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The Mapping of Highly-Repeated DNA Families and Their Relationship to C-Bands in Chromosomes of *Secale cereale*

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Abstract. The relationship between the chromosomal location of heterochromatin C-bands and of four non-homologous repeated sequence families constituting 8 to 12% of total rye DNA has been investigated in chromosomes of rye (*Secale cereale*) by in situ hybridisation. Three rye varieties, a set of rye disomic additions to wheat and a triticale were studied. Only centromeric and nucleolar organizer region (NOR) associated C-bands failed to display hybridisation to at least one of the sequences and many telomeric blocks of heterochromatin contained all four repeated sequence families. Both between-variety differences in the chromosomal distribution of repeated sequences, and intravarietal heterozygosities were frequently noted and are probably widespread. – Previously reported deletions of heterochromatin from King II rye chromosomes added to the Holdfast wheat complement were correlated with deletions of some, but not all, of the highly repeated sequence families. A previously unreported loss of some families from King II rye chromosome 4R/7R in a Holdfast wheat genetic background was detected. This loss was not associated with complete deletion of a C-band. A deletion has also probably occurred from the short arm telomere of 4R/7R in the triticale variety Rosner. It is suggested that the families of repeats in rye telomeric heterochromatin which are absent from wheat are selected against in the wheat genetic background.

Introduction

C-bands were first shown to contain highly repeated DNA sequences when repeated sequence DNA was hybridised in situ to mouse chromosomes (Pardue and Gall, 1970; Jones, 1970). Many subsequent studies have substantiated the general hypothesis that heterochromatin consists mainly of arrays of highly repeated DNA sequences (John and Miklos, 1979).

In a previous paper we have reported the molecular cloning and characterisation of members of four different repeated sequence families localised

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in heterochromatin in *Secale cereale* (Bedbrook et al., 1980a). These families together account for approximately 8 to 12% of the DNA in the genome and most of the DNA in the telomeric heterochromatin. Three of the families are essentially absent from *Secale silvestre*, which has about 55% less DNA in telomeric heterochromatin than *S. cereale*.

In order to characterise in more detail the repeated sequence composition of different rye C-bands with respect to the four specific repeated sequences, rye chromosomes with well characterised C-banding patterns have now been studied by in situ hybridisation. The characterised rye chromosomes are those of the rye cultivars King II, UC90 and Petkus Spring and the triticale variety Rosner (Singh and Röbbelen, 1975; Darvey and Gustafson, 1975; Lelley et al., 1978; Bennett and Gustafson, unpublished). Singh and Röbbelen (1976) reported that in three of the seven lines where the different individual rye chromsome pairs of King II are present in the genetic background of the wheat variety Holdfast (Riley and Macer, 1966), heterochromatin (C-bands) has been lost. These Holdfast-King II rye addition lines were therefore also included in our studies.

Materials and Methods

Plant Genotypes. The wheat-rye addition series described by Riley and Chapman (1958) and Riley and Macer (1966) were used. The wheat (*Triticum aestivum*) is Holdfast and the rye (*Secale cereale*) chromosomes were derived from the variety King II. The rye varieties studied were King II, Petkus Spring and UC90 each maintained as inbred populations. The triticale variety studied was Rosner.

In situ Hybridisation Probes. The tritium labelled probes consisted of complementary RNA made from the recombinant plasmids pSC 210, pSC 34, pSC 119 and pSC 33 described in Bedbrook et al. (1980a). These plasmids contain sequences from the rye genome which belong to unrelated families with 480, 610, 120 and 630 base pair repeating units respectively. The 630 base pair family is partially homologous with two other minor families with repeating units of 120 and 356 base pairs (Bedbrook et al. 1980a).

In some of the early experiments, the sites of the 480 base pair family sequences were detected by hybridisation with ^{3}H cRNA prepared from pSC 74. This plasmid contains only a part of the 480 base pair repeating unit but gives identical hybridisation results to pSC 210.

In situ Hybridisation. Root tip metaphase chromosome preparations were made and hybridised with the tritium labelled cRNA probes as described in Bedbrook et al. (1980a) with the following modifications. The treatment of the slides with 2 × SSC at 70° C followed by ethanol was omitted. Denaturation was accomplished by dipping slides into water at 65–70° C for 30 s after the application of 6 µl of the hybridisation solution to the slide and sealing on the acid washed coverslip with Dunlop rubber gum. 500,000–100,000 cpm in 3 × SSC 50% formamide were usually applied per slide. Hybridisation was for 12–16 h at 37–39° C. The six washes at the end of the hybridisation after the RNase step, were in 2 × SSC/0.1% SDS and extended over 3–5 h. Suitable slides were exposed for up to 60 days. Chromosomes were photographed on a Vickers M41 photomicroscope using Kodak SO115 film. Most of the karyotypes were established by organising the 14 chromosomes into 7 homologous pairs using at least two good metaphase chromosome spreads from at least two slides made from two different seedlings of each genotype. The karotypes are arranged in the figures with the pair of nucleolus organiser (NOR) chromosomes on the left and the remaining chromosome pairs in apparent order of decreasing arm ratio, except for the rye addition line chromosomes

in Fig. 2 which are ordered on the basis of their known homoeology to wheat chromosomes i.e. 1R, 2R, 3R, 4R/7R, 5R, 6R and 7R/4R.

Results

We describe first the in situ hybridisation patterns of each of the four cloned DNA sequences to the variety King II and then to the individual King II chromosomes maintained in the wheat variety Holdfast. The in situ hybridisation patterns to the other rye cultivars and Rosner triticale are described later.

The repeated sequence families have been designated below and in the figures by the lengths of their unit repeats (see Materials and Methods).

In situ Hybridisation Patterns to King II Rye Chromosomes (Fig. 1a) Using:

The Sequence from the 480 bp Family

This sequence hybridised to both arms of all chromosomes, though a heterozygosity for very weak labelling was observed with one chromosome in one individual. The two most heterobrachial chromosome pairs showed interstitial sites of hybridisation slightly proximal to the telomere; one of them also showed hybridisation at the telomere.

The Sequence from the 120 bp Family

All chromosomes showed strong sites of hybridisation with this sequence, and again the sites were predominantly telomeric. However, clear interstitial sites were also visible, and the most conspicuous of these sites were in the long arms of the most unequal armed chromosomes (designated 5R, 6R and 4R on the basis of the addition line studies – see later) and of chromosome 1R which carries the nucleolus constriction. The strong interstitial hybridisation sites correspond with known C-bands (Darvey and Gustafson, 1975). The three median chromosomes did not show strong interstitial sites, but all showed hybridisation to both telomeres. It is important to bear in mind that the number of sites detected in all the experiments is heavily dependent on the hybridisation conditions and the autoradiographic exposure time (cf. Figs. 1a and 5a).

The Sequence from the 610 bp Family

This sequence hybridised exclusively to telomeric sites. The NOR chromosome (1R) showed hybridisation at both telomeres. Two other chromosomes, median or submedian, showed hybridisation at both telomeres while the remaining four showed hybridisation at only one telomere. On the more unequal armed chromosomes it was the telomere of the short arm which showed hybridisation.

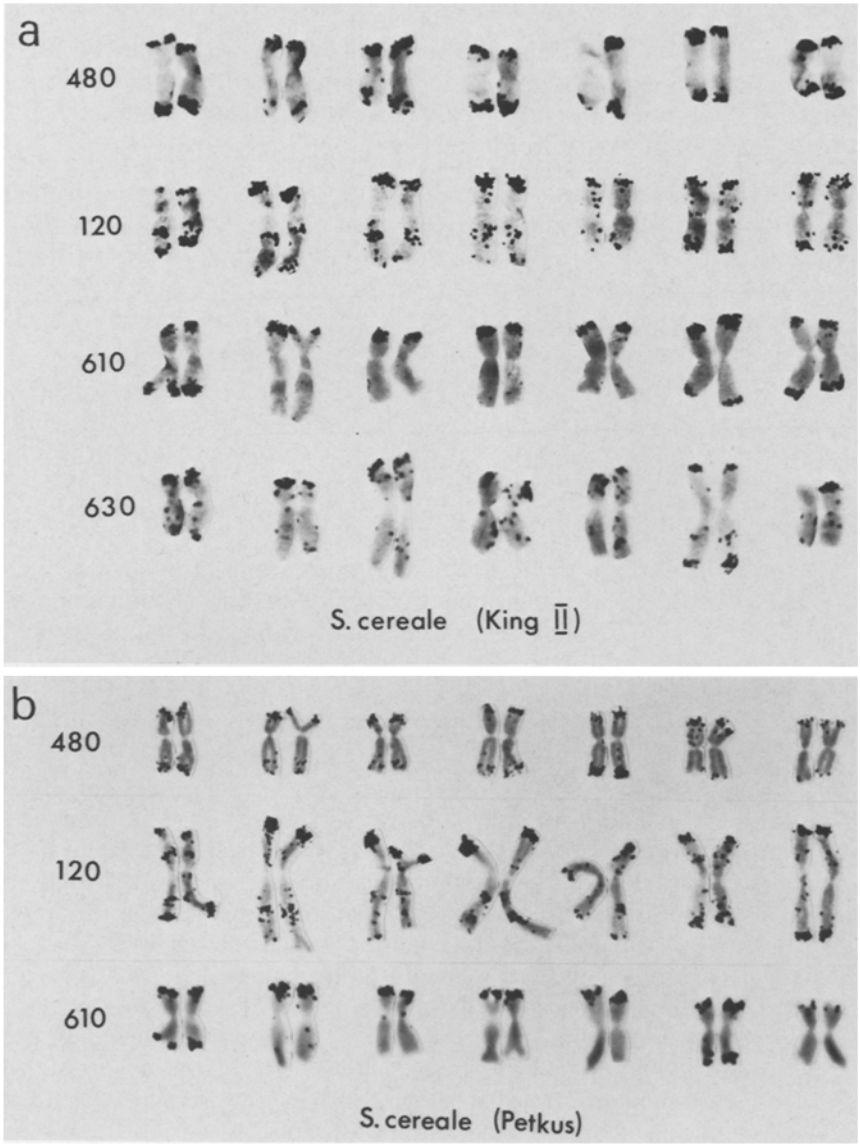

Fig. 1. a In situ hybridisation of ^{3}H cRNA to chromosomes of *S. cereale* var. King II. 480 bp family sequence: 65,000 cpm, 14 days exposure. 120 bp family sequence: 65,000 cpm, 28 days exposure. 610 bp family sequence: 60,000 cpm, 21 days exposure. 630 bp family sequence: 200,000 cpm, 36 days exposure. **b** In situ hybridisation on ^{3}H cRNA to chromosomes of *S. cereale* var. Petkus. 480 bp family sequence and 610 bp family sequence: as in Fig. 10A, B of Bedbrook et al. (1980a). 120 bp family sequence: 80,000 cpm, 21 days exposure

The Sequence from the 630 bp Family

All chromosomes showed hybridisation to this sequence. Again the hybridisation was predominantly telomeric. At least one pair showed an apparent interstitial site, while other pairs showed a less specific distribution of silver grains down the length of the chromosome arm.

In situ Hybridisation Patterns of King II Chromosomes Maintained as Single Chromosomes in Holdfast Wheat; A Comparison with Diploid Rye King II Chromosomes (Fig. 2a)

Chromosome 1R

This chromosome is the smallest rye chromsome (Gustafson and Bennett, 1976) and carries the NOR. It shows heterochromatin at both telomeres in addition to a strong C-band at the NOR, and a weaker interstitial C-band in the long arm (Darvey and Gustafson, 1975). All four repeated DNA sequences hybridised to both telomeres, and the 120 bp repeat hybridised to an interstitial site in the long arm. The labelling pattern for all sequences in the addition line chromosome was very similar to that of the NOR chromosome in King II rye (Fig. 1a).

Chromosome 2R

Chromosome 2R often has the highest DNA content of all rye chromsomes (Gustafson and Bennett, 1976). Singh and Röbbelen (1976) showed a loss of heterochromatin from the shorter arm of this chromosome (their chromosome III) in this wheat-rye addition line. The 610 or 630 base pair family sequences did not hybridize to this chromosome (Fig. 2a). The 480 bp sequence hybridised only to the long arm telomere, while the 120 bp sequence showed hybridisation to both telomeres. In King II rye, the sequences from the 610, 630 and 480 bp families hybridised to at least one telomere of all the chromosomes while that from the 120 bp pair family hybridised to both telomeres of all chromosomes (Fig. 1). This clearly suggests that 610, 630 and 480 base pair family sequences but not all the 120 base pair family sequences have been deleted from the short arm telomere of chromosome 2R in the wheat-rye addition line.

Chromosome 3R

Although called 3R for convenience in this paper, recent evidence suggests that this addition line chromosome is not 3R of King II but contains arms of two other rye chromosomes (T.E. Miller, personal communication). Both the 630 and 610 bp family repeated sequences hybridised at both telomeres in approximately equal amounts. The 480 bp family sequence hybridised strongly at one telomere, and very weakly (in three separate experiments) at the telomere of the shorter arm. Considerable interstitial hybridisation occurred with the 120 bp family repeated sequence in addition to strong

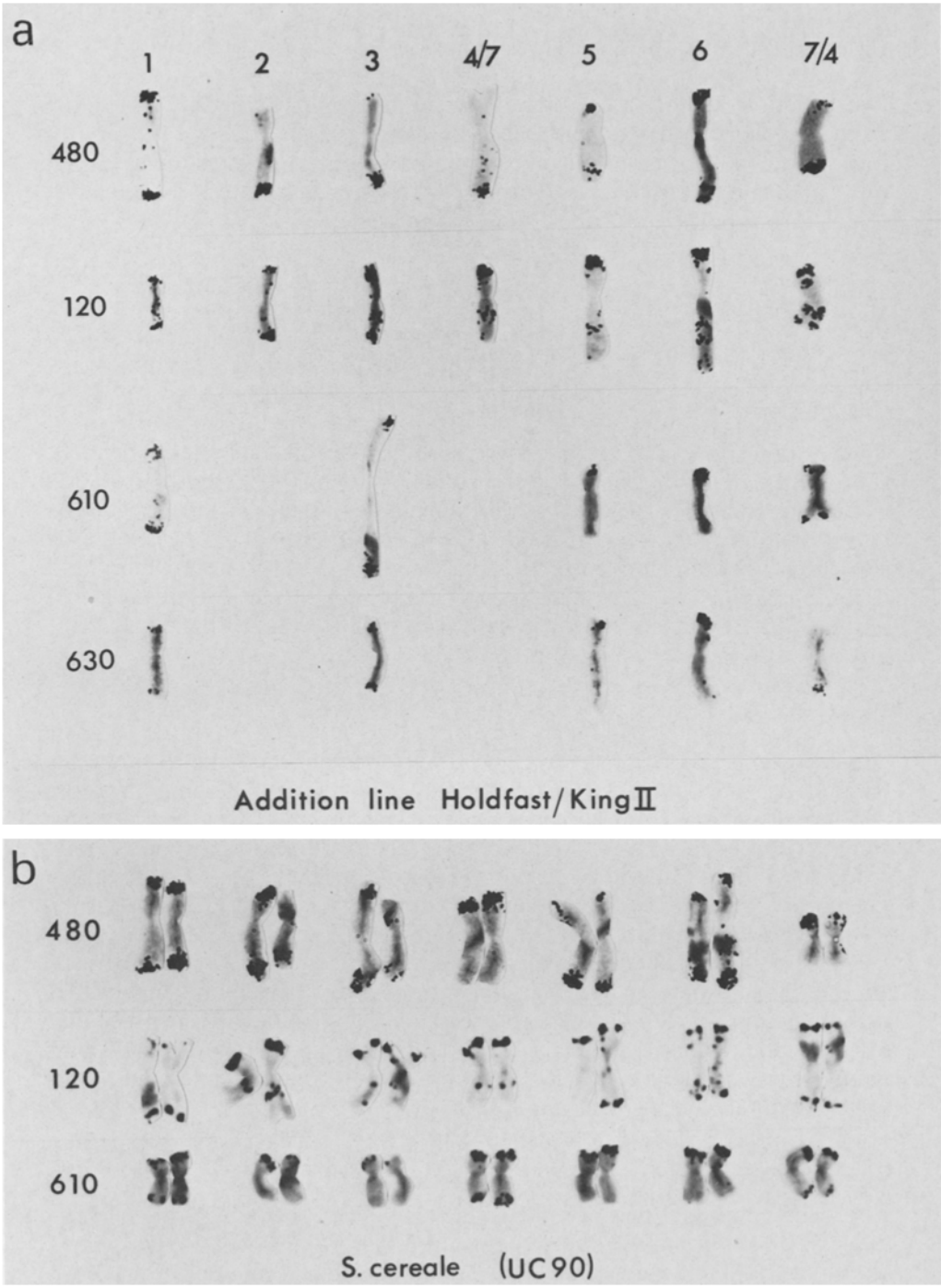

Fig. 2. a In situ hybridisation of ^{3}H cRNA to King II rye chromosomes in wheat-rye addition line plants. The conditions used for each hybridisation were not identical. 480 bp family sequence: 110,000 cpm/slide, 14 days exposure except for 5R, which was 65,000 cpm/slide, 14 days exposure. 120 bp family sequence: 90,000 cpm/slide exposure, 21 days exposure. 610 bp family sequence: 90,000 cpm/slide, 14 days exposure except for 5R and 7R/4R which were 60,000 cpm/slide, 14 days exposure, 630 bp family sequence: all 200,000 cpm/slide, 28 days exposure. **b** In situ hybridisation of ^{3}H cRNA to chromosomes of *S. cereale* var. UC90. 480 bp family sequence: 65,000 cpm/slide, 21 days exposure. 610 bp family sequence: 60,000 cpm/slide, 14 days exposure

hybridisation at both telomeres. It seems likely therefore that a deletion from the short arm telomere has caused the loss of nearly all copies of the 480 bp sequence without substantially reducing the number of copies at this telomere of the 120, 630 or 610 bp repeated sequences.

Chromosome 4R/7R

No hybridisation with the 630 or 610 bp family repeated sequences was detected on this chromosome in three separate experiments. The 480 bp family sequence showed hybridisation to the long arm telomere, while the 120 bp family sequence showed strong hybridisation to an interstitial site on the long arm which closely correlates with the location of a frequently observed C-band (Darvey and Gustafson, 1975). The in situ hybridisation results strongly suggest that 630, 610 and 480 but not 120 base pair family sequences have been deleted from the short arm of chromosome 4R/7R. However, this chromosome was not reported to have lost a C-band in the stocks studied by Singh and Röbbelen (1976).

Chromosome 5R

This is the most unequal-armed rye chromosome in many stocks (Heneen, 1962). All four repeated sequence probes hybridised to the telomere of the short arm. The 480 bp family sequence hybridised to a sub-telomeric position in the long arm, while the 120 bp sequence hybridised to a different interstitial site nearer the centromere. Both these sites within the long arm correlate with frequently observed C-banding sites (Darvey and Gustafson, 1976; Lelley et al., 1977). This hybridisation pattern matches that of a chromosome of King II rye.

Chromosome 6R

This is also a very unequal armed chromosome. Again, sequences from all four families hybridised to the short arm telomere. The 480 bp family sequence hybridised to the telomere of the long arm and a subtelomeric site, while the 120 bp sequence hybridised weakly to the telomere of the long arm, and more strongly to two sites on the proximal side of the centre of the long arm. These interstitial sites in the long arm have also been noted in the C-banded karyotype (Darvey and Gustafson, 1975). A chromosome with a similar hybridisation pattern is present in King II rye.

Chromosome 7R/4R

This chromosome was reported by Singh and Röbbelen (1976) to have a deletion of a large telomeric C-band from the shorter arm. The 480 bp sequence family hybridisation is consistent with this in that there was strong hybridisation at the longer arm telomere but a very small (though consistently found) hybridisation at the other telomere. A curious result was

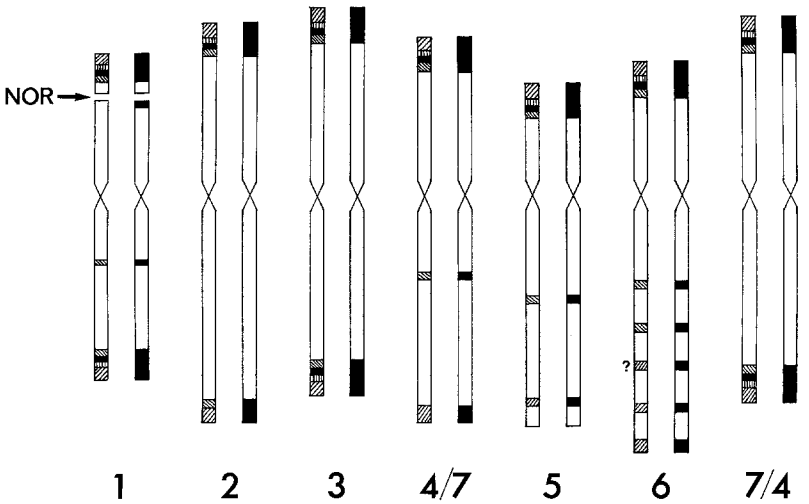

Fig. 3. Schematic representation of C-bands and in situ hybridisation for King II. The right hand member of each chromosome pair shows the C-band heterochromatin and the left hand member shows the sites of hybridisation of the members of the 480 bp (▨), 610 bp (▥), 120 bp (▧) and 630 bp (■) families. The representation of the families at the telomeres as non-interspersed arrays (blocks) is not proven – neither is the order of the arrays with respect to one another. The interstitial sites shown are those definitively recognised in King II (Fig. 1a). Others were recognised in the King II chromosomes in a wheat background (Fig. 2a). Darvey and Gustafsom (1975) reported a C-band at this position in King II. We have not seen hybridisation at this site in King II but have in two other cultivars

obtained in three different seedlings on hybridisation with the 610 bp family repeat. One of the pair of rye chromosomes showed two telomeres labelled, and the other only one. This could be due to one of the homologous chromosomes having suffered an additional deletion event. However, it is very surprising that such a heteromorphism should occur in three different plants from an inbred line. The 630 bp repeat showed hybridisation to only one telomere. Hybridisation with the 120 bp sequence revealed interstitial sites in each arm as well as sites at both telomeres.

The above results on King II chromosomes are summarised for convenience in Figure 3. The predominant hybridisation to rye chromosomes with all four sequences is to sites of telomeric heterochromatin. However, the 120 bp family is also present in interstitial sites. These sites are usually (e.g. in 1R, 4R, 5R and 6R) but not always (7R) in positions where small interstitial C-bands have been recorded (Darvey and Gustafson, 1975; Lelley et al., 1977). Some of the interstitial hybridisation in 7R with this sequence occurs at sites not previously reported to show C-bands in these stocks but where they have been observed in other stocks (Darvey and Gustafson, 1975). The two interstitial sites (5R and 6R) of the 480 bp sequence correspond with C-bands. Clearly, the correlation of the sites of these sequences with heterochromatin is very good (Fig. 3). Only the C-bands at the NOR and at the centromeres fail to show hybridisation to these sequences.

Comparison of the Hybridisation Patterns of King II, UC90 and Petkus Rye Varieties Using:

The Sequence from the 480 bp Family

The karyotpyes of Petkus (Fig. 1b) and UC90 (Fig. 2b) are broadly similar to but differ in detail from that of King II (Fig. 1a). Quantitative differences in labelling between different chromosomes are most obvious in Petkus, possibly because in the UC90 and King II slides the emulsion was saturated over the telomeres. Only UC90 lacked hybridisation sites at both telomeres of all pairs of chromosomes.

Not all chromosomes could be readily paired. There was also variation between chromosomes in different plants of the same variety. For example, in some UC90 plants the nucleolus organiser (satellited) chromosome was heterozygous for the block of 480 base pair sequences (see Fig. 4a). This correlates with a heterozygosity for C-band size sometimes found at this position (A.G. Seal, personal communication). All three varieties had chromosomes with hybridisation patterns typical of chromosomes 5R and 6R of the Holdfast/King II addition lines, i.e. with interstitial label just proximal to the telomere of the long arm. All three varieties showed some label on all pairs of chromosomes. The finding of quantitative differences between varieties is consistent with the quantitative variation for C-bands which is found within and between cultivars (Darvey and Gustafson, 1975; Lelley et al., 1977; Weimarck, 1975; Giraldez et al., 1979).

The Sequence from the 120 bp Family

All three varieties showed similar but not identical labelling patterns. The two most median chromosomes had major hybridisation sites at both telomeres as did the nucleolus organiser chromosomes. At least three of the remaining chromosomes in all varieties showed major sites of interstitial labelling on the long arm. From the addition line results (Fig. 2a) these would be chromosomes 3R, 4R/7R, 5R and 6R. The two interstitial hybridisation sites in the long arm of addition line chromosome 6R were not visible in the varieties, except perhaps in Petkus, probably due to over contraction of the chromosomes. Fewer King II chromosomes showed interstitial long-arm hybridisation sites than expected from the addition lines. Some interstitial sites appeared to be present in the short arms of Petkus chromosomes. Some heterozygosities were apparent, for example in the fifth UC90 pair from the left (Fig. 2b) and also possibly in the nucleolus organiser chromosome of King II (Fig. 1a).

The Sequence from the 610 bp Family

This sequence labelled three pairs of King II and UC90 chromosomes at both telomeres, but only two pairs in Petkus. Otherwise the three varieties were all very similar in their absence of interstitial sites of hybridisation.

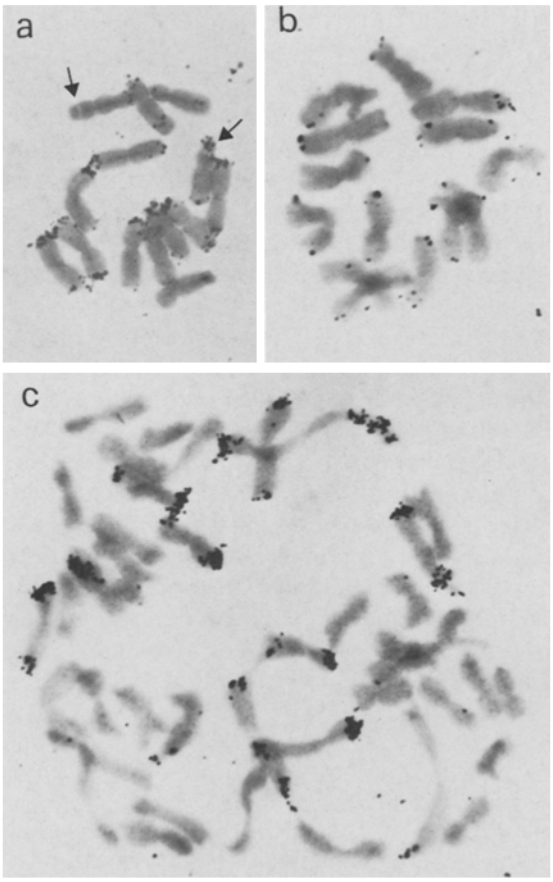

Fig. 4. (**a**) In situ hybridisation of 65,000 cpm of ^{3}H cRNA from 480 bp family sequence to UC90 chromosomes. Exposure time 14 days. *Arrows* indicate two NOR chromosomes which show heterozygosity on the satellited arm. (**b**) In situ hybridisation of 200,000 cpm of ^{3}H cRNA from 630 bp family sequence to UC90 chromosomes. Exposure time 30 days. (**c**) In situ hybridisation of 65,000 cpm of ^{3}H cRNA from 480 bp family sequence of triticale var. Rosner. Exposure time 21 days

In UC90 a quantitative heterozygoisty was apparent in the second pair from the left (Fig. 2b).

The Sequence from the 630 pb Family

This sequence was not hybridised to Petkus chromosomes. There is a considerable difference in the quantity of silver grains between UC90 and King II after hybridisation with similar amounts of probe which may reflect differential amounts of this sequence in their DNA (see Fig. 1a and Fig. 4b). Nevertheless, in UC90 the sequence still appears predominantly telomeric.

Hybridisation of Rosner Triticale Rye Chromosomes Using:

The Sequence from the 480 bp Family

This sequence hybridised strongly to five pairs of chromosomes, and weakly to one additional pair (Fig. 4c). Giemsa C-banding has failed to identify

the presence of chromosome 2R (Merker, 1975). Crosses of Rosner with hexaploid wheat show 15 bivalents (see Gustafson and Bennett 1976). A possible inference from these results is that there is a translocation between 2D and 2R, which has lost telomeric heterochromatin or 2R may have been substituted by chromosome 2D. There is no report of any abnormality in the other rye chromosome pairs. For this reason we were surprised to note that after in situ hybridisation with the 480 bp sequence only five pairs of chromosomes were strongly labelled. The sixth pair was slightly sub-median and showed weak hybridisation at the long arm telomere.

The Sequence from the 610 bp Family

This sequence also hybridised to no more than five pairs of rye chromosomes (data not shown). Only the nucleolus organiser chromosome of the rye complement showed label at both telomeres.

The Sequence from the 630 bp Family

The hybridisation pattern of this sequence is shown in Bedbrook et al. (1980a). Five chromosome pairs are labelled, only one of them at both telomeres.

The Sequence from the 120 bp Family

The hybridisation pattern with this sequence (Fig. 5a) is more complex to interpret because the sequence is also highly reiterated in the wheat chromosomes, although to a lesser extent than in rye (Bedbrook et al., 1980a). Fourteen chromosomes showed blocks of very intense labelling, including six which have patterns which correspond to those of 4R, 5R and 6R (Fig. 2a). The amount of label over the rye chromosomes is much greater than illustrated in Figs. 1 and 2. Many more minor hybridisation sites are visible. This emphasizes the fact that for some repeated sequence families, the conditions of hybridisation and length of autoradiographic exposure time can have a major effect on the apparent distribution of the sequences in the genome. It is known that the 120 base pair family is complex and contains a number of variant sequences which have become translocated and reamplified to varying extents (Flavell et al. 1980; Bedbrook, et al., 1980b). These variant forms are in lower copy number than the major tandem arrays and may constitute the additional minor sites observed in Fig. 5a.

In summary, it appears that Rosner rye chromosomes, like the rye chromosome pairs in the Holdfast wheat background, may have lost some blocks of highly repeated DNA though this is difficult to conclude unequivocally owing to the heterogeneous parentage of the rye chromosomes in Rosner. Deletions from telomeres of chromosome 2R have occurred for the 480, 610 and 630 bp but not the 120 bp sequences or the 2R chromosome has been replaced by chromosome 2D. On the basis of arm ratios, and position

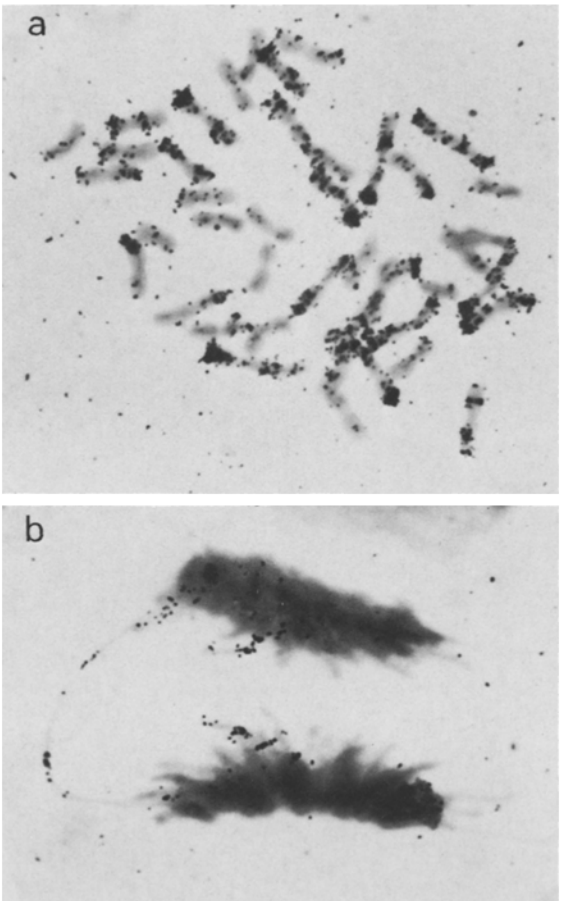

Fig. 5. (**a**) In situ hybridisation of 110,000 cpm of ^{3}H cRNA from 120 bp family sequence to chromosomes of triticale var. Rosner. Exposure time 28 days. (**b**) In situ hybridisation of ^{3}H cRNA from 480 bp family sequence to root tip cell of Chinese Spring/Imperial addition line 6R. No colchicine pretreatment was applied to the root tip which was spread to make this slide. The long arm of rye chromosome 6R contains one telomeric and 2 interstitial sites of hybridisation with this sequence. In situ hybridisation to the anaphase bridge proves that the bridge is formed from a rye chromosome. 65,000 cpm of cRNA were added to the slide. Exposure time was 14 days

and strength of labelling the Rosner chromosome which showed only very slight hybridisation to the 480 bp sequence (arrowed) appears to be 4R/7R. This chromosome has probably also lost the 480, 610 and 630 but not the 120 bp repeats from the telomere on the short arm. However, the C-band on the telomere of the short arm of this chromosome is still present (A.G. Seal, personal communication).

Discussion

The Relationship between Arrays of Highly Repeated Sequences and C Bands

These results show very clearly that a strong correlation exists between the locations of four repeated DNA families and C-bands in rye chromo-

somes (Fig. 3). They confirm that many of the larger telomeric blocks of heterochromatin contain sequences from all four families and that the heterochromatin on different chromosomes contains sequences from the same families (Bedbrook et al., 1980a). Of the C-bands previously described, only those at the centromeres and at the NOR site on chromosome 1R lack sites of discernible hybridisation for these four sequences. The distribution of sites of in situ hybridisation to interphase nuclei strongly resembles the pattern of C-banding regions recorded by Singh and Röbbelen (1975), including the apparent fusion of blocks of heterochromatin. Each previously reported interstitial C-band probably contains the 120 bp family or the 480 bp family. The only possible exception to this (apart from the NOR site) is that four interstitial bands have been reported on the long arm of chromosome 6R of King II (Darvey and Gustafson 1975), but only three hybridisation sites are illustrated in King II chromosome 6R (Fig. 2a). Perhaps studying less contracted chromosomes would uncover the additional site. However, chromosome 6R of *Secale cereale* var. Imperial contains two closely juxtaposed interstitial sites of hybridisation to the 480 bp sequence (data not shown), as does 6R of Rosner (Fig. 4c). These two sites, together with two sites for the 120 bp sequence, would make four interstitial sites on the long arm of 6R in some ryes (Giraldez et al., 1979). Chromosomes 3R and 7R/4R were not reported by Singh and Röbbelen (1976) to have interstitial C-bands in the addition line set but did show interstitial sites of hybridisation for the 120 bp family sequence (Fig. 2a). Interstitial C-bands are often "*of erratic appearance both within and between cells on the same slide*" (Bennett et al., 1977) so the lack of clear evidence for an interstitial C-band where there is a site of hybridisation is probably due to difficulties with the C-banding technique.

The variations in sites of hybridisation between the cultivars agrees in general with the variation in C-banding patterns. For example, Petkus showed interstitial sites of hybridisation for the 120 bp sequence on the short arms of four chromosomes (Fig. 1b) including the NOR chromosome 1R. Interstitial C-bands on short arms including 1R have been reported for some inbred ryes by Giraldez et al. (1979). Comparisons of the intervarietal hybridisation patterns and C-band patterns suggest that although the amounts of highly repeated sequences at a site may vary considerably within and between cultivars, the positions of the sites are relatively invariant.

Four of the King II rye chromosomes have suffered deletions of repeated sequences during or after their introduction into Holdfast wheat. Three of these deletions were recognised as losses of heterochromatin by Singh and Röbbelen (1976). The results reported here show the loss of heterochromatin to be due unequivocally to loss of DNA. However, it is very interesting that losses of particular highly repeated sequence families can occur without the complete disappearance of heterochromatin. It has been confirmed that the 4R/7R rye chromosome in the wheat background has not lost telomeric heterochromatin on the short arm (A.G. Seal, personal communication), though a reduction in amount seems likely. Studies are in

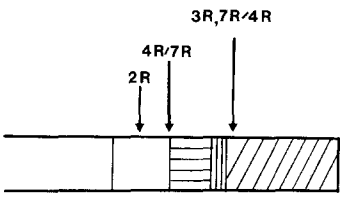

Fig. 6. A suggested arrangement of blocks of repeated DNA families at the telomeres of rye chromosomes. The arrangement is the simplest that can be derived from the deletions in King II chromosomes 2R, 4R/7R, 7R/4R and 3R in the King II/Holdfast wheat addition lines (see text). The suggested proximal ends of the deletions are shown by the *arrows*. The order of the 610 and 630 p.b. families could be reversed

progress to investigate the extent of the reduction. The significance of this for breeders of triticale as a grain crop is that selection for reduced amounts of heterochromatin may be more efficient than selection for the total loss of heterochromatin blocks (see Bennett, 1977; Müntzing, 1979).

When the number of telomeres which show labelling is compared between the addition line rye chromosomes and the King II parental set for each sequence, the numbers are 9 against 13 for the 480 bp repeat, 8 against 10 for the 610 bp repeat and 7 against 10 for the 630 bp repeat. These differences are presumably due is deletions of DNA. However May and Appels (1980) have suggested that many of the apparent losses of heterochromatin from rye chromosomes involve translocations with wheat chromosomes. While this may be true in some addition lines or triticales, the chromosomes in this Holdfast/King II set do not carry translocations on the basis of chromosome pairing assays (V. Chapman, personal communication).

One might ask how it is possible for heterochromatin DNA to be deleted so as to lose all of the 480 bp sequences, e.g. in $2R^s$ and $4R^s$, or nearly all of them, e.g. in $3R^s$ and $7R/4R^s$, without removing all or some of the other three families of repeats. The simplest explanation is that each family of sequences is in a separate block, the different blocks are adjacent to one another and the deletions arise by chromosome breakage within or between different blocks of repeats. Because examples exist (chromosome 3R, Fig. 2a) where the 480 bp family sequences are deleted while the 610, 630 and 120 bp family sequences remain and other examples (chromosomes 2R and 4R/7R, Fig.2a) here the 480, 610 and 630 bp family sequences are deleted but the 120 bp family sequences remain, the simplest arrangement of the blocks is where the 480 bp sequences are the most distal and the 120 bp family sequences the most proximal. This is illustrated in Fig. 6 together with possible breakpoints in the chromosomes which have suffered deletions. Clearly this simplest arrangement may not be appropriate for all the telomeric blocks of heterochromatin.

Each family of repeats may constitute one of the knobs recognised by Lima-de-Faria (1952) at the telomeres of pachtyene chromosomes. In support of this suggestion we sometimes see silver grains after hybridisation with the 610 bp repeat or the 120 bp repeat which are localized just in from the end of the chromosome (though one can never exclude the possibility of telomeres doubling back on themselves in such condensed chromosomes).

The Use of the Repeated Sequence Families for Chromosome Identification

In situ hybridisation with sequences from the 610, 630 and 480 bp families is probably less useful for chromosome identification and karyotype construction than good C-banding which shows up interstitial sites. However, sequences from the 120 bp family look more promising, but it may not always be possible to distinguish 2R, 3R and 7R. A mixed hybridisation with the 120 bp and 480 bp repeats would easily distinguish 4R, 5R and 6R.

The Distribution of the Arrays of Repeats in the Karyotype

The 120 bp, 480 bp and possibly the 630 bp repeated sequence arrays each exist separately from the other two families when they are at interstitial sites. In other species of *Secale* (see accompanying paper), there are examples of the 610 bp sequence at locations where 630 bp and 480 bp sequences were not detected. However, the only site of telomeric heterochromatin where the 120 bp sequence was not detected is on the long arms of 4R/7R, and so the 610 bp sequence has not been found at a site where the 120 bp sequence is absent.

Why are the arrays of repeats clustered *together* predominantly at the telomeres? These might be the positions at which the arrays of heterochromatin are most easily tolerated or alternatively, of course, the sites at which they serve a useful function.

There is good evidence (reviewed in John and Miklos, 1979) that blocks of heterochromatin at the telomeres of *Atractomorpha* (Miklos and Nankivell, 1976), *Cryptobothrus* (John, 1981) and *Drosophila* (Yamamoto and Miklos, 1978) interfere with distal chiasma formation, causing it to occur at more proximal sites. However in rye, chiasmata usually form close to the blocks of telomeric heterochromatin (Jones 1978) and genetic factors also influence chiasma frequency and hence position.

The distribution of these repeat families in the karyotype may also be a consequence of how the chromosomes physically interact and exchange sequences in the nucleus. For example, it is known that the blocks of telomeric heterochromatin on non-homologous chromosomes of rye lie close to one another at the opposite end of the nucleus to all the centromeres (Appels et al., 1978; Singh and Röbbelen, 1975). Telomere association is seen in leptotene bouquets (Thomas and Kaltsikes, 1976) and may be important for replication of telomeres (Holmquist and Dancis, 1979). Furthermore Bennett (1982) has recently described a model, and evidence to support it, for the arrangement of *S. cereale* chromosomes in the nucleus. The chromosomes of haploid sets have an ordered disposition that can be represented as a linear chain, with chromosomal order within the chain depending upon chromosome arm length. The following pairs of chromosome arms are associated in the chain: 1L3L; 3S7S; 7L2L; 2S4S; 4L6L; 6S5S; (S = short arm,

L=long arm). One end of the chain therefore consists of 1S and the other of 5L. When the arrays of repeated sequences on associated arms are compared, they are often found to be more similar than between non-associated arms. For example, arrays of the 120 bp, 610 and 630 pb families are at all telomeres except on the long arms of chromosomes 4R/7R, 6R and 5R. The long arms of 4R/7R and 6R are adjacent to one another and 5RL is at the end of the chain not associated with another chromosome.

If the positions of arrays of sequences in the chromosome complement are a consequence of how the chromosomes are organised in the nucleus and can thereby interact, then this would also explain how these repeats spread between chromosomes in the first place, how they evolve in a concerted way and possibly how they become fixed in populations so rapidly (Dover, 1981, 1982; Flavell, 1982). It is now obviously important to study the spacial disposition of chromsomes and sequences in other species. It should be noted that in other *Secale* species the repeated sequence patterns of the telomeres are different from those in *S. cereale* (Jones and Flavell, 1982).

The Effects of Tandem Arrays of Repeats and Heterochromatin

Heterochromatin is likely to have different effects depending on position, chromosome and species. In some genotype-environment interactions it may be of no selective significance. In other situations it may greatly influence chromosome biology. Consequently it is erroneous to search for a universal role of heterochromatin and highly repeated sequence DNA. The disappearance of 480 bp, 630 bp and 610 bp sequences from the telomeres of two pairs of rye chromosomes in Rosner triticale and the loss of the rye specific repeats from four out of the seven pairs of chromosomes when present as single additions to wheat is consistent with this conclusion because the deletions imply that these rye repeats are selected against in the genetic background of wheat. Such selection may be because they cause a reduction in chromosome pairing at meiosis (Thomas and Kaltsikes, 1974; Merker, 1976) and grain shrivelling (Bennett, 1973, 1977). It is possible that the variation on which this selection acts is generated by a tendency of the rye heterochromatin, which is late replicating, to give rise to chromosome "*bridges*" during anaphase. Resolution of such bridges by chromosome breakage in germ line cells could then give rise to loss of telomeric DNA segments in subsequent progeny. An anaphase bridge involving rye chromosome 6R in the King II/Holdfast addition line is clearly illustrated by in situ hybridisation in Fig. 5b. The deletion of rye sequences from the 480, 610 and 630 bp families but not the 120 bp families in the wheat genetic background is interesting because the former three families are essentially absent from wheat whereas the 120 bp family is present in wheat and is found at the telomeres of up to eleven pairs of chromosomes.

The heterozygosities in amounts of repeated DNA which have been observed are in keeping with the heteromorphisms for heterochromatin already reported in rye (Lelley, et al. 1978; Weimarck, 1975; Giraldez et al.,

1979). The heteromorphisms may occur frequently because arrays of repeated sequences readily undergo deletion and amplification. If the arrays of repeats in heterochromatin do regulate some cellular activity then the continual production of heteromorphisms would serve as a way of continually generating variation within a population.

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