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

J.D.G. Jones and R.B. Flavell

Plant Breeding Institute, Trumpington, Cambridge CB2 2LQ, Great Britain

**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...
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 chromosome 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 3H 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 karyotypes 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.