Theor Appl Genet (1985) 70:66–71

Identification of the chromosomes showing neocentric activity in rye

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Received September 21, 1984
Communicated by R. Riley

Summary. Chromosomes showing neocentric activity were identified with the aid of the C-banding technique in an inbred line of rye, 1940-129 of cv. 'Stålråg'. The frequency of cells with neocentrics varied from 34 to 35.7% at first anaphase, and from 32 to 68% at second metaphase. In most cells only one chromosome showed the activity. It always belonged to the group 4-5-6R, and in cells where individual identification of chromosomes was possible the activity was invariably located at the telomere of the arm 4RS. When in rare cases two or three chromosomes were active, one chromosome again belonged to the group 4-5-6R, the other was identified in four cells as a member of the group 2-3-7R, but it is possible that all the telomeres with a prominent C-band can occasionally show the activity. It is assumed that certain telomeric regions remain in the active state at both meiotic divisions and offer secondary accumulation sites for kinetochoric proteins.

Key words: Neocentric activity – C-heterochromatin – Meiosis – Rye – C-banding

Introduction

Considerable attention has been focused on the late replicating heterochromatic regions located at the telomeres of rye chromosomes following observations that these regions may be closely related to the occurrence of aberrant endosperm nuclei and the extent of grain shrivelling in triticale (Bennett 1977). However, one of the oldest effects of the telomeric heterochromatin known in rye, the neocentric activity, has not yet been examined with the aid of modern molecular or cytological techniques.

Neocentric activity (Darlington 1965) is a secondary kinetic activity of the chromosomes. It is known to occur in certain inbred lines of rye: 'Weihenstephaner Winterroggen O_1' (Kattermann 1939) and 'Stålråg' (Prakken and Müntzing 1942; Östergren and Prakken 1946; Rees 1955; Heyward 1962). Spindle fibers attach to one or more telomeres simultaneously with the normal centromere, usually at both meiotic divisions but not at mitosis. As a result, the neocentric active sites move first towards the poles, co-orientate with the centromeres and other neocentric active sites in the same bivalent, and produce tension in the chromosomes at both meiotic divisions. The intensity of the phenomenon and the proportion of cells with neocentrics are very variable. There are striking differences between plants of the same line and even anthers of the same plant (Rees 1955). On the other hand, the neocentric telomeres obviously have a special structure, since in F_1 hybrids between lines with and without neocentric activity, only two chromatid ends are active (Prakken and Müntzing 1942), and the differences between the lines have a genetic basis. When Prakken and Müntzing (1942) conducted crossing experiments between lines with different intensities, the F_1 plants always showed an intermediate activity. According to Heyward (1962) the control is of a polygenic nature.

The purpose of the present study was to analyse the mitotic karyotype and to identify the neocentrics in meiotic cells with the aid of C-banding technique in a high intensity line examined by Prakken and Müntzing (1942).

Materials and methods

Two inbred lines, 1940-129 (subline 3-3) and 1940-131 (23), which were developed from cv. 'Stålråg' and had been selfed about 30 generations (Heneen 1962), were used for this study. All the meiotic stages were available from three plants, designed as 129-1, 129-2 and 131-1. The kernels used in this investigation were harvested in 1967 (line 129) and 1974 (line 131), and germinated on moist blotting paper in Petri dishes in October 1982. The plants were grown in a growth chamber (16 h light). At the 3–4 leaf stage the plants were transferred to

a refrigerator (8 h light, 5–9 °C) for 4 weeks, then returned to the growth chamber. Fixations were made during the summer 1983.

A C-banding method described earlier (Viinikka and Nokkala 1981) was used, except that the heads were fixed with a 3 : 1 ice-cold mixture of methanol and acetic acid, since after that fixative the spindle was preserved, and it was possible to examine the chromosomes and spindle fibers with the aid of a phase contrast microscope before squashing the material. Both premeiotic and root-tip mitoses were used for the identification of the banding patterns. A treatment of 0.05% colchicine for 3 h and a maceration with 0.5% pectinase (from *Aspergillus*, Sigma) for 2 h, were used on the root tip cells only.

C-banded karyotypes of the mitotic chromosomes of line 129 was based on an analysis of nine cells at premeiotic mitosis and six root tip cells. Chromosomes were measured and all the visible bands were recorded. Bands present in over half of the 30 chromosomes were used as marker bands in the identification of the meiotic chromosomes.

Results

Expression of neocentric activity

The prophase movements of the chromosomes appeared to be entirely normal. As described by Thomas and Kaltsikes (1976) heterochromatic telomeres obviously move along the nuclear envelope to form a single chromocenter. At pachytene the heterochromatic regions move apart again from each other. Neocentric activity was observed for the first time at the prometaphase stage. Neocentric active telomeres seem to be connected with the poles very early – even before the other bivalents have reached the division plane. Neocentrics were discernible in rod as well as in ring bivalents at first metaphase but due to the swelling caused by the C-banding technique, more suitable stages for the analysis of neocentrics were first anaphase and second metaphase. At first anaphase, about one third of the cells of both plants of line 129 showed neocentric activity (Table 1). But at second metaphase there was a clear difference between the plants (32% and 68%). Similar differences, caused by unknown physiological factors have been described by Rees (1955).

There was only one neocentric active chromosome in the majority of the cells (Figs. 1 a, 2 a–b, 3 a–c), but in four cells of this sample two chromosomes were active (Figs. 1 b and 3 d). In addition, rare cases with three or four active chromosome ends were seen in other preparations (Figs. 1 c and 3 e).

In line 131, only one very questionable case of neocentric activity was observed among 100 cells at second metaphase which corresponds to the classification made by Prakken and Müntzing (1942). According to them, this line belonged to the category 'traces of T-chromosomes?'.

Identification of the chromosomes

The C-banding patterns of the mitotic chromosomes in line 129 (Fig. 2 c) resembled rather closely the generalized karyotype of rye (Sybenga 1983). On chromosome 1R, the band adjacent to the satellite is very prominent, and hence this chromosome could be easily identified. Of the chromosomes with major bands on both telomeres (2-3-7R), chromosome 7R can be distinguished on the basis of the two minor bands on the long arm, one in the middle of the arm and the other adjacent to the terminal band. In addition, usually chromosome 2R is larger than chromosome 3R. The group of submetacentric chromosomes without a major band on the telomere of the long arm (4-5-6R) appeared to be the most important in this study. Chromosome 5R has no bands on the telomere of the long arm, but there is a heterochromatic region near the end of the arm that often is visible as a double band, and an intercalary band in the middle of the arm. The long

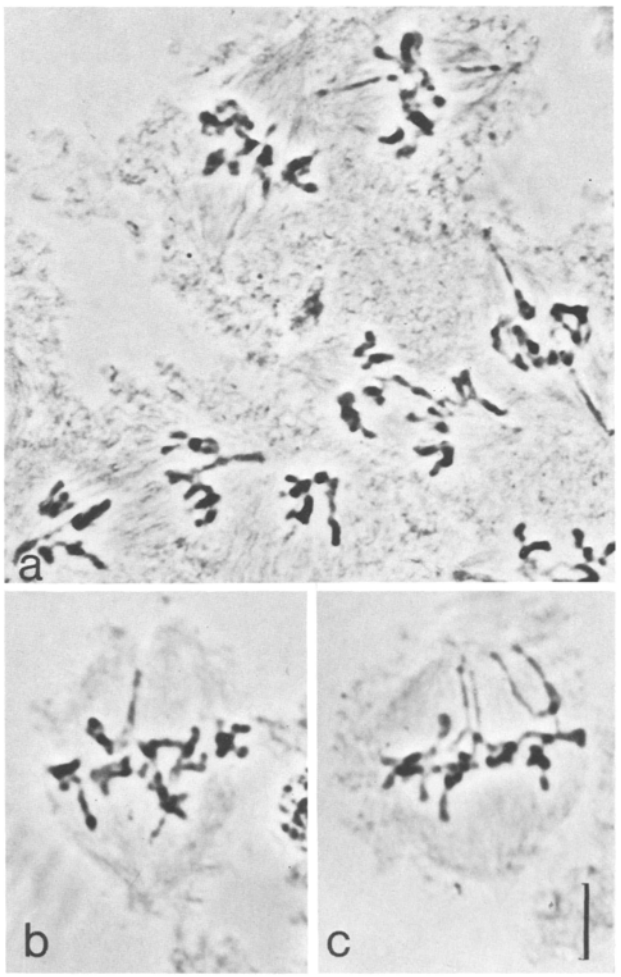

Fig. 1 a–c. Unstained chromosomes at MII with neocentric activity in one chromosome (**a**), in three chromosomes (**b**), and in both telomeres of one chromosome (**c**). Bar is equal to 10 μm

Table 1. Frequency of cells showing neocentric activity

Plant	A I					M II				
	No. of neoc. act. chromosomes			% (cells)	Total	No. of neoc. act. chromosomes			% (cells)	Total
	0	1	2			0	1	2		
129-1	33	17	–	34.0	50	34	15	1	32.0	50
129-2	27	15	–	35.7	42	16	31	3	68.0	50
131-1	–	–	–	–	–	99	1 (?)	–	1.0	100

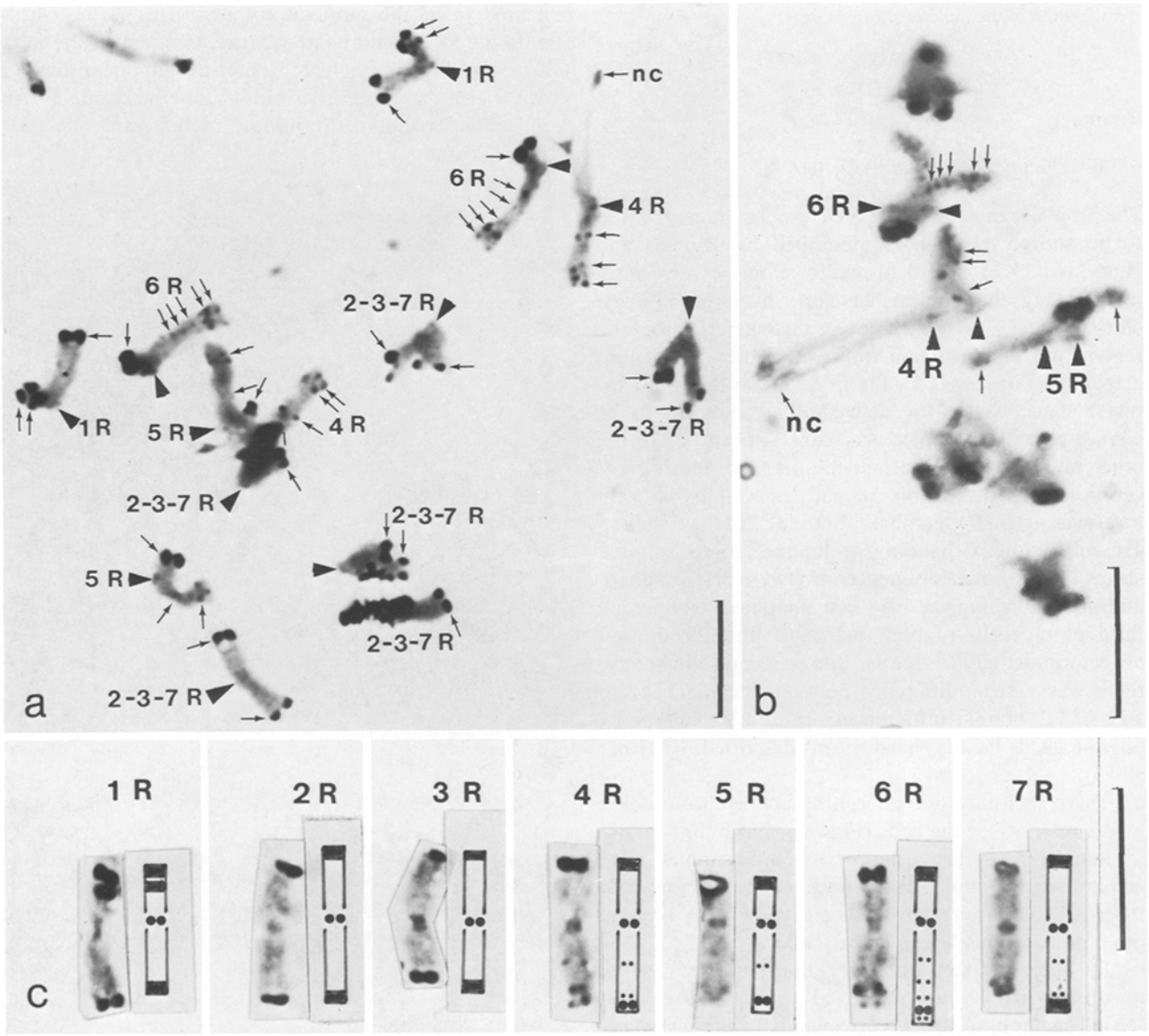

Fig. 2a–c. Identification of the chromosomes showing neocentric activity at AI (**a**) and MII (**b**) as compared with the C-banded mitotic karyotype of line 129 (**c**). *Large triangles* show the position of centromeric dots, *small arrows* point to the marker bands, *nc* = site of neocentric activity. Bars equal to 10 μm

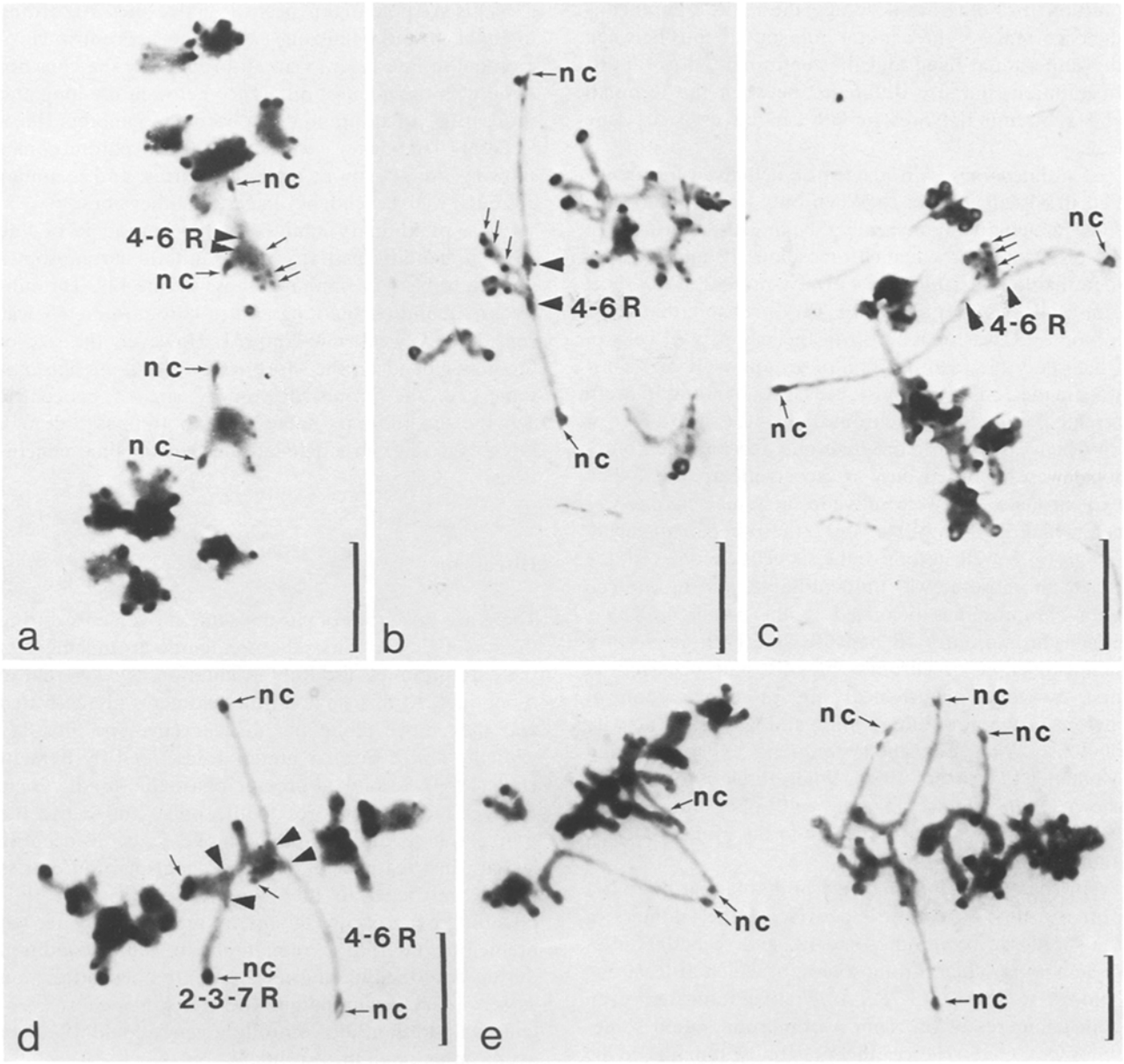

Fig. 3a–e. Neocentric activity in one chromosome pair at AI (**a**), in one chromosome at MII (**b, c**), in two chromosomes at MII (**d**), and in two adjacent cells at MII with two and three neocentrics (**e**). Bars equal to 10 μm

arm of chromosome 4R has a telomeric band and a subtelomeric band of nearly equal size, in addition to a clear intercalary band near the middle of the arm. Chromosome 6R also has a terminal and a subterminal band, of which the subterminal is the larger one, and usually three weaker bands between the subterminal band and the centromeric dots.

Individual rye chromosomes are unequivocally identifiable at mitosis, but only rarely at meiosis. The first metaphase bivalents are known to be especially difficult to identify. However, certain cells at first anaphase were observed to be suitable material for the identification (Fig. 2a) and some second metaphase cells could also be used (Fig. 2b). Fortunately, these are the same stages where the neocentric activity is most conspicuous.

The meiotic chromosomes can easily be divided into three groups: 1R, 2-3-7R and 4-5-6R. In most cells, chromosome 5R was distinguishable from the group 4-6R. In this study, chromosomes 4R and 6R were individually identified only in cases where the banding patterns of both chromosomes were clearly visible. Since the terminal, subterminal and intercalary band in the middle of the long arm have nearly identical

positions in both chromosomes, the main difference is the occurrence of three minor interstitial bands between the subterminal band and the centromeric dots on 6R. In addition, the size difference between the terminal and subterminal bands on 6R can be used in some cells.

Chromosomes with neocentric activity were identified in 85 cells (Table 2). When only one chromosome showed activity, it invariably belonged to the group 4-5-6R. Moreover, when chromosomes 4R and 6R were identifiable, the activity was always present at the short arm of 4R. Consequently, it is very probable that chromosome 4R was active also in the majority of cells in which only the identification of groups 4-6R or 4-5-6R was made. Also, in most cells the banding pattern resembled more that of chromosome 4R (Fig. 3 a–c).

Clear cases with more than one neocentric chromosome were recorded only at second metaphase. When two chromosomes were active in the same cell, one was again identified as 4R or 4-6R, the other belonged to the group 2-3-7R in four of the six cells. In one cell, the other chromosome was unidentifiable, and in one cell both chromosomes belonged to the group 4-6R, i.e. either chromosomes 4R and 6R were both active or a nondisjunction of chromosomes 4R had occurred at first division. Disturbances are rather common at meiosis of the most inbred lines, and neocentric activity obviously increases their frequency (Prakken and Müntzing 1942; Rees 1955). When three chromosomes showed the activity in one cell, one chromosome belonged to the group 4-6R, one to the group 1-2-3-7R and one was unidentifiable.

In conclusion, it seems very probable that there is a primary site for neocentric activity on the telomere of the short arm of chromosome 4R, and secondary sites of activity in other chromosomes, of which at least one belongs to group 2-3-7R. As Fig. 1c indicates, even both telomeres of the same chromosome could sometimes be active. Hence, the possibility remains to be tested that actually all the telomeric regions with major C-bands are potential neocentric active sites. According to Prakken and Müntzing (1942) the neocentric chromosome in line 129 was 'in all probability the chromosome with the greatest difference between the long and short arms'. In addition, they observed in another line a 'general T-tendency' with up to six chromosomes showing the activity at their short arms, and a similar although weaker tendency in certain other lines.

In a preliminary analysis of the karyotype of line 131, the banding patterns on the mitotic chromosomes seemed to be very similar to those in line 129. The subterminal band on the long arm of chromosome 4R was considerably weaker in line 131. However, the size of the major band on the short arm of the same chromosome, i.e. the proposed primary site of neocentric activity, was about the same size in both lines, although more exact measurements are needed for final conclusions.

Table 2. Identification of chromosomes with neocentric activity

No. of neoc. act. chromosomes	Chromosome types	No. of cells
1	4–5–6R	3
	4–6R	52
	4R	23
2	2–3–7R + 4–6R	3
	2–3–7R + 4R	1
	4–6R + 4–6R	1
	4–6R + ?	1
3	1–2–3–7R + 4–6R + ?	1

Discussion

There are two types of chromosome movements during the meiotic cycle. First, the premeiotic arrangement of the chromosomes, the Rabl orientation, is converted to a bouquet. At this stage all the telomeres are activated and they move along the nuclear envelope, possibly with the aid of fibrillar material described by Bennett et al. (1979). Second, at prometaphase the spindle fibers connect the kinetochores to the poles and cause the prometaphase and anaphase movements. Neocentric activity has features of both mechanisms. Since it is usually restricted to the two meiotic divisions, it is possible that certain telomeric regions activated at premeiotic interphase remain in an active condition during both meiotic divisions, and are able to function as secondary accumulation sites for kinetochoric proteins. In addition, the controlling genes could affect an overproduction of these proteins.

In maize, neocentric activity depends on the presence of an abnormal chromosome K10 with an extra knob and adjacent euchromatic region (Rhoades 1952, Fig. 4.1). The K10 knob is capable of inducing neocentric activity in all the other knob regions containing the same 185 bp repeat sequence that is the main component of the knob K10 itself (Peacock et al. 1981). Obviously there are additional sequences in the K10 knob that are responsible for the induction of neocentric activity. Similarly in rye, the neocentric activity is restricted to the major heterochromatic blocks. However, one of the rye chromosomes, i.e. 4R, seems to be far more often active than the other telomeric regions. In maize there is a close correlation between neocentric activity, preferential segregation and the presence of a knob on the chromosome arm, and the degree of preferential segregation depends on the size of the knob (Peacock et al. 1981). In accordance with this, the primary site of rye could contain more of the repetitive sequences corresponding to the 185 bp repeat of maize. Another possibility is that the controlling genes are located mainly in

chromosome 4R adjacent to, or interspersed, in the primary site of activity.

In rye, neocentric activity has been observed only in inbred lines, but at least of two different origins. Hence, it is very probable that the repetitive sequences and controlling genes (including variants for overproduction) were both present already in the population plants. During inbreeding, suitable combinations of controlling genes may have been selected, and changes may have also occurred in the repetitive sequences, until the situation exemplified in this study is formed.

Variants of controlling genes could be present in other varieties of rye, and they can be activated, e.g. in hybrid combinations. Neocentric activity is known to occur in *Bromus* species hybrids (Walters 1952). In this connection it may be significant that the loss of telomeric heterochromatin from different rye chromosomes has different effects on the regularity of early endosperm development in triticales, and chromosomes 4R and 6R are the most important in this respect (Bennett and Gustafson 1982; Gustafson et al. 1984).

Acknowledgement. The author wishes to thank Professor Arne Lundqvist for the seed samples used in this study.

References

Bennett MD (1977) Heterochromatin, aberrant endosperm nuclei and grain shrivelling in wheat-rye genotypes. Heredity 39:411–419

Bennett MD, Gustafson JP (1982) The effect of telomeric heterochromatin from *Secale cereale* on triticale (×Triticosecale). 2. The presence or absence of blocks of heterochromatin in isogenic backgrounds. Can J Genet Cytol 24: 93–100

Bennett MD, Smith JB, Simpson S, Wells, B (1979) Intranuclear fibrillar material in cereal pollen mother cells. Chromosoma 71:289–332

Darlington CD (1965) Cytology. J and A Churchill, London, pp 714–715

Gustafson JP, Lukaszewski AJ, Skovmand B (1984) Heterochromatin content and early endosperm development in 42-chromosome spring triticale. Can J Genet Cytol 26: 85–90

Heyward MD (1962) Genetic control of neocentric activity in rye. Heredity 17:439–441

Heneen WK (1962) Chromosome morphology in inbred rye. Hereditas 48:182–200

Kattermann G (1939) Ein neuer Karyotyp bei Roggen. Chromosoma 1:284–299

Östergren G, Prakken R (1946) Behaviour on the spindle of the actively mobile chromosome ends of rye. Hereditas 32: 473–494

Peacock WJ, Dennis ES, Rhoades MM, Pryor AJ (1981) Highly repeated DNA sequence limited to knob heterochromatin in maize. Proc Natl Acad Sci USA 78:4490–4494

Prakken R, Müntzing A (1942) A meiotic peculiarity in rye, simulating a terminal centromere. Hereditas 28:442–482

Rees H (1955) Genotypic control of chromosome behaviour in rye. Heredity 9:93–116

Rhoades MM (1952) Preferential segregation in maize. In: Gowen JW (ed) Heterosis. Iowa State College Press, Ames, pp 66–80

Sybenga J (1983) Rye chromosome nomenclature and homoeology relationships. Workshop report. Z Pflanzenzücht 90:297–304

Thomas JB, Kaltsikes PJ (1976) A bouquet-like attachment plate for telomeres in leptotene of rye revealed by heterochromatic staining. Heredity 36:155–162

Viinikka Y, Nokkala S (1981) Interchromosomal connections in meiosis of Secale cereale. Hereditas 95:219–224

Walters MS (1952) Atypical chromosome movement in meiotic anaphase of *Bromus pitensis*×*B. marginatus*. Am J Bot 39: 619–625