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Variation in Nuclear DNA in the Genus *Secale*

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Abstract. Estimates of the 4C DNA amount per nucleus in 16 taxa of the genus *Secale* made by Feulgen microdensitometry ranged from 28.85 picograms (pg) in *S. silvestre* PBI R52 to 34.58 pg in *S. vavilovii* UM 2D49, compared with 33.14 pg in *S. cereale* cv. "Petkus Spring" which was used as a standard. Giemsa C-banding patterns showed considerable interspecific and intraspecific variation and several instances of polymorphism for large telomeric C-bands. The proportion of telomeric heterochromatin in the genome ranged from about 6% in *S. silvestre* and *S. africanum* to about 12% in cultivated rye. A detailed comparison of nine taxa showed no overall relationship between 4C DNA amount and the proportion of telomeric heterochromatin in the genome. However, evidence is presented which strongly supports the notion that the major evolutionary change in chromosome structure in *Secale* has involved the addition of heterochromatin at, or close to, the telomeres. It is suggested that saltatory amplification events at telomeres were initially responsible for each large increase in DNA amount. Subsequently unequal crossing over between homologues may have played an important secondary role by extending the range of variation in the amount of heterochromatin at a given telomere, while crossing over between non-homologues may have provided a useful mechanism allowing an increase in the DNA amount at one telomere to be distributed between chromosomes.

Introduction

Secale cereale L. (rye) has several desirable agronomic characters which are absent from, or poorly expressed in, *Triticum turgidum* L. var *durum* (tetraploid wheat) and *Triticum aestivum* L. em. Thell. (hexaploid bread wheat). Rye and wheat are related species which can hybridize (for a review see Gustafson, 1976). Moreover, disomic wheat-rye chromosome substitution lines show that rye chromosomes can compensate for some missing wheat chromosomes (Gupta,

1971), and that wheat chromosomes can be substituted for rye chromosomes in 42-chromosome triticale (Gustafson and Zillinsky, 1973; Merker, 1975; and Darvey and Gustafson, 1975). However, there is no apparent pairing between wheat and rye chromosomes at meiosis either in F_1 hybrids or in colchicine induced amphidiploids. Consequently attempts to introduce desirable genes from rye into wheat were switched to attempts to combine wheat and rye genomes in amphidiploids (triticales) selected for stable bivalent formation at meiosis.

While considerable progress has been made in improving the meiotic stability of hexaploid triticales (Gustafson and Qualset, 1975), the best lines still have about 7–10% aneuploids among their progeny (Gustafson, 1976) besides exhibiting unstable nuclear development in their endosperms (Bennett, 1973; Kaltsikes et al., 1975). Both at meiosis and during early endosperm development the rate of nuclear development in rye is significantly slower than in wheat (Bennett et al., 1971; Bennett and Kaltsikes, 1973; Bennett et al., 1975). It was therefore suggested that nuclear instability in triticale at both these stages may result from the interaction of genomes which control development at different rates at those stages in the wheat and rye parents (Bennett et al., 1971; Bennett et al., 1975).

It is well established that DNA amount per diploid genome and the rate of cell development are positively correlated in higher plants (Van't Hof and Sparrow, 1963; Bennett, 1971; Van't Hof, 1975). Rye has about 33% more DNA than the largest of the diploid wheat genomes in tetraploid or hexaploid wheat. It has been suggested, therefore, that the slower rate of nuclear development in rye than in wheat may be caused by the different DNA contents of their diploid genomes (Bennett, 1973).

In view of a possibly important relationship between DNA amount and nuclear stability in triticale amphidiploids, it was decided to screen the genus *Secale* in an attempt to find genotypes whose 4C DNA amount per genome is closer to that of the diploid wheat genomes than that of cultivated rye. With the exception of the genus *Hordeum* (Bennett and Smith, 1971), investigations of species in other genera in the Gramineae have shown considerable intrageneric variation in 4C DNA amount (Bennett and Smith, 1976). Consequently the chances of finding *Secale* taxa with lower DNA amounts than *S. cereale* spp. *cereale* seemed high.

Rye chromosomes differ from those of tetraploid and hexaploid wheat not only in the amount of DNA, but also in the amount of heterochromatin stained as C-bands which they contain. Rye chromosomes characteristically possess large C-bands at one or both telomeres, as well as some intercalary C-bands (Bennett, 1973; Merker, 1973; Sarma and Natarajan, 1973; Gill and Kimber, 1974a; Verma and Rees, 1974; Vosa, 1974; Darvey and Gustafson, 1975; Weimarck, 1975; Schlegel and Friedrich, 1975; Singh and Röbbelen, 1975). Wheat chromosomes possess no major C-bands at or near their telomeres, but, they do possess small intercalary C-bands (Gill and Kimber, 1974b). Telomeric heterochromatin in rye is late replicating at S (Lima-de-Faria and Jaworska, 1972; Ayonoadu and Rees, 1973). It has therefore been suggested that the presence of large segments of late replicating chromatin at the telomeres of rye chromosomes, but not of wheat chromosomes, may be a cause of nuclear instability in triticale

amphidiploids (Bennett, 1973; Gustafson and Bennett, 1976). Consequently, it was also decided to screen the genus *Secale* for genotypes with amounts of telomeric heterochromatin closer to that of wheat chromosomes.

Most of the experiments reported in the present work were conducted prior to July, 1975 independently at the Plant Breeding Institute, Cambridge and the University of Manitoba, Winnipeg. Comparison of the two sets of experimental results in July, 1975 showed them to be similar and complementary. It was decided, therefore, to cooperate in completing the research programme and to present all the results in a joint paper.

Materials and Methods

1. Materials

Detailed accounts of the taxonomy and cytotaxonomy of the genus *Secale* L. are given by Vavilov (1917), Roshevitz (1948), Jain (1960), and Stutz (1972). The genus consists of from four to twelve species (depending upon the criteria used for species definition). Consequently a reasonably comprehensive comparative study is not too difficult. All taxa in the genus are diploids with 14 chromosomes, and all can be intercrossed to yield at least partially fertile hybrids.

In the present work taxa are defined as in Stutz (1972) who divided the genus into six species. Accessions of all six species, comprising twelve taxa, were examined (Table 1). These were taken from the collections of rye accessions maintained at the Plant Breeding Institute, Cambridge, and the University of Manitoba, Winnipeg.

The *Secale* taxa studied in the present work are all cross-pollinators. Such taxa are difficult to maintain pure. While all reasonable precautions have been taken to prevent contamination (for example, repeatedly bagging ears together of just two strains of the same accession which cross-fertilize), nevertheless, some cross-fertilization between accessions in the respective collections may have occurred. This may well be one reason for the observation of some of the polymorphism reported in the present work. At the same time it should be emphasized that, with the exception of *S. silvestre* UM 2D20 (which was suspected to be misidentified or contaminated – see Dedio et al., 1969), care was taken to select accessions for study whose morphological appearance agreed with that of the type species or variety.

Table 1. *Secale* accessions studied in the present work. (N.B. Accessions studied only at Cambridge have no asterisk, while those studied only at the University of Manitoba have a single asterisk. Accessions studied both at Cambridge and at the University of Manitoba have two asterisks)

Annuals

S. cereale L. ssp. *cereale* cv. "Petkus Spring"**
S. cereale L. ssp. *afghanicum* (Vav.) Roshev. (PBI R8)
S. cereale L. ssp. *ancestrale* Zhuk. (PBI R27 & UM 2D38**)
S. cereale L. ssp. *dighoricum* (Vav.) Roshev. (PBI R40 & UM 2D39*)
S. cereale L. ssp. *segetale* (Zhuk.) Roshev. (PBI R41; UM 2D56 & UM 2D37*)
S. silvestre Host. (syn. *S. fragile*) (PBI R52; UM 2D20 & UM 2D59*)
S. vavilovii Grossh. (PBI R11; UM 2D49** & UM 2D116**)

Perennials

S. africanum Stapf. (UM 2D127**)
S. anatolicum Boiss. (PBI R98; UM 2D18*)
S. montanum Guss. spp. *montanum* (PBI R15; UM 2D23*; UM 2D35**)
S. montanum ssp. *dalmaticum* Vis. (UM 2D19)
S. montanum spp. *kuprijanovii* Grossh. (PBI R35 & UM 2D34*)

2. Methods

i. Nuclear DNA Measurements

Seeds were germinated on Whatman No. 1 filter paper moistened with distilled water in plastic Petri-dishes. Root-tips about 1–2 cm in length were fixed in freshly made 3:1 absolute ethyl alcohol: glacial acetic acid (v/v) for about 20 h at room temperature. Thereafter they were hydrolysed in 1 N HCl at 60° C for 10 min, stained in leuco-basic fuchsin (pH 3.6) for 2 h, and then given three 10 min washes in SO_2 water (McLeish and Sunderland, 1961). Darkly stained root-tips were excised and squashed in a drop of 45% acetic acid. Estimates of nuclear DNA content were made using a Vickers M86 microdensitometer within 3 to 5 h of squashing. For each accession ten 4C nuclei, judged to be at mid-prophase, were measured on each of three slides. Each slide was made using a root-tip from a different seedling. The absorption of each nucleus was estimated as the mean of three readings. All results obtained in arbitrary relative absorption units were converted to absolute amounts using *Secale cereale* cv. "Petkus Spring" as a calibration standard (4C = 33.14 picograms (pg), Bennett and Smith, 1976). With the exception of *S. silvestre* PBI R52 whose nuclear DNA content was estimated in three separate experiments, and *S. africanum* UM 2D127 which was estimated in two separate experiments, estimates for other accessions were made only once.

ii. C-banding

a) At Cambridge. Actively growing roots (1–2 cm long) were excised from seeds germinated on moist filter paper in plastic Petri dishes kept at 23° C in the dark. After pretreatment for 3.5 h in saturated 1-bromonapthalene they were fixed in 1:3 ethyl alcohol:glacial acetic acid (v/v) for 24 h, and then softened by 2–3 h treatment with 45% acetic acid. Excised root-tips were then squashed in 45% acetic acid using coverslips coated with a very thin layer of albumen. Coverslips were soaked off in absolute ethyl alcohol and subsequently kept in absolute alcohol for 1 h prior to air drying. They were then immersed in saturated $Ba(OH)_2$ solution at 45° C, for 5 min (this solution having been previously maintained at this temperature in an oven for up to several hours). The preparations were then washed in several changes of distilled water before incubation in $2 \times$ SSC at 60° C for 30 min, and then washed again in distilled water. A 1% solution of Gurr's R66 Giemsa solution in buffer at pH 6.8 was used for staining. When the correct degree of staining was obtained, as judged by microscopic examination, preparations were washed in distilled water and air dried. Denaturation, renaturation, and staining were all carried out in disposable plastic Petri-dishes. Dried preparations were mounted on microscope slides using Euparal. Photographs were taken with a Vickers M41 microscope using Kodak SO-410 film in conjunction with a daylight blue filter.

b) At the University of Manitoba. 1. Germinating seedlings were transferred to a dish containing filter paper soaked in prefixative and left for 3.5–4 h in a germination cabinet at $18 \pm 1°$ C. Prefixative was prepared by adding 0.005 gm 8-hydroxyquinoline to 10 cc of deionized water. The solution was heated until the 8-hydroxyquinoline dissolved then cooled to room temperature, and added to 10 cc of deionized water containing 0.01 gm colchicine.

2. Seedlings were rinsed in a water bath at $1 \pm 1°$ C (distilled water + ice) for a minimum of 10 min (prolonged washing decreases the proportion of seedlings which fail to produce new roots due to the toxicity of prefixative).

3. The root-tips were placed in 1.5% aceto-orcein and left for 15–20 h in a refrigerator. They were then transferred to a solution comprising nine parts of 1.5% aceto-orcein to one part of 2 N HCl (N.B. the relative amount of HCl is very important) for 3 h at 21–24° C. After rinsing the material twice in distilled water the root-tips were replaced in 1.5% aceto-orcein for a few minutes.

4. The meristematic zone was excised and mounted in a drop of 45% acetic acid using a subbed microscope slide (5–10 g of gelatin and 0.5 g of chrome alum in 1000 ml of distilled water) and a coverslip cleaned with silicone treated eyeglass tissue. The cover slip was then removed by the CO_2 freezing method.

5. The slide was air dried for 1–2 h before successive rinses in 75% ethyl alcohol (15–20 min), 95% ethyl alcohol ($1^1/_2$ h), and absolute ethyl alcohol (10 min). [N.B. The procedure in 5 has been found the most suitable for triticale and rye, and is not necessarily suitable for other materials (see Results, p. 155–158)].

6. Slides were dried for 1–2 d in a desiccator containing calcium chloride.

7. Slides were then transferred for 5.5 min to a saturated $Ba(OH)_2$ solution kept at 20–25° C in a water bath. [N.B. The $Ba(OH)_2$ solution was prepared by adding boiled distilled water, cooled to approximately 80° C, to crystals of barium hydroxide in a conical flask. The flask was shaken well and the supernatant quickly poured off. More distilled water at 80° C was added to the crystals of barium hydroxide remaining on the bottom of the flask. The flask was filled to the top, shaken well and sealed. It was then stood at room temperature for about 3 h until the solution became clear with a barium carbonate precipitate and barium hydroxide crystals on the bottom].

8. Deionized water was then run into the barium hydroxide solution until the barium hydroxide was replaced. Then the slides were transferred into a clean dish and rinsed twice with warm, distilled, deionized water at 45° C for approximately 2 min.

9. Slides were transferred to 2 × SSC at room temperature and allowed to incubate. After 15 min they were transferred to a fresh solution of 2 × SSC at room temperature and placed in an oven at 70–80° C. When the temperature of the solution reached 50–52° C the oven was readjusted to 45–52° C and the slides incubated at this temperature for 45–60 min. (N.B. While the temperature of the 2 × SSC solution may fall to 45° C during this period, it is important that it should not exceed 52° C).

10. Slides were removed from the oven and quickly dipped once into deionized water at room temperature, before being stained immediately in Leishman's diluted 1:4 in phosphate buffer (pH 6.8) for 5–30 min. (N.B. It was also possible to air dry the slides and stain them the next day. In general, slides air-dried prior to staining required a longer time in the stain. Slides were checked frequently during staining to ensure that the desired density and contrast was obtained).

11. After staining slides were dipped once in deionized water and air-dried overnight.

12. Finally slides were transferred to xylene for 24 h before a coverslip was mounted with "Permount".

Photographs were taken using a Zeiss photomicroscope with Kodak high contrast copy film 5069 in conjunction with a green filter.

iii. Karyotype Analysis, and Measurements of Chromosome Length and the Relative Size of Heterochromatin Bands

A high contrast Electrochrome TV monitor (EVM-11) was attached to a Zeiss photomicroscope via a Sony video camera (AVC-3260). The length of each arm, and the length of each telomeric C-band of heterochromatin was measured for each chromosome from the TV monitor using a pair of callipers. As the staining methods used resulted in variable staining of intercalary bands no attempt was made to measure their sizes. The karyotypes of the nine accessions (Fig. 4) were obtained from measurements of chromosomes in approximately 10 cells per accession.

Before C-banding methods were developed, the seven chromosomes of cultivated rye were classified using the numbers 1 to 7 (Gaspar, 1973), I to VII and the letters A to G (see Darvey, 1973) using various criteria. Recently the C-banding patterns were described for the seven chromosomes of cultivated rye numbered 1R, 2R, 3R, 4R/7R, 5R, 6R, and 7R/4R (Darvey and Gustafson, 1975), classified according to their tentative assignment to homoeologous groups with respect to bread wheat (Darvey, 1973). As there is no generally agreed classification of rye chromosomes, it was decided to number the chromosomes of cultivated rye 1 to 7 in the present work, according to Darvey and Gustafson (1975), for two reasons. First, this classification clearly identifies each of the seven chromosomes using C-banding as its criterion. Second, in our opinion, the classification of rye chromosomes according to cytogenetic criteria will prove the most useful in the long term. It is realized that current knowledge of the homoeology of wheat and rye chromosomes is probably incomplete, and that some rye chromosomes may bear segments homoeologous with more than one wheat group, since there are at least three known translocations involved in the differentiation of wheat and rye. Consequently, the classification of rye chromosomes used in the present work

should be regarded primarily as a means of identifying the seven chromosomes, and should not be interpreted as implying full homoeology of those chromosomes with wheat groups.

In an attempt to classify the chromosomes of the other *Secale* taxa according to their homoeology with those of cultivated rye, the chromosomes were tentatively numbered 1 to 7 according to their relative similarity with respect to C-banding patterns and arm ratios to the chromosomes of cultivated rye (Figs. 3 and 4). Using these criteria it was easily possible to distinguish seven different chromosomes in each taxon. It is recognised that this classification is probably subject to some error, particularly that associated with the occurrence of several translocations within the genus. However, we believe this classification to be a good approximation allowing of the most meaningful comparisons possible between taxa until the distribution of translocations is better understood.

Results

1. 4C Nuclear DNA Content

Estimates of the 4C DNA amounts for sixteen taxa are presented in Table 2a. The analysis of variance for these data (Table 2b) clearly shows that there are large significant differences between species over and above those between plants within species. Estimates for single accessions ranged from 28.85 pg (*Secale silvestre* PBI R52) to 34.58 pg (*S. vavilovii* UM 2D49), the estimate for the latter being almost 20 per cent higher than the estimate for the former. These minimum and maximum estimates are about 87 and 104%, respectively, of the estimate for *S. cereale* cv. "Petkus Spring".

Assuming the taxonomy of *Secale* given by Stutz (1972) is correct, then the present results also show examples of intraspecific variation. Thus, *S. cereale* spp. *afghanicum* PBI R8 (33.22 pg) is significantly ($P<0.001$) higher than *S. cereale* spp. *dighoricum* PBI R40 (31.52 pg); *S. vavilovii* UM 2D49 (34.58 pg) is significantly higher ($P<0.001$) than PBI R11 (32.94 pg); and *S. silvestre* UM 2D20 (33.59 pg) is significantly higher ($P<0.001$) than for PBI R52 (28.85 pg). It should be noted that Dedio et al. (1969) indicated that *S. silvestre* UM 2D20 (which gave the higher 4C DNA estimate in the present work) was incorrectly identified. The present results may be interpreted as supporting this view. There is, however, no reason for believing that any other accessions have been misidentified and, therefore, the present results indicate that intraspecific variation in 4C nuclear DNA content may be widespread in *Secale* species.

2. Heterochromatin Bands

Only in *Secale cereale* cv. "Petkus Spring" and in *S. africanum* UM 2D127 were heterochromatin banding patterns studied both at Cambridge and at the University of Manitoba in the same accession. Comparison of the results for these two accessions showed complete agreement for banding patterns obtained using the different C-banding techniques, and therefore, it is assumed that all C-banding patterns obtained in both laboratories are comparable.

Table 2a. The DNA amount per 4C nucleus (in picograms) of 16 *Secale* taxa

Taxon	Mean		p[a]
Annuals			
1. *S. cereale* ssp. *cereale* cv. "Petkus Spring"	33.14		standard
2. *S. cereale* ssp. *afghanicum* PBI R8	33.22		n.s.
3. *S. cereale* ssp. *ancestrale* PBI R27	31.78		**
4. *S. cereale* ssp. *ancestrale* UM 2D38	31.75		**
5. *S. cereale* ssp. *dighoricum* PBI R40	31.52		***
6. *S. cereale* ssp. *segetale* PBI R41	32.78		n.s.
7. *S. cereale* ssp. *segetale* UM 2D56	33.18		n.s.
8. *S. silvestre* PBI R52	29.59		***
	27.78	28.85[b]	***
	29.19		***
9. *S. silvestre* UM 2D20	33.59		n.s.
10. *S. vavilovii* PBI R11	32.94		n.s.
11. *S. vavilovii* UM 2D49	34.58		**
12. *S. vavilovii* UM 2D116	34.06		n.s.
Perennials			
13. *S. africanum* UM 2D127	29.95	29.68[b]	***
	29.41		***
14. *S. montanum* ssp. *montanum* PBI R15	32.82		n.s.
15. *S. montanum* ssp. *montanum* UM 2D35	33.49		n.s.
16. *S. montanum* ssp. *kuprijanovii* PBI R35	31.85		**

[a] Estimates for each taxon are compared with *S. cereale* cv. "Petkus Spring" using L.S.D.s between taxa means, and differences significant at the 5, 1 and 0.1% levels are indicated by one, two or three asterisks, respectively ($L.S.D._{(5\%)}=0.94$; $L.S.D._{(1\%)}=1.24$; $L.S.D._{(0.1\%)}=1.58$)

[b] The DNA contents of *S. silvestre* PBI R52 and *S. africanum* UM 2D127 were estimated in three and two separate experiments, respectively, and the result for each experiment and the overall mean is given

Table 2b. Analysis of variance for 4C DNA data presented in Table 2a

Item	d.f.	M.S.	V.R.	P
Between species	18	107.655	40.58	0.001
Between plants within species	38	2.653	3.30	0.001
Between cells within plants within species	513	0.804		

It has been shown by varying the technique that the centric, telomeric, or both centric and telomeric C-bands are differentially stained in *Allium* (Fiskesjö, 1974). The method used regularly at the University of Manitoba is set up to show the terminal and intercalary bands, but not the small centric bands, of rye chromosomes while still showing some of the more prominent centric bands of wheat chromosomes in triticale. Using this method in the present work small centric bands were often not distinguished. This implies no disagreement with the results of Singh and Röbbelen (1975) who found small centric bands in *S. cereale* and *S. vavilovii,* since by altering the technique (step 5, air dry for 1 h; and, rinse in absolute EtOH for 25 min; step 6, air dry for

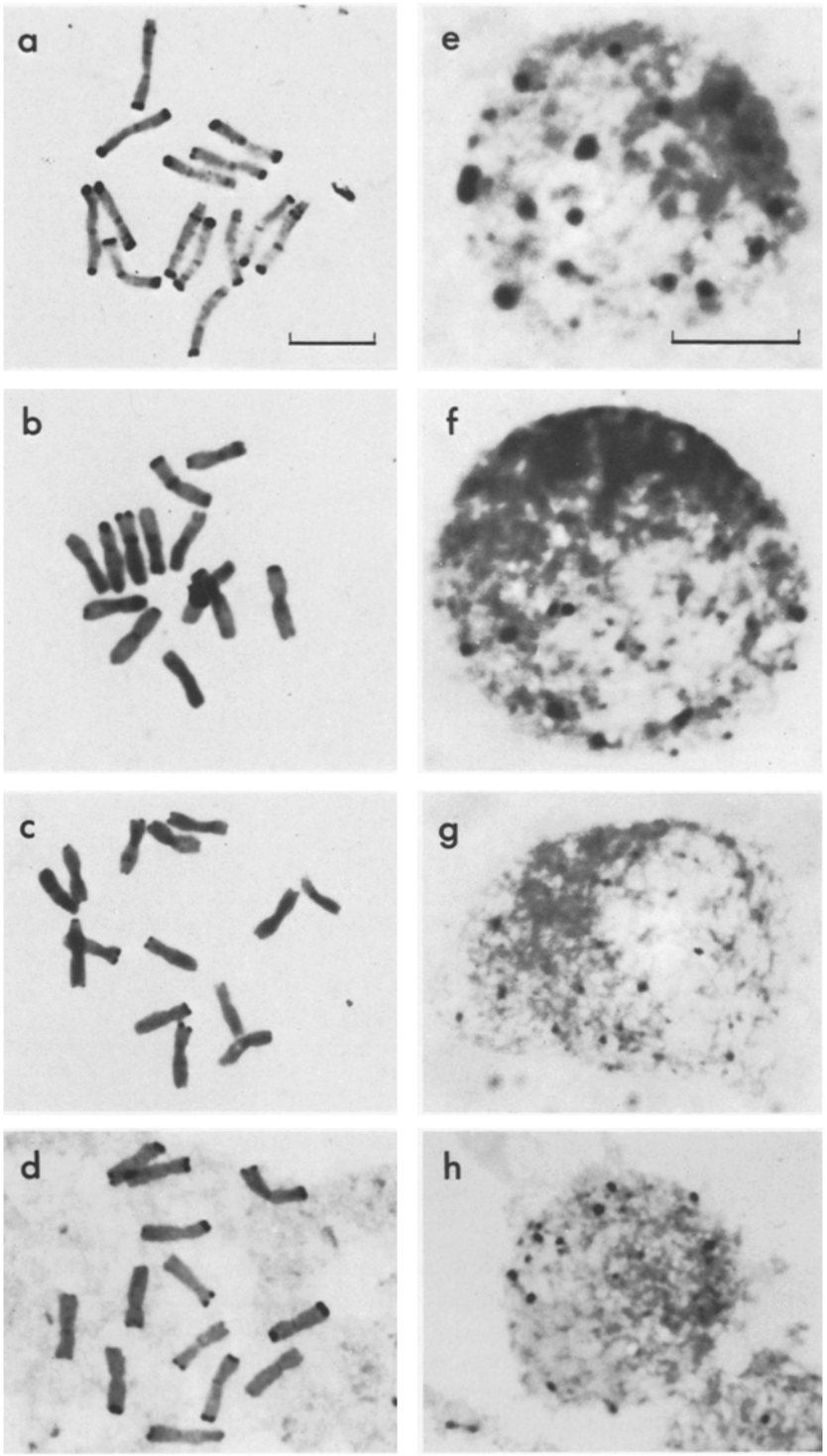


Fig. 1a–h. C-banding in root-tip metaphase (**a–d**) and interphase (**e–h**) nuclei of *Secale cereale* cv. "Petkus Spring" (**a**, **e**); *S. africanum* UM 2D127 (**b**, **f**); *S. silvestre* UM 2D49 (**c**, **g**); and *S. vavilovii* UM 2D49 (**d**, **h**). Note the correspondence between the amount of C-banding in metaphase and interphase nuclei of the same species. Micrographs **b–d**, **h** are the same magnification as **a**, while the rest are the same as **e**. Bars 10 μm long are shown

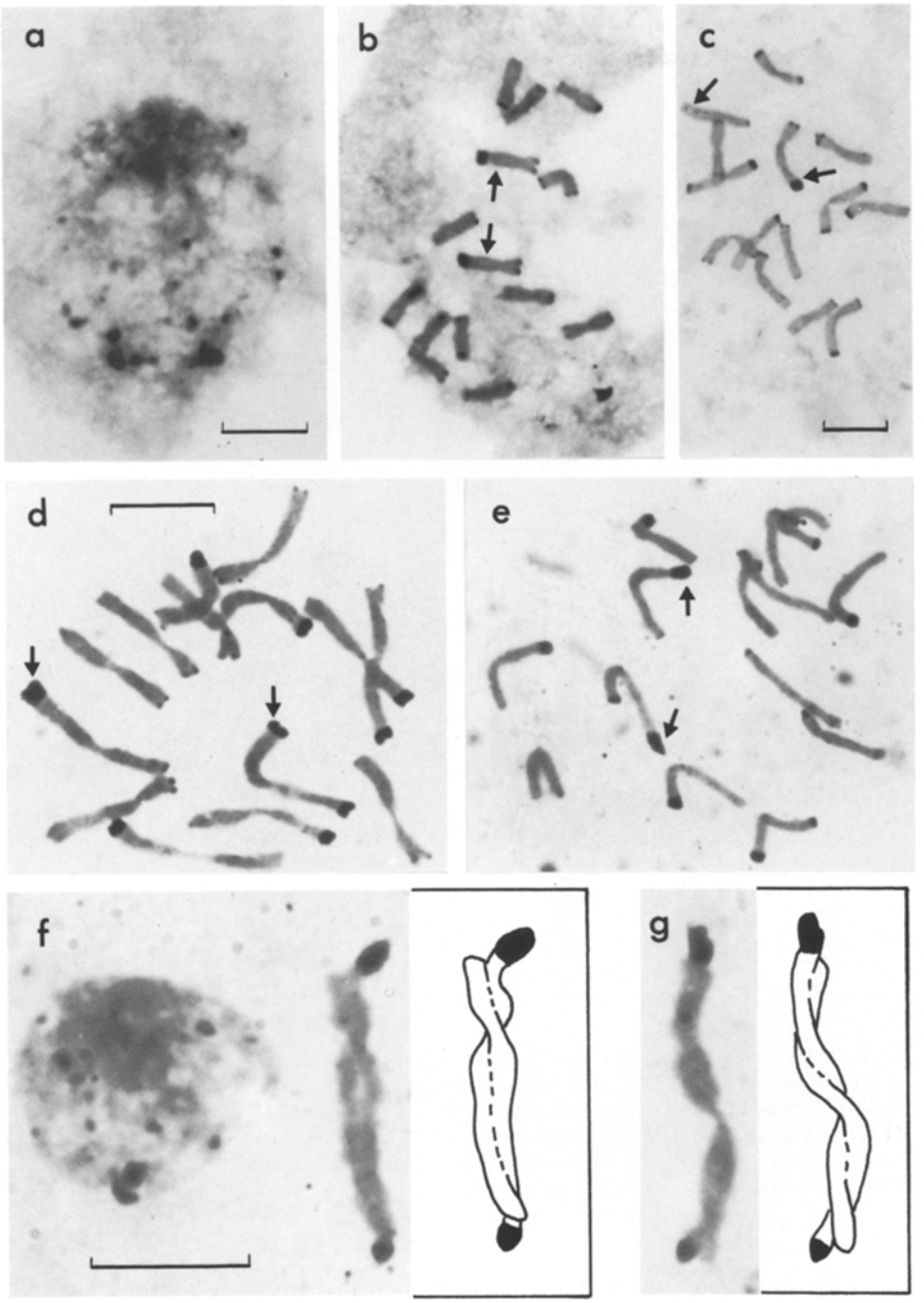


Fig. 2a–g. C-banding of interphase and metaphase root-tip chromosomes (**a–e**) and of meiotic chromosomes (**f, g**) showing polymorphism or heteromorphism for large terminal C-bands. **a–c** Cells from the same root-tip of *S. kuprijanovii* UM 2D34 either homomorphic for a large band on the short arm of chromosome 6 (arrowed) at interphase (**a**) and metaphase (**b**), and heteromorphic for this band (**c**). **d, e** Polymorphism between two plants of *S. dalmaticum* UM 2D19 (note also that arrowed chromosomes are heteromorphic for telomeric bands on one arm). **f, g** Diplotene bivalents in *S. anatolicum* PBI R98 heteromorphic for C-bands at both telomeres (note that the homologue with the large C-band projects beyond the telomere of the homologue with a small, or no, telomeric C-band), together with an anther wall interphase nucleus for comparison (**f**). Scale bars are 10 μm long. Micrographs **a** and **b** are the same magnification, as are **d** and **e**, and **f** and **g**

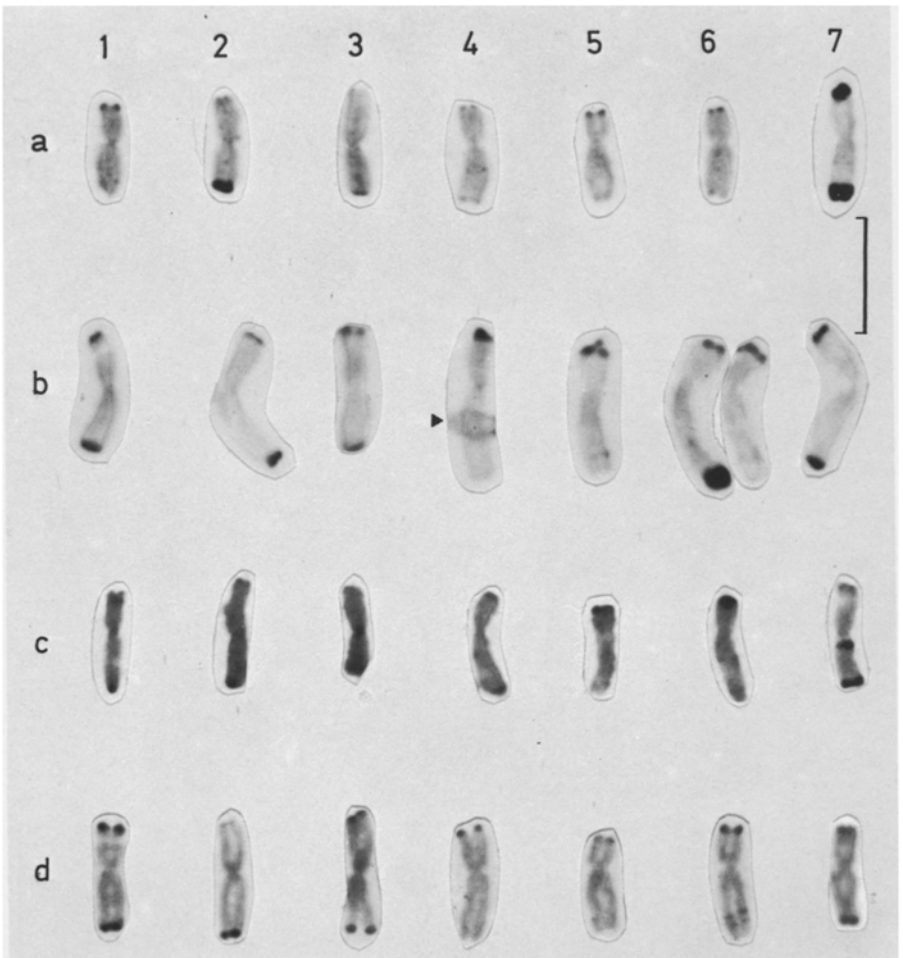
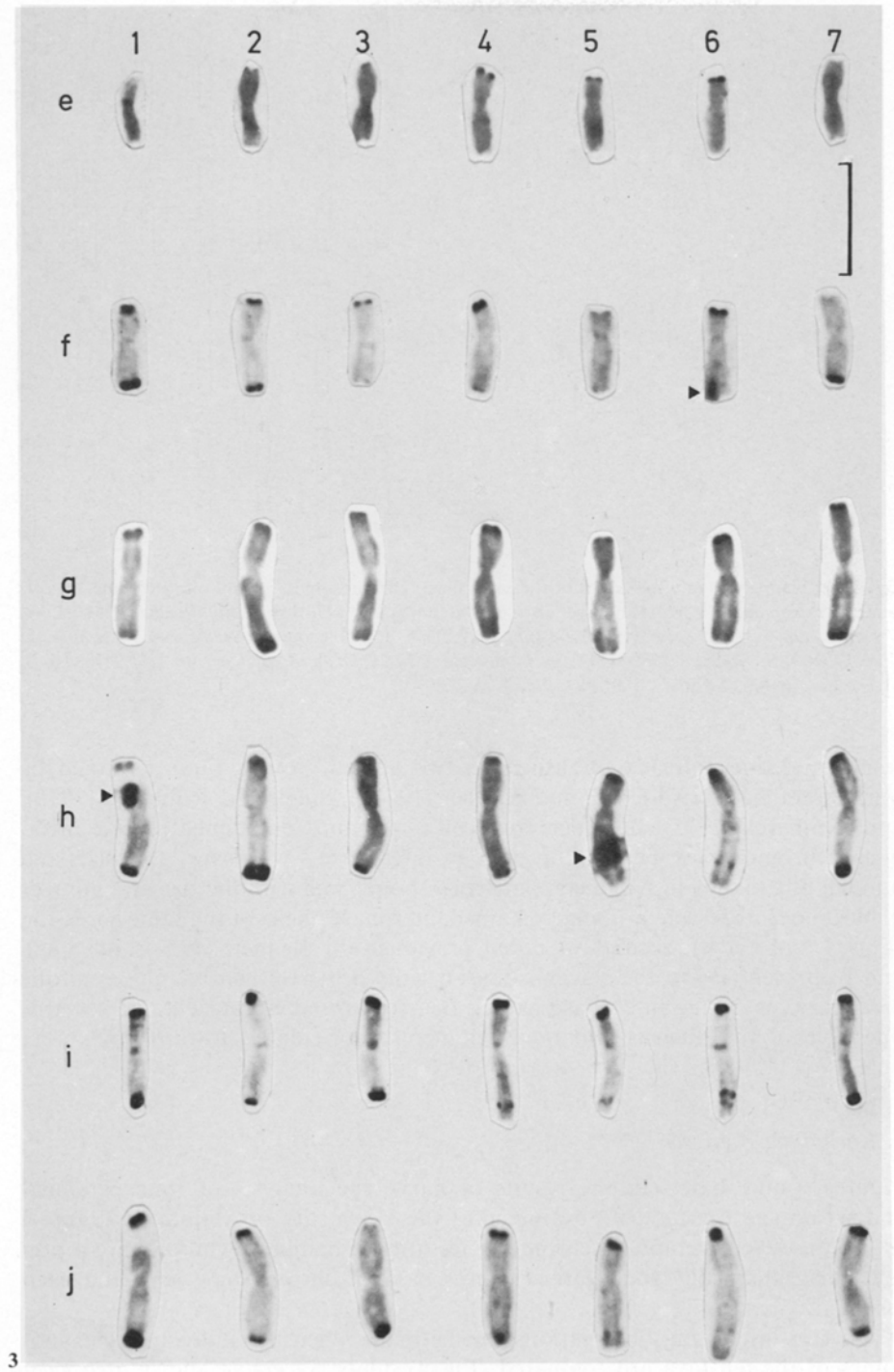


Fig. 3a–j. Comparative Leishman C-banding in the seven chromosomes in root-tip metaphase cells. **a–d** Four perennial *Secale* taxa; **a** *S. montanum* UM 2D23; **b** *S. kuprijanovii* UM 2D34; **c** *S. africanum* UM 2D127; **d** *S. anatolicum* UM 2D18. **e–j.** Six annual *Secale* taxa; **e** *S. silvestre* UM 2D59; **f** *S. vavilovii* UM 2D49; **g** *S. segetale* UM 2D37; **h** *S. dighoricum* UM 2D39; **i** *S. cereale* cv. "Petkus Spring"; **j** *S. ancestrale* UM 2D38. Scale bar is 10 μm long. (N.B. Overlapping chromosomes and artifacts are indicated by a ▶)

12 h; step 10, stain for 30–45 min) small centric bands on *S. vavilovii* and *S. cereale* (Fig. 1a) were obtained by the present authors without altering the appearance of the other bands. It is important to note, therefore, that the presence of centric bands on rye chromosomes can apparently vary independently in the constant presence of telomeric and intercalary bands, depending on the technique used.

Before presenting a detailed account of the present results for the distribution and size of C-bands, it seems worthwhile noting agreement between the present



3

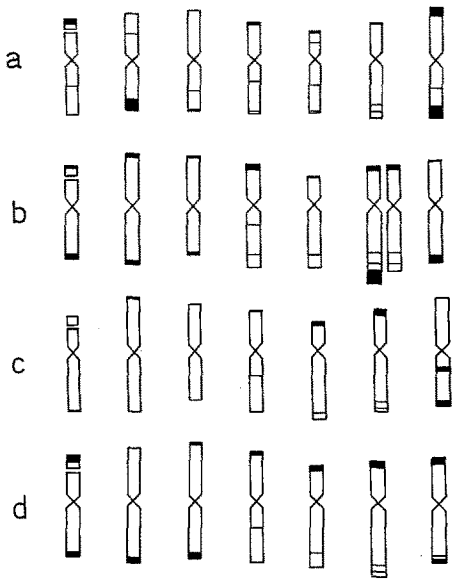


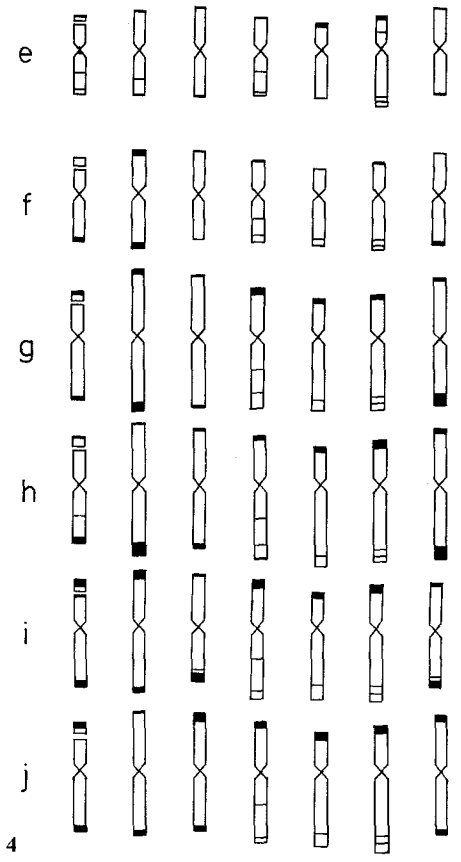
Fig. 4a–j. Karyotype analysis of root-tip metaphase chromosomes. **a–d.** Four perennial *Secale* taxa. **a** *S. montanum* UM 2D23 (see also Gustafson et al., 1976); **b** *S. kuprijanovii* UM 2D34; **c** *S. africanum* UM 2D127; **d** *S. anatolicum* UM 2D18, **e–j.** Six annual *Secale* taxa. **e** *S. silvestre* UM 2D59; **f** *S. vavilovii* UM 2D49; **g** *S. segetale* UM 2D37; **h** *S. dighoricum* UM 2D39; **i** *S. cereale* cv. "Petkus Spring"; **j** *S. ancestrale* UM 2D38

results and those already published on two general points. First, as noted by Verma and Rees (1974), Gill and Kimber (1974a), Singh and Röbbelen (1975), and Weimarck (1975) chromocentres seen as C-bands on stained mitotic metaphase chromosomes persist throughout interphase. Moreover, the size and number of C-bands seen at metaphase corresponds well with the size and number of chromocentres seen in similarly stained interphase nuclei of the same accession (Figs. 1 and 2a, b). Second, as noted previously by Bennett (1973) and Singh and Röbbelen (1975), the chromosomes usually preserve their anaphase mitotic arrangements during interphase having their centromeres coorientated towards one pole of the nucleus and their telomeres coorientated towards the other pole.

3. Karyotype Analysis

Figures 3 and 4 present the results of karyotype analysis of four perennial and six annual *Secale* taxa, examined at the University of Manitoba. Figure 3 shows the seven metaphase chromosomes after C-banding, while Figure 4 presents diagramatically the relative lengths of each chromosome arm and each terminal C-band.

Several interesting observations can be made when the individual chromosomes of the ten taxa are compared (Fig. 4). Only *S. cereale* and *S. africanum* constantly had intercalary bands on the satellited arm of chromosome 1, that



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of *S. cereale* being large while that of *S. africanum* was very small as noted by Singh and Röbbelen (1975). They noted an intercalary band on the satellited arm of chromsome 1 in *S. montanum,* but this was not observed in the present study (Fig. 4) or by Gustafson et al. (1976).

Chromsome 2, a sub-median chromosome, had either one or two terminal C-bands. In taxa with one terminal C-band this was always on the long arm, in taxa with two terminal C-bands the larger was always on the long arm except in *S. cereale* where it was on the short arm. This change in banding pattern may indicate that chromosome 2 was involved in one of the translocations known to have occurred during the evolution of *S. cereale.*

Chromsome 3, a median chromosome, varied greatly between taxa and ranged from no visible terminal C-bands (*S. africanum*) to two large terminal C-bands (*S. ancestrale*) (Fig. 4). Chromosome 3 of *S. africanum* in the present study differed from that observed by Singh and Röbbelen (1975) in not having terminal C-bands.

Chromosomes 4, 5 and 6, all sub-median, varied little in relation to each other. Thus, chromosome 5 was the most sub-median followed by chromosome 6, while chromosome 4 was the least sub-median of the three. Apart from

the polymorphism for chromosome 6 of *S. kuprijanovii* described below, the major variation within and between taxa was in the relative sizes of the terminal C-bands on the short arms of these three chromosomes. A striking instance of polymorphism in *S. kuprijanovii* was seen for the long arm of chromosome 6 which was either homomorphic for the presence of a very large terminal C-band or heteromorphic (Fig. 2b, c). Surprisingly, cells homomorphic and heteromorphic for chromosome 6 were observed in each squash made from a single root-tip taken from five individual plants. In the present study no cell was seen in which chromosome 6 was homomorphic for the absence of the band on the long arm. No similar instance of polymorphism was observed for any chromosome in any other taxon studied.

Chromosome 7 varied more between taxa than any other chromosome both in total size and the size of terminal C-bands, which ranged from two small bands (e.g. *S. silvestre*) to two large bands (e.g. *S. montanum*). Intermediate patterns included a large and a small band with the large band occurring on the long (e.g. *S. dighoricum*) or the short (e.g. *S. anatolicum*) arm.

Many intercalary bands were of erratic appearance both within and between cells on the same slide. Consequently no systematic analysis was made of the intercalary bands. However, a few intercalary bands which were of generally constant appearance are illustrated in Figure 4. For instance, chromosome 6 was the same in all taxa as it was in *S. cereale* (Darvey and Gustafson, 1975) in that from 1 to 6 intercalary bands were observed, but only two were of general appearance.

The present results agree with those of Singh and Röbbelen (1975) both in showing considerable interspecific variation for C-banding in the genus *Secale*, and in ranking species in the same order with respect to the relative amounts of heterochromatin in their complements. However, in *S. vavilovii* the dark band on the long arm of chromosome 4, and the intercalary band on the short arm of chromosome 1, noted by Singh and Röbbelen (1975), were not observed using the University of Manitoba technique. In general the differences between the present results and those of Singh and Röbbelen (1975) should not be viewed as contradictory, since such differences are most probably due to either variations in technique or intraspecific variation between accessions.

The present results show instances of polymorphism similar to those previously noted by Vosa (1974), Weimarck (1975), Darvey and Gustafson (1975), and Singh and Röbbelen (1975). Moreover, the present work shows that polymorphism was not restricted to small bands since polymorphisms for very large telomeric bands are reported for *S. kuprijanovii* (Fig. 2b, c), *S. dalmaticum* (Fig. 2d, e), and *S. anatolicum* (Fig. 2f, g). Although polymorphism is not uncommon in *Secale* taxa, its occurrence is not so great as to render C-banding patterns useless as diagnostic features of different species. Indeed the polymorphisms found in cultivated rye, where numerous accessions have been analysed, has not obscured the existence of a basic pattern, highly characteristic of the species. The number of accessions analysed for each taxon in the present work was small and, therefore, the karyotypes presented should not be regarded as definitive. Nevertheless, they are probably close approximations to the basic pattern for each taxon studied.

4. Nuclear DNA Amount and the Proportion of Telomeric Heterochromatin

In view of the results presented in the previous sections it is interesting to question whether the variation in 4C nuclear DNA content is related to variation in either chromosome size or the size of segments stained as C-bands. Qualitative visual assessment gives a clear impression that there may be a positive relationship between 4C nuclear DNA content and the amount of telomeric C-bands. For instance, *S. silvestre* and *S. africanum*, which both have low DNA amounts, also have the least telomeric heterochromatin.

A well-known positive correlation between chromosome size and nuclear DNA content exists when species from the same genus, with widely different nuclear DNA amounts, are compared (Rees et al., 1966; Jones and Rees, 1968a; Bullen and Rees, 1972; Pegington and Rees, 1971). However, a comparison of the relative total lengths of the haploid complement with the 4C DNA amount for nine *Secale* taxa (Fig. 5A) shows no significant relationship ($P=0.58$; $r=0.21$). Similarly, a comparison of the relative total length of the complement represented by telomeric C-bands with the 4C nuclear DNA content (Fig. 5B) for the nine taxa also shows no significant relationship ($P=0.33$; $r=0.37$).

Total metaphase chromosome volume per cell can vary by up to about 390% within a higher plant species (Pierce, 1937). Such variation, which occurs despite a constant 4C DNA amount, reflects the level of metabolic activity and occurs naturally (during ageing) as well as in response to various environmental treatments (Bennet and Rees, 1969; Bennett, 1970; Bennett et al., 1972). Thus, the absence of a relationship between chromosome length and nuclear DNA content for *Secale* taxa is not unexpected in view of the narrow range of variation in 4C nuclear DNA content (approximately 20%) compared with the large range of variation in total metaphase chromosome volume per cell seen in a single inbred line of *S. cereale* (up to 117%) (Bennett and Rees, 1969), and the large range of interphase chromosome volumes (up to 116%) noted by Gaspar (1973) for five of the nine *Secale* taxa used in the present comparison. Clearly intravarietal variation in chromosome size may obscure a relationship between 4C nuclear DNA content and the size of telomeric C-bands in *Secale*, making an alternative test of a possible relationship between these two characters desirable.

It has been shown that intraspecific variation in the total volume per cell of metaphase chromosomes with a constant 4C DNA amount involves corresponding changes in the size of each individual chromosome (Bennett, 1970). Thus, the proportion of the total length of the haploid complement made up of telomeric C-bands should remain relatively constant despite intraspecific variation in chromosome size between cells not involving variation in the 4C DNA amount. The present results show that the range of 4C DNA amounts in the genus *Secale* was small (about 20%), but that the total length (L.) of the haploid complement was always large compared with the total length of terminal C-bands (t. C-band 1.) (Table 3). Thus, the proportion of the total length of the haploid complement made up of telomeric C-bands should remain relatively constant, but decrease slightly, if increasing the 4C DNA amount

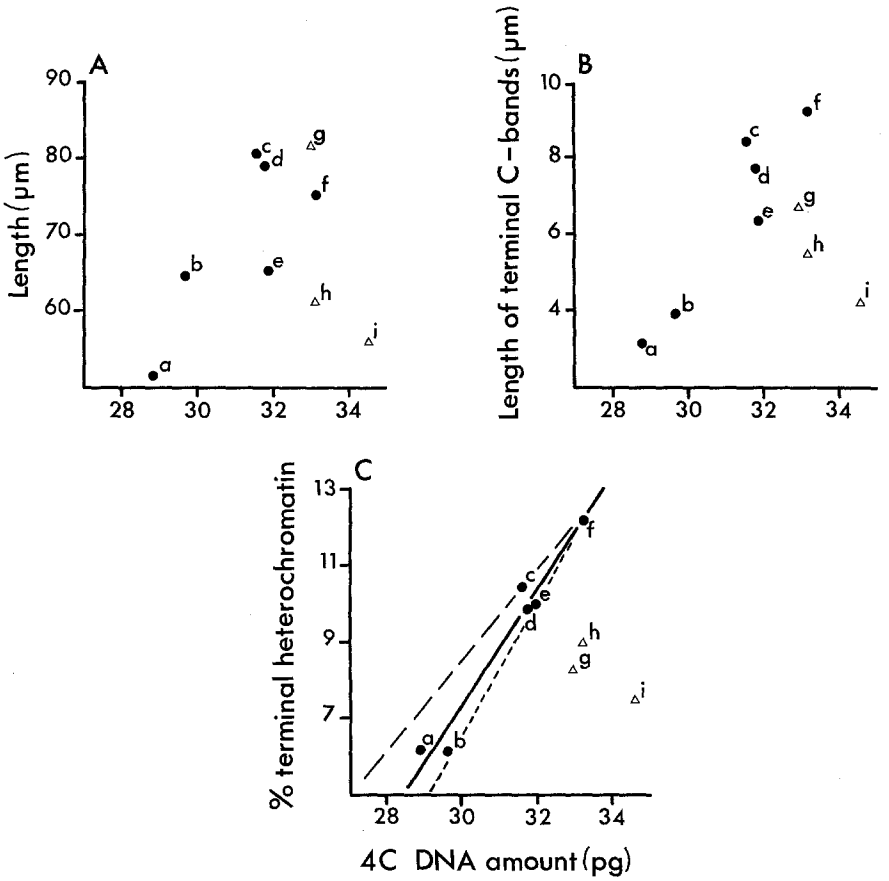

Fig. 5A–C. The relationship between the 4C nuclear DNA amount (see legend to Table 4) and: **A** the total length of the haploid complement; **B** the total length of terminal C-bands in the haploid complement; **C** the proportion of the total length of the haploid complement made up of terminal C-bands (as a percentage). (N.B. Values of L.; t.C-band 1.; and % t.C-band 1. plotted in (A), (B) and (C), respectively, are taken from Table 3). Key: *Group one taxa*: a *S. silvestre*; b *S. africanum*; c *S. dighoricum*; d *S. ancestrale*; e *S. kuprijanovii*; f *S. cereale*. *Group two taxa*: g *S. segetale*; h *S. montanum*; i *S. vavilovii*. (-----) Expectation assuming the evolution of cultivated rye involved the progressive addition of telomeric heterochromatin with a DNA density equal to that of euchromatin. (– –) Expectation assuming the evolution of cultivated rye involved the progressive addition of telomeric heterochromatin with a DNA density 1.5 times that of euchromatin. (——) The regression line for group one species

by 20% in *Secale* was achieved by adding DNA mainly or entirely to any chromosome segments other than telomeric C-bands. However, the proportion of the total length of the haploid complement made up of telomeric C-bands should be considerably larger in *Secale* taxa with high 4C DNA amounts only if the increase in 4C DNA amount per nucleus results mainly or entirely from an increase in the size and DNA content of segments stained as C-bands. Therefore, by comparing the proportion of the total length of the haploid complement made up of terminal C-bands in nine *Secale* taxa (Table 3) with

Table 3. Chromosome length (L) and the total length of the terminal C-bands per chromosome (t. C-band 1.), and the latter expressed as a percentage of the former (% t. C-band) in the haploid complements of 9 *Secale* taxa. (N.B. Lengths of chromosomes and telomeric C-bands are given in μm)

Taxon	Chromosome							Haploid Complement
	1	2	3	4	5	6	7	
1. *S. silvestre* UM 2D59								
L	6.60	7.80	8.13	6.80	6.53	8.03	7.75	51.64
t. C-band 1.	0.43	0.45	0.28	0.23	0.63	0.70	0.45	3.17
% t. C-band	6.52	5.77	3.44	3.38	9.65	8.72	5.81	6.14
2. *S. africanum* UM 2D127								
L	8.35	10.50	8.50	9.05	8.75	9.03	10.33	64.51
t. C-band 1.	0.63	0.40	0.23	0.20	0.53	0.70	1.25	3.94
% t. C-band	7.54	3.81	2.71	2.21	6.06	7.75	12.10	6.11
3. *S. dighoricum* UM 2D39								
L	9.65	12.75	11.25	11.70	11.30	11.48	12.48	80.61
t. C-band 1.	0.98	1.70	0.95	0.78	0.93	1.00	2.08	8.42
% t. C-band	10.16	13.33	8.44	6.67	8.23	8.71	16.67	10.45
4. *S. ancestrale* UM 2D38								
L	9.75	11.88	11.40	11.50	10.98	12.08	11.68	79.27
t. C-band 1.	1.50	1.05	1.13	0.70	1.00	0.98	1.43	7.79
% t. C-band	15.38	8.84	9.91	6.09	9.12	8.11	12.24	9.83
5. *S. kuprijanovii* UM 2D34								
L	8.13	10.20	8.93	9.40	8.23	10.75[a]	9.55	65.19
t. C-band 1.	1.05	0.95	0.55	0.53	0.30	2.00[a]	1.08	6.46
% t. C-band	12.92	9.31	6.16	5.64	3.65	18.60[a]	11.31	9.91
6. *S. cereale* cv. "Petkus Spring"								
L	9.93	11.88	10.50	11.50	10.30	11.25	10.13	75.49
t. C-band 1.	1.48	1.70	1.78	0.90	0.93	1.00	1.45	9.24
% t. C-band	14.90	14.31	16.95	7.83	9.03	8.89	14.31	12.24
7. *S. segetale* UM 2D37								
L	9.65	13.73	13.13	11.65	10.52	11.00	12.23	81.91
t. C-band 1.	0.78	1.55	0.65	0.78	0.73	0.55	1.70	6.74
% t. C-band	8.08	11.29	4.95	6.70	6.94	5.00	13.90	8.23
8. *S. montanum* UM 2D23								
L	8.25	8.95	9.25	8.38	7.42	8.50	10.50	61.25
t. C-band 1.	0.70	1.25	0.38	0.25	0.25	0.25	2.38	5.46
% t. C-band	8.48	13.97	4.11	2.98	3.37	2.94	22.67	8.91
9. *S. vavilovii* UM 2D49								
L	7.30	9.20	8.25	7.50	6.98	8.00	8.70	55.93
t. C-band 1.	0.75	1.30	0.50	0.28	0.40	0.25	0.70	4.18
% t. C-band	10.27	14.13	6.06	3.73	5.73	3.13	8.05	7.47

[a] The figures for *S. kuprijanovii* chromosome 6 are for the homomorphic variant with a large telomeric band present on the short arm of both homologues

their 4C nuclear DNA contents, it should be possible to test whether or not the present variation in 4C DNA amount involves large proportional changes in the DNA content of telomeric C-bands.

The proportion of the length of the complement made up of terminal C-bands ranged from about 6.1% in *S. africanum* UM 2D127 to about 12.2% in *S. cereale* cv. "Petkus Spring" with the other taxa showing continuous variation for intermediate proportions (Table 3). The proportion for *S. cereale* cv. "Petkus

Spring" (12.2%) is in good agreement with Verma and Rees (1974) who estimated that the C-bands occupied 14% of the total chromosome area at metaphase of rye with two B-chromosomes.

Analysis showed no significant relationship ($P=0.21$; $r=0.46$) between the proportion of the length of the haploid complement represented by terminal C-bands and 4C nuclear DNA content for the nine *Secale* taxa analysed. However, an alternative way of interpreting these results is to compare how the points plotted on Figure 5C compare with expectation assuming that *S. cereale* cv. "Petkus Spring" (the species with the highest proportion and amount of telomeric heterochromatin) was derived from an ancestral form with little or no telomeric heterochromatin, by the progressive addition of telomeric heterochromatin. The dotted line on Figure 5C shows the expectation for increasing the proportion of heterochromatin from zero to 12.24% assuming that heterochromatin has the same DNA density as euchromatin. However, heterochromatin often has a higher DNA density than euchromatin. No precise measurement of the relative densities of DNA in euchromatin and heterochromatin are available, but Jones and Rees (1968b) estimated the relative density at metaphase of DNA in supernumerary B-chromosomes of *S. cereale* (which are known to be more heterochromatic than A-chromosomes) and A-chromosomes to be 1.5 and 1.0, respectively. Thus, the dashed line on Figure 5C shows the expectation for increasing the proportion of heterochromatin from zero to 12.24% assuming that the DNA density in heterochromatin is 1.5 times that in euchromatin. Figure 5C shows that points for five of the eight remaining taxa fall on, or between, the two expectation lines, while the points for the other three taxa depart greatly from expectation, but all in the same direction. Thus, with respect to expectation, the taxa are divided into two groups. Those in group one (which fit expectation) are *S. silvestre, S. africanum, S. dighoricum, S. ancestrale, S. kuprijanovii,* together with *S. cereale* (the species on which the expectation is based). The regression line for these six taxa ($P=0.005$; $r=0.94$) is shown (Fig. 5C) for comparison with the two expectation lines. The taxa in group two (which depart greatly from expectation) are *S. segetale, S. montanum* and *S. vavilovii.* It should be noted that this division of *Secale* taxa into two groups does not appear to correspond with previously emphasized taxonomic divisions within the genus, such as between cleistogamous and allogamous taxa, or between annuals and perennials. Thus, any taxonomic implications of the present division must be viewed with caution.

Figure 6 gives separate plots for each of the seven chromosomes of the haploid complement for the proportion of the length of the chromosome represented by terminal C-bands against the 4C nuclear DNA content for nine *Secale* taxa. Analysis for all nine taxa in groups one and two showed (Table 4) no significant relationship between 4C DNA amount and the proportion of total length of a single chromosome made up of terminal heterochromatin ($P<0.31$; $r>\pm 0.33$) except for chromosome 2 ($P=0.003$; $r=0.87$). However, analysis for the six taxa in group one shows a significant relationship ($P<0.05$; $r>0.84$) between the proportion of the total length of a single chromosome made up of terminal heterochromatin and 4C DNA amount per cell for chromosomes 1, 2, 3, and 4 (Table 5), but not for chromosome 5 ($P=0.90$; $r=-0.07$),

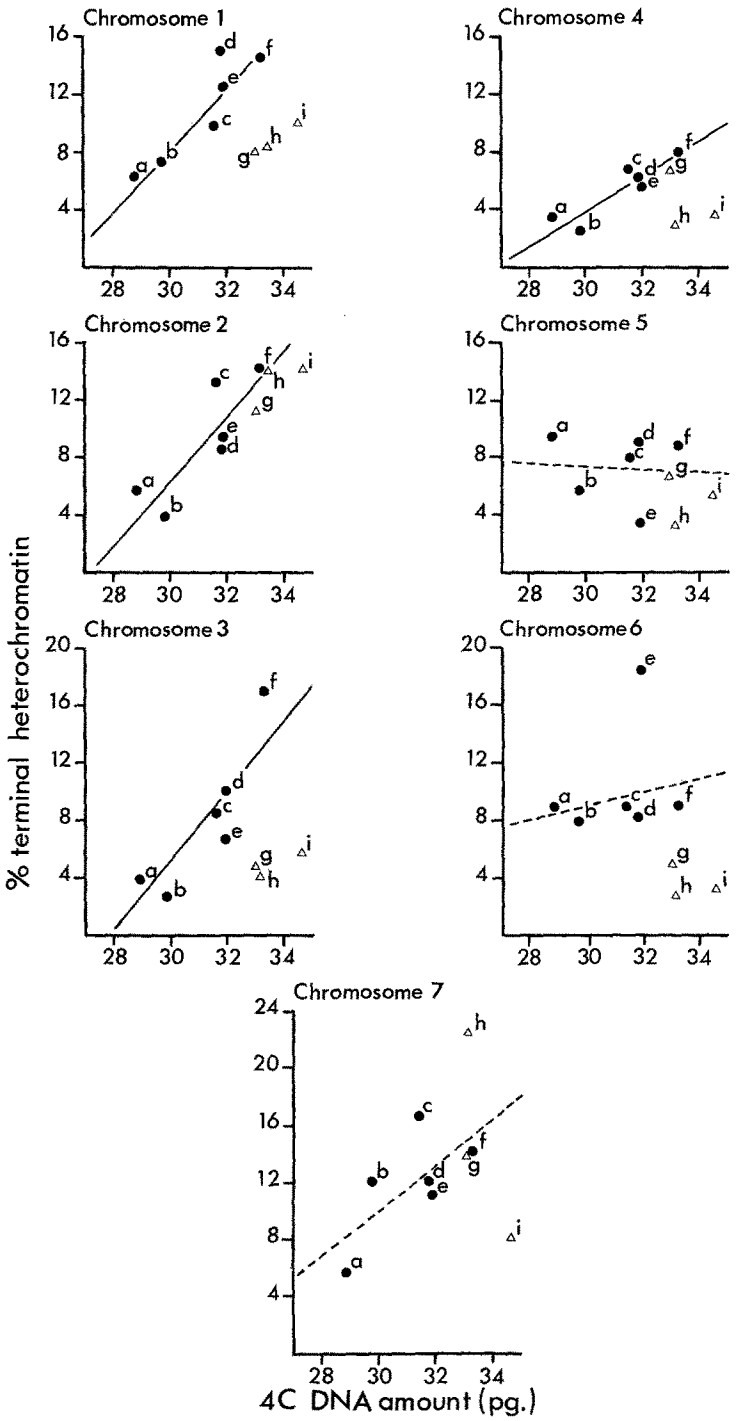

Fig. 6. The relationship between 4C nuclear DNA content (see legend to Table 4) and the proportion of the length of each individual chromosome made up of terminal C-bands (i.e. values of % t. C-band 1. for individual chromosomes taken from Table 3). (N.B. The regression line for taxa in group one is shown as a solid line when significant at the 5% level, and as a broken line otherwise. The key to the taxa is the same as for Fig. 5)

Table 4. A comparison of the proportion of the length of each chromosome made up of terminal heterochromatin (% t. C-band 1.—from Table 3) with 4C nuclear DNA amount (from Table 2) for nine *Secale* taxa. (N.B. % t. C-band 1. values are compared with 4C DNA values for the same accession for *S. africanum* 2D UM127; *S. ancestrale* UM 2D38; *S. cereale* cv. "Petkus Spring"; and *S. vavilovii* UM 2D49. Otherwise % t. C-band 1. values are compared with 4C DNA values for *S. silvestre* PBI R52; *S. dighoricum* PBI R40; *S. kuprijanovii* PBI R35; the mean 4C DNA value for *S. segetale* PBI R41 & UM 2D56; and the mean 4C DNA value for *S. montanum* PBI R15 & UM 2D35)

Chromosome	MS		DF	F Ratio	P	r
1	Regression	12.0876	1	1.18	0.3131	0.380
	Error	10.2348	7			
2	Regression	89.7714	1	21.02	0.0025**	0.866
	Error	4.2698	7			
3	Regression	21.2062	1	1.11	0.3267	0.371
	Error	19.0788	7			
4	Regression	3.6320	1	0.91	0.3703	0.340
	Error	3.9642	7			
5	Regression	5.6467	1	1.03	0.3431	−0.359
	Error	5.4626	7			
6	Regression	22.3036	1	1.03	0.3419	−0.360
	Error	21.4662	7			
7	Regression	22.7315	1	0.95	0.3618	0.346
	Error	23.8878	7			

Table 5. A comparison of the proportion of the length of each chromosome made up of terminal heterochromatin (% t. C-band 1.—from Table 3) with 4C nuclear DNA amount (from Table 2) for the six *Secale* taxa in Group one. (N.B. The 4C values used are as stated in the legend to Table 3)

Chromosome	MS		DF	F Ratio	P	r
1	Regression	58.1081	1	18.76	0.012*	0.908
	Error	3.0961	4			
2	Regression	60.4478	1	10.21	0.0330*	0.848
	Error	5.9188	4			
3	Regression	105.0652	1	13.54	0.0211*	0.879
	Error	7.7542	4			
4	Regression	18.6685	1	20.83	0.0102*	0.916
	Error	0.8958	4			
5	Regression	0.1206	1	0.10	0.8997	−0.067
	Error	6.7012	4			
6	Regression	5.7815	1	0.28	0.6219	0.258
	Error	20.3116	4			
7	Regression	32.5400	1	3.89	0.1197	0.702
	Error	8.3581	4			

chromosome 6 (P=0.622; r=0.26) and chromosome 7 (P=0.120; r=0.70). Examination of the plots for individual chromsomes (Fig. 6) shows that the distribution of group two taxa for chromosomes 1, 3, and 6 is very similar to their distribution in the corresponding plot for the whole complement (Fig. 5C), i.e. they all lie together well below the regression line for group

one taxa. For the remaining chromosomes, there is a general tendency for group two taxa to correspond with their distribution in Figure 5C.

These results indicate that interspecific variation in the proportion of terminal heterochromatin in the complement of group one is, in general, associated with corresponding proportional variation involving at least four of the seven chromosomes. Similarly, the lower proportion of heterochromatin in the complement of group two than in group one with corresponding 4C DNA amounts (Fig. 5C) reflects a smaller proportion of terminal heterochromatin in several chromosomes.

The highly significant positive relationship between the proportion of the complement represented by terminal heterochromatin and the 4C nuclear DNA content for the six species in group one (Fig. 5C) provides compelling evidence in support of the notion that the increases in 4C nuclear DNA content in this group of *Secale* taxa has involved a large increase in the amount of telomeric heterochromatin. Strong supporting evidence for this conclusion comes from a comparison of DNA amount per diploid genome in *Secale* and in species from related genera in the Gramineae, and from biochemical studies of nuclear DNA from *S. cereale* cv. "Petkus Spring" and *S. silvestre* PBI R52 (see Discussion, p. 170, 171). Additional support is provided by observations of C-banded diplotene bivalents in plants of *S. anatolicum* PBI 98 polymorphic for a large terminal C-band (Fig. 2f, g) showing that the extra DNA of the large terminal C-band projects unpaired beyond the telomere of its homologue with a smaller or no telomeric C-band.

Because the relative densities of the DNA in segments seen as C-bands and other segments is unknown, it is impossible to estimate exactly how much of the variation in 4C nuclear DNA content is accounted for by variation in the amount of telomeric heterochromatin. Nevertheless, meaningful calculations based on reasonable assumptions can be made. Given the regression for group one (Fig. 5C), and assuming that within the complement of a single taxon the distribution of DNA is directly proportional to chromosome length, and that the density of DNA in C-bands and other segments is the same, then about 57% of the increase in 4C DNA amount for taxa in group one would be accounted for by the increase in the proportion of the complement represented by bands of telomeric heterochromatin. However, as heterochromatin frequently has a higher density than euchromatin, it is reasonable to suppose that the increase in the amount of heterochromatin has accounted for a somewhat higher percentage of the increase in 4C DNA amount.

The evolution of group two species has also clearly involved a considerable increase in 4C DNA amount since wheat and *Secale* diverged from their common ancestor. However, the three taxa in group two differ from those in group one by having a lower proportion of terminal heterochromatin than a group one species with a corresponding 4C nuclear DNA amount. For instance, if *S. vavilovii* UM 2D49 (34.58 pg) were in group one, it would be expected to have about 14% terminal heterochromatin, but it has only about 7.5%. Thus, a considerable part of the extra DNA in group two species is not present in telomeric C-bands but in euchromatin. It would be premature to speculate whether the additional euchromatin in group two species is at the telomeres

or, whether it was added as euchromatin or derived by the conversion to euchromatin of previously heterochromatic segments.

Discussion

1. Variation in Secale Chromatin in Relation to Triticale

As noted in the introduction the present work was undertaken primarily to screen the genus *Secale* for taxa whose chromosomes match those of wheat more closely than those of *S. cereale* cultivars previously used as parents for triticale; i.e. for chromosomes with lower 4C DNA amounts and less terminal heterochromatin. The present work shows clearly that there are naturally occurring *Secale* taxa with significantly less DNA per chromosome than cultivated rye, and naturally occurring *Secale* taxa with significantly less telomeric heterochromatin than cultivated rye. This and the strong correlation between 4C DNA amount and the amount of telomeric heterochromatin found for *Secale* taxa in group one (Fig. 5C) suggests that it may be relatively easy to obtain strains of cultivated rye whose chromosomes have both a reduced DNA content and less telomeric heterochromatin for use as rye parents in triticale breeding.

2. 4C DNA Amount in Secale in the Context of the Gramineae

Extensive surveys of DNA amounts in several genera of the Gramineae (Bennett and Smith, 1976) indicate that DNA amount per diploid genome varies considerably within the family, and often within the genus. For instance, variation in DNA amount per diploid genome has been reported in *Aegilops* (14%, Furuta, 1970), *Avena* (37%, Bennett and Smith, 1976) *Briza* (55%, Murray, 1975), *Bromus* (72%, Bennett and Smith, 1976), *Lolium* (43%, Jones and Rees, 1967), *Phalaris* (78%, Kadir, 1974), *Poa* (40%, Bennett and Smith, 1976), *Sorghum* (65%, Paroda and Rees, 1971) and *Triticum* (41%, Bennett and Smith, 1976). Only in Hordeum, (4%, Bennett and Smith, 1971) was no significant intrageneric variation found. Thus, the present results for *Secale* are in general agreement with previous results for the Gramineae in showing intrageneric variation in 4C DNA content per diploid genome. However, the range of variation for this character (about 20%) is small compared with that previously noted for other genera, some of which are listed above. This, and the fact that there are no natural polyploid *Secale* species supports the view that the genus *Secale* is of recent origin.

Secale contains some of the largest diploid genomes known in the Gramineae. Indeed, out of the 186 naturally occurring taxa listed by Bennett and Smith (1976) only five (*Briza maxima*, 43.2 pg; *B. media*, 33.8 pg; *B. minor*, 29.2 pg; *Aegilops bicornis*, 29.3 pg; and *Ae. triaristata*, 31.0 pg) have larger diploid genomes than the smallest *Secale* genome known (*S. silvestre*, 28.9 pg), while only two (*B. maxima* and *B. media*) have amounts larger than the largest *Secale* genome (*S. vavilovii* UM 2D49, 34.6 pg). DNA contents for diploid

Secale genomes (28.9–34.6 pg) are similar to, or larger than, the largest values in the ranges of the two closely related genera with which *Secale* taxa readily hybridize, namely, *Aegilops* (14.5–31.0 pg) and *Triticum* (19.7–27.6 pg), and overlap slightly with the former but not the latter. Thus, from a comparison of DNA amounts in *Secale* taxa with those of species in the genera with which it is most closely related, it may safely be assumed that its evolution has involved an increase in nuclear DNA amount from the time *Secale* diverged from its common ancestor with wheat.

3. The Mechanism Responsible for DNA Variation in Secale

Ever since the large ranges of genome sizes and DNA contents in higher plants were first recognised there has been continuing discussion regarding possible mechanisms which may give rise to increases in DNA amount per chromosome (Rees and Jones, 1972). Among the mechanisms proposed are: duplications (Wilson and Sparrow, 1960), differential polyteny (Martin and Shanks, 1966; Rothfels and Heimberger, 1968), cryptopolyploidy (Sparrow and Nauman, 1973), chromosome fusion by Robertsonian translocation (Jones, 1974), saltatory replication (Flavell et al., 1974), and a large number of unequal crossing-over events (Smith, 1976). It is unlikely that the same mechanism is responsible for all the variation in DNA amount per chromosome and per genome in higher plants, and therefore it would be unwise to draw general conclusions from the present results regarding the source of such variation. Nevertheless, it seems worthwhile considering which of the mechanisms listed above offers the most likely explanation for the present variation in *Secale*.

The total variation in 4C nuclear DNA amount within *Secale* is estimated to be about 20% spread among the chromosomes. Moreover, the variation, which appears to be continuous, is largely due to variation in the amount of heterochromatin located at, or close to, the telomeres. Consequently, chromosome fusion by Robertsonian translocation, differential polyteny, and cryptopolyploidy are all excluded, while duplications, saltatory replication, and a large number of unequal crossing-over events are all theoretically possible explanations for the variation in *Secale*.

Biochemical analyses of the types and arrangement of highly repeated sequences in *S. cereale* cv. "Petkus Spring" and *S. silvestre* PBI R52 (Flavell and Smith, personal communication) have shown that *S. cereale* has very highly repeated sequences (i.e. present in about 50,000 to 250,000 copies) not amplified in *S. silvestre*, while the very highly repeated sequences of *S. silvestre* are all found in *S. cereale*. Furthermore, the highly repeated sequences found in *S. cereale* but not in *S. silvestre* are clustered in the chromosomes and appear to occupy about 10% of the 4C DNA amount in *S. cereale*. They are thus good candidates for being major constituents of the telomeric heterochromatin not found on *S. silvestre* chromosomes. The fact that *S. cereale* contains highly reiterated sequences not amplified in *S. silvestre* (with a lower DNA amount) mitigates very strongly against duplications of large chromosome segments being responsible for the addition of DNA at the telomeres of *Secale* taxa in group

one, since if duplications were responsible they would not introduce new highly reiterated sequences, but slightly increase the reiteration of already highly repeated sequences.

While unequal crossing-over and saltatory amplification are both possible explanations of the observed variation in telomeric DNA in *Secale* the short time scale imposed by the supposed recent origin of *Secale* makes the former very unlikely. However, a saltatory amplification event producing tens of thousands of copies is more plausible to account for the relatively recent addition of telomeric heterochromatin. Moreover, saltatory replication could explain the addition of DNA at one or more telomeres in *Secale*. Instances are known in which the DNA content of one, or several, chromosomes has been significantly increased in somatic cells of higher plants and although the mechanisms responsible are not understood, they presumably involve saltatory amplification types of event. Perhaps the best known example of a sudden increase in the DNA content of a single chromosome is the occurrence of megachromosomes in *Nicotiana* [due to differential additional replication of a prominent block of heterochromatin (Gerstel and Burns, 1967; Collins et al., 1970)], while Durrant's flax genotrophs provide an intriguing example of an increase in DNA involving many chromosomes. In the latter, divergence of nuclear DNA content of up to 16% (i.e. similar in proportion, though not in amount, to that reported in *Secale* in the present work) was induced in somatic cells during five weeks of different environmental treatments (Evans et al., 1966; Evans, 1968).

While unequal crossing-over between homologues at meiosis is unlikely to have been responsible for the initial large scale addition of heterochromatin at a given telomere, nevertheless, it could have played an important role in extending the range of variation in the amount of heterochromatin at that telomere subsequent to a saltatory amplification event. The recent cytological demonstration of somatic recombination between homologues (Huttner and Ruddle, 1976) raises the possibility that unequal crossing-over between homologues might also occur in somatic cells. It seems worthwhile also considering whether crossing-over between non-homologues might provide a useful mechanism allowing an increase in the amount of telomeric DNA at one telomere to be distributed between chromosomes.

Crossing-over between non-homologues involving segments bearing major genes is usually suppressed since the resulting translocations are either extremely rare or lethal. However, ectopic pairing of heterochromatic segments containing the same satellite DNA on non-homologous chromosomes is known to occur (Mayfield and Ellison, 1975). Provided the telomeres of non-homologous chromosomes possess highly repeated sequences in common, as seems highly likely, then crossing-over could involve non-homologous chromosomes. Provided the repeated sequences involved were distal with respect to all major genes then no genetic recombination or imbalance should result. Thus, given the conditions just mentioned, crossing-over between non-homologues would allow an increase in the amount of heterochromatin at one telomere to become translocated to the telomeres of other chromomes. The observed spatial arrangement of rye chromosomes appears to provide ample opportunity for crossing-over at the telomeres of homologues and non-homologues in both somatic and meiotic

cells. Thus, during the meiotic cycle there is a pronounced bouquet stage in *S. cereale* during which all the telomeres frequently become associated in a single large chromocentre (Thomas and Kaltsikes, 1976), while a reduction in the number of chromocentres at interphase compared with metaphase in somatic cells in *S. cereale* is generally attributed to the fusion of telomeres (Singh and Röbbelen, 1975; Godin and Stack, 1975).

While there is no evidence proving conclusively that unequal crossing-over occurs at the telomeres in rye, there are several observations which would be explained if it did occur. These include the observation of polymorphism for telomeric C-bands within inbred lines of *S. cereale* (Weimarck, 1975), and the occurrence of "modified" rye chromosomes in inbred hexaploid triticales which have lost or have a smaller C-band at a given telomere (Gustafson and Bennett, 1976). It is realized that breakage and loss of telomeric heterochromatin are alternative explanations of these observations.

The observed variation in *Secale* prompts two questions. First, what mechanism is responsible for the addition of heterochromatin at a single telomere? Second, what is the explanation for the addition of heterochromatin at telomeres on many or all of the chromosomes in the complement? While a single mechanism can answer both questions by acting at one or many telomeres, it is also possible that one mechanism results in the addition of heterochromatin at one telomere while another distributes it between chromosomes. Thus, it is suggested that the variation in the amount of telomeric heterochromatin in *Secale* has probably resulted mainly in the first instance from saltatory amplification events on one or more chromosomes, but that subsequently unequal crossing-over between homologues, and perhaps non-homologues, may have played an important secondary role in distributing the newly amplfied sequences to telomeres throughout the karyotype.

Kranz (1957) stated that *Secale* is an actively expanding genus in which evolutionary research offers great promise and interest. The present work supports this view. By coupling the cytochemical and biochemical techniques of quantitative microdensitometry, C-banding, in situ hybridisation and repeated sequence analysis it should be possible to investigate the timing, frequency and nature of variation in the type and amount of DNA at single known telomeres, and to test directly which of the possible mechanisms is responsible.

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