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# Effects of constitutive heterochromatin and genotype on frequency and distribution of chiasmata in the seven individual rye bivalents*

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**Summary.** Number and distribution of chiasmata were studied in the single pair of homologous rye chromosomes in 29 chromosomal $F_1$ hybrids between the seven disomic wheat rye addition lines of 'Chinese Spring'/'Imperial' and five selected inbred genotypes of cultivated rye by using the differential Giemsa staining technique. The results indicate that the number and position of chiasmata is independent from the amount and position of C-heterochromatin. Genotype had an effect on chiasma number, whereas chiasma distribution within bivalents appeared to be determined by morphological features of chromosomes. Late replicating DNA in constitutive heterochromatin may delay the separation of half bivalents if chiasmata are formed between them and the centromere.

**Key words:** Rye – C-heterochromatin – Giemsa staining – Chiasma frequency – Chiasma distribution – Chromosome addition lines

## Introduction

Chromosomes of cultivated rye, *Secale cereale* L., possess large amount of constitutive heterchromatin mainly in terminal position (Merker 1973). It has been frequently proposed that constitutive heterochromatin interact with the formation and positioning of chiasmata (Miklos and Nankivell 1976; Santos and Giraldez 1978; John and Miklos 1979; Loidl 1979).

In addition, it has been suggested that interactions of constitutive heterochromatin in rye with wheat chromosomes in wheat/rye hybrids interfere with meiotic pairing of rye chromosomes (Bennett and Kaltsikes 1973; Thomas and Kaltsikes 1974; Merker 1976). But recent data on rye from Benavente and Orellana (1984), Orellana et al. (1984) and Drögemüller and Lelley (1984) cast doubt on the general validity of these suggestions.

The unequal distribution of C-heterochromatin between and within the single chromosomes of cultivated rye makes this species especially suitable for investigating the relationship between C-heterochromatin and meiotic behaviour of chromosomes.

The present study was conducted to determine the influence of constitutive heterochromatin on the formation and distribution of chiasmata in the seven pairs of rye chromosomes and to distinguish it from the influence of the genotype or from morphological features of the chromosomes.

## Materials and methods

The seven wheat/rye addition lines of 'Chinese Spring'/'Imperial' which were originally developed by Driscoll and Sears (1971) were used as female parents in crosses with five inbred rye genotypes selected from the collection of the Plant Science Department, University of Manitoba, Winnipeg. The inbred lines named 1a, 2a, 3c, 3h and 5e have been studied cytogenetically in detail as were their C-banding patterns (Lelley et al. 1978). The Giemsa karyotype of the 'Chinese Spring'/'Imperial' addition series has been chosen as a standard for the banding pattern of the seven rye chromosomes (Sybenga 1983).

Each addition line was crossed with all five inbred genotypes, resulting in 35 cross combinations. In the $F_1$'s mitotic selection was made for 29 chromosomal plants by the conventional Feulgen technique. In those plants only the added rye chromosome and its homologue in the respective inbred line were disomic. Due to the application of Giemsa staining technique in meiosis (Gustafson et al. 1976), the single

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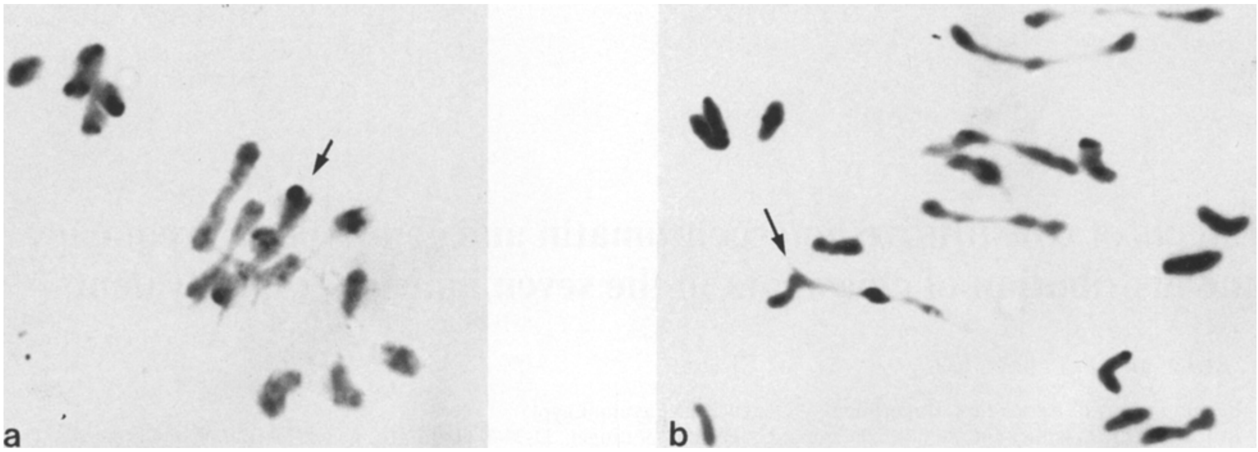

**Fig. 1 a, b.** A rye bivalent in metaphase I of the hybrids between rye chromosome addition lines of 'Chinese Spring' wheat and inbred lines of rye. **a** Hybrid between addition line 5 and inbred line 3c showing an easily identifiable rod rye bivalent (*arrow*) with stretched regions between centromeres and the chiasma. secondary band of constitutive heterochromatin can be seen behind the chiasma. **b** Heteromorphic bivalent of a hybrid between addition line 5R and inbred line 3h (*arrow*). Most of the long arm of 5R is deleted. Chiasma is formed in the short arm with the large telomeric C-band

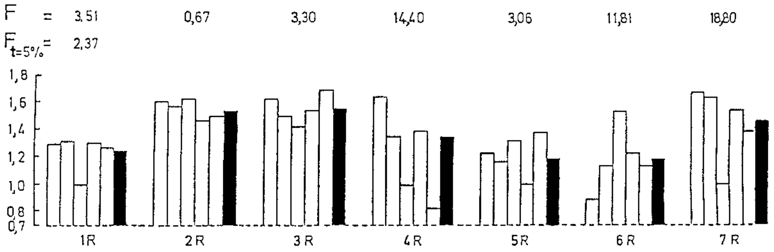

**Fig. 2.** Graphic representation of the average number of chiasmata formed in the bivalent between each of the seven added rye chromosome and its homologue in the five inbred rye genotypes 1a, 2a, 3c, 3h, 5e. Black column represents the mean of the five combinations of each addition line. F-values of these combinations are given

homologous rye bivalent was clearly distinguishable from bivalents which were occasionally formed between homoeologous wheat chromosomes (Fig. 1 a). At least 30 PMC's from 2 or 3 plants in each $F_1$ combination were analysed at metaphase I. The meiotic study focused on the two homologous rye chromosomes examining whether (1) they were paired, if so (2) how many chiasmata were formed and (3) where chiasmata occurred in relation to the location of a Giemsa band, i.e. whether they were terminal or interstitial. Differences were tested for significance by the F-test and the Scheffe-test. This work was started in 1981 in Göttingen and continued later in El-Fayum.

## Results

The average number of chiasmata which were formed in the single rye bivalent in each of the 35 different cross combinations is shown in Fig. 2. The percentage of PMC's in which the rye chromosomes failed to pair or paired as a rod or a ring bivalent and the proportion of terminal and interstitial chiasmata for each of the seven addition lines are given in Table 1. The highest percentage of PMC's showing a potential failure of the two homologous rye chromosomes to pair was found in 4R (11.4), while the lowest (4.8) was found in 3R.

Scheffe tests showed highly significant differences (Table 2) between overall averages of chiasmata in the single rye bivalent in crosses of the seven addition lines and the five rye inbred genotypes (black columns in Fig. 2) in 10 out of 21 cases. The highest average number of chiasmata in a rye bivalent (1.55 and 1.57) was between the added rye chromosomes 2R and 3R and their respective homologues in the inbred lines (Fig. 2). As expected, the same two rye chromosomes occurred most frequently (58.1% and 62.0%, respectively) as ring bivalents (Table 1). With an average of 1.2 chiasmata per bivalent, the lowest pairing intensity was demonstrated by the two added rye chromosomes 5R and 6R (Fig. 2). The single chiasma in these two chromosomes was found on the long chromosome arm in more than 95% of cases. These arms do not possess a primary telomeric C-band, but characteristic secondary bands can be seen regularly in the interstitial position. These bands contain definitely less constitutive heterochromatin than primary bands which occur in these two chromosomes in the telomeres of the short arm. However, the chiasma was formed regularly on the short arm of 5R with the telomeric heterochromatin in

**Table 1.** Mean percentages of different pairing configurations and the percentages of terminal and interstitial chiasmata in the single bivalent formed in the $F_1$ hybrids between the seven disomic 'Chinese Spring'/'Imperial' addition lines and the five inbred genotypes of rye

| Addition line | Added rye chromosome | Two homologous chromosomes | | | Chiasmata | |
|---|---|---|---|---|---|---|
| | | Not paired | Paired as | | Terminal | Interstitial |
| | | | rod | ring | | |
| 1 | 1R | 6.9 | 60.6 | 32.5 | 76.5 | 23.5 |
| 2 | 2R | 5.8 | 36.1 | 58.1 | 89.0 | 11.0 |
| 3 | 3R | 4.8 | 33.3 | 62.0 | 88.6 | 11.4 |
| 4 | 4R | 11.4 | 44.6 | 44.1 | 85.6 | 14.4 |
| 5 | 5R | 10.7 | 59.5 | 29.0 | 41.3 | 58.7 |
| 6 | 6R | 9.9 | 59.3 | 30.7 | 30.3 | 69.7 |
| 7 | 7R | 6.5 | 46.8 | 46.6 | 47.3 | 52.7 |

a particular plant of the hybrid 5R×3h where a great portion of the long arm of 5R was deleted (Fig. 1 b).

It was frequently observed that in the rod bivalents of 5R and 6R the chromosomes were highly stretched in the region between the centromere and the interstitially positioned chiasma, whereas the chromatin behind the chiasma to the telomere was still normally contracted (Fig. 1 a). Within the combinations of a specific addition line with the five inbred rye genotypes, differences in chiasma frequency in the single rye bivalent were significant, with the exception of chromosome 2R (see F-values in Fig. 2).

Chiasmata were most frequently localized in terminal positions in chromosomes 2R and 3R (89.0% and 88.6%, respectively) (Table 1), while the highest frequency of interstitial chiasmata occurred in chromosomes 5R and 6R (58.7% and 69.7%, respectively).

## Discussion

The combination of each of the six added rye chromosomes, except 2R, with five different inbred rye genotypes resulted in a significantly different average number of chiasmata in the single bivalent formed between the added chromosome and its respective homologue in the inbred genotype. This difference can only be the consequence of the different genotypic constitution of the inbred lines, since the wheat genotype ('Chinese Spring') was the same in all combinations.

There are remarkably fewer chiasmata in the heterozygous bivalents in the present study than in the seven rye homozygous bivalents, i.e. the seven 'Chinese Spring'/'Imperial' addition lines, found by Drögemüller and Lelley (1984). Heterozygous rye chromosomes in crosses between inbred lines always show an increase in chiasma frequency (Rees and Thompson 1956). Thus the reduction in our material can only be the result of genetic interactions between the rye chromosomes and the wheat background. A similar observation was made by Lelley (1981) in wheat/rye hybrids.

The differences in the average of chiasmata in crosses between the same inbred genotype and different addition lines, e.g. 1R×3c (1.02) compared to 6R×3c (1.52) or 3R×5e (1.66) to 4R×5e (0.80) in Fig. 2, indicate different genetic interactions between a specific rye genotype and the seven added rye chromosomes. The chromosomes with the highest (2R and 3R) and the lowest (5R and 6R) average chiasma frequencies have very similar karyomorphological properties and this may indicate a correlation between number and distribution of chiasmata and chromosome morphology. Chromosomes 2R and 3R possess an almost median localized centromere, whereas those of 5R and 6R are close to subterminal (Sybenga 1983). In chromosomes 5R and 6R, chiasmata are regularly formed in the long arm; less than 5% of the formed chiasmata occurred in their short arms. But the formation of chiasma was 100% shifted to the short arm in a particular plant of the cross combination 5R×3h, where the long arm of chromosome 5R was deleted. This indicates the pronounced influence of chromosome arm length on the competition for chiasmata if the genetic potential for chiasma number is low, e.g. chromosomes having the shortest arms in the rye genome (5R or 6R) have less chance to form chiasmata in both arms than the more median ones. Similar results were obtained by Giraldez and Orellana (1979) and Naranjo and Lacadena (1980). This may explain why chromosome 5R and 6R each with a total length comparable to chromosomes 2R or 3R, form many fewer chiasmata under the same genetic conditions. Direct cytological observations allowed us to exclude the possibility that terminal or subterminal chiasmata on the short arms of chromosomes 5R and 6R were already dissolved at the time of examination.

Another reason for the marked reduction of chiasma formation on the short arms of chromosomes

**Table 2.** Significances between the means (black columns in Fig. 2) of the seven addition lines, 1–7, using the Scheffe test

| | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 1 | -- | -- | | | | -- |
| 2 | | | | ++ | ++ | |
| 3 | | | ++ | ++ | ++ | |
| 4 | | | | | | |
| 5 | | | | | | -- |
| 6 | | | | | | -- |

5R and 6R could be the large telomeric heterochromatic bands in these arms. John and Miklos (1979) found that in Drosophila and several grass-hopper genera these bands affect the formation of chiasmata in their vicinity. A high proportion of terminally-formed chiasmata in chromosomes 2R and 3R which possess large amounts of constitutive heterochromatin in their telomeres (Sybenga 1983) was found in the present paper (Table 1). It strongly suggests that positional distribution of chiasmata within a bivalent is independent of the presence and position of C-heterochromatin in rye. In addition, regular formation of chiasmata occurred in the short arm of 5R having the large telomeric block of C-heterochromatin, when a major portion of its long arm, was deleted. Benavente and Orellana (1984) and Orellana et al. (1984) excluded any effect of rye heterochromatin on pairing of chromosomes in rye and in wheat/rye derivatives. Similarly, our data do not indicate that C-heterochromatin disrupts the cytology of wheat/rye hybrids. However, this has been postulated by several authors (see Müntzing 1979).

The only effect of C-heterochromatin on the meiotic behaviour of chromosomes may be to delay the separation of half bivalents by interstitial heterochromatin, as proposed for *Allium flavum* (Loidl 1979) and in *Chorthippus biguttulus* (Santos and Giraldez 1978). The rod bivalents with an interstitial chiasma are stretched between the chiasma and the centromere (Fig. 1a), whereas the chromatin between the chiasma and the telomere is highly contracted. Interstitial secondary bands were frequently visible right at the start of the highly contracted region behind the chiasma.

We assume that secondary interstitial C-bands are not barriers preventing chiasma terminalization. Rather, the late replication of their frequently repeated DNA sequences (Lima-de-Faria and Jaworska 1972), delay the separtion of sister chromatids of half bivalents and thereby retard the dissolution of any chiasmata formed between the centromere and the C-band.

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