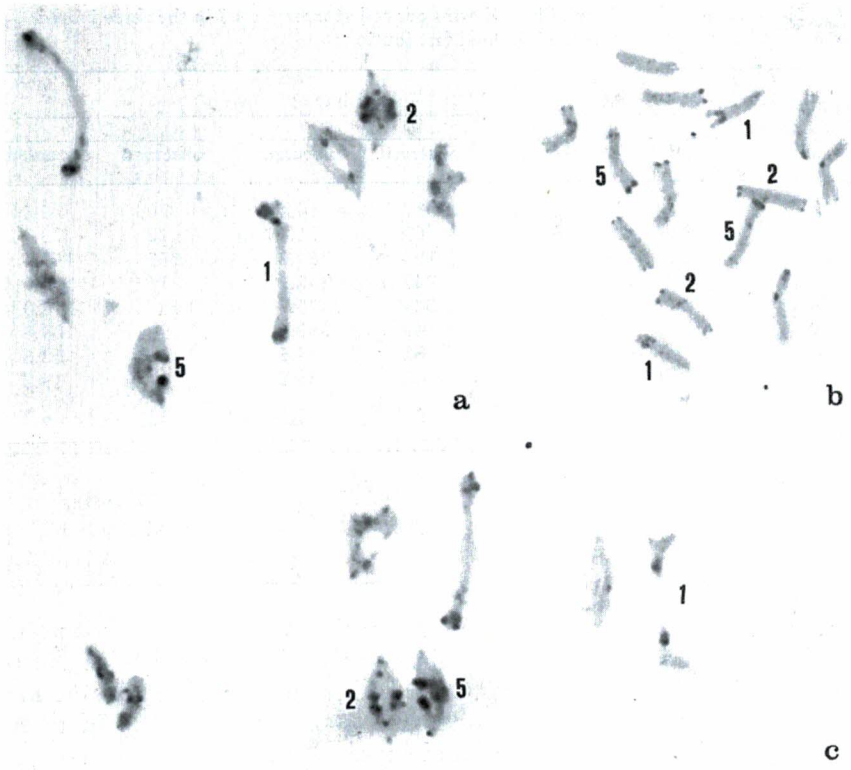

Fig. 1a—c Mitotic and meiotic C-banded cells of inbred line E. a and c Metaphase I cells in which the identified chromosome pairs show different configurations. b Mitotic metaphase plate. Bars represent 10 μ

quency of bonds than long arms, there is no direct relationship between the probability of being bound and the length. For instance, chromosomes 5R and 6R of inbred line P have a similar length and arm length ratio (GIRALDEZ et al. 1979), but differ considerably in bound arm frequency in the short arm.

(3) For a specific chromosome arm, there are differences between lines in the frequency of bound arms. These differences are not correlated with the overall frequency of bound arms of the different lines, i.e., there are significant differences between lines in the relative contribution of each chromosome arm to the total number of bonds. This also indicates that the probability of being bound is not directly related to arm length.

Table 4 shows the distributions of bound arms per cell at metaphase I in the three inbred lines compared with the expected distributions calculated from the known probabilities for each chromosome pair to form a ring bivalent (R), an open bivalent (Ol+Os) and a univalent pair (U) (*Table 2*). (For the non distinguishable chromosomes the average probabilities of ring open bivalent and univalent pair were considered.) In these theoretical distributions it is assumed that, for a given chromosome pair, the probabilities of being in

Tab. 2 Frequencies of the different metaphase I configurations (R, ring bivalents; Ol, open bivalents with the long arm bound; Os, open bivalents with the short arm bound; U, univalent pairs) for the identified chromosomes in the three inbred lines P, M and E (9, 10 and 8 plants per line respectively). 100 metaphase I cells per plant. Contingency χ^2 tests for independence between band formation in the two arms of each identified chromosome are included.

Chromosome	Metaphase I configuration	Inbred line		
		P	M	E
1R	R	495	420	312
	Ol	247	464	307
	Os	100	50	84
	U	58	66	97
Contingency χ^2		0.681 n.s.	0.799 n.s.	0.894 n.s.
2R	R	591	491	380
	Ol	237	240	275
	Os	46	194	93
	U	26	75	52
Contingency χ^2		1.796 n.s.	2.234 n.s.	1.838 n.s.
5R	R	587	457	532
	Ol	255	409	214
	Os	26	75	32
	U	22	59	22
Contingency χ^2		5.399 sig.	0.477 n.s.	3.518 n.s.
6R	R	170	—	—
	Ol	672	—	—
	Os	16	—	—
	U	42	—	—
Contingency χ^2		1.811 n.s.		
Remaining bivalents (pooled)	Ring bivalents	1778	2330	2020
	Open bivalents	861	1515	997
	Univalent pairs	61	155	183

a ring, open or univalent pair is the same in all cells and that all chromosome pairs behave independently. In the three inbred lines, the differences between the observed and the expected values are significant.

3. Bound arm loss during metaphase I

One of the basic assumptions under which most theoretical distributions of bonds per cell are made is that the probability for a bivalent arm to be bound is the same in all PMCs at metaphase I. However, it has been suggested (SYBENGA 1967, GIRALDEZ and LACADENA 1976) that in inbred lines of rye there is a loss of bonds during metaphase I. This would produce between cell differences in respect of the probability for a bivalent arm to be bound.

Tab. 3 The number of bonds formed by each identified bivalent arm in the three lines. The probability for each bivalent arm to form a bond appears between brackets. Contingency χ^2 tests concern the relative contribution of each chromosome arm to the total number of bonds.

Inbred line	Chromosome arm 1R S	1R L	2R S	2R L	5R S	5R L	6R S	6R L	Remaining bivalents (pooled)	Total number of bonds	Total number of cells
P	595 (0.661)	742 (0.824)	637 (0.708)	828 (0.920)	623 (0.692)	852 (0.948)	186 (0.206)	842 (0.936)	4417 (0.818)	9722	900
M	470 (0.470)	884 (0.884)	685 (0.685)	731 (0.731)	532 (0.532)	866 (0.866)	–	–	6175 (0.772)	10343	1000
E	396 (0.495)	619 (0.773)	473 (0.591)	655 (0.818)	564 (0.705)	746 (0.932)	–	–	5037 (0.787)	8490	800
χ^2 (P–M)	22.26**	4.78*	0.036	12.56**	13.16**	0.823			7.489**		
χ^2 (P–E)	16.75**	0.65	6.748	3.31	0.36	0.003			5.507*		
χ^2 (M–E)	0.14	8.54*	7.91**	2.47	17.01**	0.859			0.068		

* = Significant ($0.05 > p > 0.01$)
**= Highly significant ($0.01 > p$)

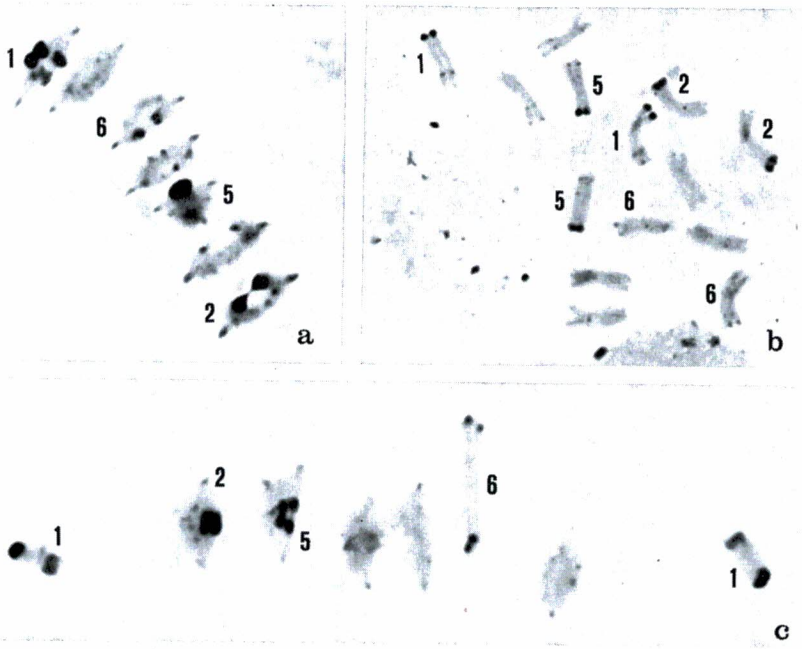

Fig. 2a—c Mitotic and meiotic C-banded cells of inbred line P. a and c Metaphase I cells in which the identified chromosome pairs show different configurations. b Mitotic metaphase plate. Bars represent 10 μ

Tab. 4 Distributions of numbers of bound arms per cell at metaphase I in the three inbred lines compared to the corresponding expected distributions calculated from the probabilities of each chromosome pair to be forming a ring bivalent, an open bivalent (Ol + Os) or a univalent pair *(Table 2)*.

Number of bound arms per cell	Inbred line P observed	P expected	M observed	M expected	E observed	E expected
14	31	11.7	17	10.8	20	15.6
13	90	83.3	69	62.5	72	72.8
12	171	199.2	154	160.0	152	154.2
11	241	251.9	247	240.8	215	197.9
10	204	198.1	229	255.7	157	173.0
9	95	104.8	149	164.2	96	109.5
8	48	38.8	81	76.5	51	53.3
7	16	10.1	42	25.8	24	17.4
6 or less	4	2.1	12	3.7	13	6.3
x^2	45.491		26.057		15.631	
	0.001 > p		0.001 > p		0.05 > p > 0.01	
d.f.	7		7		7	

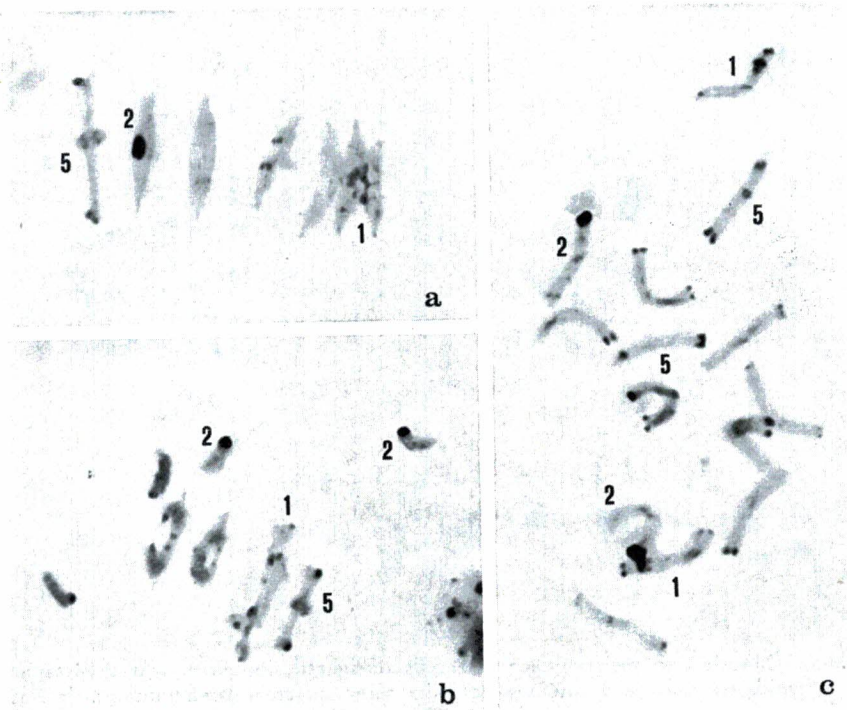

Fig. 3a—c Mitotic and meiotic C-banded cells of inbred line M. a and b Metaphase I cells in which the identified chromosome arms show different configurations. c Mitotic metaphase plate. Bars represent 10μ

In order to examine this possibility, anthers of inbred lines E and P were stained using the Feulgen method, the four different pollen sacs of each anther were separated under a dissection microscope and squashed on different slides. Using this procedure, the degree of synchrony of the PMCs in each slide was increased. The developmental stage of cells at metaphase I within a sac was estimated using the index of REES and NAYLOR (1960):

$$I = \frac{100 - a + b}{2}$$

in which a and b are the percentages of PMCs earlier or later than metaphase I respectively. Metaphase I indices were obtained from 50 to 100 cells per pollen sac. A coincidence of PMCs in earlier and later stages than metaphase I was never found within the same pollen sac.

The numbers of ring bivalents, open bivalents and univalent pairs were scored in twenty-five metaphase I cells of each pollen sac. *Figure 4* shows the mean number of bound arms per cell plotted against the metaphase I index (transformed to angles) in each pollen sac. In both lines, significant negative correlations were found.

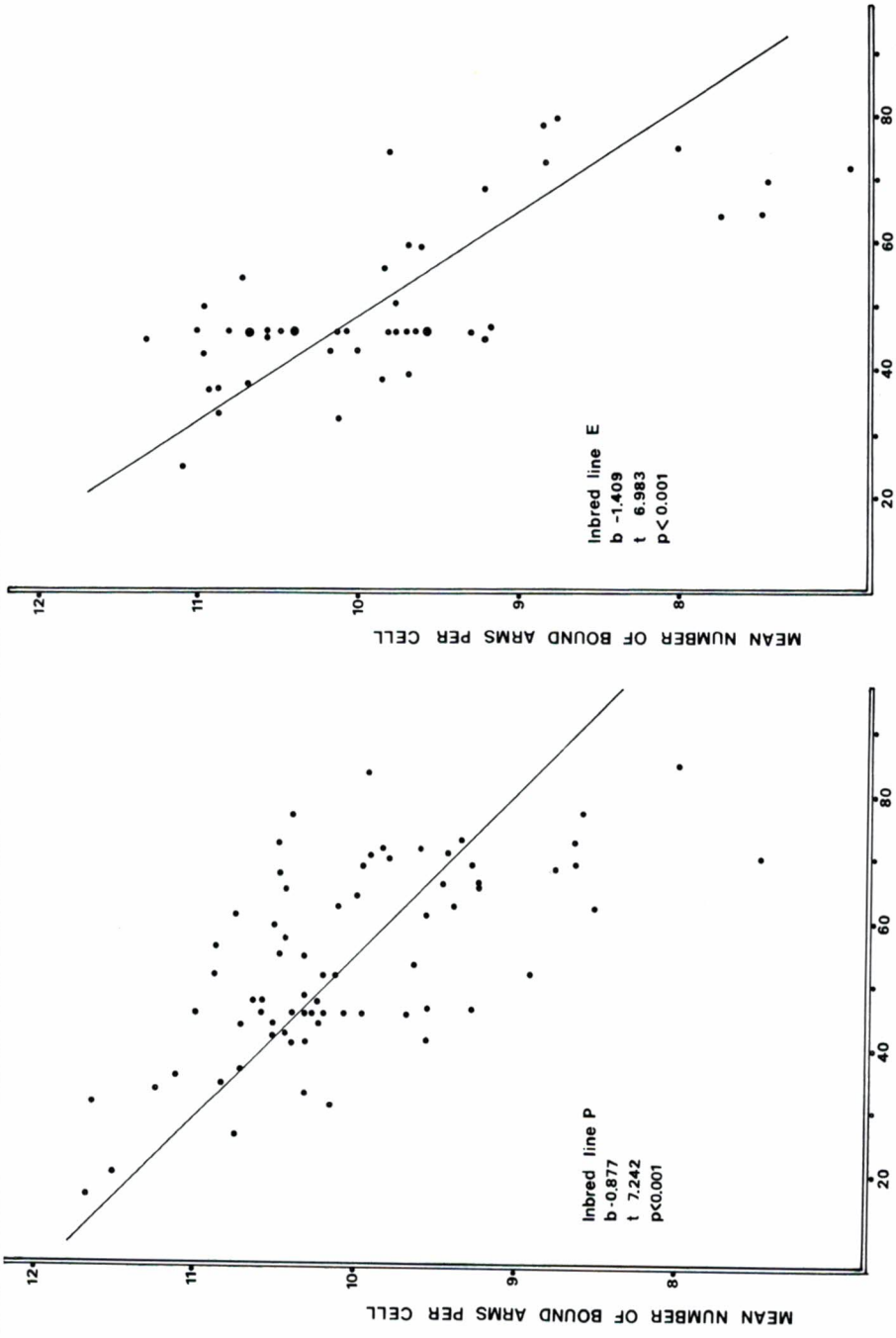

Fig. 4 The mean number of bound arms per cell plotted against the metaphase I index (transformed to angles) of pollen sacs of inbred lines P and E

As metaphase I proceeds, there is a significant decrease in the frequency of ring bivalents per cell (line E: $b = -0.773$, $t = 5.501$, $p < 0.001$; line P: $b = -0.638$, $t = 5.685$, $p < 0.001$) and a significant increase in the number of univalents pairs per cell (line E: $b = 0.636$, $t = 8.505$, $p < 0.001$; line P: $b = 0.241$, $t = 5.557$, $p < 0.001$).

Discussion

Concerning the distributions of bound arms per cell, two of the basic assumptions under which a binomial series can be expected are not fulfilled: The probability of being bound is not the same for all chromosome arms of the complement and the probability for a bivalent arm to be bound is not the same in all cells (even when only anthers having all cells at metaphase I are considered, a large between anther variation can be found, *Fig. 4*). In spite of this, there is a good fit between the observed distributions of bound arms per cell and the corresponding binomial series (*Table 1*). However, when a theoretical distribution is made in which between arm differences are taken into account, the fit is lost (*Table 4*).

These discrepancies may be explained if the two basic assumptions that are not fulfilled produce apposite effects in the actual bound arms per cell distribution. Then, let p be the probability of being bound in each of the two arms of bivalent 1, let $(1+n)p$ and $(1-n)p$ be respectively the probabilities for the two arms of bivalent 2 to be bound. In both bivalents 1 and 2 the average probability for a bivalent arm to be bound is p. If there is no interference across centromeres, then the probability for bivalent 1 to be open will be:

$$O_1 = 2p(1-p)$$

and the probability for bivalent 2 to be open:

$$O_2 = (1+n)p\,[1-(1-n)p] + (1-n)p\,[1-(1+n)p] = 2p(1-p)+2p^2n^2$$

as $0 < n < 1$, then, $O_2 > O_1$

As a consequence, the between arms differences in the frequency of being bound would lead to a bonds per cell distribution with a smaller variance than the one expected under a binomial series.

On the other hand, the average probability for a bivalent arm to be bound decreases as metaphase I proceeds (*Fig. 3*). This produces a heterogeneous cell population in which the variance of the distribution of bound arms per cell would be higher than that of the corresponding binomial series.

The opposite effects of the between arms differences and the cell heterogeneity would allow the distributions of bound arms per cell to fit the corresponding binomial ones. When the effect of the between arms differences is corrected in part, i.e., when an expected distribution is made in which the between arms differences are taken into account, the fit with the observed data is lost due to the increase of variance produced by the cell heterogeneity (*Table 3*).

The fit between observed bound arms per cell distributions and corresponding Poisson series has been studied on other occasions in rye. Probably, the best documented cases are those of SYBENGA (1960) and JONES (1967). In both cases, the comparisons made with normal plants showed a significant departure from the Poisson series, whereas in plants with translocations (SYBENGA 1960) and plants of abnormal genotype (JONES 1967) the distributions of bonds per cell followed a Poisson distribution. Although in these cases the fit can be attributed to change of the control system for chiasma formation in rye, the possibility of an effect similar to the one presented here cannot be discarded.

The results obtained in this work are an example of how the fit with theoretical mathematical models need not imply that their basic assumptions are actually fulfilled. This same material was studied by GIRALDEZ and LACADENA (1978) by traditional staining procedures. In that instance the conclusion that all seven pairs of homologues have similar chiasma frequencies was justified, because the expected and observed distributions of bound arms per cell were the same. From the present work it now seems that such a conclusion is no longer tenable.

There are two aspects of the present work that are worthy of discussion. These are the reasons for the between bivalent arm differences in the frequency of bonds and the nature of the bonds that are lost during metaphase I.

Evidently, both questions are related to one another. If a bond at metaphase I is considered as the result of at least one chiasma, the loss of bonds could be considered as a "late terminalization" taking place during metaphase I. However, there is evidence (ORELLANA and GIRALDEZ 1983) indicating that some of the bonds appearing at metaphase I are actually non-chiasmatic, i.e., they are a remnant of prophase pairing, with no chiasmata formed between the two chromosome arms involved. In this case, the loss of bonds cannot be considered as terminalization. Anyway, the existence of bound arm loss at metaphase I increases the difficulty of establishing the reason for the between bivalent arm differences in the frequency of bonds since these differences can be due to a different probability of pairing (or chiasma formation), or to a different probability of loss.

The results concerning the between arm differences in the frequency of being bound (*Tables 2* and *3*) indicate that such differences cannot be explained satisfactorily by the different chromosome arm lengths nor by the presence or absence of telomeric C-heterochromatin. For instance, the differences in the short arm of chromosomes 5R and 6R of line P (which are similar in length) could be related to the higher amount of telomeric C-heterochromatin present in chromosome 5R (*Fig. 2*). However, the relative frequency of bonds in the long arm of chromosome 1R of line P (having a big block of telomeric C-heterochromatin) is lower than that in the same chromosome arm of line M (without telomeric C-heterochromatin) and similar to that of line E (without telomeric C-heterochromatin (*Table 3*).

As suggested by SYBENGA (1976) and GIRALDEZ and SANTOS (1981), pairing preferences could be explained by efficiency or activity differences between chromosomes. The differences in relative bound arm probability between

lines for the same chromosome arm can be due to such differences in activity of efficiency which would not be related necessarily with chromosome length or C-heterochromatin constitution.

Zusammenfassung

Endbindungen in Metaphase I und Häufigkeit von Crossover im Roggen

II. Inzuchtlinien

Mittels einer Giemsa-C-Banden-Technik konnten in der Meiose von drei Inzuchtlinien des Roggens verschiedene Chromosomenpaare identifiziert werden, die Desynapsis zeigten. Untersucht wurde die Wahrscheinlichkeit von Endbindungen in spezifischen bivalenten Chromosomenarmen. Andererseits ließ der Vergleich der Anzahl von Endbindungen je Zelle in verschiedenen Abschnitten der Metaphase I das Vorkommen von Endbindungsausfall während dieses Stadiums erkennen. Es wird gefolgert, daß 1. signifikante Unterschiede in der Endbindungshäufigkeit zwischen bivalenten Armen innerhalb von Inzuchtlinien sowie innerhalb bivalenter Arme zwischen Inzuchtlinien vorkommen, die weder durch Unterschiede der Armlänge noch ihres C-Heterochromatin-Gehalts erklärt werden können, 2. gute Übereinstimmung besteht zwischen der beobachteten Verteilung der gebundenen Arme je Zelle und einer entsprechenden binomialen Verteilung trotz der Tatsache, daß zwei der Grundannahmen, unter denen eine binomiale Serie erwartet werden kann, nicht zutreffen (die Wahrscheinlichkeit einer Bindung ist nicht in allen bivalenten Armen dieselbe und die Wahrscheinlichkeit für einen bivalenten Arm, eine Bindung einzugehen, ist in den verschiedenen Metaphase-I-Zellen einer Inzuchtlinie wegen Endbindungsausfalls nicht gleich). Der Grund für die Übereinstimmung ist, daß die beiden Faktoren bezüglich der Varianz der Verteilung gebundener Arme je Zelle in ihren Wirkungen entgegengesetzt sind.

This work has been partially supported by a grant of the Comisión Asesora de Investigación Científica y Técnica of Spain.

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